Protection Of Abasic Sites During DNA Replication

Apurinic and apyrimidind (abasic or AP) sites are the most common DNA lesions that have the ability to block replicative polymerases. Stem cell-specific 5-hydroxymethylcytosine-binding protein (HMCES) covalently crosslinks to the AP site and prevents genome instability. An HMCES DNA-protein crosslink (DPC) with an AP site within a 3? overhang DNA is able to shield the Ap site from endonucleases and error-prone polymerases. The conserved SOS-response associated peptidase (SRAP) domain of HMCES and its Escherichia coli ortholog YedK are able to mediate lesion recognition by interaction with a single strand- and double-strand-containing DNA structures found in DNA damage sites. AP site is being protected by a stable thiazolidine linkage, which causes HMCES DPC stability.

Human HMCES contains a single domain called SRAP proteins, which is 38.5% identical to that of E. coli YedK. KAREEM 2019 SRAP is a protein made up of an invariant cysteine, a conserved histidine, and glutamic acid and existed inside a putative catalytic pocket. A positively charged surface is available next to this pocket. DNA binding depends on the arginine lined on this surface. KAREEM 2019 Regarding binding to DNA, both HMCES and YedK strongly prefer ssDNA compare to dsDNA ligands. Wang 2019 These DNA-protein crosslink (DPSs) are exposed to DNA damage that generates AP sites. KAREEM 2019AP site is repaired by base excision repair (BER), which is dependent on an intact DNA duplex. Not only base loss happens in ssDNA more rapidly than dsDNA, but also dsDNA AP sites that have not been repaired are converted into ssDNA. AP sites and replicative polymerase will stall at the AP site and leave a 3’ dsDNA-ssDNA junction PETRIA 2019. It has been revealed that AP are typical DNA lesion and lead to hypersensitivity of HMCES cells to DNA damaging agents. KAREEM 2019 DPC formation in cells is dependent on the HMCES catalytic cysteine and DNA binding surface. Therefore, HMCES is known as a sensor of AP sites and covalently modifies the ssDNA AP site to generate a DPC. KAREEM 2019 Here, we discuss a recent discovery of HMCES as a sensor which shield AP sites in ssDNA at replication forks.

Main

DNA glycosylase and glycosylase/abasic (AP) lyases are the enzymes that initiate the base excision repair pathway. In such a process, they recognize the damaged target base and catalyze the breakage of the base?sugar glycosyl bond. DPC can be formed by covalently binding of AP site to PARP-1 during alkylating agent-induced base excision repair (BER). This complex formation depends on existence of natural AP sites in DNA and single-strand DNA incision Rajendra 2014 It has been showed that detecting of HMCES DPC in cells is comfortable,KAREEM 2019, its incubation does not change the percentage of crosslinked protein, and only a small peptide-DNA linkage remains stable after proteolysis of the DPC with proteinase K. PETRIA 2019 Altogether suggest HMCES DPC as a stable chemical linkage and irreversible in physiological conditions. PETRIA 2019

Both the Trp81Glu substitution at dsDNA-interaction site A and Arg4Ala substitution at dsDNA-interaction site B are essential for substrate DNA binding the HMCES SRAP domain with 3? overhang DNA which contains an AP site. In order to structure a reactive aldehydic form of the AP site which has the ability to crosslink to HMCES, the 3’ overhang to obtain a deoxyuridine

(dU) at position 9 of the longer DNA strand was designed. Levon 2019 The AP site is located above Cys 2 which is at the N terminus of the protein. Addition of an N-terminal GST fusion protein inhibits DPC formation. KAREEM 2019In order to crosslinking happens between AP and Cys2 of HEMES, the N- terminal methionine needs to be removed to expose the NH2 of Cys2 and produce a catalytically active form of HMCES. Cys2 and AP react in ring-opened aldehyde to form a thiazolidine DNA-protein crosslink. Levon 2019

Cys2 belongs to a cluster of three conserved residues that includes Glu 105 and His 160 PETRIA 2019 This kind of evolutionary conserved residues cause DNA and protein sides of the thiazolidine linkage to be stable. PETRIA 2019 The electron density is located clearly the AP in the ring-opened form, so HMCES reacts with the aldehyde in the ring-open way. KAREEM 2019 In addition, a continuous density between C3? and this cysteine side chain has been showed. KAREEM 2019

