composite microstructure of the building units, which we imaged by EBSD down to the submicron scale and which is essential for the mechanical strength and self-sharpening ability of the calcite teeth. There is direct as well as indirect evidence supporting the paradigm [6] that most if not all of the biocalcites grow from amorphous precursors. Crystallographic textures vary from weakly cylindrical (in coralline red algea) via the very frequent strongly cylindrical textures in shell valves to 3-dimensional single crystal-like coherence.

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Keywords: biomineralisation, microstructure, texture

MS.15.1

Acta Cryst. (2011) A**67,** C49

Structural and functional analysis of the ISW1a chromatin remodeling complex

Kazuhiro Yamada, Timothy D. Frouws, Brigitte Angst, Kyoko Schimmele, David F. Sargent and Timothy J. Richmond. *Institute of Molecular Biology and Biophysics, ETH-Honggerberg, 8093, Zurich (Switzerland).* E-mail: yamada@mol.biol.ethz.ch

Saccharomyces cerevisiae (Yeast) ISW1a is classified into the Imitation Switch (ISWI) subgroup of the ATP-dependent chromatin remodeler superfamily including the SWI2/SNF2-, CHD1- and INO80-subgroups, each with various chromosomal processing activities. The main function of ISW1a is the repression of gene expression by an ATP-dependent modulation of nucleosome positioning around the promoter region. ISW1a is comprised of two subunits, Isw1 and Ioc3, with a total mass of 220 kDa. Isw1 contains the N-terminal ATPase domain of 633 amino acids as its catalytic motor domain connected by a linker of 133 amino acids to the C-terminal domain composed of three consecutive subdomains (Hand, Sant and Slide: "HSS"). This HSS region, comprising 300 amino acids, has been shown by many biochemical studies to be the DNA binding module of Isw1. The Ioc3 subunit is composed of 787 amino acids and has a significant amino acid similarity to yeast Esc8, which is implicated in gene silencing. Ioc3 is as yet poorly characterized, with only one biochemical study suggesting it to be a general DNA binding protein. Its domain composition and structure are entirely unknown. Here we present the crystal structures of ISW1a lacking the ATPase domain (ΔATPase) as a HSS-Ioc3 complex, either without DNA (resolution 3.25Å) or with two DNA segments bound independently to Ioc3 and HSS (resolution 3.6 Å). The structures reveal four fundamental aspects for the ISWIa complex. (1) The complex is 'L'-shaped (approximately 130 x 80 x 60 Å) with a large, tight interface between HSS and Ioc3 (buried surface area 5303 Å^2). (2) The novel protein fold architecture of Ioc3 has two major protruding subdomains: a domain composed entirely of loops functionally involved with the Sant subdomain interaction, and a domain containing a five-helix bundle potentially involved in nucleosome recognition. (3) Ioc3 has a novel recognition motif and binds the major groove of DNA with some sequence specificity. This motif is formed from two loops originating from two widely separated regions of the primary sequence. (4) Sant and Slide are structurally similar subdomains, each composed of three-helix bundles. Together the two subdomains bind a second copy of DNA, but in a topologically unique manner: the first helix of Sant lies across the DNA minor groove while with Slide the third helix contacts the DNA and is aligned parallel to the minor groove. Neither of these interactions shows any sequence specificity.

In further studies, EM analysis of a complex of ISW1a with a nucleosome revealed two forms of HSS-Ioc3-mononucleosome complexes. By interpreting the cryo-EM 3D images of these two complexes on the basis of our atomic-resolution structures of the nucleosome core particle and of the HSS-Ioc3-DNA complex, it was apparent that the HSS-Ioc3 complex had opposite orientations with respect to the nucleosome in the two forms (with either the Hand subdomain or Ioc3 bound to the nucleosome). These two structures can be integrated into a model with one HSS-Ioc3 complex located on linker DNA between two adjacent nucleosomes (a di-nucleosome). Using biochemical mobility assays with ISW1a (with ATPase) complexed with a di-nucleosome, we have shown that the ATPase domain of ISW1a drives the uni-directional relocation of the DNA wrapped around a nucleosome. Taken together, our data allows us to describe the entire remodeling mechanism of ISW1a for nucleosomes in the higher order chromatin structure.

Keywords: chromatin, nucleosome, structure

MS.15.2

Acta Cryst. (2011) A**67,** C49

Combinatorial readout of histone H3 modifications specifies localization of ATRX to heterochromatin

Sebastian Eustermann,[a] Ji-Chun Yang,[a] Martin Law,[b] Rachel Amos,[b] Clare Jelinska,[b] Lynda Chapman,[a] Richard Gibbons,[b] Daniela Rhodes,[a] Douglas Higgs[b] and David Neuhaus,[a] [a]*Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge (UK).* [b]*Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford (UK).* E-mail: se@mrc-lmb.cam.ac.uk

Accurate read-out of chromatin modifications is essential for eukaryotic life. Mutations in the X-linked ATRX gene cause a mental retardation syndrome [1], while wild-type ATRX protein targets pericentric and telomeric heterochromatin for deposition of the histone variant H3.3 [2] via a largely unknown mechanism. Here, we show that the ADD domain of ATRX, where most syndrome-causing mutations occur, engages the N-terminal tail of histone H3 through two rigidly oriented binding pockets, one for unmodified Lys4, the other trimethylated Lys9. *In vivo* experiments show that this combinatorial readout is required for ATRX localization, with recruitment enhanced by a third interaction via heterochromatin protein 1 (HP1) that also recognizes trimethylated Lys9. The cooperation of ATRX ADD domain and HP1 in chromatin recruitment results in a tripartite interaction that may span neighbouring nucleosomes and illustrates how the "histone-code" is interpreted by a combination of multivalent effector-chromatin interactions.

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Keywords: Epigenetic Memory, Disease Mechanism, Multivalent Chromatin Interactions

MS.15.3

Acta Cryst. (2011) A**67,** C49-C50

Molecular mimicry in the assembly of chromatin protein complexes

Andrea Mattevi, Marcello Tortorici, Simona Pilotto, Daniele Bonivento, Giuseppe Ciossani, Aristotele Karytinos, *Department of Dept. Genetics and Microbiology, University of Pavia (Italy).* E-mail: andrea.mattevi@unipv.it

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.

Supported by AIRC and Fondazione Cariplo

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Key-words: chromatin, transcription, protein_complex

MS.15.4

Acta Cryst. (2011) A**67,** C50

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

Mariko Ariyoshi,[a] Junji Otani,[b] Kyohei Arita,[b] Mariko Kinoshita,[b] and Masahiro Shirakawa,[b] [a]*Institute for Integrated Cell-Material Sciences, Kyoto University, (Japan).* [b]*Graduate Scholl of Engineering, Kyoto University, (Japan).* E-mail: ariyoshi@moleng.kyoto-u.ac.jp

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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Keywords: biomolecule, DNA, protein

MS.15.5

Acta Cryst. (2011) A**67,** C50-C51

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

Na Yang, Hao Hu, Rui-Ming Xu, *National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, (China).* E-mail: yangna@moon.ibp.ac.cn

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.