certain base preferences. Base 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 *phi*X-type primosome assembly.

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Keywords: replication, primosome, single-stranded DNA binding protein

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

Roman Fedorov[a], Anita Coetzee[b], Gregor Witte[a], Ute Curth[a], Dietmar J. Manstein[a], *[a]Institute of Biophysical Chemistry, Medical School, 30625 Hannover, Germany. [b]Bruker AXS B.V*. E-mail: manstein@mh-hannover.de

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.

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Keywords: SSB, replication, flexible-regions

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Crystal Structure of SUMO1-conjugated Thymine DNA Glycosylase

Masahiro Shirakawa[1,3,7,8], Daichi Baba[1], Nobuo Maita[1,2], Jun-Goo Jee[3], Yasuhiro Uchimura[4], Hisato Saitoh[4], Kaoru Sugasawa[5], Fumio Hanaoka[5,6], Hidehito Tochio[1], Hidekazu Hiroaki[1], [1]*Graduate School of Integrated Science, Yokohama City University, Japan.* [2]*Japan Biological Informatics Consortium, Japan.* [3]*RIKEN Genomic Sciences Center, Yokohama, Japan.* [4]*Department of Regeneration Medicine, Institute of Molecular Embryology Genetics, Kumamoto University, Japan.* [5]*Cellular Physiology Laboratory, Discovery Research Institute, RIKEN, Japan.* [6]*Graduate School of Frontier Biosciences, Osaka University, Japan.* [7]*Graduate School of Engineering, Kyoto University, Japan.* [8]*CREST, Japan Science and Technology Corporation, Japan.* E-mail: shirakawa@moleng.kyoto-u.ac.jp

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 crash 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

Joanna Timmins, Ingar leiros, David R. Hall, Sean McSweeney, *Macromolecular Crystallography Group, European Synchrotron Radiation Facility, B.P. 220, 6 rue Jules Horowitz, 38043 Grenoble, France.* E-mail: timmins@esrf.fr

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 Cys_4 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

Daichi Baba[1], Nobuo Maita[1,2], Jun-Goo Jee[3], Yasuhiro Uchimura[4], Hisato Saitoh[4], Kaoru Sugasawa[5], Fumio Hanaoka[5,6], Hidehito Tochio[1], Hidekazu Hiroaki[1], Masahiro Shirakawa[1,3,7,8], *[1]Graduate School of Integrated Science, Yokohama City University, Japan.* [2]*Japan Biological Informatics Consortium, Japan.* [3]*RIKEN Genomic Sciences Center, Yokohama, Japan.* [4]*Department of Regeneration Medicine, Institute of Molecular Embryology Genetics, Kumamoto University, Japan.* [5]*Cellular Physiology Laboratory, Discovery Research Institute, RIKEN, Japan.* [6]*Graduate School of Frontier Biosciences, Osaka University, Japan.* [7]*Graduate School of Engineering, Kyoto University, Japan.* [8]*CREST, Japan Science and Technology Corporation, Japan.* E-mail: baba@tsurumi.yokohama-cu.ac.jp

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

SUMO modification over the TDG dissociation from DNA has not been revealed yet. Simultaneously, the functional difference between SUMO-1 and SUMO-2/3 modification is also unclear if it exists. Here we solved the crystal structure of TDG central region in conjugated to SUMO-2 (SUMO2-TDG). Within the structure we've determined, TDG and SUMO-2 are interacted intermolecularly through both covalent and non-covalent interactions as seen in the crystal structure of SUMO1-TDG (as will be presented by Shirakawa et al.). From the mutational analyses, we could not find the difference in SUMO / TDG interactions between SUMO-1 and SUMO-2/3. These observations strongly suggest the equivalence of functional consequence of the modification among SUMO isoforms. The effect of mutations over the DNA binding activity of SUMO2-TDG is under examination.

Keywords: SUMO, ubiquitin, DNA repair

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Crystal Structure of Leucine Zipper Protein Hy5 Complexed with DNA

Toshiyuki Shimizu[a], Hiroki Adegawa[a], Hiroshi Hashimoto[a], Matsui Minami[b], Mamoru Sato[a], [a]*Graduate School of Integrated Science, Yokohama City University of Japan,* [b]*Plant Functional Genomics Research Group, Genomic Sciences Center, RIKEN Yokohama Institute*. E-mail: shimizu@tsurumi.yokohama-cu.ac.jp

The basic leucine tipper (bZIP) transcription factor Long Hypocotyl 5 (HY5) is a positive regulator of photomorphogenesis, which acts downstream of the light receptor network and directly affects the transcription of light-induced genes [1]. HY5 activity is controlled by a key negative regulator, Constitutive Photomorphogenic 1 (COP1), an ubiquitin ligase that targets HY5 for degradation in dark-grown conditions. HY5 is a 168–amino acid protein representing a member of a class of basic leucine zipper (bZIP) DNA binding proteins. HY5 is involved in light regulation of transcriptional activity of the promoters containing the G-box (CACGTG). We also show Hy5 bind to the CRE-sequence (TGACGTCA).

To clarify DNA recognition mechanism of Hy5, we have tried to determine the structure of Hy5 complexed with DNA containing CRE-sequence with X-ray crystallographic analysis. Crystals suitable for analysis were obtained at 293 K by hanging drop vapor-diffusion method. The structure is determined with molecular replacement using the combined model of CREB-CRE and Jun-CRE complex. The DNA recognition mechanism will be discussed.

