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Keywords: phage, protein complexes, protein-protein interactions

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

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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.

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

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

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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

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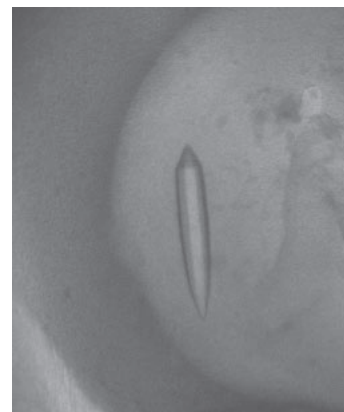
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The structural analysis of Rpn14 as the molecular-chaperone for eukaryotic 26S proteasome assembly

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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



required to clarify the role of Rpn14 in 26S proteasome assembly.

Keywords: proteasome, chaperones, assembly

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3D structure of small dense LDL; Application of low-resolution diffraction and *ab initio* methods

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Until now, no three-dimensional structural data of the 'especially bad' small dense (sd-LDL) particles existed. Our previously published X-ray structure [Lunin et al. 2001] and the one obtained by Orlova et al. [1999] (cryo EM) correspond to the larger LDL-2 particles. Both papers showed that LDL particles have a cylindrical respectively ellipsoidal shape and are not spherical particles as commonly assumed. In the particle core, a system of flat layers was observed. At present, we are able to grow crystals of native, human LDL particles from all LDL subfractions, except LDL-4. Whereas LDL-1 to LDL-3 (large LDL) crystallize in space group C2, LDL-5 and LDL-6 (sd-LDL) crystallize in space group P2(1). By special methods we were able to collect 100% complete datasets of LDL crystals in a resolution range of 300-27Å. A simple analysis of the parameters of the reduced cells shows that large-LDL and sd-LDL differ predominantly in the short axis of the crystallographic unit cell (large-LDL: $a=180$ Å, sd-LDL: $a=143$ Å). These results lead to the hypothesis that sd-LDL particles, if approximated as cylinders, have a height reduced by 37 Å compared to large-LDL. As the repeating distance of smectic layers of LDL cholesterol esters is also 37 Å, the observed reduction of one cell axis could correspond well to the disappearance of one layer of cholesterol esters in the particle core. This is perfectly supported by the now available electron density maps calculated from LDL-5 and LDL-6 datasets by *ab initio* methods were in fact the disappearance of one layer inside the particle core is visible. While differences in core structure are obvious, the analysis of the particle surface (ApoB fold) is more complicated due to the dense packing of the crystals, and still in progress.

Keywords: lipoproteins, *ab-initio* structure determination, low resolution

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The hexameric structures of the archeal secretion ATPase revealed by X-ray crystallography and SAXS

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Secretion ATPase superfamily is a widely conserved ATPase family that play the central role in many bacterial and archaeal macromolecular transport and filamentous subunit assembly mechanisms; such as type II secretion, type IV secretion, natural competence, type IV pilin, and archaeal flagellar systems. They

commonly form hexameric ring structure, by which they would function as a motor via the conformational transition upon ATP hydrolysis. Here, we report the crystal structure of the archaeal secretion ATPase, GspE from *Archaeoglobus fulgidus*, complexed with the non-hydrolysis ATP analog, AMP-PNP. It represents the alternating open and closed subunit conformations within a hexameric ring. The active site conformation bound with AMP-PNP and Mg^{2+} in the closed form reveals the catalytically active conformation. Solution structures analyzed by small angle x-ray scattering (SAXS) showed that the gamma-phosphate of AMP-PNP can lock the closed conformation to form the symmetric all-closed hexamer. In contrast, SAXS ADP structure revealed the asymmetric hexameric ring similar to crystal structure with AMP-PNP, suggesting the mixture of the active closed conformation and inactive open conformations. Together with these results, we propose the conformational transition mechanism upon ATP hydrolysis and its catalytic cycle, which would be common for all secretion ATPase superfamily.

Keywords: ATPase, hexameric ring, SAXS

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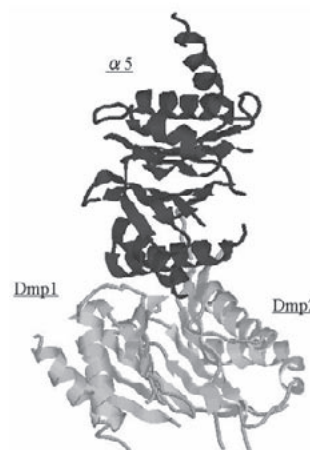
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Crystal structure of a chaperone complex that contributes to the assembly of yeast 20S proteasomes

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Eukaryotic 20S proteasomes are composed of two α -rings and two β -rings, which form an $\alpha\beta\beta\alpha$ stacked structure. Here we describe a proteasome-specific chaperone complex, designated Dmp1-Dmp2, in budding yeast. Dmp1-Dmp2 directly bound to the $\alpha 5$ subunit to facilitate α -ring formation. In Δ -dmp1 cells, α -rings lacking $\alpha 4$ and decreased formation of 20S proteasomes were observed. We also determined the crystal structure of Dmp1-Dmp2 Δ loop- $\alpha 5$ complex and illustrated an intermediate state of proteasome assembly. A model of Dmp1-Dmp2 interacting with the α -ring was generated by superimposed the $\alpha 5$ subunit from Dmp1-Dmp2 Δ loop- $\alpha 5$ on the structure of the 20S proteasome (PDB ID code 1RYP). Since the $\beta 2$, $\beta 3$ and $\beta 4$ subunits of the proteasome are thought to attach to the α -ring during the primary stage of β -ring assembly, the steric hindrance caused by $\beta 4$ and Dmp1 must trigger the release of Dmp1-Dmp2 from the α -ring during the attachment of the β subunit onto the α -ring.



Keywords: proteasome, chaperones, macromolecular X-ray crystallography