to discover CARM1 specific inhibitors and their binding mode. Our last results concerning the development of news selective inhibitors of PRMTs will be also presented.

Keywords: chromatin, methylation, inhibitor

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Towards the Molecular Mechanism of Holliday Junction Resolution

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Four-way DNA junctions (Holliday junctions) are key intermediates formed during homologous recombination, which elegantly explain how the exchange of genetic material between two DNA molecules can occur. Their resolution back to two separate duplexes is catalysed by various classes of DNA junction-resolvases, which specifically cleave Holliday junctions, but critically have no nucleolytic activity against double stranded DNA. Junction resolution proceeds via the sequential nicking of the DNA backbone at two positions close to the centre of the junction and productive resolution is ensured by a significant enhancement of the rate of nicking of the second DNA strand relative to the first. Previously, we have solved the crystal structure at 3.2 Å of a complex between a slow cleaving mutant of Endonuclease I (the resolvase from bacteriophage T7) and a synthetic Holliday junction to ascertain the structural basis for junction specificity[1]. The structure reveals the DNA junction to be bound in a highly distorted conformation relative to the free junction, the protein interacts solely with the DNA backbone suggesting that junction recognition is structure rather than sequence specific, and the active site structure suggests that an activated water molecule is positioned for in-line attack with the scissile phosphate. We have recently solved the structure of wild-type EndoI and are using this crystal system to understand the molecular basis of the increased rate of DNA nicking for the second strand by attempting to follow junction cleavage in the crystals and cryo-trapping the complex at different stages of the reaction. We have also crystalllised Endonuclease I with a junction pre-nicked in one of the strands mimicking a partially cleaved intermediate.

[1] J.M. Hadden, A.C. Declais, S.B. Carr, , D.M.J. Lilley, S.E.V. Phillips *Nature* **2007** *449*, 621-624

Keywords: holliday junction, resolvase

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Structural insight into maintenance methylation by mouse DNA methyltransferase 1

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In mammals, genomic DNA is often methylated at the fifth position of the cytosine base in CpG sequences. This DNA methylation, which is one of the major epigenetic modifications, plays a crucial role in development, genome stability, X-chromosome inactivation, and silencing of retrotransposons. Once the DNA methylation patterns are established, they are maintained by DNA methyltransferase 1 (Dnmt1), which ensures the transmission of lineage-specific DNA methylation patterns during replication. Dnmt1 preferentially methylates the hemimethylated state of DNA that appears just after replication or repair. To do this, Dnmt1 interacts both with proliferating cell nuclear antigen (PCNA), a factor that is a prerequisite for replication, and Np95/Uhrf1, a factor that is necessary for the maintenance of DNA methylation and that binds hemimethylated DNA at replication foci. Thus, the Dnmt1 is responsible for the propagation of methylation patterns to the next generation via its preferential methylation of hemimethylated CpG sites in the genome; however, how Dnmt1 maintains methylation patterns is not fully understood.

In the present study, we determined the crystal structure of the large fragment (291-1620) of mouse Dnmt1 and S-adenosyl-L-methionine (AdoMet) or S-adenosyl-L-homocystein (AdoHcy) complexes [1]. Data sets were collected on BL44XU beamline at SPring-8 (Hyogo, Japan) with a MX225-HE Charge Coupled Device detector (Raynonix). The crystal structure of Dnmt1(291 – 1620) showed a distinct multidomain structure comprising the replication foci targeting sequence (RFTS), a zinc-finger-like motif, two tandemly connected bromo-associated homology domains, and the catalytic domain. Notably, the RFTS responsible for targeting Dnmt1 to replication foci is inserted into the DNA-binding pocket, indicating that this domain must be removed for methylation to occur. Upon binding of AdoMet, the catalytic cysteine residue (C1229) undergoes a conformation transition to a catalytically competent position. For the recognition of hemimethylated DNA, Dnmt1 is expected to utilize a target recognition domain that overhangs the putative DNA-binding pocket. Recently, the crystal structure of a shorter Dnmt1 fragment in complex with unmethylated DNA has been reported by Song et al [2]. The DNA in reported structure is positioned away from the catalytic center, leading them to propose that the deduced structure represents the auto-inhibition form of Dnmt1 protecting from *de novo* DNA methylation. In contrast, our present crystal structure, although not containing DNA, but including the RFTS and the methyl group donor AdoMet, shows three striking features. First, the RFTS is inserted into the catalytic pocket such that DNA cannot access the catalytic center. Second, the CXXC motif is in a position where the DNA binds in the structure reported by Song et al. Last, the complex with AdoMet causes C1229 to flip toward the target cytosine. This residue is expected to form a covalent bond with the sixth position of the target cytosine base. The results clearly indicate that multiple structural changes must take place for faithful maintenance DNA methylation.

[1] Takeshita et al., *PNAS* **2011**. [2] Song et al., *Science* **2011**

Keywords: crystallography, structure, methylation

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Crystallographic studies of DNA minor groove binding drugs

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Targeting the minor groove of DNA through small molecules has long been considered an important recognition strategy in biology [1].

A wide range of minor groove binding ligands (MGBLs) with good sequence discrimination ability are of interest as potential therapeutic agents in variety of human diseases such as cancer, along with anti-bacterial and/or anti-parasitic activities. These MGBLs are highly selective to A:T base pairs of the minor groove of the DNA. Some of these compounds are in phase III clinical trials while some are currently in use for their veterinary applications.

