

certain base preferences. Based on the PriB structural information and biochemical studies, we propose that the potential tetramer formation surface and several other regions of PriB may participate in protein-protein interaction during DNA replication. These findings may illuminate the role of PriB in *phiX*-type primosome assembly.

[1] Li, J.-H., Chan, T.-W., Huan, C.-Y., Che, S.-U., W, H.-N., Chang M.-C., Hsiao C.-D., *J. Biol. Chem.*, 2004, **279**, 50465.

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Structure of a Dimeric Single-stranded DNA Binding Protein from *Thermus aquaticus*

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Single-stranded DNA binding (SSB) proteins are involved in DNA replication, repair and recombination. While not showing pronounced sequence specificity they bind strongly to single-stranded DNA (ssDNA) but not to double-stranded DNA (Meyer und Laine 1990). This results in stabilizing the ssDNA, preventing hairpin formation and holding it in a suitable conformation for the action of other proteins involved in e.g. DNA replication. There exist several structural classes of SSB proteins ranging from monomers, homodimers, heterotrimers to homotetramers which all have oligonucleotide/oligosaccharide binding folds (OB-fold) in common (Murzin 1993; Suck 1997). One of these classes is formed by the homotetrameric SSB proteins which occur in eubacteria like *E. coli* and in eukaryotic mitochondria. These proteins contain one OB-fold per monomer resulting in four DNA binding sites in each homotetramer. Recently, SSB proteins were identified in the bacterial *Thermus* group that share homologies to the tetrameric SSB proteins, but the monomers are twice the size compared to those of the homotetrameric SSBs. These proteins contain two OB-folds per monomer and it could be shown that they form dimers in solution (Dabrowski et al., 2002; Eggington et al., 2004). Thus, the principle of four DNA binding sites per functional unit also is conserved in these bacterial SSB proteins. In this work we have expressed, crystallized and solved the structure of the SSB protein from the thermophilic bacterium *Thermus aquaticus*. New insights, based on the structural information, will be discussed in the context of the SSB function in thermophilic bacteria.

[1] Dabrowski S., Olszewski M., Piatek R., Brillowska-Dabrowska A., Konopa G., Kur J., *Microbiology*, 2002, **148**(10), 3307-15. [2] Dabrowski S., Olszewski M., Piatek R., Kur J., *Protein Expr Purif*, 2002, **26**(1), 131. [3] Eggington J. M., Haruta N., Wood E. A., Cox M. M., *BMC Microbiol*, 2004, **4**(1), 2. [4] Meyer R. R., Laine P. S., *Microbiol Rev*, 1990, **54**(4), 342-80. [5] Murzin A. G., *Embo J.*, 1993, **12**(3), 861-7. [6] Suck D., *Nat Struct Biol*, 1997, **4**(3), 161.

Keywords: SSB, replication, flexible-regions

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Crystal Structure of SUMO1-conjugated Thymine DNA Glycosylase

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SUMO (Small ubiquitin-like modifier) is post-translationally

attached to many proteins and regulates the function or localization of modified proteins. Thymine DNA glycosylase (TDG), which plays an integral role in base excision repair of G-U or G-T mismatch DNA, is shown to be modified by SUMO-1 or SUMO-2/3. SUMO modification of TDG promotes the TDG dissociation from its product DNA containing an abasic site. However, the molecular mechanism of the SUMOylation-induced DNA release of TDG has not been revealed yet. Here we determined the crystal structure of SUMO-1 modified TDG central region (SUMO1-TDG). The structure revealed that TDG and SUMO-1 interact with each other through both covalent and non-covalent interactions, and these interactions likely induce the structural rearrangement of the C-terminal region of TDG. This induced structural change includes the alpha-helix formation, which protrudes from the body of the protein and seems to make a steric clash with DNA bound to TDG. Thus, this steric clash seemingly enhances the release of the product DNA from TDG. Results from biochemical assays using a series of TDG mutants support this structure and function model for molecular mechanism of SUMOylation-dependent TDG dissociation from DNA.

Keywords: SUMO, DNA repair, protein modification

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Crystal structure of *Deinococcus Radiodurans* RecO

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The RecFOR pathway has been shown to be essential for DNA repair through the process of homologous recombination in bacteria and, recently, to be important in the recovery of stalled replication forks following UV irradiation. RecO, along with RecR, RecF, RecQ and RecJ, are principal actors in this fundamental DNA repair pathway.

Here we present the three-dimensional structure of RecO from the extreme-radiation resistant bacterium, *Deinococcus radiodurans*. The crystal structure of *D. radiodurans* RecO (drRecO) reveals possible binding sites for DNA and for the RecO-binding proteins within its three discrete structural regions: an amino-terminal oligonucleotide/oligosaccharide binding (OB) domain, a helical bundle and a Cys₄ zinc finger motif. Furthermore, drRecO was found to form a stable complex with RecR and to bind both ssDNA and dsDNA. Mutational analysis confirmed the existence of multiple DNA binding sites within the protein.

Keywords: DNA repair, zinc finger motif, X-ray crystal structure

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Crystal Structure of SUMO2-conjugated Thymine DNA Glycosylase

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SUMO (Small ubiquitin-like modifier) is post-translationally attached to many proteins and regulates the function or localization of modified proteins. TDG (Thymine DNA glycosylase), which plays an important role in base excision repair of G-U or G-T mismatch DNA, is shown to be modified by SUMO-1 or SUMO-2/3. SUMO modification of TDG promotes the TDG dissociation from its product DNA containing an abasic site. However, the molecular mechanism of