Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan, [3]Kyoto University, 54 Kawara-machi, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan, [4]The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan, E-mail：fukai@iam.u-tokyo.ac.jp

Vesicular traffic during exocytosis is regulated by Rab GTPase, Sec4p in yeast. Sec2p is a guanine nucleotide exchange factor (GEF) for Sec4p, and the N-terminal 160 residues of Sec2p are sufficient for the GEF activity. Since this region of Sec2p shows no sequence similarity to any other GEFs with known structures, the GEF mechanism by Sec2p has remained unknown. To elucidate this nucleotide exchange mechanism by X-ray crystallography, we crystallized three constructs of the native Sec2p (Sec21-160p, Sec218-160p, and Sec231-160p) and three constructs of the selenomethionine (SeMet)-labeled Sec2p [Sec231-160p, Sec231-160p (M115L), and Sec231-160p (M115L, K121M, T142M)]. These six crystals diffracted to 8.8, 4.8, 2.6, 4.0, 3.3, and 3.0 Å resolutions, respectively. The data set of the SeMet-labeled Sec231-160p (M115L, K121M, T142M) crystal was processed for SAD phasing, producing an interpretable map after density improvement. The atomic model of the Sec2p GEF domain was refined to an Rfree value of 28.9%. Unexpectedly, the Sec2p GEF domain consists of a homodimeric, parallel coiled coil that extends over 180 Å. Pull-down and guanine nucleotide exchange assay using a series of deletion and point mutants of Sec2p unveiled the catalytic residues for its GEF activity and the Sec4p binding site.

Keywords: vesicle membrane fusion, GTP-binding proteins, membrane trafficking

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Structure and inhibition of Arf GTPases

Jacqueline Cherfils[1], Jean-Christophe Zeeh[1], Mahel Zeghouf[1], Valerie Biou[1], Vanessa Buosi[2], Aurelien Thureau[2], Carine van Heijenoort[2], Eric Guittet[2]
[1]CNRS, Laboratoire d'Enzymologie et Biochimie Structurales, Batiment 34, CNRS, Avenue de la Terrasse, Gif-sur-Yvette, -, 91198, France, [2]Institut de Chimie des Substances Naturelle, CNRS, Avenue de la Terrasse, Gif-sur-Yvette, 91198 France, E-mail：cherfils@lebs.cnrs-gif.fr

Transport of proteins and membranes sustains all aspects of cellular life. It is therefore associated to major cellular processes such as signalling, morphology and division and it is also sensitive to subversion by pathogens. Small GTP-binding proteins (GTPases) of Arf families are major player in cellular traffic, where they tag and organize membranes for specific trafficking events. They are activated by a family of guanine nucleotide exchange factors (GEFs) that carry a catalytic domain (the Sec7 domain), which stimulates the exchange of the tightly bound GDP nucleotide for GTP. Structural studies have elucidated the exchange mechanism of the Sec7 domain, yet its exquisite ability to discriminate between closely related Arf isoforms remains unexplained. Combining X-ray crystallography, NMR and the use of small molecular weight inhibitors, we identify structural dynamics as a previously overlooked aspect of Arf GTPases functions.

Keywords: GTPase, guanine nucleotide exchange factor, traffic

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Atomic model of Rab27a:Exophilin4/Slp2-a complex: Structural studies on vesicular transport

Leonard M.G. Chavas[1,2], Kentaro Ihara[2], Masato Kawasaki[2], Ryuichi Kato[2], Tetsuro Izumi[3], Soichi Wakatsuki[2]
[1]The University of Manchester, Faculty of Life Sciences, Oxford Road, Manchester, Lancashire, M13 9PT, UK, [2]Structural Biology Research Center (IMSS-PF-KEK), 1-1 Oho, Tsukuba, Ibaraki, 305 0801, Japan, [3]Department of Molecular Medicine, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi 371-8512, Japan, E-mail：Leonard.Chavas@manchester.ac.uk

Cycling between active and inactive conformations, Rab small GTPases coordinate vesicular trafficking within eukaryotic cells by collaborating with a set of effectors. Rab27a/b isoforms regulate numerous exocytotic pathways, and their dysfunction causes human immunodeficiency known as Griscelli syndrome. Exophilin4/Slp2-a localizes on the phosphatidylserine-enriched plasma membrane of melanocytes and its N-terminal Rab27 binding domain (RBD) specifically recruits Rab27a on the surface of melanosomes prior to docking and fusion events. To clarify the characteristic features for the selective binding of Rab27 to eleven distinct effectors, high resolution structures of both inactive GDP-bound and active GppNHp-bound Rab27 in complex with RBD of Exophilin4/Slp2-a were determined. While drastically reordering its switch and interswitch elements under nucleotide exchange, Rab27 moulds a compatible surface for recognizing Exophilin4/Slp2-a, presenting some particularities at the interface of the Rab27 effectors structural motif (S/T)(G/L)xW(F/Y)2 on account of modulating effector affinity for Rab27. The observed structural complementation in the interacting surfaces of Rab27a and Exophilin4/Slp2-a sheds light on the disparities among Rab27 effectors and untangles the general mechanism for their recruitment.

Keywords: membrane trafficking, Rab GTPase, macromolecular assemblies

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Crystal structure of *E.coli* MacA reveals the assembly of the tripartite bacterial efflux pump

Soohwan Yum, Shunfu Piao, Yongbin Xu, Nam-Chul Ha
Pusan National University, College of Pharmacy and Research Institute for Drug Development, Pusan National University, Busan 609-735, Korea, Busan, Busan, 609-735, Korea (S), E-mail：royyum@pusan.ac.kr

Periplasmic membrane fusion proteins (MFPs), including MacA and AcrA, are an essential component of multidrug efflux pumps in Gram-negative bacteria. They play a crucial role in bridging the outer membrane porin TolC and two distinctive types of inner membrane transporters. MacA and AcrA form the MacA-MacB-TolC and AcrA-AcrB-TolC efflux pumps, respectively, in Escherichia coli. Although the crystal structures of two MFPs have been reported, the functional form and the mechanistic role of MFPs are only vaguely understood. Here, we show that MacA forms a funnel-like hexameric assembly with a central channel whose diameter is similar to that of TolC and a conical mouth that appears to accommodate the periplasmic end of MacA. In accordance with the results of biochemical experiments, we propose a structural model for how MFP induces the opening of the central channel of TolC in the periplasmic space of Gram-negative bacteria. Based on the complementing available structures and information, realistic models for the tripartite multidrug efflux pumps are proposed, which visualizes how MFPs link TolC and inner membrane transporters bridging the inner and outer membranes.

Keywords: MFP, efflux pump, MacA

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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

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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

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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.

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