OP345

Arabidopsis thaliana and wheat AP endonucleases contain the NIR function

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Aim of the study: Apurinic/apyrimidinic (AP) endonucleases are important DNA repair enzymes involved in two overlapping pathways: DNA glycosylase-initiated base excision (BER) and AP endonuclease-initiated nucleotide incision repair (NIR). In the BER pathway, AP endonucleases cleave DNA at AP sites and 3'-blocking moieties generated by DNA glycosylases, whereas in NIR, the same AP endonucleases incise DNA 5' to a wide variety of oxidized bases. The flowering plant Arabidopsis thaliana contains three genes encoding homologues of major human AP endonuclease 1 (APE1): Arp, Ape1L and Ape2. It has been shown that all three proteins contain AP site cleavage and 3'-repair phosphodiesterase activities. However, it was not known whether the plant AP endonucleases contain the NIR activity.

Material and Methods: To characterize the DNA repair activities involved in the BER and NIR pathway we have used affinity-purified the *A. thaliana* and wheatproteins from *E. coli* expressing the His-tagged form of atArp and wArp. To examine DNA substrate specificity of plant AP endonucleases, DNA cleavage activities of atARP and wARP towards 30-mer THF•T and αdA•T duplexes (in which THF and αdA residues were in position 11) were measured under reaction conditions optimal for the hAPE1-catalyzed AP endonuclease and nucleotide incision activity, respectively. The *Arabidopsis thaliana* mutant lines SALK_021478 (*arp–/–*), harboring T-DNA insertions in the ARP gene were used to test the sensitivity to MMS, *t*-BuO2H and H2O2 exposure. Here, we report that ARP proteins from Arabidopsis and common wheat (*Triticum aestivum*) contain NIR and 3'→5' exonuclease activities in addition to their AP endonuclease and 3'-repair phosphodiesterase functions.

Results: The steady-state kinetic parameters of reactions indicate that Arabidopsis ARP cleaves oligonucleotide duplexes containing α-anomeric 2'- deoxyadenosine (αdA) and 5,6-dihydrouridine (DHU) with efficiencies (kcat/KM = 134 and 7.3 μM–1•min–1, respectively) comparable to those of the human counterpart. However, the ARP-catalyzed 3'-repair phosphodiesterase and 3'→5' exonuclease activities (kcat/KM = 314 and 34 μM–1•min–1, respectively) were about 10-fold less efficient as compared to those of hAPE1. Expression of ARP greatly reduces the sensitivity of AP endonuclease-deficient *Escherichia coli xth nfo* and *Saccharomyces cerevisiae Δapn1 Δapn2* strains to both alkylating and oxidizing agents. Furthermore, homozygous *A. thaliana arp-/-* mutant exhibits high sensitivity to methyl mehtansulfonate and *tert*-butyl hydroperoxide, but not to H_2O_2, suggesting that ARP is a major AP endonuclease that removes abasic sites and specific types of oxidative DNA base damage. Taken together, these data establish the presence of the NIR pathway in plants and suggest its possible role in the repair of DNA damage generated by oxidative stress.

Keywords: DNA repair; nucleotide incision repair; base excision repair; AP endonuclease.