Keywords: bacterial pili, isopeptide bonds, oligomer assembly

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### Recognition of an unusual peroxisomal targeting signal 1 by the import receptor Pex5p

Krisztian Fodor, Nicole Holton, Simon Holton, Matthias Wilmanns
EMBL Hamburg Outstation, Structure Biology Unit, Notkestr. 85, Hamburg, Hamburg, 22607, Germany, E-mail：fodor@embl-hamburg.de

The structure of the peroxisomal receptor Pex5p in the presence of a small model cargo revealed a large conformational change of the receptor upon cargo binding (Stanley et al., 2006). It, however, remained unknown whether the binding was cargo-specific and only indirect methods were applicable to test cargo activity during the translocation process. In order to investigate whether the previously observed type of cargo binding is generally applicable, we have determined the structure of Pex5p in complex with alanine-glyoxylate aminotransferase (AGT). The complex reveals how the unusual C-terminal KKL receptor recognition motif can be accommodated within the previously characterized central binding cavity. The present structure, similarly to the Pex5p-mSCP2 complex, reveals a secondary interaction site. From the receptor side the motif that participates in the interaction is the very same than the one in the Pex5p-mSCP2 structure. A common feature of all the available Pex5p crystal structures is that one of the seven TPR repeats of the receptor (TPR4) is not visible and thus can be considered as a highly flexible part of the molecule. Since most TPR proteins participate in various interactions it would not be surprising if the TPR4 segment was serving as an interaction site and stabilized by a binding partner in a later step of peroxisomal translocation. Important to note that TPR4 and the secondary binding motif are located on the opposite sides of the receptor. It is intriguing to hypothesize that secondary interactions are playing a role in the correct orientation of the cargo to ensure the accessibility of the TPR4 region during translocation process. Our structural and biochemical data also consistently show that AGT remains fully active when bound to the receptor.

Keywords: peroxisome, transport, Pex5p

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### Crystal structure of L-CKS from *Haemophilus influenzae* in complex with KDO

Hye-Jin Yoon[1], Min-Je Ku[1], Bunzo Mikami[2], Se Won Suh[1]
[1]Department of Chemistry, Seoul National University, Gwanak-gu,, Seoul, 151-742, Korea (S), [2]Graduate School of Agriculture, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan, E-mail：yoonhj@snu.ac.kr

The enzyme 3-deoxy-manno-octulosonate cytidylyltransferase (CMP-KDO synthetase; CKS) catalyzes the activation of 3-deoxy-D-manno-octulosonate (or 2-keto-3-deoxy-manno-octonic acid, KDO) by forming L-CKS. In order to determine the structure of L-CKS from *H. influenzae*, we have crystallized it by hanging drop vapour-diffusion method at 296 K. The crystal of L-CKS is orthorhombic, belonging to the space group $P2_12_12_1$ with unit cell parameters of $a$ = 48.42, $b$ = 82.61, $c$ = 115.71 Å. The presence of two monomers in the asymmetric unit gives a reasonable $V_M$ of 2.05 $Å^3$ $Da^{-1}$, with a solvent content of 40.0%. We determined the crystal structure of L-CKS from *H. influenzae* in complex with KDO at 2.30 Å resolution by the multiwavelength anomalous diffraction method. The overall protein structure is similar to that of K-CKS from *E. coli*. The C-terminal alpha-helix (Ala230-Asn254) of monomer A has a unique conformation. The structure of L-CKS from *H. influenzae* in complex with KDO will be useful in structure-based inhibitor design.

[1] Ku et al. (2003) *Acta Crystallog Sect D* 59, 180-182.
[2] Jelakovic et al. (2001) *J Mol Biol* 312, 143-155.