The anomeric C1? carbon of the AP site bond covalently to the ?-amino nitrogen and the side chain sulfur of Cys2. When AP aldehyde C1? carbon by Cys 2 ?-NH2 generates a Schiff base intermediate by nucleophilic attack, it causes Cys 2 sulfhydryl group attack C1? and form a thiazolidine ring. PETRIA 2019 YedK DPC formation is diminished by removing thiol from C2A mutant KAREEM 2019

As in the reaction between cysteine and aldehydes, the equilibrium favors thiazolidine wang 2019 compared to Schiff base, no DNA lyase activity resulted from ?-elimination of the Schiff base intermediate can be observed. PETRIA 2019, both the C2A and C2S mutant showed DNA lyase activity when incubated with ssDNA containing an AP site, which is reduced when crosslinking happens in the presence of sodium cyanoborohydride (NaBH3CN). NaBH3CN is a reducing agent which causes the Schiff base intermediate stabilization. PETRIA 2019 Therefore, in this mechanism, the Schiff base intermediate is captured as a result of the nucleophilic attack to the cysteine thiol.PETRIA 2019

DNA also interacts with SRAP inside the dsDNA interaction site, which contains Gly3, Arg4, Pro 46, Asp 47, Trp128. This interaction site is evolved to bind duplex DNA, which represents a potential binding site for 5’overhang DNA. Levon 2019 A crystal structure of YedK linked noncovalently to an ssDNA oligomer containing a C3-spacer in place of the AP site and a protein in the non-covalent complex identical to that of DPC. Wang 2019 In this non-covalent complex, the DNA at the 5’ end is positioned identical to that of the DPC structure, while its 3’ end is mobile, which results in a crystal packing difference between the two complexes. Both these structures show that the SRAP domain accommodates dsDNA on the 3’ side of the AP site.PETRIA 2019 On the 3’ side, all nucleobases of the AP site are stacked in a B-DNA conformation and the residues adjacent to the AP site stacks on the surface of the protein against Pro40 and Ile74 which forms a highly conserved ‘shelf’ which stabilize a base pair 3’ to the AP site. Conservations in this region show that binding to AP sites in the context of a 3’-truncated ssDNA-dsDNA junction is an important feature. PETRIA 2019

A 1.6-Å resolution crystal structure of E. coli YedK covalently crosslinked to a heptamer ssDNA containing an AP site showed that a positively charged channel is formed inside the core ?-sheet that structures the ssDNA phosphoribosyl backbone along one face of the protein. PETRIA 2019 This backbone is kinked and twisted by 90° at the AP site and cause the nucleobases of each flanking trinucleotide be orthogonal to one another which explains why SRAP disfavors binding to dsDNA. levon 2019 Among the SRAP domains, residues inside the

DNA-binding channel are the most highly conserved ones which shows conservation of DNA-binding modality. PETRIA 2019 Both YedK and HMCES prefer to bind to ssDNA and the mutation of conserved amino acid diminishes DNA binding for both proteins. Wang 2019

The crystal structure of SRAP-DNA complex that contains a three-nucleotide overhang at the 3? end shows that SRAP binds to both 3'end of DNA and blunt-end of another DNA, while both interaction surfaces were highly conserved. SRAP binding to 3'end happens by a hydrophobic shelf created by Tr 81 and Phe92 and interact with the duplex segment of DNA at the ssDNA-dsDNA junction. Levon 2019 Therefore, as DNA polymerase stall at an AP site leaves a 3?-truncated nascent strand with a 5?-overhanging template, SRAP proteins should function at a stalled replication fork. HMCES is efficient at binding and crosslinking to an AP site immediately adjacent to the 3? ssDNA-dsDNA junction as to ssDNA but not at the presence of dsDNA on the 5'end PETRIA 2019

To conclude, it has been revealed that HMCES shield AP sites from further processing by exonucleases during DNA replication and promote genome stability. Cells detect DNA lesions by using HMCES as sensors. HMCES recognizes AP sites in ssDNA and modifies the lesion to generate a DPC. HEMCES and SRAP-containing proteins crosslink to AP sites at 3' end and it has substrate preference because of DNA formation happens when polymerases stall at Ap sites. The thiazolidine ring involves the NH2 of Cys2 and acts as a sink for AP sites and prevents strand breaks. This thiazolidine DPC link and SRAP-AP-DNA crosslink cause genome stability and improve organism fitness.