techniques for efficient collection and data extraction from thermodynamic literature. The integrated databases enable us to analyze many aspects of biomolecular function.

2S1-5 DDBJ, PDBj, KEGG, CBRC のウェブサービスによる連携

Integration of bioinformatics web services provided by DDBJ, PDBj, KEGG and CBRC

Toshiaki Katayama (Human Genome Center, Institute of Medical Science, University of Tokyo)

The number of web service providers in the field of bioinformatics is increasing every year. In theory, these services are interoperable and independent of specific computer languages. However, each service uses its own definition of data types and method naming conventions. Moreover, these services are often not usable by specific computer languages.

This lack of interoperability prevents these services from becoming popular among end users and developers alike and they are underutilized as an infrastructure of their daily research. Especially, there are several web service providers in Japan such as DDBJ, KEGG, PDBj and CBRC but none of these are integrated in any way.

For this reason, at DBCLS (Database Center for Life Science), we started to develop a new integrated web service, TogoWS, which translates user requests for the appropriate web service providers. TogoWS supports all major Japanese web services and several foreign web services in both REST and SOAP/WSDL style. We also provide documents and sample programs to utilize all functionalities of the domestic web services and regularly check their availability to provide stable service.

In this symposium, we will explain why this virtual integration of domestic web services is needed, how our integrated service is achieved in cooperation with the four providers, and what our service can help to develop infrastructure for life sciences.

2S1-6 統合DBのインパクトと将来への期待

Expect for Database Center for Life Science

Haruki Nakamura. (Institute for Protein Research, Osaka Univ. / Database Center for Life Science)

MEXT Integrated Database project has started since the fiscal year of 2006, and the Database Center for Life Science (DBCLS) was founded in Tokyo in April 2007, as a core institute of the project. Now, the portal site (<http://lifesciencedb.jp/>) is open, and it replies to many different kinds of queries in the entire life science field. The aim of this project is to integrate all of the life science databases constructed in Japan as well as in the world, using the most advanced informatics technologies. The integrated database is not only the unified database, but several additional advantages should be provided by integration, even if the actual different kinds of databases are not physically unified in a single database center. We manage the protein tertiary structure database, PDBj (<http://www.pdbj.org/>), and expect the integrated database not only as the archives of life science data, but also as the institute that provides all digital (text) and analog (picture, sound, movie, etc) knowledge about life science, which can be quickly queried. In particular, many current experimental methods in the life science fields should also be archived with the standardized description and ontology, as well as the observed data.

2S2-1 超高速1蛍光分子追跡法が明らかにしたコンパートメント化された細胞膜内でのリン脂質のホップ拡散

Super-speed single fluorescent-molecule tracking revealed hop diffusion of a phospholipid in the compartmentalized plasma membrane

Takahiro Fujiwara (1,2), Shinji Takeuchi (4), Takaaki Mizushima (4), Kazuhide Hanaka (4), Kokoro Iwasawa (1,2), Kenichi Suzuki (1,2), and Akihiro Kusumi (1,2,3). (1: Membrane Mechanisms Project, ICORP-JST; 2: Institute for Integrated Cell-Material Sciences (iCeMS); 3: Institute for Frontier Medical Sciences, Kyoto University; 4: Imaging Division, Photon Limited)

Previously, using single-particle tracking at a temporal resolution of 0.025 ms, employing 40 nm- ϕ colloidal gold probes, we have shown that virtually all of the molecules incorporated in the plasma membrane undergo hop diffusion in the plasma membrane, which is parceled up into apposed domains by the actin-based membrane skeleton (MSK) as well as a variety of transmembrane proteins anchored to and aligned along the MSK. However, the 40 nm- ϕ gold probes are problematic because they can be susceptible to steric hindrance and can crosslink target molecules, necessitating many control experiments. To circumvent these problems, here, we developed a super-speed high-sensitivity camera system, which allows us to observe single fluorescent-molecule probes at a time resolution of 0.1 ms, improving the presently available best system by a factor of 30. A CMOS 2D-sensor was employed, and the signal intensity at the sensor was increased 10-fold by directly coupling the sensor to the image intensifier with an optical fiber bundle, and the thermal noise was $\approx 10\times$ reduced by cooling. This camera system gave the position determination accuracy for single fluorescent molecules of ≈ 35 nm at a 0.1 ms time resolution. A phospholipid, DOPE, tagged with a fluorescent dye Cy3 in the T24-cell plasma membrane was found to undergo hop diffusion over the 110-nm compartments with a mean residency time of 10 ms, in agreements with the high-speed gold-particle tracking data. In conclusion, lipid hop diffusion was confirmed without using gold probes.

