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Keywords: coordination polymers, ligand conformation, semi flexible ligands.

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Crystal structures of histone chaperone CIA/ASF1-containing complexes

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The nucleosome, which is a fundamental repeating unit of chromatin, consists of about 200 base pairs of DNA and a histone octamer. Since the interactions between the histone proteins and DNA in the nucleosome hamper enzymes' access to DNA, disassembly of nucleosomes is required for nuclear reactions such as transcription. Histone chaperones, which facilitate nucleosome assembly and disassembly, are therefore considered to play a critical role in transcription. Indeed, biological analyses have suggested that the histone chaperone CIA/ASF1, which is the most conserved histone chaperone in eukaryotes, is involved in histone eviction at the promoter regions in the transcriptional activation process. Other biochemical and biological studies have revealed that a critical signal of transcription activation is histone acetylation, which seems to function as a signal for the nucleosome disassembly in transcription. The acetylation signal is therefore likely to be transferred to histone chaperone CIA/ASF1. However, its molecular mechanism has remained elusive. In 2002, the Horikoshi group showed that CIA/ASF1 physically and genetically interacts with the double bromodomain (DBD) of CCG1 in the TFIID complex [1]. This result suggests that the interaction between CIA/ASF1 and DBD plays a key role in connecting the histone acetylation and site-specific nucleosome disassembly.

In order to elucidate this molecular mechanism at the atomic level, we determined the crystal structures of two molecular complexes containing CIA/ASF1. Initially, we determined the crystal structure of the CIA/ASF1–H3–H4 complex at 2.7 Å resolution [2]. This crystal structure showed that CIA/ASF1 interacts with the histone H3–H4 dimer and that the interaction inhibits nucleosome formation, suggesting that this complex occurs in the nucleosome disassembly process. In addition, the genetic analysis suggested that the interaction between CIA/ASF1 and histone H3 is involved in the transcription initiation process in yeast.

Next, we determined the crystal structure of the CIA/ASF1–DBD complex at 3.3 Å resolution [3]. The genetic analysis, combined with structural information, showed that the interaction between CIA/ASF1 and DBD is also involved in the transcription initiation process in yeast. A ChIP analysis using a structurally designed DBD mutant suggested that CIA/ASF1 is recruited to promoter regions through the interaction with DBD and induces site-specific histone eviction around the promoter regions, leading to transcriptional activation. Our biochemical results showing that CIA/ASF1 can change its interacting partner from DBD to the histone H3-H4 dimer also supports this model. This is the first structure-based model of the biological signaling from histone modifications to structural change of the nucleosome (hi-MOST model) [3].

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Methylation by CARM1: A structure based approach to design inhibitors and peptide mimics

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Post-translational methylation of arginine is a widespread epigenetic modification found in eukaryotes that is catalyzed by the protein arginine methyltransferases (PRMTs). PRMTs have been implicated in a variety of biological processes, such as regulation of transcription, translation and DNA repair. At least nine members of PRMTs (PRMT1 to PRMT9) have been identified and classified into two main classes. Coactivator-associated arginine methyltransferase 1 (CARM1, also known as PRMT4) was identified as an enhancer of the transcriptional activation by several nuclear hormone receptors. CARM1 is a crucial protein involved in many biological processes including the regulation of chromatin structure and transcription via methylation of histones and many transcriptional cofactors. Since deregulation of these processes appears to be implicated in the pathogenesis of different diseases such as human cancers, CARM1 and other PRMTs represent potential new targets for which compounds can be developed and that can be exploited to provide new therapies against cancer. As such, understanding the detailed mechanism of action of this protein at the structural level is important and has implications ranging from pure structural information to potential way of regulating gene expression via inhibitor design. Understanding the mechanism of action of CARM1 at the atomic scale is therefore crucial both for fundamental biology and pharmacological applications.

The work presented here combine chemistry, molecular modeling and X-ray crystallography with the aim to address two challenges in the field. The first one is to understand at the atomic level the mode of binding of substrate/product arginine-containing peptides, reflecting states prior and subsequent to methylation. The second one is to design, synthesize and improve by structure based-drug design compounds that can inhibit CARM1 methylation activity. We have previously solved several crystal structures corresponding to three isolated modules of CARM1. Crystal structures of the CARM1 catalytic module revealed large structural modifications and have shown that the NH_2-terminal and the COOH-terminal end of CARM1 catalytic module contain molecular switches that may inspire how CARM1 regulates its biological activities by protein-protein interactions. Moreover, our recent structural and functional studies have shown that peptides outside the catalytic core of CARM1 are essential for substrate binding and recognition.

Extensive works on PRMTs did not succeed yet to reveal the mode of mode of binding of substrate/product arginine-containing peptides, reflecting states prior and subsequent to methylation. This is certainly due to the weak or transient nature of the peptide/enzyme interaction. We have developed a novel approach to tackle this challenge and therefore understand how CARM1 and more generally PRMTs recognize and bind their peptide substrate. We have synthesized analogs or mimics of the transition state of the methylation reaction where the substrate/product is linked to the S-adenosyl-L-methionine cofactor. Full detailed analysis of new structures of CARM1 in the presence of substrates mimics will be presented. We have used the same approach

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.

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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.

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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].