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