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Order and disorder in base excision DNA repair

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Motivation and Aim: Structures of many polypeptides possess intrinsically disordered regions, which may be important for protein–protein and enzyme–substrate interactions, protein intracellular localization, etc. [1, 2]. A number of such proteins may be found in base excision repair (BER), a pathway that purges our genome of most lesions generated in DNA by spontaneous and induced oxidation, deamination, alkylation, and base loss [3, 4]. However, the roles of the disordered regions in these proteins are mostly unknown.

Methods and Algorithms: We have used X-ray crystallography and computational methods (molecular dynamics, coarse-grained Monte Carlo simulations, *ab initio* folding, molecular docking, etc.) to determine experimentally or predict structures of several human and bacterial DNA glycosylases and apurinic/apyrimidinic (AP) endonucleases, the enzymes that catalyze the first two steps of the BER pathway. Direct enzyme kinetics, binding and substrate perturbation methods combined with site-directed mutagenesis were used to verify functional predictions made based on the structural information.

Results: Three main structural classes of DNA glycosylases are α/β-fold uracil–DNA glycosylase-like (UNG-like) proteins (*Escherichia coli* Ung and Mug, human UNG, TDG, and SMUG1), helix–hairpin–helix (HhH) motif containing proteins (*E. coli* Nth, MutY, and AlkA, human OGG1, NTHL1, MUTYH, and MBD4), and helix–two-turn–helix (H2TH) motif containing proteins (*E. coli* Fpg and Nei, human NEIL1, NEIL2, and NEIL3) [4, 5]. AP endonucleases belong to exonuclease–endonuclease–phosphatase (EEP) structural family (*E. coli* Xth, human APEX1) and triose isomerase barrel (TIM barrel) structural family (*E. coli* Nfo) [4, 5]. Most human homologs have extended N- and C-terminal tails while their bacterial counterparts are limited to the catalytic core. Even in two-domain bacterial H2TH glycosylases Fpg and Nei, the linker between the domains is well-ordered and restructures itself for substrate DNA binding. Surprizingly, the most disordered region in Fpg and Nei was their substrate-binding pocket, which may explain profound substrate promiscuity of these enzymes in terms of the lesions recognized. The terminal tails in human DNA glycosylases and APEX1 are predicted as disordered and are not seen in the X-ray structures but molecular modeling reveals their tendency to exist in several conformations rather than be completely disordered. Some of these conformations are well suitable for facilitating DNA binding or protein multimerization on DNA. Several human DNA glycosylases (NEIL2, MBD4, MUTYH) possess long internal unstructured regions, which we found to possess autoinhibitory properties possibly relieved upon interaction with other BER participants or with specific DNA elements such as methylated CpG sites.

Conclusion: Disordered regions in DNA glycosylases and AP endonucleases are involved in non-specific DNA binding, discrimination of damaged *vs* undamaged bases, and regulation of enzyme activity. Unlike truly intrinsically disordered regions, many of them likely populate a limited number of distinct conformations, which could have specific functional roles.

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