Keywords: antibacterial target, CKS, KDO

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### Towards the structural basis for bacterial two-partner secretion

Hye-Jeong Yeo, Takeshi Yokoyama
University of Houston, Biology and Biochemistry, 369 Science & Research Bldg 2, Houston, TX, 77204, USA, E-mail：hyeo@uh.edu

In pathogenic Gram-negative bacteria, many virulence factors are secreted via the two-partner secretion (TPS) pathway, which consists of an exoprotein called TpsA and a cognate outer membrane translocator called TpsB. The HMW1 and HMW2 adhesins are major virulence factors in nontypeable Haemophilus influenzae and are prototype TPS pathway exoproteins. A key step in the delivery of HMW1 and HMW2 to the bacterial surface involves targeting to the HMW1B and HMW2B outer membrane translocators by an N-terminal region called the secretion domain. To understand the structural basis for bacterial TPS, we aim to determine the crystal structures of the component proteins in the H. influenzae TPS pathway. The crystal structure of the HMW1 pro-piece (HMW1-PP), a region that contains the HMW1 secretion domain, reveals a large right-handed beta-helix fold. Comparison of HMW1-PP and the Bordetella pertussis FHA secretion domain (Fha30) displays limited amino acid homology but shared structural features, suggesting that diverse TpsA proteins have a common structural domain required for targeting to cognate TpsB proteins. Our progress on the project will be presented and discussed.

Keywords: two-partner secretion, beta-helix, HMW1 adhesin



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### Structure and function of the human histone chaperone CIA complexed with the bromodomain from TFIID

Yusuke Akai[1,2], Naruhiko Adachi[1], Yohei Hayashi[3], Masamitsu Eitoku[3], Norihiko Sano[3], Norio Kudo[2], Masaru Tanokura[2], Masami Horikoshi[3], Toshiya Senda[4]
[1]JBIRC, JBIC, Aomi 2-42, Koto-ku, Tokyo, 135-0064, Japan, [2]Grad. Sch. of Agri. and Life Sciences, The Univ. of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-8657, Japan, [3]IMCB, The Univ. of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-0032, Japan, [4]BIRC, AIST, Aomi 2-42, Koto-ku, Tokyo, 135-0064, Japan, E-mail：y-akai@aist.go.jp

Histone modifications frequently function as a mark to induce nucleosome structure changes in a site-specific manner. Although many domains that specifically recognize histone modification have been identified, the molecular mechanism of the change in the nucleosome structure induced by histone modification remains elusive. We have therefore studied the functional interaction between the histone chaperone CIA, which has histone-$(H3\text{-}H4)_2$-tetramer disrupting activity, and an acetylated histone-recognizing

double-bromodomain of the CCG1 subunit in the TFIID complex (DBD(CCG1)). This interaction should be a key to connecting the histone acetylation and the nucleosome structure change. Here we report the crystal structure of the CIA-DBD(CCG1) complex at 3.3 Å resolution. Although we crystallized the complex by mixing equimolar amounts of CIA and DBD(CCG1), the crystal structure showed that one DBD(CCG1) molecule binds two CIA molecules in two distinct sites. The GST pull-down assay with point mutants of DBD(CCG1) at either binding site also suggested that DBD(CCG1) has two binding sites for CIA in solution. The crystal structure of the CIA-DBD(CCG1) complex suggested that an acetylated lysine residue can interact with DBD(CCG1) in the complex. In addition, the superposition of the CIA/ASF1 in the CIA-histone-H3-H4 complex [Natsume *et al.*, Nature (2007)] onto the CIA in the present complex showed that the histone H3-H4 dimer competes with DBD(CCG1) for CIA through steric hindrance. These observations suggest that (i) the recruitment of the CIA-DBD(CCG1) complex to the acetylated histone and (ii) the histone acetylation-induced nucleosome structure change are mediated by interactions among CIA, DBD(CCG1), and acetylated histones.

Keywords: histone chaperone, bromodomain, nucleosome assembly/disassembly

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### Structural studies of human PCNA mutant, REV6-1

Asami Hishiki[1], Hiroshi Hashimoto[1], Yuji Masuda[2], Shinya Saijo[3], Aya Serizawa[1], Kenji Kamiya[2], Haruo Ohmori[4], Toshiyuki Shimizu[1], Mamoru Sato[1]

[1]Yokohama City University, International Graduate School of Arts and Sciences, 1-7-29 Suehiro-cho Tsurumi-ku, Yokohama, Kanagawa, 230-0045, Japan, [2]Hiroshima University, [3]Japan Synchrotron Radiation Research Institute, [4]Kyoto University, E-mail：asami@tsurumi.yokohama-cu.ac.jp

