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

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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

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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

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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

protein crystallized in space group $P2_1$, with unit-cell parameter a = 34.538, b = 56.772, c = 71.710 Å. The overall structure contains three alpha-helix and four beta-strands. In refined model, we can suggest that the very electrostatically positive surface of SMRD is a possible site of interaction between SMRD and DNA substrate. To define the mechanism of DNA recombination and repair, this structural insight will have to be complemented by new cell-based and complexed approaches.

Keywords: SMRD, B3BP, nicking endonuclease

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Crystal structure of the Fab fragment of antibody against *p*-bronophenylalanine

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p-Boronophenylalanine (BPA) is used to the treatment of brain tumors as one of the boron carriers of boron neutron capture therapy (BNCT). The monoclonal antibody against BPA was prepared and named “anti-BPA”. Anti-BPA with high specificity is expected to be a potent and effective tool in order to elucidate the intra/extra cellular distribution and mode of action of BPA. The monoclonal antibody is also useful to perform the specific monitoring and analytical immunoassay system of BPA including determination of BPA concentration. We have started an analysis for the crystal structure of anti-BPA to clarify the structural features participating in antigen recognition of anti-BPA. Fab fragment of anti-BPA was prepared by papain digestion. Fab was purified by MonoQ column. The solution of Fab was concentrated to 8 mg/ml in 20 mM Tris-HCl (pH 7.5) prior to crystallization. Fab was crystallized by sitting drop vapor diffusion method at 293 K using PEG4000 as a precipitant. A data set was collected to 3.0 Å resolution from a frozen crystal using synchrotron radiation of wavelength 1.0 Å at PF. The crystal belongs to the rhombohedral space group $R3$ with unit-cell parameters a = b = 160.17, c = 306.19 Å. Molecular-replacement calculations were carried out with the program *Molrep* using structure of anti-(4-hydroxy-3-nitrophenyl) acetate antibody as a search model. Refinement and manual modifications of the model structure are currently in progress.

Keywords: antibody, boron compound, crystal structure

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Structural studies of the cytochrome c_z from the green photosynthetic bacterium *Chlorobium tepidum*

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Green sulfur photosynthetic bacteria have a reaction center (RC) with a Fe-S cluster as the terminal electron acceptor. The RC consists of five subunits: PscA containing a special pair (P840), PscB containing Fe-S clusters A and B (F_A/F_B), PscC containing a heme c (cytochrome c_z), PscD binding to the FMO, and the BChl-a protein FMO. Two molecules of cytochrome c_z bind to the RC and each of them has been reported to directly transport an electron from cytochrome bc_1 to the P840. Cytochrome c_z is supposed to consist of an N-terminal transmembrane domain and a C-terminal periplasmic domain which contains one heme c. In order to determine the crystal structure of the functional domain, we constructed a soluble variant of cytochrome c_z from the green sulfur photosynthetic bacterium *Chlorobium tepidum* (residues 111-206; C-cyt c_z). We determined the crystal structure of oxidized C-cyt c_z by the Fe-SAD method and refined to 1.3 Å resolution. The N-terminal 20 residues of C-cyt c_z are disordered and additional 8 residues form a loop structure. This feature may explain the flexibility between the transmembrane and the periplasmic domains of cytochrome c_z, which makes it possible to mediate the direct electron transfer between cytochrome bc_1 and RC. C-cyt c_z shows structural similarities with cytochrome c_{551} from *Pseudomonas aeruginosa* and cytochrome c_6 from *Monoraphidium braunii*. Despite of the overall structural similarities with the class I cytochrome proteins, the coordination pattern of the heme c iron is different between C-cyt c_z and other members in this class. On the other hand, unusual paramagnetic NMR shifts were observed for the oxidized form of C-cyt c_z. This may be attributed to the unique coordination environment of the heme c as revealed from the crystal structure.

Keywords: cytochromes, photosynthesis-related proteins, paramagnetic NMR

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Isopeptide bonds stabilize Gram-positive bacterial pilus structure and assembly

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Bacterial pili are long, slender appendages that mediate adhesion to host cells, and thus promote colonisation and infection. Gram positive organisms such as Streptococcus pyogenes have extremely thin (~30 Å) pili, formed by covalently-linked pilin subunits. We have determined the crystal structure of the major S. pyogenes pilin protein, and have derived an atomic model for pilus assembly which explains the extraordinary strength and stability of this structure. The 2.2 Å crystal structure reveals an extended structure comprising two all-beta domains. The molecules associate in columns through the crystal, providing a model for pilus assembly and for the location of the inter-subunit covalent bonds. The structure also revealed novel intramolecular crosslinks in each subunit, in the form of isopeptide bonds linking Lys and Asn side chains. Located at strategic places in the fold, these give strength and stability. Mutagenesis shows that they are generated by an intramolecular reaction involving adjacent Glu residues. Mass spectrometric analyses of purified pili confirm both the intramolecular isopeptide bonds and the intersubunit isopeptide bonds suggested by the crystal packing. Our results provide a model for the assembly of GAS pili, in which self-generated intramolecular isopeptide bonds complement the sortase-catalyzed intermolecular bonds. Database searches indicate that internal isopeptide crosslinks also exist in other proteins where mechanical strength and stability are needed, notably in other surface proteins of Gram-positive organisms.