factor (EGF)-like module, whereas β-NX has a single LG domain. Recently several groups have reported crystal structure of β-NX and NL complex revealing a unique binding with 2:2 stoichiometry. However these crystal structures do not provide significant insights into the synaptic signal transduction triggered by β-NX/NL interaction. We determined the crystal structure of β-NX/NL complex at 3.5Å resolution in different crystal form, possibly mimicry their molecular clustering of synaptic cleft. We also report the crystal structure of a single repeat segment of α- NX at 2.3Å resolution (i.e. LG-EGF-LG segment). Comparison of these structures stolongly suggested that α- and β-NX have the different clustering mechanism mediated by the interaction with their ligands NL.

Keywords: structures of biomolecules, cell adhesion, complexes

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Preliminary X-ray analysis of MEK1/ERK2 complex

Setsu Nakae, Takayoshi Kinoshita, Ikuyo Yoshida, Toshiji Tada
Osaka Prefecture University, Graduate School of Sciences, 1-1, Gakuen-cho, Naka-ku, Sakai, Osaka, 599-8531, Japan, E-mail：nakae07@b.s.osakafu-u.ac.jp

The Ras/Raf/MEK (mitogen-activated protein kinase) /ERK (extracellular-signal- regulated kinase) pathway plays a key role in governing cell proliferation, differentiation and survival. The pathway represents an attractive drug target in proliferative diseases. The aim of the present study is to clarify the mode of the interaction between MEK and ERK by analyzing the crystal structure of MEK1/ERK2 complex. A detailed study of the three-dimensional structure of the complex should provide information to design novel and target-specific drugs against proliferative diseases. MEK1 and ERK2 were expressed in *E. coli* with GST tags at their N-termini. The lysate including GST-tagged MEK1 was centrifuged to produce a crude extract, which was then loaded onto a GST-affinity column. By on-column cleavage using precision protease, the desired protein was obtained. Subsequent purification by anion exchange chromatography on a MONO Q column yielded two peaks which were assigned as homologous MEK1. ERK2 was purified by a similar procedure and also split into two peaks on a MONO Q column. For each of four complexes obtained by combining two MEK1 and two ERK2, conditions of crystallization were searched using the commercially available sparse-matrix screening kits. Optimization of crystallization conditions for X-ray crystallography is currently progress.

Keywords: MEK, ERK, crystallization

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Structural biology of a nuclear import of proteins by transportin 1

Mamoru Sato, Tsuyoshi Imasaki, Toshiyuki Shimizu, Hiroshi Hashimoto, Hanako Ishida, Daisuke Kamei, Michiyuki Yamada
Yokohama City University, International Graduate School of Arts and Sciences, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa, 230-0045, Japan, E-mail：msato@tsurumi.yokohama-cu.ac.jp

The transport of macromolecules between the nucleus and the cytoplasm through nuclear pore complexes (NPCs) is mediated via several transport pathways by transport receptors that are most commonly members of the importin-β family. Transport receptors form complexes with their transport substrates (cargoes) through cognate nuclear localization signals (NLSs) for import substrates or nuclear export signals (NESs) for export substrates, and target substrates to NPC components termed nucleoporins. Transport directionality and interactions between the transport receptor and substrate are regulated by RanGTP and, in the nuclear import system, binding of RanGTP to the receptor in the nucleus is associated with substrate dissociation. Of the several transport pathways, the best characterized is an import pathway mediated by importin-β (karyopherin-β1). Transportin 1 (Trn1) (karyopherin-β2) is a transport receptor that belongs to the importin-β family and has 24% sequence similarity to importin-β. Here we describe four crystal structures of human Trn1 in a substrate-free form as well as in the complex with three NLSs (hnRNP D, JKTBP and TAP, respectively). Our data have revealed that (i) Trn1 has two sites for binding NLSs, one with high affinity (Site A) and one with low affinity (Site B), and NLS interaction at Site B controls overall binding affinity for Trn1, (ii) Trn1 recognizes the NLSs at Site A followed by conformational change at Site B to interact with the NLSs, and finally, (iii) a long flexible loop, characteristic of Trn1, interacts with Site B, thereby displacing transport substrate in the nucleus. These studies provide deep understanding of substrate recognition and dissociation by Trn1 in import pathways.