2S2-2 膜骨格と細胞膜との3次元相互作用: フリーゼエッチ電子線トモグ

ラフィーによる可視化

Three dimensional interplay of the membrane skeleton with the plasma membrane as visualized by freeze-etch electron tomography

Nobuhiro Morone (1), Takahiro K. Fujiwara (2), Rinshi S. Kasai (2), Shigeki Yuasa (1), Jiro Usukura (3), Akihiro Kusumi (2). (1: Dept. Ultrastructural Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry; 2: Membrane Mechanisms Project, International Cooperative Research Project (ICORP), Japan Science and Technology Agency, Institute for Integrated Cell-Material Sciences and Institute for Frontier Medical Sciences, Kyoto University; 3: Divn. Integrated Projects, EcoTopia Science Institute, Nagoya University)

Currently, we have visualized the three dimensional interplay of the membrane-associated part of the cytoskeleton ("membrane skeleton") with the plasma membrane by freeze-etch electron tomography. The reconstructed structures consist of 0.85-nm-thick, consecutive sections made ~ 100 nm from the cytoplasmic surface of the rapidly-frozen, deeply-etched, platinum-replicated upper plasma membrane of the cultured cell. The membrane skeleton primarily consists of actin filaments and actin-associated proteins, covers almost the entire cytoplasmic surface, and is closely linked to clathrin-coated pits and caveolae. Previously, we found that the molecules in the plasma membrane undergo short-term confined diffusion within a domain and long-term hop diffusion between the domains. The membrane skeletal filaments that are closely apposed to the cytoplasmic surface (within 10.2 nm) are likely to form the boundaries of the membrane compartments responsible for the temporary confinement of membrane molecules. The distribution of the membrane-skeleton mesh size (as determined by electron tomography) and that of the compartment size (determined from high-speed single particle tracking) agree well in two cell types with very different sizes (200 and 50 nm for NRK and FRSK cells). Our correlative analysis supported that the plasma membrane is parceled up into domains by the coralling effects of the membrane skeleton and its associated transmembrane proteins (the membrane-skeleton fence and membrane-skeleton anchored-protein picket models).

2S2-3 Self-organization of the actin machinery for protrusion - biophysics and computational model

Self-organization of the actin machinery for protrusion - biophysics and computational model

Gary G. Borisy (Marine Biological Laboratory, U.S.A.)

2S2-4 Mapping the molecular dynamics of clathrin mediated endocytosis

Mapping the molecular dynamics of clathrin mediated endocytosis

Christien Merrifield (1), Marcus Taylor (1), and David Perrais (2). (1: MRC Laboratory of Molecular Biology, UK; (2): CRS UMR 5091 et Université Bordeaux 2, Institut Francois Magendie, France)

Clathrin mediated endocytosis is the principal mechanism by which cells internalize cell surface receptors. It can be thought of as a molecular machine that concentrates receptors into patches at the plasma membrane and bends the membrane into a vesicle that pinches off. Here I will describe how we are using evanescent field microscopy and novel assays to measure key events in clathrin mediated endocytosis including membrane curvature / coated pit invagination, membrane scission and the recruitment of endocytic proteins to sites of scission. Using these tools we are constructing an accurate spatial and temporal model of the endocytic machine in action.

2S2-5 New insights into the formation and function of caveolae

New insights into the formation and function of caveolae

Robert G. Parton (Institute for Molecular Bioscience, University of Queensland, Australia)

Caveolae are flask-shaped pits of the cell surface, an abundant morphological feature of many mammalian cells. Caveolins, the major proteins of caveolae, play a crucial role in the formation of caveolae. Mutations in caveolins are associated with breast cancer and with a number of muscle diseases, including limb girdle muscular dystrophy. We have studied how caveolin-lipid interactions generate the unique architecture of the caveolar domain by studying caveolae formation in caveolin-null fibroblasts by light and electron microscopy upon expression of mammalian caveolins (caveolin-1 and caveolin-3), specific mutants of these proteins, or non-mammalian caveolins (from *C. elegans* and the honey bee, *Apis mellifera*). Our recent studies have identified a new family of coat proteins which regulate caveolae formation. PTRF-cavin family members regulate association of caveolin with caveolae and identify a cellular mechanism to regulate caveolar and non-caveolar functions of caveolins¹. These studies have implications for understanding the distinct cell-type specific roles of caveolin in disease conditions. 1. Hill et al, 2008. Cell 132:113-124.

2S3-1 結晶構造が明らかにするべん毛モーターの機能

Molecular mechanism of the bacterial flagellar motor revealed by the crystal structures

Katsumi Imada (Dept. Frontier Biosciences, Osaka Univ.)

The bacterial flagellum is a filamentous organelle for cell motility. It is driven by a reversible rotary motor, which is embedded in the cell membrane at the base of the filament and powered by the transmembrane electrochemical gradient of a specific ion. The motor is composed of the rotor and the stator, and torque is