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Keywords: DNA-protein complexes, transcription factor, leucine zipper

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Crystal Structures Restriction Endonuclease *Eco*O109I DNA Bound to Divalent Metal

Tsuyoshi Imasaki[a], Hiroshi Hashimoto[a], Toshiyuki Shimizu[a], Keiko Kita[b], Mamoru Sato[a], [a]*Graduate School of Integrated Science, Yokohama City University.* [b]*Graduate School of Agiculture, Kyoto University.* E-mail: ima@tsurumi.yokohama-cu.ac.jp

Over 3,000 type II restriction endonucleases have been discovered. They require divalent metals (such as Mg^{2+} or Mn^{2+}) as cofactors with their activity. Most type II restriction endonucleases have activity under the existence of Mg^{2+} or occasionally Mn^{2+}. The restriction endonuclease does not support catalysis with Ca^{2+} and Ba^{2+}, however, it forms a stable protein-metal-DNA complex without cleaving DNA.

*Eco*O109I is a type II restriction endonuclease and recognizes a palindromic sequence RGGNCCY (R = A,G: Y = T,C) and the enzyme cleaves the sequence between the second and third base, and produces leaving 5'-overhanging ends under the existence of Mg^{2+} or Mn^{2+}. In contrast, Ba^{2+} does not support catalysis. The structures of *Eco*O109I DNA-free and *Eco*O109I DNA-complex have been determined [1]. The structures of *Eco*O109I DNA-complex have one metal ion per the active site. To explore how *Eco*O109I uses divalent metal ions, we determined the crystal structure of *Eco*O109I with its cognate DNA substrate containing Ba^{2+} or Mn^{2+} at 1.6 Å resolution. In the Ba^{2+} bound structure, DNA stays intact and one Ba^{2+} was found per active site, whereas in the Mn^{2+} structure, DNA was cleaved and three Mn^{2+} were found per active site.

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Keywords: protein structures, DNA-protein complexes, endonucleases

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Sense/Antisense Open Reading Frames and the Amino Acid Composition of Ribosomal Proteins

Robert Huether[1], William L. Duax[1,3], Lukas Habegger[1], Vladimir Pletnev[2], Sonjay Connare[1], [1]*Structural Biology Dept. Hauptman-Woodward Medical Inst., 73 High St., Buffalo, NY 14203-1186.* [2]*Inst. Bioorg. Chem., RAS Moscow.* [3]*SUNY, Dept. of Structural Biology, Buffalo, NY 14260.* E-mail: bhuether@hwi.buffalo.edu

By combining information on three dimensional structures and conservation of amino acid sequences in families of ancient proteins it is possible to trace the evolution of the genetic code and the amino acid composition of proteins. We discovered a pattern of multiple open reading frames (ORFs) and amino acid bias in *Streptomyces coelicolor*. Of particular interest was the high incidence of sense/antisense ORFs (SASORFs) and the absence of Trp and Cys residues in ribosomal proteins. The L1 proteins from the 50S ribosomal subunit were selected for further analysis. Of 125 sequences of L1 proteins in the SWISS-PROT TrEMBL database, 50% are missing Trp, 64% are missing Cys and 35% are missing both. Those from archaea rarely have Trp and/or Cys residues whereas those from eukaryote usually have both. Comparison of the amino acid sequences of the 125 50SL1 proteins reveals that Cys is not conserved in any sequence position at greater than 8%. In 37 structures a Trp residue resides in a common position on the surface of L1 in bacteria but not archaea. These finding suggest that SASORFs, severe codon bias and absence of Trp and Cys residues are hallmarks of ancient enzymes that have been little altered by millions of years of evolution or lateral genes transfer.

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Keywords: open reading frames, codon bias, ribosome

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Structure of XPF Endonuclease from *A. pernix*

Judith Murray-Rust[a], Matthew Newman[a], Andrew Fadden[a], John Lally[a], Phillip Knowles[a], Maureen Biggerstaff[a], Malcolm White[b], Neil Q McDonald[a], [a]*Structural Biology Laboratory, Cancer Research UK, London WC2A 3PX.* [b]*Centre for Biomolecular Sciences, University of St Andrews, Fife KY16 9ST, UK*. E-mail: murrayru@cancer.org.uk

The XPF structure-specific endonucleases form part of the nucleotide excision repair machinery which is required to detect and remove bulky DNA lesions caused e.g. by UV light. XPF acts on splayed DNA substrates by cutting one strand of the duplex upstream of a 3' flap. XPF family members have a catalytic nuclease domain connected by a linker sequence to tandem DNA-binding HhH domains. Eukaryotic XPFs also have a N-terminal SF2-like helicase domain and form heterodimers with smaller partners such as ERCC1. Archaea have either a short form of XPF regulated by PCNA or a long form that has an active helicase domain.

We have solved the structure of the XPF homodimer from *A. pernix* both alone and bound to a DNA duplex. The flexibility of the linker allows the nuclease and $(HhH)_2$ domains to dimerise independently. On binding DNA a large interdomain rearrangement takes place, resulting in an asymmetric complex. This is the first structure of an essentially intact XPF, and provides insight into how XPF can recognise branched DNA substrates.

Keywords: endonucleases, DNA repair, protein-DNA complexes