FA1-MS9-T01

Large ring ATPases that organize mitotic chromosomes. Christian Haering, *EMBL, Heidelberg*

Two multi-subunit protein complexes named cohesin and condensin are key components of the cell's mitotic and meiotic chromosome segregation machineries. Cohesin physically links the replicated sister chromatids and thereby allows their bi-polar orientation on the mitotic spindle. Once all sister chromatids have been successfully bi-oriented, cleavage of one of cohesin's subunits by the site-specific protease separase releases their linkage and triggers chromosome movement to the poles. Condensin is essential for holding chromosomes in a compact shape during their movements and thereby prevents them from getting entangled or trapped in the middle of the dividing cell.
Both complexes are built upon two specific proteins of the Structural Maintenance of Chromosomes (SMC) family that bind to each other via hetero-dimerization domains at one end of 40-50nm long coiled-coils. The ABC ATPase head domains situated at the other ends of the coiled coils are connected by a third protein that is a member of the so-called kleisin protein family and recruits additional HEAT-repeat containing subunits to the complex.
I will present evidence that cohesin and condensin bind chromosomes using a unique mechanism, namely by topologically entrapping them inside the large tripartite ring structure formed by their SMC and kleisin proteins, and discuss how cycles of ATP binding and hydrolysis by the SMC head domains may drive the conformational changes required for the organization of mitotic chromosomes.

FA1-MS9-T02

Structural and evolutionary insights into the vesicle fusion machinery. Dirk Fasshauer, *Max-Planck Institut of Biophysical Chemistry, Göttingen.*

Transport of cargo between organelles in eukaryotic cells is mediated by vesicles that bud from a donor compartment and specifically fuse with an acceptor membrane. Currently, it is becoming clear that the underlying molecular machineries involved in the principal aspects of vesicular trafficking are highly conserved, not only between different species but also between different vesicle trafficking steps. In all steps, the central machinery involved in the fusion process is composed of members of the SNARE protein family. Distinctive, heterologous sets of SNARE proteins anchored in the vesicle and target membrane are thought to assemble in a zipper-like fashion into a four-helix bundle, providing the energy to mediate fusion of the two bilayers. Although SNAREs are sufficient to drive membrane fusion when inserted into liposome membranes, this minimal machinery is organized and controlled by additional factors *in vivo*. Members of the cytosolic Sec1/Munc18 (SM) family of proteins have been established as essential factors in different intracellular transport steps, during which they functionally interact with the SNARE machinery. To come to a better understanding of the molecular events during vesicle fusion, we focus on a detailed structural, kinetic, thermodynamic, and phylogenetic characterization of the core vesicle fusion machinery.

FA1-MS9-T03

Self-Association of Quality Control Components in the ER. Jennifer Hanna[a,b], Anja Schütz[a], Franziska Zimmermann[a], Thomas Sommer[a], Udo Heinemann[a]
[a]*Max Delbrück Center for Molecular Medicine, Berlin, Germany.* [b]*Leibniz Graduate School of Molecular Biophysics.*
E-mail: j.hanna@mdc-berlin.de

To avoid accumulation of misfolded proteins in the endoplasmic reticulum, unfolded and misfolded proteins are disposed of by a pathway called ER-associated degradation. First, misfolding is recognized and distinguished from unfolded, but folding competent states. Misfolded proteins are then transported into the cytosol, where they are ubiquitinated and degraded by the proteasome. The Hrd complex is one of the protein complexes at the ER membrane which play a central role in this pathway. We have determined the structure of a protein-protein interaction domain of the quality control protein Yos9, a lectin which recognizes misfolded proteins by their sugar modifications. In this crystal structure, the domain adopts a dimeric arrangement. Here, we report that *in vitro*, the domain does not confer binding to the Hrd complex on its own, as was suggested by previous experiments. Instead, we propose that the domain is important for self-association of the protein, which is thought to promote binding to the Hrd complex.

