pumps are proposed, which visualizes how MFPs link TolC and inner membrane transporters bridging the inner and outer membranes.

Keywords: MFP, efflux pump, MacA

P04.14.330

Acta Cryst. (2008). A**64**, C334

Structural basis of regulatory inactivation of DnaA

Qingping Xu[1,2], Ashley M. Deacon[1,2], Ian A. Wilson[2,3]
[1]Stanford University, Stanford Synchrotron Radiation Laboratory, 2575 Sand Hill Road MS99, Menlo Park, CA, 94025, USA, [2]Joint Center for Structural Genomics, http://www.jcsg.org, [3]The Scripps Research Institute, La Jolla, California, E-mail：qxu@slac.stanford.edu

Regulatory inactivation of DnaA (RIDA) is an important mechanism to prevent over initiation during bacterial chromosomal replication. RIDA is dependent on the protein Hda and the sliding clamp of DNA polymerase III. Hda, a member of the AAA+ ATPase superfamily and a homolog of DnaA, can trigger the transformation of DnaA from the active DnaA-ATP form into the inactive DnaA-ADP, when bound to the sliding clamp loaded on the duplex DNA. The crystal structure of the dimeric Hda from Shewanella Amazonensis SB2B was determined to 1.75 Å resolution by X-ray crystallography. The arrangement of the two domains in Hda differs dramatically from that of DnaA, despite structural similarities within each domain. A CDP molecule, bound to Hda, anchors the two domains in a conformation which promotes dimer formation. The Hda dimer displays a novel mode of oligomeric assembly for AAA+ proteins in which the arginine finger (Arg 161), that is crucial for ATP hydrolysis, is fully exposed. The structure suggests that Hda has not retained the ability to bind and hydrolyze nucleotide triphosphates. However, the dimer arrangement is compatible with an Hda-DnaA interaction, similar to the DnaA self-assembly at the origin of replication, which allows Hda to hydrolyze DnaA-ATP through a typical AAA+ type mechanism. Two sliding clamp binding motifs at the N-terminus (13-18) of Hda form an antiparallel β-sheet at the dimer interface. This surface could interact with the sliding clamp through hydrophobic interactions. A model for the Hda-DnaA, sliding clamp and duplex DNA complex is proposed. The JCSG is funded by NIGMS/PSI, U54 GM074898. SSRL operations are funded by DOE BES, and the SSRL SMB program by DOE BER, NIH NCRR BTP and NIH NIGMS.

Keywords: regulatory inactivation of DnaA (RIDA), Hda, DNA replication initiation

P04.14.331

Acta Cryst. (2008). A**64**, C334

Crystallographic analysis of the Phycobilisome antenna complex: Assembly and disassembly of a giant

Noam Adir[1], Liron David[1], Monica Dines[1], Merav Klartag[1], Ailie McGregor[1], Eleonora Sendersky[2], Rakefet Schwarz[2]
[1]Technion, Technion City, Schulich Faculty of Chemistry, Technion, Haifa, N/A, 32000, Israel, [2]The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel., E-mail：nadir@tx.technion.ac.il

In cyanobacteria and red algae, the major light harvesting pigment-protein complex is the phycobilisome (PBS), an enormous complex with a molecular weight of 3-7MDa. Three dimensional structures of isolated components have been determined by X-ray crystallography for a variety of species; however our understanding of the overall structure of the PBS is still dependent on low resolution studies. We describe here high resolution structural information obtained on isolated components of the PBS from the thermophilic cyanobacterium T. vulcanus: trimeric phycocyanin (1.4Å), rod phycocyanin (MW of ~500kDa) in the presence of linker proteins (1.8 Å) and trimeric allophycocyanin (2.9 Å). These structures shed light on the process of PBS energy transfer mechanisms, on its ability to self-assembly and on its resistance to thermal or chemically induced denaturation. We have isolated, purified, functionally characterized and crystallized intact PBS of a molecular of greater than 3MDa. The crystals diffract to at least 3Å and we will describe our progress in determining its structure. Under certain conditions of nutrient starvation, the PBS is disassembled in an ordered manner, and its components are degraded as a secondary source of metabolites. We report here the determination of the three-dimensional structures of the NblA protein (2.5Å), an essential component in the disassembly process, from two cyanobacterial species (T. vulcanus and S. elongatus). Random mutagenesis in the S. elongatus protein shows that critical residues affecting the disassembly process in vivo can be found at internal positions as well as at the polypeptide termini leading to a model for the NblA proteins mode of action which is different than previously suggested.

