FA1-MS01-O1

The Recognition of Endocytic Signal Sequences by the AP2 Complex. Philip Evans[a], David Owen[b] Bernard Kelly[b], Airlie McCoy[b], Lauren Jackson[b]. [a]*MRC laboratory of Molecular Biology, Cambridge, UK.* [b]*Cambridge Institute for Medical Research, Cambridge, UK.*
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Membrane proteins are packaged for transport between the different membrane compartments of eukaryotic cells into small vesicles formed by an elaborate system of cytoplasmic proteins. Selection of cargo for vesicle formation at the plasma membrane (endocytosis) is generally mediated directly or indirectly by the heterotetrameric clathrin adaptor complex AP2, which binds short sequence recognition motifs of two types, YxxΦ (tyrosine-based motif, where Φ is a hydrophobic residue) and [DE]xxxLL (acidic dileucine motif). The structure of the 200kDa AP2 "core" crystallised in the absence of peptides [1] showed a closed conformation, with the known YxxΦ binding site on the μ2 subunit blocked. We have now determined the structure of a complex with an acidic dileucine peptide, which binds to the small σ2 subunit in a site previously occluded by the N-terminus of the β2 subunit [2]. A conformational change in the helical solenoids of the large α and β2 subunits opens up this site, but leaves the YxxΦ blocked: a further conformational change is required to make a fully active conformation.

[1] B.M.Collins, A.J.McCoy, H.M.Kent, P.R.Evans and D.J.Owen, *Cell,* 109, 523-535, **2002**. [2] Bernard T. Kelly, Airlie J. McCoy, Kira Späte, Sharon E. Miller, Philip R. Evans, Stefan Höning and David J. Owen. Nature, 456, 976-979, **2008**.

Keywords: membrane associated proteins; protein-peptide interactions; conformational change

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Structural Basis for the Regulated Protease and Chaperone Function of DegP. Tim Clausen. *Institute of Molecular Pathology, IMP, Vienna, Austria.*
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All organisms have to precisely monitor the folding state of cellular proteins. The heat-shock protein DegP is a protein quality control factor in the bacterial envelope that is involved in eliminating misfolded proteins and in the biogenesis of outer membrane proteins (OMPs). To investigate the molecular basis of these dual activities we have isolated and characterized a DegP/OMP complex. OMP binding transforms hexameric DegP into large, catalytically active 12- and 24-meric multimers. Structural analysis of these particles revealed that DegP represents a novel high-symmetry packaging device, whose central compartment is used to sequester proteins. However, the inner cavity serves antagonistic functions. While encapsulation of folded OMP protomers is protective and might allow safe transit through the periplasm, misfolded proteins are eliminated in the molecular reaction chamber. Oligomer re-assembly and concomitant activation upon substrate binding may also be critical to regulate other HtrA proteases implicated in protein folding disease.

[1] Krojer, T., *Nature*, **2008**, 453, 885.

Keywords: protein-ligand complexes; protein crystallography; electron microscopy

FA1-MS01-O3

Functional Architecture of RNA Polymerase I. Sebastian R. Geiger[a], Claus-D. Kuhn[b], Sonja Baumli[c], Marco Gartmann[a], Jochen Gerber[d], Stefan Jennebach[a], Thorsten Mielke[e], Herbert Tschochner[d], Roland Beckmann[a], Patrick Cramer[a]. [a]*Gene Center Munich, Ludwig-Maximilians-Universität München, Germany.* [b]*Cold Spring Harbor Laboratory, Cold Spring Harbor, USA.* [c]*Department of Biochemistry, University of Oxford, Oxford, UK.* [d]*Institut für Biochemie, Genetik und Mikrobiologie, Universität Regensburg, Regensburg, Germany.* [e]*Max-Planck Institute for Molecular Genetics, Berlin, Germany.*
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Synthesis of ribosomal RNA (rRNA) by RNA polymerase I (Pol I) is the first step in ribosome biogenesis and a regulatory switch in eukaryotic cell growth. Pol I activity accounts for up to 60% of all nuclear transcription and the product rRNA represents up to 80% of all cellular RNA. Pol I has a molecular weight of 590 kDa and comprises 14 subunits. Despite the recent progress in obtaining the crystal structure of the complete RNA polymerase II (Pol II), a detailed atomic model for Pol I is still missing. Here we describe the Pol I architecture, determined by a combination of cryo-electron microscopy of the 14-subunit Pol I and X-ray crystallography of the subcomplex A14/43, which resulted in the hybrid structure of Pol I. The crystal structure of A14/43 reveals structural differences in comparison to its RNA polymerase counterparts and elucidates a specific binding interface with the Pol I core enzyme and the Pol I initiation factor Rrn3. The Pol I-specific subunits A49 and A34.5 form a heterodimer near the enzyme funnel that acts as a built-in elongation factor and is related to the Pol II-associated factor TFIIF. In contrast to Pol II, Pol I has a strong intrinsic 3'-RNA cleavage activity, which is dependent on the C-terminal domain of subunit A12.2. The functional roles defined for the subunits, together with the Pol I hybrid structure, enable a comprehensive structure-function analysis of rRNA transcription and processing.[1] [2]

[1] Kuhn C.D., Geiger S.R., Baumli S., Gartmann M., Gerber J., Jennebach S., Mielke T., Tschochner H., Beckmann R., Cramer P., *Cell*, **2007**, 131, 1260. [2] Geiger S.R., Kuhn C.D., Leidig C., Renkawitz J., Cramer P., *Acta Cryst.*, **2008**, F64, 413.

Keywords: RNA polymerase I; ribosome biogenesis; transcription