Keywords: Quality control, glycoprotein, ER associated degradation

FA1-MS9-T04

The crystal structure of the ERGIC-53/MCFD2 transport receptor complex. Edvard Wigren, Jean-Marie Bourhis, Inari Kursula, Jodie E. Guy, Ylva Lindqvist. *Dept. of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.*
E-mail: Edvard.Wigren@ki.se

The membrane bound glycoprotein receptor ERGIC-53 in complex with the co-receptor MCFD2 (multiple coagulation factor deficiency 2) mediates export of the blood coagulation proteins, factor V (FV) and factor VIII (FVIII), from the Endoplasmic Reticulum (ER) to the Golgi complex. This calcium-dependent complex serves as a specific cargo receptor for the selective packaging of FV and FVIII into COPII-coated vesicles that bud from the ER for transport to the Golgi. Mutations in MCFD2 or ERGIC-53 cause a mild form of hemophilia known as combined deficiency of factors V and VIII (F5F8D). In order to facilitate understanding of the function of the ERGIC-53/MCFD2 transport complex and the mechanism by which mutations in the proteins cause F5F8D, we have determined the structure of the carbohydrate recognition domain (CRD) of ERGIC-53 in complex with MCFD2.
Comparison of our structure with previous uncomplexed structures of the proteins shows that MCFD2 undergoes conformational changes upon complex formation, but no major structural changes can be detected in ERGIC-53. The interaction interface of MCFD2 involves most of the F5F8D-causing mutations known to date, clarifying the means by which several of these mutations result in inefficient secretion of FV and FVIII. The structure provides new information on

the organisation of the components of a transport receptor complex in the secretory pathway. Knowledge of these transport processes may ultimately lead to new strategies for the treatment of other inherited and acquired diseases in which protein secretion is impaired

Keywords: Glycoprotein transport, ER quality control, Protein complex

FA1-MS9-T05

Structural basis for the membrane targeting of the exocyst complex. Masami Yamashita[a], Kazuo Kurokawa[b], Yusuke Sato[a], Atsushi Yamagata[a], Hisatoshi Mimura[a] Azusa Yoshikawa[a], Ken Sato[c], Akihiko Nakano[b,d], Shuya Fukai[a]. [a]*Synchrotron Radiation Research Organization and Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan.* [b]*RIKEN Advanced Science Institute, Japan.* [c]*Graduate School of Arts and Science, The University of Tokyo, Japan.* [d]*Graduate School of Science, The University of Tokyo, Japan*.
E-mail: kk087351@mgs.k.u-tokyo.ac.jp

Cell polarization is a critical process for differentiation and proliferation in eukaryotes. A prominent example of the polarization is budding in yeast, during which required proteins and lipids are carried in secretory vesicles and transported to the bud tip by exocytosis. The exocyst complex is a large hetero-octameric protein complex critical for cell polarization, which regulates exocytosis in yeast and mammal. The exocyst complex is composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84. The exocyst complex tethers secretory vesicles to specific regions of the plasma membrane by recognizing small GTPase and phosphoinositide $PI(4,5)P_2$ on the membrane. Although how the exocyst complex recognizes both small GTPase and $PI(4,5)P_2$ on the plasma membrane has been unknown, the GTPase and $PI(4,5)P_2$ binding region of exocyst subunit has been identified. For instance, the N-terminal region of yeast Sec3 (Sec3-N) is sufficient for the binding to the small GTPase Rho1 and $PI(4,5)P_2$. Here we have determined the crystal structure of yeast Sec3-N in complex with Rho1 at 2.6 Å. The crystal structure exhibits that Sec3-N adopts pleckstrin homology (PH) fold, despite having no detectable sequence homology with other PH domains of known structure. Furthermore, clusters of conserved basic residues constitute a positively charged cleft, which was identified as a binding site for $PI(4,5)P_2$. The structure and structure-based site-directed mutagenesis studies *in vitro* and *in vivo* show that the small GTPase and $PI(4,5)P_2$ regulate the localization of the exocyst complex at the specific site of plasma membrane in a complementary fashion[1].

[1] Yamashita M *et al*., *Nature Struct. Mol. Biol.*, 2010, 17, 180-186.

Keywords: protein X-ray crystallography, protein transport, protein structures