All cells are continually exposed to a large variety of external and internal agents that damage their DNA. DNA replication in eukaryote is a highly coordinated process involving many proteins that work cooperatively to ensure the accurate and efficient replication of DNA. Although replicative DNA polymerases δ/ε synthesize genomic DNA in normal condition, the replicative polymerases are not able to synthesize DNA using damaged nucleotide as a template because of their high fidelity. In that situation, translesion synthesis (TLS) polymeases alternatively synthesize DNA by error-free or error-prone manners. PCNA, a member of sliding clamp family and ring-shaped homotrimer, not only stimulates processive DNA synthesis by the replicative polymerase, Polδ, but also provides the central scaffold to which TLS polymerases bind to gain access to the replicative machinery stalled at the lesion site and to execute lesion bypass synthesis. Actually, PCNA is characterized as one of *REV* genes' products affecting UV-induced mutagenesis in *Saccharomyces cerevisiae*. Furthermore, the *rev6-1* mutant strain produces a G178S substitution in PCNA (REV6-1) and shows deficiency in TLS. Although the side chain of substituted serine appears to show an unfavorable steric interaction because the G178 residue is located in a $\beta$-sheet at the interface between the monomers composing trimer, structural features of REV6-1 are still unclear. To clarify the influence to oligomerization of PCNA by only one-residue-substitution, we investigate the difference of structural and functional features between human PCNA and REV6-1 by several methods including crystallography, and discuss the comparisons of their behaviors in solution and crystal.

Keywords: DNA replication, structural biology, protein crystallography

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### Uranyl mediated photocleavage in proteins

Annasara Dahlstroem, Marc Schiltz

EPFL, LCR, LCR-IPMC-FSB-EPFL, Lausanne, VD, 1015, Switzerland, E-mail：annasara.dahlstrom@epfl.ch

Uranyl ions absorb light in the UV-visible region and have been found to bind to and cause photo-cleavage in biomolecules such as proteins and nucleic acids. In DNA, the uranyl ion binds to the phosphate backbone of the molecule and can potentially cleave the molecule between any base pair along the chain. In proteins, this binding and thereby the induced structural changes, are much more specific. One of many uranyl-binding proteins is porcine pancreatic elastase (PPE). In this study, elastase-crystals have been soaked in a uranyl-containing buffer and several data sets have been collected in house and at the synchrotron at both room temperature and at 100K. The uranyl binds with high occupancy in a single position close to the well-known calcium/sodium binding-site. Only small conformational changes compared to the native structure are seen upon ligand binding. These changes originate from amino acids moving either to accommodate space for (Tyr71) or to co-ordinate the ligand (Glu59, Glu69). However, the sodium ion is not moving from its native site. In addition, two acetates bind to the uranyl. After irradiation (400nm, 2h), the observed structure is still that of the native structure. However the density of one amino acids in the direct proximity of the uranyl ion, Asn63, has completely disappeared. The densities of the acetates have also degraded and only the positions of the coordinating oxygens are visible.

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Nielsen, P.E., Jeppesen, C., Buchardt, O., Uranyl salts as photochemical agents for cleavage of DNA and probing of protein-DNA contacts. FEBS Letters 1988, 235, 122-124.

Keywords: photo-induction, protein-ligand complexes, protein X-ray crystallography

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### Water-soluble chlorophyll-binding protein from *Chenopodium album*

Akira Uchida, Takayuki Ohtsuki, Isao Oonishi

Toho University, Department of Biomolecular Science, Miyama 2-2-1, Funabashi, Chiba, 274-8510, Japan, E-mail：auchida@biomol.sci.toho-u.ac.jp

Water-soluble chlorophyll-binding proteins (WSCPs) have been found in some terrestrial plants. WSCPs can be categorized into two classes according to photoconvertibility. The absorption spectra of class I WSCPs change drastically on exposure to visible light, whereas those of class II WSCPs do not change at all. The amino acid sequences of classes I and II WSCP show no similarity to each other. The physiological function of WSCPs is not known although it is supposed to be a chlorophyll (Chl) carrier. So far, only the structures of class II WSCP have been determined because of high sensitivity