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Structural and Functional Studies on Nucleotide Excision Repair From Recognition to Incision

Maintenance of the correct genetic information is crucial for all living organisms because mutations are the primary cause of hereditary diseases, as well as cancer and may also be involved in aging. The importance of genomic integrity is underscored by the fact that 80 to 90% of all human cancers are ultimately due to DNA damage[1]. Among the different repair mechanisms that have evolved to protect the genome[2], nucleotide excision repair (NER) is a universal pathway found in all organisms. NER removes a wide variety of bulky DNA adducts including the carcinogenic cyclobutane pyrimidine dimers induced by UV radiation, benzo(a)pyrene-guanine adducts caused by smoking and the guanine-cisplatin adducts induced by chemotherapy[3]. The importance of this repair mechanism is reflected by three severe inherited diseases in humans, which are due to defects in NER: xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy[4].

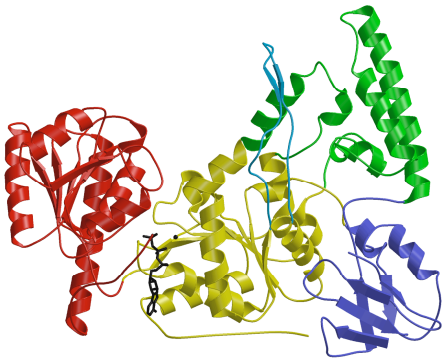

Figure 1: Structure of UvrB. Domains 1a, 1b, 2, and 3 are color coded in yellow, green, blue and red, respectively. The β-hairpin bridging the gap between domains 1a and 1b is shown in cyan. The ATP molecule bound at the interface between domains 1 and 3 is shown in all-bonds representation[5]

UvrB plays a pivotal role in this repair cascade by initially interacting with UvrA in the damage recognition process and then guiding the DNA from recognition to incision by complex formation with UvrC. Finally, it is involved in repair synthesis, ensuring that no gapped DNA intermediates are released before the repair pathway is completed. Thus UvrB was one of our primary targets for structural and biochemical analysis. We solved the crystal structure of UvrB (Figure 1) at the same time as two other labs[6, 7] and identified it as a protein with two helicase-like domains and two additional domains unique to repair proteins[5]. Distinguishing it from helicases, UvrB features a β-hairpin that extrudes from one domain and obstructs the space occupied by the translocated strand in true helicases. Based on our crystal

structure, we suggested that UvrB uses this β -hairpin to clamp down on one DNA strand, thereby tightly binding to damaged DNA. To test this hypothesis we deleted the tip of the β-hairpin and showed that this renders UvrB inactive. The mutant does not form a stable UvrB-DNA pre-incision complex and is inactive in UvrABC-mediated incision[8, 9]. These and subsequent experiments suggest that adding the β-hairpin to the general helicase scaffold is necessary for UvrB's ability to recognize and tightly bind to damaged DNA. In a parallel approach we have determined the structure of a UvrB variant, which for the first time revealed the structure of UvrB's domain 2 that is critical for the interaction with UvrA[10]. Site directed mutagenesis and subsequent analysis of highly conserved residues on the surface of this domain led to the identification of residues that are required for the formation of a productive UvrA-UvrB interaction and are thus essential for damage recognition.

UvrC is the endonuclease in prokaryotic nucleotide excision repair that is responsible for the 3' and 5' incision events to remove the damage containing oligonucleotide. No structural information was so far available for UvrC with the exception of the C-terminal Helix-hairpin-Helix domain, which is responsible for DNA binding.

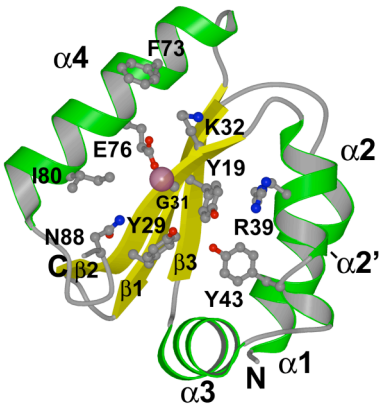

Figure 2: Ribbon presentation of the N-terminal endonuclease domain of UvrC from *Thermotoga maritima*. Conserved residues are shown in ball-and-stick representation and a bound divalent cation implicated in catalysis is shown as a magenta sphere.

We have solved the structure of the N-terminal catalytic domain of UvrC at 1.5 Å resolution (Figure 2)[11]. This domain catalyzes the 3' incision reaction and we have shown that it shares homology with the catalytic domain of the GIY-YIG family of intron-encoded homing endonucleases. The structure revealed a patch of highly conserved residues surrounding a catalytic magnesium-water cluster, suggesting that the metal binding site is an essential feature of UvrC and all GIY-YIG endonuclease domains. Our structural and biochemical data strongly suggest that the N-terminal endonuclease domain of UvrC utilizes a novel one-metal mechanism to cleave the phosphodiester bond.

