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Crystallographic and calorimetric studies with nuclear transport of DNA repair proteins

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The DNA damage can occur by several agents which may promote instability of the genome leading to several diseases such as cancer, neurological disorders, immunodeficiencies and premature aging. To avoid this serious consequences, cells have evolved a number of DNA repair pathways, which carry out the process in multiple steps to repair specific DNA damage, and maintain the integrity of the genome. The nuclear import is a pre requisite for the functions of DNA repair proteins and their correct location is essential. The classical nuclear import pathway is the best characterized and, probably the most used protein import mechanism to the cell nucleus, which involves the binding of the cargo protein via nuclear localization sequence (NLS) recognized by the importin-α protein (Impα). Classical nuclear localization sequences are targeting signals that link the cargo proteins to the Impα import receptor. They are formed by one or two basic clusters of amino acid residues, termed monopartite or bipartite NLSs. Aiming to understand the structural basis of the nuclear import process of DNA repair proteins, we have used X-ray crystallography and calorimetric tools to study the interaction between Impα and the NLS regions of each protein. Five different complexes have been studied, which cover the most important DNA mechanisms: i) base excision repair (BER) - FEN1 protein, ii) nucleotide excision repair (NER) - XPG protein, iii) mismatch repair (MMR) - PMS2/MLH1 heteodimer and iv) non-homologous end joining (NHEJ) - Ku70/Ku80 heterodimer. We demonstrated that all these proteins are able to be transported to the cell nucleus by classical nuclear import pathway using monopartide or bipartite NLS sequences. However, we demonstrated that, for many of these proteins, the NLS regions are different from the previously described data in literature. In addition, new structural features related to minor and linker NLS binding regions were observed, which may give specificity for the transport of different DNA repair proteins.

References:

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Structural characterization and comparison of crystallization behaviour of selected haloalkane dehalogenases

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Halogenated aliphatic compounds represent one of the largest groups of environmental pollutants. Haloalkane dehalogenases are responsible for one of the key reactions in the bacterial degradation of various halogenated pollutants. Apart from applications in bioremediation, haloalkane dehalogenases can be potentially applied in biosensing of pollution, biosynthesis, cellular imaging and protein immobilization. These enzymes catalyze the cleavage of a carbon-halogen bond in haloalkanes with water as the sole co-substrate, resulting in formation of a halide ion, a corresponding alcohol, and a proton. The role of conformational flexibility has been well established in connection with the accessibility of the active site, the binding of substrates and ligands, and release of products, stabilization and trapping of intermediates, orientation of the substrate into the binding cleft or adjustment of the reaction environment.

Several haloalkane dehalogenase structures were determined by X-ray diffraction analysis of enzymes' crystals, providing a good theoretical framework for their modification by protein engineering. Crystallization conditions for haloalkane dehalogenases DhaA from Rhodococcus rhodochrous NCIMB 13064, LinB from Sphingobium japonicum UT26, and DbeA from Bradyrhizobium elkanii USDA94 and their mutant variants were compared and analyzed. Analysis of crystallization cocktails revealed common components for the majority of compared dehalogenases such as divalent cations such as calcium or magnesium, and medium size polyethylene glycols (PEGs) 3350 or 4000 as well as almost neutral pH. Instead of X-ray diffraction analysis our model systems have been also investigated by other structural methods such as neutron crystallography, time-resolved crystallography and hydrogen-deuterium exchange mass spectrometry. Based on carefully designed experiments and by combination of the information obtained from different, but complementary, techniques we will be able to get inside into (i) conformational changes of selected enzymes upon their interactions with substrates, (ii) location of hydrogen atoms inside the enzyme active site and the access tunnels and (iii) protonation state of catalytic residues of the enzymes during their catalytic cycles.

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Keywords: haloalkane dehalogenases, crystallization, structure