GENETIC DISSECTION OF THE BIOCHEMICAL ACTIVITIES OF HUMAN DNA REPAIR PROTEIN, APE1

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Introduction. Human apurinic/apyrimidinic endonuclease 1 (APE1) is a key DNA repair enzyme involved in both base excision repair (BER) and nucleotide incision repair (NIR) pathways. In the BER pathway, APE1 cleaves DNA at AP sites and 3'-blocking moieties generated by DNA glycosylases. In the NIR pathway, APE1 incises DNA 5' to a number of oxidatively damaged bases. Here we propose to identify and characterize critical amino acids of APE1 involved in either BER and/or NIR functions by using the alignment of the known three-dimensional (or tertiary) structures of Xth family AP endonucleases including the *Methanothermobacter thermautotrophicus* Mth212, *Bacillus subtilis* ExoA (1), *E. coli* Xth and human APE1 proteins (2).

Materials and methods. Crystallization of the *Bacillus subtilis* ExoA protein and characterization of its substrate specificities on DNA containing various modified residues. Structural alignment of tertiary conformations of four homologous AP endonucleases Mth212, ExoA, Xth and APE1. Construction of NIR-deficient APE1 mutants and their characterization.

Results. We obtained 3D structure of ExoA and identified amino acid residues in APE1 that affect its function in either the BER or NIR pathway. Biochemical characterization of APE1 carrying single G231S and T268D amino acid substitutions revealed that all mutants exhibited greatly reduced NIR and 3'->5' exonuclease activities, but were capable of performing BER functions.

Conclusions. Taken together, these data further substantiate the role of NIR as a distinct and separable function of APE1 that is essential for processing of potentially lethal oxidative DNA lesions.

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References.

1. Shida, T., Ogawa, T., Ogasawara, N. and Sekiguchi, J. (1999) Characterization of Bacillus subtilis ExoA protein: a multifunctional DNA-repair enzyme similar to the Escherichia coli exonuclease III. *Biosci. Biotechnol. Biochem.,* **63,** 1528-1534
2. Mol, C.D., Izumi, T., Mitra, S. and Tainer, J.A. (2000) DNA-bound structures and mutants reveal abasic DNA binding by APE1 and DNA repair coordination. *Nature,* **403,** 451-456.