Keywords: importin, nuclear transport, nuclear pore complex

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Structures of starch binding domain of R. oryzae glucoamylase reveal an amylosic binding model

Jung-Yu Tung[1], Dah-Tsyr Margaret Chang[2], Wei-I Chou[2], Yuh-Ju Sun[1]
[1]National Tsing Hua University, Bioinformatics and Structural Biology, Room 603, Life Science Building II , 101, Section 2 Kuang Fu Road, Hsinchu, Taiwan, 300, Taiwan, [2]Institute of Molecular and Cellular Biology, National Tsing Hua University, E-mail：d934605@oz.nthu.edu.tw

Glucoamylase hydrolyzes starch and polysaccharides to β-D-glucose. Rhizopus oryzae glucoamylase (RoGA) consists of two functional domains, an N-terminal starch binding domain (SBD) and a C-terminal catalytic domain, which are connected by an O-glycosylated linker. The SBD of RoGA belongs to the carbohydrate binding modules (CBMs) family 21 (RoGACBM21). The crystal structures of SBD and the complexes with a cyclic carbohydrate, β-cyclodextrin and a linear carbohydrate, maltoheptaose were determined at 1.25, 1.8, and 2.3 Å resolution, respectively. The overall structures of SBD belong to a β-sandwich fold with an immunoglobulin-like architecture. Two carbohydrate binding sites, sites I and II, were determined on the surface of SBD, where site I is a flat and broad hydrophobic binding region created by the aromatic residues, Trp47, Tyr83, and Tyr94; site II is a protruded and narrow binding environment formed by Tyr32 and Phe58. Besides the hydrophobic interaction, two unique polyN loops comprising consecutive asparagines also participate in the sugar binding. To elucidate the mechanism of polysaccharide binding, a number of mutants were constructed and characterized by the quantitative binding isotherm and Scatchard analysis. In addition to sites I and II, a continuous binding surface through Tyr67 and Tyr93 might be essential for long-chain polysaccharide binding. An amylosic binding model for RoGA was proposed.

Keywords: starch binding domain, Rhizopus oryzae glucoamylase, cyclodextrin

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Crystal structure of the Sec4p:Sec2p complex in the nucleotide exchanging intermediate state

Yusuke Sato[1,2], Shuya Fukai[1], Ruichiro Ishitani[2], Osamu Nureki[2]
[1]the University of Tokyo, Institute of Molecular and Cellular Biosciences, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-0032, Japan, [2]Tokyo Institute of Technololgy, 4259 Nagatsuta-cho, Midori-ku, Yokohama-shi, Kanagawa, 226-8501, Japan, E-mail：yusato@bio.titech.ac.jp

Exocytosis is a basic membrane traffic event including transport, docking, and fusion of secretory vesicles. The yeast Rab GTPase, Sec4p, regulates vesicular transport in exocytosis by oscillating between the active GTP-bound and inactive GDP-bound states. Sec2p is a guanine nucleotide exchange factor (GEF) for Sec4p, which catalyzes GDP release to promote GDP-GTP exchange for Sec4p activation. The N-terminal region (residues 1-160) of Sec2p is necessary and sufficient for its GEF activity while it shows no sequence homology to any other GEFs with known structures. The crystal structure of S. cerevisiae Sec2p revealed that the Sec2p GEF domain folds into a parallel dimeric coiled coil. To elucidate its GDP/GTP exchange mechanism, we have determined the crystal structure of the GEF domain of S. cerevisiae Sec2p in a complex with the nucleotide-free Sec4p. Upon complex formation, the Sec2p helices approach each other, and the switch I and switch II regions of Sec4p are largely deformed, to create a flat hydrophobic interface that snugly fits the surface of the Sec2p coiled-coil. These drastic conformational changes disrupt the interactions between switch I and the bound guanine nucleotide, which facilitates the GDP release. In mammals, two GEFs, GRAB and Rabin3, are known as orthologs of Sec2p. The putative Rab binding regions (corresponding to residues 96 to 124 of Sec2p) share 93% similarity between Rabin3 and GRAB. In spite of this similarity, the specificity for Rab GTPases differs between Rabin8 and GRAB; Rabin3 exchanges GDP for GTP on Rab8, but not on Rab3A, while GRAB exchanges GDP for GTP on Rab3A. To elucidate their selectivity for Rab subfamily GTPases, crystalization screening of the Rab8:Rabin3 and Rab3A:GRAB complexes is now under way.

