The structure and mechanism of two proteins involved in DNA repair: UvrA and RNase H2

Marcin Nowotny

Laboratory of Protein Structure, International Institute of Molecular and Cell Biology, Warsaw, Poland

DNA in the cell constantly incurs chemical damage which must be repaired. One of the most general mechanisms of this repair is Nucleotide Excision Repair (NER) pathway. Its main feature is the ability to recognize and repair a wide spectrum of different DNA lesions. In bacteria the first protein in this pathway is a dimeric ABC ATPase called UvrA. Its role is to locate the site of the DNA lesion. In order to elucidate the mechanism of DNA damage detection by UvrA, we solved its crystal structure in complex with a modified DNA. In the structure, the protein does not interact with the DNA modification site itself but binds the duplex on both sides of the lesion. The DNA in the complex is deformed: bent, stretched and unwound. These types of deformation are induced by various types of DNA modifications. We therefore propose that UvrA uses them for indirect readout of the presence of the damage.

Another type of DNA damage occurs during replication and involves misincorporation of single ribonucletides. They are mutagenic and have to be removed. The only enzyme that can initiate this process is RNase H2. While bacterial and archaeal RNases H2 are monomeric, all eukaryotic RNases H2 comprise three subunits – the catalytic one and two auxiliary ones. Mutations of the human enzyme lead to a severe autoimmune disorder called Aicardi-Goutières syndrome (AGS). In order to elucidate the mechanism of action of RNases H2, we have determined structures of a bacterial enzyme in complex with nucleic acid substrate. They revealed the mechanism by which the enzyme specifically recognizes its substrate and a unique nucleic acid-assisted mechanism of catalysis. The structures showed that two key metal ions are involved in the hydrolysis reaction. We have also determined a structure of human RNase H2 complex. The catalytic subunit resembles closely the monomeric archaeal and bacterial RNases H2 with the characteristic RNase H fold. The auxiliary subunits form an intertwined dimer adopting a triple barrel fold. We mapped the positions of all 29 mutations found in the AGS patients. Possible effects of these mutations on the protein's stability and function will be discussed.