

Repair of DNA damage induced by alkylating agents in *Arabidopsis thaliana*

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Methyl methanesulfonate (MMS) is an alkylating agent that reacts with DNA causing extensive methylation of the N7 position of guanine residues, among other alkylation damages. Base excision repair (BER) is a critical pathway in cellular defence against DNA damage, but its relevance in N7-methylguanine (N7-meG) repair remains unknown. BER is initiated by DNA glycosylases that recognize and excise damaged bases, generating abasic (apurinic/aprimidinic, AP) sites. AP sites may be processed by either AP endonucleases or AP lyases, but the factors that influence the participation of one or other type of enzyme are still unknown. Our research group has previously demonstrated that the DNA 3'-phosphatase ZDP of *Arabidopsis thaliana* is involved in the BER pathway, and ZDP deficient plants are hypersensitive to MMS. This suggests that the repair of DNA damage induced by MMS may be ZDP dependent in *Arabidopsis*.

In this work we developed an experimental system allowing *in vitro* mimicking of repair of damage induced by alkylating agents in the genome of *A. thaliana*. As a DNA substrate we used a double stranded oligonucleotide with a N7-meG residue in a defined position. Our results show that N7-meG spontaneously hydrolyzes generating an AP site. The processing of these AP sites in *zdp* mutants leads to an accumulation of 3'-P repair intermediates. In contrast, such intermediates are not observed in *fpg* mutants. These results suggest that FPG processes the AP sites arisen by spontaneous depurination of N7-meG, generating 3'-P intermediates that are hydrolyzed to 3'-OH by ZDP. We also studied why ARP, the main AP endonuclease in *Arabidopsis*, has a secondary role in this process. We found that FPG shows a marked preference for AP sites paired with C, whereas ARP shows preference for AP sites paired with G. Altogether, our results suggest that the base opposite the lesion is an important factor determining whether BER proceeds by an AP endonuclease or an AP lyase-dependent sub-pathway, thus establishing the involvement of specific proteins in subsequent steps of the repair process.