Nucleic acids require structured water molecules in order to maintain their stability, polymorphism and flexibility of the duplex DNA. The binding of ligands into the minor groove of DNA involves the displacement of the native (drug-free) structured water molecules. These ligands have been studied extensively over the last two decades using a number of methods. However, for many of these systems understanding regarding water interactions and protonation states of the DNA-ligand complex and components remains unclear. This critical information is important for understanding the stability and recognition of DNA ligand complexes. Crystallographic methods have been used to determine the molecular structure of small molecules bound to DNA sequences, in order to better understand the details of molecular recognition by DNA.

Some of the MGBLs have shown to be effective inhibitors of a number of minor and major groove binding protein-DNA interactions (e.g. OTF 1, *Antp* HD, HMGA2, etc.) [2]. Studies have been done with major groove binding transcription factor NF-κB which will add additional detail towards the biological significance and activity for the MGBLs. In this project we aim to build a library of information relating drug-DNA-water interactions to sequence specificity and drug design using X-ray crystallography as well as kinetics (e.g. from SPR) data and gel shift assays. This information is valuable for rational drug design in future.

[1] B. Nguyen, S. Neidle, W.D. Wilson *Accounts of Chem. Res* **2008** *42(1)*, 11-21. [2] R.E. Speight, D.J. Hart, J.M. Blackburn *J. Mol. Recognit* **2002** *15*, 19-26.

Keywords: crystallography, DNA, drug

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Specificity and efficiency in activity of anti-HIV actinohivin for sugar binding

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In order to overcome the present multi-drug resistance problem in treating HIV/AIDS pandemic, a new lectin actinohivin (AH) was found to exhibit a potent anti-HIV activity through binding the high-mannose type glycans (HMTG) which are bound to gp120 of HIV. X-Ray analyses of AH and its complex with α(1,2)-mannobiose (M_2) which is the terminal end of the three branches (D1, D2 and D3) of HMTG revealed that AH is composed of tandem repeated three structural modules associated with a *pseudo* three-fold symmetry[1]. In each module, M_2 is accommodated through the specific hydrogen bonds with D, Y and N residues equivalent between the three modules.

In this study, the structural features were revised, as seen in Fig. 1(*a*), based on which essential residues for specific inter-actions were confirmed by mutation experiments[2]. In addition, dimerization effect of AH on anti-HIV activity was examined by increasing the number of HMTG-binding sites in a molecule, because gp120 is covered with many HMTGs, as seen in Fig. 1(*b*). Several dimeric AH (AH_2) derivatives were prepared and their anti-syncytium formation and anti-HIV activities were evaluated.

D15, Y23, L25, N28 and Y32 (in module 1) and the corres-ponding residues in the other modules were identified to be essential for AH activity. Among them, D, Y and N residues participate in recognition of M_2. By superimposing the three terminal ends of D1 branches onto the bound M_2, a model of gp120 bound to several AHs has been constructed. This model suggests that the AH affinity to gp120 is amplified in col-laborative binding when it forms a dimer as a cluster. Among several AH_2s (see Fig. 1(*c*)), those linked by a head-to-tail fashon showed higher (20 folds at maximum) in anti-syncytium formation activity and (2~30 folds) in anti-HIV activity than those of AH monomer. These activities vary depending on the linker sequence. Therefore, the anti-HIV activity of AH can be improved as a microbicide to prevent HIV transmission.

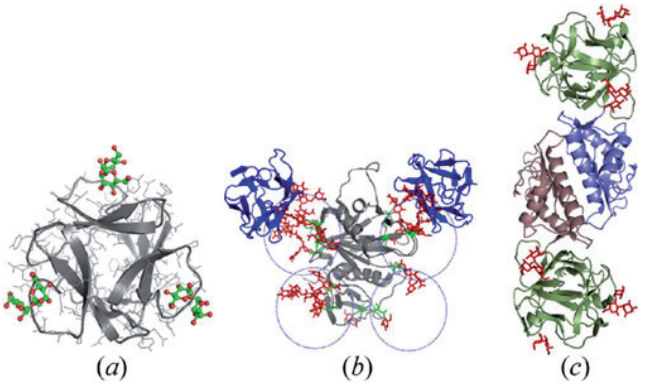

Fig. 1. Three M_2s bound in the three modules of AH (*a*), a model of several AHs bound to gp120 (*b*), and an image of designed AH_2 associated to each other through dimerization domains (*c*).

[1] Tanaka, H., *et al*., *Proc. Natl. Acad. Sci. USA* **2009** *106*, 15633-15638. [2] Takahashi, A., *et al*., *J. Antibiot*. **2010,** 2010.106.

Keywords: X-ray structure, anti-HIV activity, actinohivin

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Crystal structures of DNA containing X relevant to gastro-intestinal cancer

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Red meat stimulates endogenous intestinal *N*-nitrosation of glycine and its derivatives that can induce DNA mutations by reacting with DNA to form O^6-carboxymethylguanine (hereafter X) which is associated with increased risk of gastrointestinal cancer. In order to obtain insights into the pairing geometry of DNA duplexes containing X (modified or damaged nucleotide) and to further understand its biological implications, we have determined the crystal structures of two DNA dodecamers with the sequences d(CGCXAATTCGCG) (hereafter X:C) and d(CG CGXATTCGCG) (hereafter X:T) by X-ray analyses.