Structural Studies on a High-fidelity DNA Polymerase

The accurate replication of DNA is crucial for the maintenance of a functional genome and is accomplished by high fidelity DNA polymerases. The basic architecture of the polymerase domains, the fingers, palm, and thumb, can be characterized as a “right hand” which is capable of holding DNA in its grasp[12, 13]. Family B DNA polymerases include the major replicative DNA polymerases α and δ and the replicative DNA polymerases from bacteriophages T4 and RB69. Three conformations of RB69 pol have been observed in crystal structures: the apo state without DNA[14], the editing mode forming a binary complex with DNA bound to the exonuclease site, and the replicating mode, a ternary complex with DNA and dNTP bound in the polymerase site[15]. Differences between these structures indicate the conformational changes that occur during DNA polymerization. The fingers domain undergoes the largest conformational changes during DNA polymerization. In the apo state and editing mode the enzyme is in the 'open' conformation, characterized by a rotation of the fingers domain by 60º away from the palm domain. In the 'closed' conformation residues of the fingers domain are involved in hydrogen bonding to the phosphate groups of the incoming dNTP.

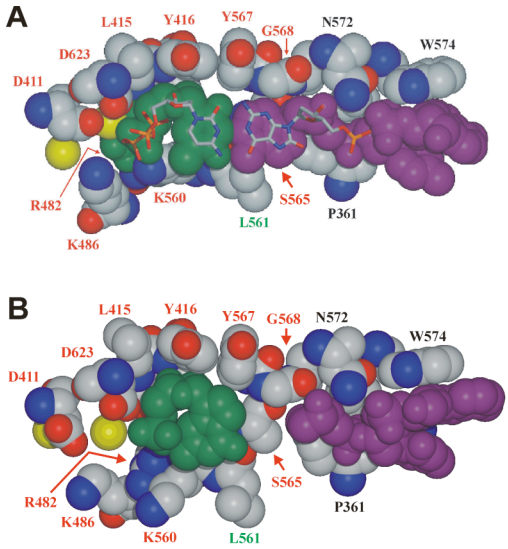

Figure 3: The active site of RB69 pol (A) superposition of the 8-oxodG:dCTP base pair (in stick mode) with the dA:dTTP base pair (space filling mode) of the replicating complex with undamaged DNA. The amino acids of the active site are shown in space filling mode as well. Residues that are highly conserved are labeled in red, residues labeled in green are type conserved and residues labeled in black are not conserved. (B) Active site of the polymerase with tetrahydrofuran and dGMP.

Replicative polymerases demonstrate high fidelity and processivity when utilizing normal DNA, however, they can be challenged with modified or damaged DNA, which has escaped the DNA repair machinery. The polymerase may then disengage from the primer/template to allow another DNA polymerase to bypass the DNA lesion

(translesion synthesis), or another damage avoidance mechanism to occur, or the replicative polymerase may perform translesion synthesis itself. These events are important in understanding the mechanism by which DNA damage can result in miscoding, leading to mutagenesis and carcinogenesis.

2'-deoxy-7,8-dihydro-8-oxoguanosine (8-oxodG) is an endogenous premutagenic lesion generated by reactive oxygen species as a result of aerobic respiration. 8-OxodG is one of the most prevalent lesions found in DNA and, if not repaired, can direct incorporation of dATP resulting in G → T transversions. About 10,000 abasic sites are created per day in each mammalian cell[16]. They arise through spontaneous depurination or by the action of DNA glycosylases during base excision repair. The natural abasic site is unstable and we have used tetrahydrofuran (3-hydroxy-2-(hydroxymethyl)tetrahydrofuran), as a model of the closed form of an abasic site. *In vivo* and *in vitro* studies have shown that dA is preferentially incorporated opposite tetrahydrofuran and that this lesion represents a block to DNA synthesis[17, 18].

We solved the crystal structures of RB69 DNA pol with DNA containing 8-oxodG and tetrahydrofuran as a model for an abasic site[19]. Based on the insights gained from these studies, we are now able to predict which new lesion/dNTP combinations will be tolerated in the active site of a family B DNA polymerase and which may be sterically too demanding to enable replication: (I) Lesions in the template strand must allow the tight steric fit of the binding pocket on the minor groove side, hence any sterically demanding groups may only protrude into the major groove. (II) Unless dealing with abasic sites, suitable incoming nucleotides or nucleotide derivatives must be provided to allow the formation of a closed ternary complex. Even if a nascent 'base pair' passes the requirements of the active site binding pocket, further extension may be inhibited because of an additional size requirement that has to be fulfilled at the template side upon DNA translocation. The presence of an abasic site, however, leads to a catalytically inactive state that is distinct from the apo or exo states of the polymerase and explains the replication blockage at the molecular level.

Published Manuscripts Funded in Part by this Grant:

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