for the first time. These observations lead to a mechanism of RapA-mediated RNAP recycling, including the RapA-facilitated release of sequestered RNAP from DNA template and the σ70-dependent removal of RapA from the RapA•CORE complex for transcription reinitiation. The derived mechanism of RapA provides a framework for further structural and biochemical investigations on, for example, how and where RNAP becomes sequestered in the PTC, the exact composition of the PTC, the DNA translocase activity of RapA and its precise mechanism, and additional factors that may contribute to the destabilization of the PTC.

Keywords: Swi2/Snf2, RapA, mechanism for RNA polymerase recycling

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Structure and function of the histone chaperone CIA/ASF1 complexed with histones H3 and H4

Ryo Natsume[1], Masamitsu Eitoku[2], Yusuke Akai[1], Norihiko Sano[2], Masami Horikoshi[2], Toshiya Senda[3]
[1]Japan Biological Informatics Consortium, Japan Biological Information Research Center, AIST Bio-IT Research Building 3F, Aomi 2-42, Koto-ku, Tokyo, 135-0064, Japan, [2]IMCB, The Univ. of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-0032, Japan, [3]BIRC, AIST, Aomi 2-42, Koto-ku, Tokyo, 135-0064, Japan, E-mail：ryo-natsume@aist.go.jp

Histone chaperones mediate the nucleosome assembly/disassembly. Since the nucleosome structure inhibits the interaction between a protein and DNA, the assembly/disassembly of the nucleosome are critical processes regulating the transcription *etc*. To elucidate the molecular mechanism of the nucleosome assembly/disassembly, we have studied the structure-function relationship of histone chaperone CIA/ASF1. CIA/ASF1 interacts preferentially with histones H3 and H4 and is involved in transcription, DNA replication, and DNA repair. Here, we report the crystal structure of CIA/ASF1 complexed with histones H3 and H4 [Natsume *et al.* Nature (2007)]. The crystal structure shows that CIA/ASF1 interacts with the histone H3-H4 dimer in a manner that inhibits the histone $(H3\text{-}H4)_2$ tetramer formation. Since the CIA/ASF1-histone-H3-H4 complex is crystallized from a solution containing CIA/ASF1 and the histone $(H3\text{-}H4)_2$ tetramer, CIA/ASF1 seems to have a histone $(H3\text{-}H4)_2$ tetramer-splitting activity. Biochemical analysis demonstrated that CIA/ASF1 splits the histone $(H3\text{-}H4)_2$ tetramer through forming the CIA/ASF1-histone-H3-H4 complex. This is the first experimental evidence for the existence of an endogenous factor that splits the histone $(H3\text{-}H4)_2$ tetramer into two histone H3-H4 dimers. This finding should have a great impact on the research of chromatin. A comprehensive *in vivo* mutational analysis using budding yeast suggested that the interaction observed in the crystal structure is of biological significance and that the CIA/ASF1-histone-H3-H4 complex occurs as an intermediate of the nucleosome assembly/disassembly process during transcription, DNA replication, and DNA repair. In this context, this study should give a new insight into the molecular mechanisms of epigenetic inheritance.

Keywords: histone chaperone, nucleosome assembly/disassembly, epigenetics

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Novel fold of VirA, a type III secretion system effector protein

Alexander Wlodawer, Jamaine Davis, Jiawei Wang, Di Zhang, Joseph E Tropea, Zbigniew Dauter, David S Waugh
National Cancer Institute, Macromolecular Crystallography Laboratory, Bldg. 536, Rm. 5, Frederick, MD, 21702, USA, E-mail：wlodawer@ncifcrf.gov

VirA is an effector protein that is injected into mammalian cells by the type III secretion system of *Shigella flexneri*. VirA was postulated to function as a papain-like cysteine protease, with its putative catalytic cysteine residue identified by mutation to alanine (Yoshida et al. Science. 314, 985, 2006). The structure of VirA was solved by single wavelength anomalous scattering, utilizing a SeMet-substituted protein expressed in *E. coli* as a fusion to MBP. Processing of the fusion product with TEV protease yielded full-length VirA (400 amino acids). The protein was crystallized using the vapor-diffusion technique. The crystals belong to the monoclinic space group *C*2, with unit-cell parameters a=150.3, b=170.9, c=46.2Å, β=104.9, and diffract to 3.0 Å resolution. With the presence of two molecules in the asymmetric unit, the Matthews coefficient (Vm) is approximately 3.2, corresponding to a solvent content of about 61%. The structure was solved using a combination of SHELXD and PHASER/BUCCANEER and was refined with PHENIX. The fold of VirA is novel and does not resemble that of any known protein, including papain. The shape of the molecule resembles the letter V, with the N-terminal 130 residues (some of which are disordered) forming one clearly identifiable domain, and the remainder of the molecule forming the other half of the V-like structure. Two long helices (286-307) stabilize a dimer observed in the crystals. The oligomeric nature of VirA in solution is being investigated by analytical ultracentrifugation. Although the expressed protein appears to cleave α-tubulin, we have not found any structural features that resemble the active sites of known proteases. Thus, the mode of action of this unusual protein needs further elucidation.

Keywords: new fold, protease, virulence

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Structural basis on small MutS-related domain of human BCL-3 binding protein

Tae Gyun Kim, Changill Ban
Pohang University of Science and Technology, Dept of chemistry #403, San 31, Hyoja-dong, Nam-gu, Pohang, Kyungbuk, 790-784, Korea (S), E-mail：crystal@postech.ac.kr

DNA recombination is involved in the appearance of new variants by incorporating of exogenous DNA or endogenous reshuffling. MutS homologs, identified in nearly all bacteria and eukaryotes, include the bacterial proteins MutS1 and MutS2 and the eukaryotic MutS homologues, and they often are involved in recognition and repair of mismatched bases and small insertion/deletions, thereby limiting illegitimate recombination and spontaneous mutation. The small MutS-related domain (SMRD) of the bacterial MutS2 family is also found in eukaryotic homologue, C-terminal regions of BLC-3 binding protein (B3BP), which interacts with BCL3 and p300/CBP. Here, we report the crystal structure of soluble SMRD, a protein of 80 amino acids, which may function as a monomeric nicking endonuclease. Data were collected and refined to 1.5 Å resolutions from a single crystal of SMRD under cryogenic conditions. The