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

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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.

[1] Chattopadhyay S. , Ang L.H., Puente P., Deng X.W., Wei N., *Plant Cell*., 1998, **10**, 673.

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

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

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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.

[1] Hashimoto H., Shimizu T., Imasaki T., Kato M., Shichijo N., Kita K., Sato M., *J. Biol. Chem*., 2005, **280**, 5605.

Keywords: protein structures, DNA-protein complexes, endonucleases

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

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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*

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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