yeast cells was estimated to be approximately five times higher than that expressed in COS7 cells. Possible reasons for this difference are discussed.

3PT120 紫膜表面の隆起構造およびバクテリオロドプシン分子構造に与える紫膜中の古細菌型脂質除去の影響

Effects of archaeal lipids removal in purple membrane upon bump structures of membrane surface and bacteriorhodopsin conformations

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Purple membrane (PM) is a membrane patch found in cytoplasmic membrane of halophilic archaeobacteria. Since PM consists of a two-dimensional crystal of membrane protein bacteriorhodopsin (bR) with some archaeal lipids, it is presumed that surface of PM is uniform planner. However, atomic force microscopy (AFM) observations for PM have revealed a lot of bump structures in the surface, showing that PM surface is not a uniform. The molecular packing of bR and the lipids, including the bump structure formation, should be determined by physicochemical interaction between bR molecules as well as bR and lipids. Our studies against the bump structures of PM have showed a large contribution of electrostatic interaction because bump curvatures were affected by pH and ionic concentrations of medium. In this work, we focus on the lipids in PM because of its highly negatively charged headgroups, and investigate removal effects of the lipids upon bump structures of membrane surface by AFM observations. AFM topography of delipidated PM demonstrated bump structures had larger curvature than those in native PM. A mean curvature was largest at 25 % delipidated PM samples, and decreased with increasing delipidation level. AFM analysis in the presence of KCl with various concentrations showed KCl concentration dependence of the bump curvature of delipidated PM was different from that of native PM. The removal effect of the lipids upon bump structures will be discussed together with these results as well as delipidation effects on bR conformations.

3PT121 EGF 受容体の活性化機構: EGFR はリガンド結合前に 2 量体として存在する

Molecular mechanism underlying activation of the EGF receptor: preformed, inactive dimer and positive cooperativity

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Epidermal growth factor receptor (EGFR) is an integral membrane protein that has an extracellular ligand binding domain, a single-path transmembrane domain, and an intracellular kinase domain. The 'dimerization model' has widely been believed as a molecular mechanism underlying the activation of EGFR, in which ligand binding induces EGFR dimers from monomers. However, we have recently shown that EGFR and its family proteins exist as a preformed, yet inactive, dimers prior to ligand binding, and have proposed a "rotation/twist model", in which ligand binding induces rotation/twist of the receptor's transmembrane domains. The rotation/twist breaks the inactive dimeric structure, and rearranges the domains to take active forms. To distinguish the two models, we examined the dimeric structure based on FLIM/FRET before ligand binding, and found that EGFR has a preformed dimeric structure. We also constructed a series of EGFR mutants in which a small point mutation or large deletion was introduced into the receptor molecule, and found that EGFR mutants with the deletion at its C-terminal region were spontaneously activated. In contrast to the wild-type EGFR with biphasic affinity for EGF, further, the mutant showed a single affinity for the ligand. These results are consistent with the 'rotation/twist' model for the mechanism of the EGFR activation. In the meeting, we shall report the results described above, and shall discuss about the positive cooperativity between the ligand-binding sites required for the activation of EGFR.

3PT122 表層ストレス応答における、PDZ ドメインによる RseP プロテアーゼ機能制御機構の解析

Analysis of a regulatory mechanism of the proteolytic function of RseP by the PDZ domains in extracytoplasmic stress response

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The *Escherichia coli* σ^E extracytoplasmic stress response monitors and responds to folding stress in the cell envelope. A protease cascade directed at RseA, a membrane-spanning anti- σ that inhibits σ^E activity, controls this critical signal-transduction system. Stress cues activate the DegS proteases to cleave RseA; a

second cleavage by the RIP protease releases RseA from the membrane, enabling its rapid degradation. Stress control of proteolysis requires that RseP cleavage is dependent on DegS cleavage. *In vitro* and structural studies found that RseP cleavage requires binding of RseP PDZ-C to the newly exposed carboxyl terminal residue (Val148) of RseA, generated by DegS-cleavage, explaining dependency (Li et al., 2009, Proc. Natl. Acad. Sci. U. S. A.). We tested this mechanism *in vivo*. Neither the C-terminal residue of DegS-processed RseA nor mutations in the putative PDZ ligand-binding regions or even deletion of entire RseP PDZ domains had significant effects on RseA cleavage *in vivo*. Indeed, strains with a chromosomal *rseP* gene deleted for either PDZ domain grew normally at all temperatures and exhibited almost normal σ^E -activation in response to stress signals. We conclude that recognition of the cleaved amino acid by the PDZ domain of RseP is unable to explain sequential cleavage *in vivo*. To explore the possibility that the PDZ domains are involved in regulation of the RseP functions through ligand-binding we are trying to identify a physiological ligand of RseP PDZ by using site-directed *in vivo* photo-cross-linking experiment.

3PT123 部分フッ素化ホスファチジルコリン小胞に再構成されたバクテリオロドプシンの液晶相における分子集合体

Molecular assembly of bacteriorhodopsin reconstituted into partially fluorinated phosphatidylcholine liposome in liquid crystalline phase

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Fluorine-containing lipids are expected to be very useful in the research area of membrane proteins. Our recent studies on physical properties of a novel partially fluorinate phosphocholine, 1,2-di(11,11,11,12,12,13,13,14,14-nonafluorotetradecanoyl)-sn-glycero-3-phosphocholine (diF4H10-PC), an analog of a common phosphatidylcholine DMPC, demonstrated that diF4H10-PC membrane shows the gel-to-liquid crystalline phase transition at $\sim 5^\circ\text{C}$ and the fluidity in the interface region is higher than that of DMPC. We succeeded in reconstituting a photoreceptor membrane protein, bacteriorhodopsin (bR), into diF4H10-PC liposome. The reconstituted bR retained the trimeric structure even after the chain melting phase transition of lipid bilayers at least up to 40°C and showed a photocycle similar to native purple membrane, which is in a striking contrast with the previous studies on bR reconstituted in DMPC*. In order to address what is responsible for retaining the trimeric structure even in the fluid phase of diF4H10-PC, bR reconstituted in a binary immiscible membrane of diF4H10-PC and DMPC was investigated by some spectroscopic methods. It has been shown that most of bR molecules are selectively reconstituted into DMPC-rich domain, suggesting bR is mostly immiscible to diF4H10-PC. It is plausible that the immiscibility between bR and diF4H10-PC is essential for the formation of bR trimers.

*Sonoyama et al., (2009), Yokoyama et al., (2010)

3PT124 Elucidation of the membrane microdomain assembly mechanism via reconstituted artificial lipid membranes using high performance proteomics

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Plasma membrane microdomains are associated with a variety of biomolecules and considered to play an important role in effective response to external stimuli. The physicochemical basis controlling the association remains incompletely understood. In this work, we investigate the role of intermolecular interactions in the assembly of proteins and lipids into the microdomain using reconstituted membranes of varying composition and physicochemical properties. We carried out high performance proteomics of the membrane microdomain and identification of the proteins to investigate component proteins in the microdomain. Using a standard procedure, the membrane microdomain was extracted from Jurkat cells (Human T-cell lymphoblast cell line) because membrane microdomains play an important role in the recognition of antigen-presenting-cells in immune system. The results of the 2D-gel electrophoresis showed about 150 spots and of these approximately 60 proteins have so far been identified using in-gel tryptic digestion and LC-MS/MS measurements. Some identified proteins such as actin and V-type ATPase have already been reported as membrane microdomain components. However, original proteins such as interferon-induced GTP-binding protein, tumor necrosis factor and sphingolipid