Opposite base specificity during DNA repair of abasic sites

M. Jordano-Raya[1,2,3], T. Roldán-Arjona[1,2,3], R.R. Ariza[1,2,3] and D. Córdoba-Cañero [1,2,3]

[1] *Department of Genetics, University of Córdoba, Córdoba, Spain*
[2] *Maimonides Biomedical Research Institute of Cordoba (IMIBIC), Córdoba, Spain*
[3] *Reina Sofía University Hospital, Spain*
E-mail: b52joram@uco.es

Abasic (apurinic/apyrimidinic, AP) sites are ubiquitous DNA lesions that arise though spontaneous base loss or by the catalytic activity of DNA glycosylases on damaged or modified bases during base excision repair. Processing of AP sites may proceed by either AP endonucleases or AP lyases, but the relative roles of these two types of enzymes are not well understood. We hypothesized that sequence flanking the AP site and/or the identity of the orphan base on the complementary DNA strand may determine the enzyme responsible for its processing. We have analyzed AP endonuclease and AP lyase activity from plant, bacteria and human on DNA substrates containing an abasic site opposite either G or C in different sequence contexts. We found that the major *Arabidopsis* AP lyase (FPG) exhibited preference for AP sites opposite C, whereas no preference for the orphan base was observed in the AP lyase activity detected in human cells extracts. In contrast, the major plant AP endonuclease (ARP), in either recombinant or native form, preferred AP sites opposite G. However, the major human AP endonuclease (APE1) did not show any significant preference for the orphan base. Interestingly, we found that bacterial AP endonucleases, such as Exo III and Endo IV behave similarly to the plant enzyme and also prefer G as the base opposite the abasic site.

Through structural and homology modelling, we searched for differentially conserved amino acids between animal, plant and bacterial AP endonucleases, focusing on those residues that may interact with the orphan base. One such residue is Met270 in human APE1, whose homologs are Arg488 in *Arabidopsis* ARP and Arg216 in *E. coli* ExoIII. Importantly, at this amino acid position most animal AP endonucleases have methionine, whereas plant and bacterial enzymes usually have arginine. To test the prediction that Arg488 has a role in the preference for the orphan base of *Arabidopsis* ARP, we generated two mutant versions in which Arg488 is changed either to glycine (R488G) or methionine (R488M). We found that both mutants proteins have lost the capacity to distinguish between cytosine and guanine on the complementary strand. Our results suggest that the preference for G opposite the abasic site is an ancestral feature of AP endonucleases, and that such specificity has been lost in the metazoan lineage.