Keywords: macromolecular assemblies, photosynthesis, biological structure-activity relationships

P04.14.332

Acta Cryst. (2008). A**64**, C334−335

A topological model of the baseplate of lactococcal phage Tuc2009

Valerie Campanacci[1], Giuliano Sciara[1], Stephanie Blangy[1], Silvia Spinelli[1], Marina Siponen[1], Stephen Mc Grath[2], Douwe van Sinderen[2,3], Mariella Tegoni[1], Christian Cambillau[1]
[1]CNRS, AFMB, case 932, 163 avenue de Luminy, Marseille, France, 13288, France, [2]Department of Microbiology, National University of Ireland, Cork, Ireland, [3]Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland, E-mail：valerie.campanacci@afmb.univ-mrs.fr

The knowledge on phages infecting gram (+) bacteria and belonging to the Siphoviridae family lags behind that accumulated for members of Myoviridae. The receptor binding proteins (RBPs) are 20-30 kDa proteins that are attached to the baseplate, a large phage organelle, located at the tip of the non contractile tail. These proteins allow the recognition of a host cell wall receptor by the phage. The 3D structures of the RBPs of three phages infecting Lactococcus lactis have been determined recently (1-3). Tuc2009 phage baseplate is formed by the products of 6 ORFs, including the RBP (4). Since phage binding to its receptor induces DNA release, it has been postulated that the baseplate might be the trigger for DNA injection. Structural features of the Tuc2009 phage baseplate were established using blue native PAGE and also size exclusion chromatography coupled to on-line UV/VIS absorbance, light-scattering and refractive index detection (MALS/UV/RI). Using this latter system, we determined the self-association and the inter-association of the baseplate components, the stoichiometry of the interacting components and we also measured their hydrodynamic radii. We will present how the results obtained with this approach and combined with literature data led us to propose a “low resolution” model of Tuc2009 baseplate (5). We will also show how this strategy could be helpful to submit relevant complexes to crystallization trials for Tuc2009 in particular and for phages in general.
1. Tremblay D.M., et al. (2006). J Bacteriol. 188 (7), 2400-10.
2. Spinelli S., et al. (2006). J Biol Chem. 281 (20), 14256-62.

3. Ricagno S., et al. (2006). J Virol. 80 (18), 9331-5.
4. Mc Grath, S., et al. (2006) J Bacteriol 188, 3972-3982
5. Sciara G., et al. (2008). J Biol Chem. 283 (5), 2716-23.

Keywords: phage, protein complexes, protein-protein interactions

P04.14.333

Acta Cryst. (2008). A**64**, C335

Structural studies on the active and inactive positive elongation factor b complexes

Ella Czarina M. Juan[1], Shun-ichi Sekine[1,2], Shigeyuki Yokoyama[1,2,3]
[1]The University of Tokyo, Department of Biophysics and Biochemistry, Graduate School of Science, 2-11-16 Yayoi, Bunkyo-ku, Tokyo, Tokyo, 113-0032, Japan, [2]Genomic Sciences Center, Yokohama Institute, RIKEN, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan, [3]RIKEN SPring-8 Center, Harima Institute, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan, E-mail：ella@biochem.s.u-tokyo.ac.jp

The positive elongation factor b (P-TEFb), which is composed of cyclin-dependent kinase 9 (Cdk9) and Cyclin T1, facilitates transcriptional elongation by phosphorylating RNA polymerase II and the negative elongation factors. P-TEFb is required for transcription of a majority of cellular genes, as well as for the expression of the HIV-1 genome. Recent studies have shown that half of nuclear P-TEFb in HeLa cells is rendered inactive by the interaction of the 7SK RNA and the HEXIM1 protein, while the other half is catalytically active and binds the bromodomain protein, Brd4. The structures of fragments of Cyclin T1 [1], HEXIM1 [2] and Brd4 [3] are available, but the macromolecular complex structures remain essential to achieve a detailed understanding of the mechanism of P-TEFb regulation. The human Cdk9, Cyclin T1, HEXIM1 and Brd4 genes were cloned, expressed in Escherichia coli and purified as histidine-tagged or GST fusion proteins. Multicrystals were obtained using standard crystallization techniques. Optimization of the crystallization conditions are ongoing, and will hopefully give single crystals suitable for X-ray diffraction experiments.
[1] Anand K., Schulte A., Fujinaga K., Scheffzek K., Geyer M., J. Mol. Biol., 2007, 370, 826-836. [2] Dames S.A., Schönichen A., Schulte A., Barboric M., Peterlin B.M., Grzesiek S., Geyer M., Proc. Natl Acad. Sci. USA, 2007, 104, 14312-14317. [3] Abbate E.A., Voitenleitner C., Botchan M.R., Mol. Cell, 2006, 24, 877-889.

