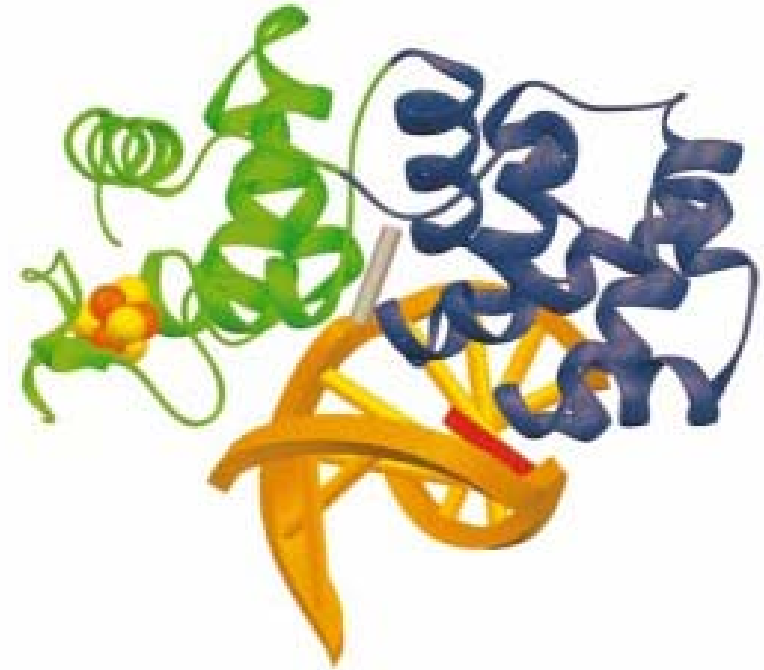


Trapping of the Hnth-Nucleosomal DNA Schiff Base intermediate

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Oxidative phosphorylation or ionizing radiation may generate oxidative species which can induce base damage in DNA. hNth, the human homologue of Endonuclease III, is a bifunctional DNA glycosylase that possesses a Base Excision Repair activity and an APlyase activity to repair damaged bases such as thymine glycol. The mechanism for this repair proceeds via a Schiff base intermediate, which can be reduced to form a covalent adduct using NaBH_4 . Using this method, hNth can be trapped in the act of repairing DNA and this complex can be used to study how DNA repair enzymes gain access to nucleosomal DNA.



J.Fromme, G.Verdine. Structure of a trapped endonuclease III-DNA covalent intermediate. EMBO J, 22, 13, 3461-3471 (2003).

Undergraduate Ken Loh (Harvey Mudd '09) carried out this trapping using *E.coli* Endonuclease III and P32 labeled DNA. DNA containing uracil was made using Polymerase Chain Reaction and Uracil DNA glycosylase in conjunction with Endo III was used to create the Schiff base intermediate for trapping. The human homologue hNth was purified using an overexpression plasmid that contained the coding sequence for hNth, a gift from Rabindra Roy of Georgetown University. The vector overexpresses the full length hNth attached to the C terminus of the GST-protein, allowing separation using a glutathione–Sepharose column through affinity chromatography. The primary goal of this project is to purify the trapped complex of hNth in the act of repairing nucleosomal DNA to investigate how DNA repair enzymes gain access to DNA which is supercoiled around histone octamers.