In the last years, we have studied a nuclear protein complex formed by the association of histone deacetylase 1, a co-repressor protein CoREST, and lysine-specific histone demethylase LSD1 that specifically acts on Lys4 of histone H3. This LSD1/HDAC/CoREST multi-enzyme module functions as a "double-blade razor" that first eliminates the acetyl groups from acetylated Lys residues and then removes the methyl group from Lys4 [1], [2]. Our structural studies of the CoREST/LSD1 complex highlighted a uniquely specific binding-site for the histone H3 N-terminal tail and a catalytic machinery that is closely related to that of other flavin-dependent amine oxidases. These insights have been critical for our efforts towards structure-based development of demethylase inhibitors. These newly designed inhibitors were evaluated with a cellular model of acute promyelocytic leukemia chosen since its pathogenesis includes aberrant activities of several chromatin modifiers. Marked effects on cell differentiation and an unprecedented synergistic activity with anti-leukemia drugs were observed [3].

It has been recently discovered that the transcription factor Snail1 binds to LSD1/CoREST and that the three proteins are over-expressed in cancer cell lines and breast tumors. Snail1 controls the epithelial-mesenchymal transition, which is essential for numerous developmental processes (including metastasis). Structure determination of the ternary complex LSD1/CoREST/Snail1 peptide has revealed that Snail1 interacts with LSD1/CoREST in a remarkable way: the N-terminal residues of this transcription factor bind in the active site cleft of LSD1 effectively mimicking the histone H3 tail. Therefore, Snail1 is a potential endogenous inhibitor of LSD1. Furthermore, this finding predicts that other members of the Snail1-related transcription factor family associate to LSD1/2 through a similar histone-mimicking mechanism [4].

A challenge for future studies will be to extend these structural investigations to visualize nucleosome binding by LSD1-containing protein complexes through biophysical methods and crystallography.

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Structural basis of the versatile DNA recognition by the methyl CpG binding domain of MBD4

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DNA methylation is one of the major epigenetic marks associated with a repressed chromatin state and gene silencing, and is involved in regulation of various physiological events such as X chromosome inactivation, embryogenesis and genomic imprinting. Cytosine methylation at the C5 position in CpG (5'-CG-3') dinucleotides is the only covalent modification in mammalian genomic DNA. The DNA methylation pattern established during developmental processes determines characteristics of each cell type. Various multi-protein complexes are recruited on the methylated CpG sites and regulate gene expression. Methyl-CpG binding domain (MBD) proteins bind to methylated CpG sites and recruit other protein factors including histone modification enzymes and chromatin remodelling factors, generally resulting in establishment of silent chromatin.

MBD4 is a unique MBD family member that contains a glycosylation domain, and plays an important role in DNA repair as a thymine glycosylase to remove T/G mismatches generated after the deamination of 5-methylated cytosine (^{5m}C). Recently, MBD4 has been shown to be involved in active DNA demethylation in mammalian cells. The active demethylation has been to date suggested to be mediated at least partly by a base excision repair pathway involving converting of ^{5m}C to thymine by the AID/Apobec family of deaminases followed by G/T mismatch repair by the DNA glycosylase MBD4 [1]. Furthermore, MBD4 has been implicated to directly facilitate demethylation by its glycosylasion activity towards the ^{5m}C base depending on its phosphorylation state [2]. Towards full understanding of the molecular mechanism underlying DNA demethylation, we characterized the DNA binding specificity of the MBD domain of mouse MBD4 (MBD_{MBD4}) and determined its crystal structure in the complex with a DNA fragment containing symmetrically methylated CpG (^{5m}CG/^{5m}CG) or its deamination product (^{5m}CG/TG).

Combined with biochemical data, our structures showed MBD_{MBD4} binds to both of symmetrically methylated ^{5m}CG/^{5m}CG and ^{5m}CG/TG mismatch sequences in B-form DNA duplex with similar affinities. MBD4 shares an essential recognition mode for ^{5m}CG/^{5m}CG with structurally known MBD domains of MBD1 [3] and MeCP2 [4]. However, the DNA binding interface of MBD_{MBD4} is modulated to enable bi-substrate recognition of ^{5m}CG/^{5m}CG and ^{5m}CG/TG. There are two key structural features for the versatile DNA recognition: flexibility of the arginine finger responsible for base recognition and the DNA binding pocket containing a water-molecule network. Moreover, our biochemical data have demonstrated the inhibitory effect of MBD_{MBD4} in the T/G mismatch glycosylation activity of its isolated glycosyalse domain. This result implies unknown molecular mechanism for the substrate hand over from MBD_{MBD4} to the glycosylase domain in the context of full-length protein.

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Structure of a CENP-A-histone H4 heterodimer in complex with chaperone HJURP

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The centromere is epigenetically specified by the histoneH3 variant Centromere Protein-A (CENP-A). In higher eukaryotes, deposition of CENP-A to the centromere requires histone chaperone HJURP (Holliday junction recognition protein). The crystal structure of an HJURP-CENP-A-histone H4 complex shows that HJURP binds a CENP-A-H4 heterodimer. The C-terminal β-sheet domain of HJURP caps the DNA-binding region of the histone heterodimer, preventing it from spontaneous association with DNA. Our analysis also revealed a novel site in CENP-A that distinguishes it from histone H3 in its ability to bind HJURP. These findings provide key information for specific recognition of CENP-A and mechanistic insights into the process of centromeric chromatin assembly.