Influence of the orphan base on AP endonuclease activity in Base Excision Repair

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Abasic (apurinic/apyrimidinic, AP) sites are ubiquitous DNA lesions that can arise from the spontaneous loss of a nitrogenous base or as intermediates during the Base Excision Repair (BER) pathway. AP sites can be processed by either AP endonucleases or AP lyases, but the relative roles of these two types of enzymes are not well understood. We hypothesized that the sequence flanking the AP site and the orphan base opposite the lesion may determine the enzyme responsible for its processing and the repair efficiency. We compared the AP incision activities of human, bacterial, and plant AP endonucleases using DNA substrates containing an abasic site opposite guanine (G), adenine (A), thymine (T), or cytosine (C). We observed no preference for the opposite base in the major human AP endonuclease, APE1. However, a strong effect of the orphan base was observed for the plant (*Arabidopsis* ARP) and the bacterial (*Escherichia coli* Exo III) orthologues, which showed their lowest efficiency on AP sites opposite C. Using structural and homology information we identified differentially conserved residues in APE1, ARP, and Exo III. Mutations of these residues resulted in significant changes in AP site processing, depending on the orphan base. Our results suggest that opposite-base specificity is an ancestral feature of AP endonucleases that may have been lost in the metazoan lineage. The lack of specificity in APE1 may be related to its ability to efficiently cleave AP sites on both single-stranded (ssDNA) and double-stranded DNA (dsDNA). In contrast, Arabidopsis ARP exhibits an inability to incise AP sites on ssDNA. We have identified specific residues responsible for discriminating between these two types of DNA substrates. Our study highlights the functional differences between human, plant, and bacterial AP endonucleases and provides new insights into the evolution of DNA repair pathways.

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