Protection of abasic sites during DNA replication by a stable thiazolidine protein-DNA crosslink

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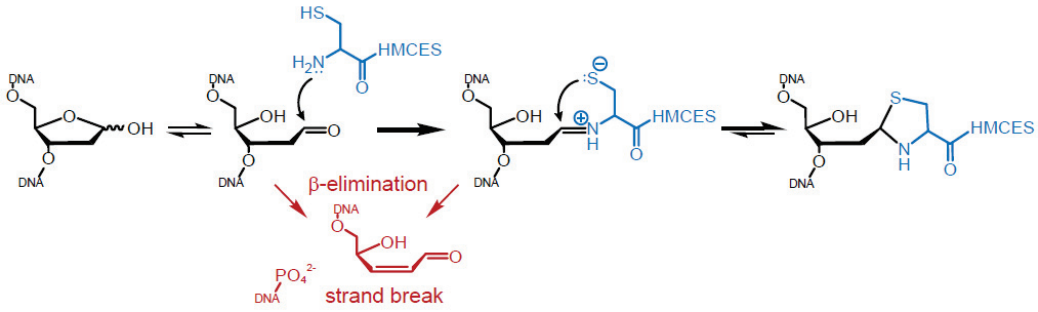
Genome instability resulting from replication stress underlies cancer. Apurinic / apyrimidinic (abasic or AP) sites are one of the most common DNA lesions and can block replicative polymerases. Until recently, the major mechanisms to overcome this replication challenge was thought to be translesion synthesis by error-prone polymerases [1]. However, a new pathway dependent on the SRAP (SOS-Response Associated Peptidase) domain protein HMCES (5-Hydroxymethylcytosine Binding, ES Cell Specific) was discovered recently that provides an alternative that improves cell viability and reduces mutation frequency [2]. HMCES recognizes and processes AP sites in the context of single-stranded DNA (ssDNA) in a manner distinct from base excision repair of AP sites in double-stranded DNA [3,4]. A HMCES DNA-protein crosslink (DPC) intermediate is thought to shield the AP site from endonucleases and error-prone polymerases. The highly evolutionarily conserved SRAP domain of HMCES and its *Escherichia coli* ortholog YedK mediate lesion recognition. Here we discover the basis of AP site protection by SRAP domains from a crystal structure of the YedK DPC together with biochemical analysis of the human and bacterial proteins. HMCES forms a stable thiazolidine linkage between a ring-opened AP site and the α-amino and sulfhydryl substituents of its N-terminal cysteine residue. The thiazolidine linkage explains the remarkable stability of the HMCES DPC, its resistance to strand cleavage, and the proteolysis requirement for resolution. Furthermore, its structure reveals that HMCES has specificity for AP sites in ssDNA at junction structures found when replicative polymerases encounter the AP site lesion.



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