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

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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].