Keywords: elongation factor complexes, RNA-protein complex, transcription regulation

P04.14.334

Acta Cryst. (2008). A**64**, C335

Structure of the Yersinia needle protein YscF in complex with its heterodimeric chaperone YscE/YscG

David S. Waugh, Ping Sun, Joseph E Tropea, Brian P Austin, Scott Cherry
Naitonal Cancer Institute, Macromolecular Crystallography Laboratory, 1050 Boyles Street, Frederick, MD, 21702, USA, E-mail：waughd@ncifcrf.gov

The plague-causing bacterium Yersinia pestis utilizes a Type III Secretion System (T3SS) to deliver effector proteins into mammalian cells where they interfere with signal transduction pathways that mediate phagocytosis and the inflammatory response. Effector proteins are injected through a hollow needle structure composed of the protein YscF. YscG and YscE act as “chaperones” to prevent premature polymerization of YscF in the cytosol of the bacterium prior to assembly of the needle. Here, we report the crystal structure of the YscEFG protein complex at 1.8 Å resolution. Overall, the structure is similar to that of the analogous PscEFG complex from the Pseudomonas aeruginosa T3SS, but there are noteworthy differences. The structure confirms that, like PscG, YscG is a member of the tetratricopeptide repeat (TPR) family of proteins. YscG binds tightly to the C-terminal half of YscF, implying that it is this region of YscF that controls its polymerization into the needle structure. YscE interacts with the N-terminal TPR motif of YscG but makes very little direct contact with YscF. Its function may be to stabilize the structure of YscG and/or to participate in recruiting the complex to the secretion apparatus. No electron density could be observed for the N-terminal 49 residues of YscF. This and additional evidence suggest that the N-terminus of YscF is disordered in the complex with YscE and YscG. As expected, conserved residues in the C-terminal half of YscF mediate important intra- and intermolecular interactions in the complex. Moreover, the phenotypes of some previously characterized mutations in the C-terminal half of YscF can be rationalized in terms of the structure of the heterotrimeric YscEFG complex.

Keywords: type III secretion, plague, tetratricopeptide repeat

P04.14.335

Acta Cryst. (2008). A**64**, C335−336

The structural analysis of Rpn14 as the molecular-chaperone for eukaryotic 26S proteasome assembly

Sangwoo Kim[1], Tsunehiro Mizushima[1,2], Yasushi Saeki[3], Takashi Yamane[2], Keiji Tanaka[3], Koichi Kato[1]
[1]Nagoya City University, Department of Structural Biology and Biomolecular Engineering, Graduate School of Pharmaceutical Sciences, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi, 467-8603, Japan, [2]Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan, [3]Tokyo Metropolitan Institute of Medical Science, Bunkyo-ku, Tokyo 113-8613, Japan, E-mail：nakada93@hotmail.com

Eukaryotic 26S proteasomes are macromolecular complex for degrading by recognition of ubiquitination proteins. 20S proteasome was composed of 7α-subunits and 7β-subunits for being associated in the order of $\alpha\beta\beta\alpha$. 19S regulatory particle (RP) was composed of base and lid complexes. Recent studies have been reported that the proteasome-chaperones were necessary for 26S proteasome assembly. Rpn14 has been reported to related stability of 26S proteasome by interaction with 19S RP. We have determined the three-dimensional structure of Rpn14 as the molecular-chaperone for eukaryotic 26S proteasome to assemble at a resolution of 2.5 Å. Recombinant protein was expressed in *E. coli*. The protein was purified by Ni-affinity, ion exchange and gel filtration chromatography. The protein crystallizes in space group $P6_4$ (a = 78.6 Å, b = 78.6 Å, c = 110.1 Å) with one 43 kDa protein monomer per asymmetric unit. Rpn14 has a globular structure consisting of a seven-WD40 repeat. Further studies on physiological analysis for the Rpn14 activity and its interaction with subunits of the proteasome will be