Keywords: GEF, GTP-binding proteins, protein complex structure

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Crystal structure of hMyD88 at 1.8 Å resolution

Jiansheng Jiang, Greg Snyder, Tsan Xiao
National Institute of Alergy and Infectious Diseases, Structural Immunobiology Section, Laboratory of Immunology, 4 Memoral Drive, Bethesda, MD, 20892, USA, E-mail：jiangji@niaid.nih.gov

MyD88 (Myeloid differentiation primary response gene 88) is one of the signaling adaptor proteins with a Toll/interleukin receptor (TIR) domain. MyD88 has been recognized as a universal adaptor for all Toll-like receptors (TLRs), except for TLR-3, to activate transcript factor NF- κ B [1]. We determined the crystal structure of hMyD88 at resolution 1.8 angstrom. The structure reveals that it may form an active signaling complex with other TIR domain containing adaptors. The conserved BB loop of the TIR domain was shown to play an important role in interaction with other TIRs [2]. The hMyD88 structure displays a different conformation of the BB loop in comparison with TIR1 and TIR2. We have observed distinct monomer and dimer species of hMyD88 in solution. hMyD88 dimer species can be reduced to monomer sizes upon addition of reducing agents, indicating that disulfides might also mediate its dimerization. We also observed a dimer formation in the crystal that utilizes the BB, DD and EE loops at the interface. Based on this structure, the models of hMyD88 in complexes with other TIRS and adaptors are proposed.
[1] O'Neil & Bowie, Nature Reviews/Immunology, 2007, 7, 353-364.
[2] Xu, Tao, Shen, Horng, Medzhitov, Manley & Tong, Nature, 2000, 408, 111-115.

Keywords: signaling adaptor, Toll/interleukin receptor, Toll-like receptor

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First structure of a kinase domain in complex with Ca^{2+}/CaM

Inaki De Diego Martinez, Jochen Kuper, Neda Bakalova, Frank Lehmann, Matthias Wilmanns
European Molecular Biology Laboratory (EMBL) - Hamburg, Hamburg Outstation, EMBL Hamburg c/o DESY, Notkestrasse 85, Geb 25a, Hamburg, Hamburg, 22603, Germany, E-mail：inaki.dediego@embl-hamburg.de

DAP kinase-1 is a CaM-regulated Ser/Thr kinase which acts as a cell death mediator and possesses tumor suppressor activity in humans. Like in other Calmodulin regulated kinases, direct binding of Calcium-activated Calmodulin (Ca^{2+}/CaM) to a Calmodulin Binding/Autoinhibitory Domain (CBD/AID), adjacent to the catalytic core, has been proposed as the mechanism to release the autoinhibion through the removal of intramolecular interactions between this domain and key residues within the catalytic cleft. We present the structure of the kinase and CBD/AID domains of human DAPK-1 (residues 1-320) in complex with Ca^{2+}/CaM, solved at 2.1 Å resolution using X-ray crystallography. The interaction occurs mainly through a hydrophobic interface generated by the collapse of Ca^{2+}/CaM around the α-helical CBD. Several changes in the catalytic domain are imposed by this new conformation, when compared with the corresponding fragment of the autoinhibited DAPK-1 structure, that explain the catalytic activation and confirms the previously proposed 'release-based' mechanism. Electrostatic contacts, some of them involving extraregulatory domains, help to stabilize the complex and may participate in additional control mechanisms. Site-directed mutagenesis in combination with a biochemical approach was used to validate the structural data and determine the kinetics of the activation process. These findings allow to understand the mechanism of regulation of CaMKs by Ca^{2+}/CaM, in the context of a biologically active, macromolecular assembly.

Keywords: apoptosis, calmodulin-mediated calcium signal transduction, protein kinases

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Novel crystal structure of red-absorbing form of cyanobacteriochrome AnPixJ-GAF2

Rei Narikawa, Norifumi Muraki, Tomoo Shiba, Genji Kurisu,