3PT178 脂質二重膜上における Def6 PH ドメインの高次構造の解析**Conformational properties of the Def6 PH domain located at the lipid bilayer surface**

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Conformations of the pleckstrin homology (PH) domain of SWAP-70-like adapter of T-cells (SLAT, also known as Def6) in solution and at the membrane surface were investigated by using circular dichroism (CD), fluorescence and NMR spectroscopy. Previously, we reported that the switch-associated protein (SWAP) -70 PH domain undergoes conformational alteration including the α -helix to random coil transition at the lipid bilayer surface. The random coil transition of the C-terminal α -helix was expected to allow water-exposed conformation of nuclear localization signal (NLS) sequence located in the C-terminal α -helix, and consequently regulates intracellular relocalization of SWAP-70. The Def6 PH domain shares high sequence homology with the SWAP-70 PH domain, but lacks NLS in the C-terminal α -helix. The CD and NMR spectra show that the β -sandwich core of the Def6 PH domain undergoes similar conformational change to that of the SWAP-70 PH domain at the lipid bilayer surface. On the contrary, the random coil transition of the C-terminal α -helix was not observed for the Def6 PH domain. The difference in the conformational properties of the C-terminal α -helices of the Def6 and SWAP-70 PH domain would relate to the absence and presence of the functional NLS in the C-terminal α -helix.

3PT179 ErbB シグナル伝達過程において Shc と Grb2 は個別に反応する
Independent response of Shc and Grb2 in the ErbB signaling revealed by FCS/FCCS and single molecule analysis

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The signaling pathway of ErbB receptors including ErbB1 - 4 is involved in diverse physiological processes such as cell proliferation, differentiation, apoptosis, and carcinogenesis. Systems-level analyses have been recently carried out for this pathway, combining conventional biochemical methods in vitro with computational simulation. However, information about spatiotemporal dynamics and reaction-kinetics of related proteins are largely lacking. It will be worthwhile to directly quantify the changes of localization, diffusion, and interaction of related proteins during the process in living cells. We have established a live cell FCS/FCCS and single molecule (SM) analysis of the proteins in the ErbB networks. Firstly, we focused on the behavior of diffusions of Shc52 and Grb2 and direct interaction between them. Complex formation between them is known to be necessary for activating Ras in the EGF (ErbB1 ligand) and HRG (ErbB3, 4 ligand) signaling process. Our analysis using live cells indicates that diffusional responses of the two cofactors to ligand stimulations were very different. Moreover, direct interaction between them was hardly found in living cells, even though interactions with ErbBs were detected for the factors. These results suggest that spatiotemporal responses of Shc52 and Grb2 with ErbB receptors are independently regulated in living cells, even details of the independent response have not been described until now. Our study will pave the way toward a new insight for the true role of Shc52 in the ErbB signaling.

3PT180 クロストークを利用したべん毛回転制御系の構築**Control of bacterial flagellar rotation via crosstalk from a non-cognate histidine kinase to the response regulator CheY**

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Escherichia coli has various two-component regulatory systems (TCS), typically consisting of a histidine kinase (HK) and a response regulator (RR) for responses to environmental changes. For instance, the chemotaxis of *E. coli* employs, as a central processing unit, a TCS consisting of CheA (HK) and CheY (RR). The activity of CheA is regulated but the four chemoreceptors in the cytoplasmic membrane in response to environmental stimuli. CheA autophosphorylates at its histidine residue and then donates its phosphoryl group to an aspartate residue of CheY. Binding of phospho-CheY to the flagellar motor induces its clockwise (CW) rotation. An *in vitro* study showed that 22 out

of 30 HKs of *E. coli* transfer their phosphoryl groups to non-cognate RRs (Yamamoto et al., 2005). In this study, we examined *in vivo* whether the activity of CheY can be regulated by non-cognate HK, BaeS. We first established a CheY expression system under the control of the arabinose-controllable promoter. In a strain (HCB437), in which all the chemotaxis genes are deleted, the more CheY is expressed, the more population of cells tumbled. We then co-expressed BaeS with CheY in the same strain. The tumbling frequency of cells co-expressing BaeS and CheY was significantly higher than that of cells expressing only CheY. Furthermore, the addition of indole enhanced tumbling frequency of cells co-expressing BaeS and CheY, but not those expressing CheY only. Thus, crosstalk between BaeS and CheY appears to occur *in vivo*, providing a system to regulate flagellar rotation.

3PT181 T 細胞活性化におけるカルシウムシグナリングと細胞内構造変化の時空間定量解析

Spatial-temporal dynamics of calcium signaling and organelles in T cell activation

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When cells are subjected to the specific signals, Ca^{2+} is released from the endoplasmic reticulum (ER) resulting store-operated calcium entry (SOCE) via calcium-release activated calcium (CRAC) channels in the plasma membrane. These channels are opened by STIM1, ER-resident calcium sensor, after depletion of intracellular Ca^{2+} stores. In T cell, the duration of this cytoplasmic Ca^{2+} increases controls the efficiency and specificity of the transcription of immune-related genes. STIM1 and Orai1, an essential pore subunit of the CRAC channel, accumulate in the area of contact between T cell and antigen presenting cells. Recently mitochondria are reported to translocate to the contact area and allow sustained Ca^{2+} entry. As STIM1 resides in ER membrane, the accumulation can cause the translocation of ER. Actually, we reported last year that ER redistributed in T cell activation. However, the relationship between Ca^{2+} release from ER, SOCE, and local cytosolic Ca^{2+} elevations in T cell activation is not known in detail. Here aiming to analyze the relationship between ER redistribution and local Ca^{2+} elevations, we visualized CRAC channels or ER redistribution using GFP fusion proteins. In addition, we visualized local Ca^{2+} entry via CRAC channels or Ca^{2+} dynamics in ER using genetically encoded calcium indicator. We will discuss spatial regulation of Ca^{2+} signaling by structural changes in T cell.

3PT182 低分子量 G タンパク質 Ras のフォトクロミック分子 PAM を用いた光制御

Photo-regulation of small G protein Ras using photochromic molecule

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Ras is one of small G-proteins known as a molecular switch. Ras performs intracellular signal transduction. Interestingly, the core nucleotide-binding motif is considerably conserved in Ras and the ATP driven motor proteins: myosin and kinesin. Therefore, it is believed that these bio-molecular machines share common molecular mechanism utilizing nucleotide hydrolysis cycle. Previously, we have incorporated photochromic molecules, 4-phenylazophenyl maleimide (PAM), into the functional site of kinesin as a photo-switching device and succeeded to regulate kinesin ATPase activities reversibly upon VIS and UV light irradiation.

In this study, we performed basic study to control the Ras function reversibly by light using photochromic molecules. First, to observe the nucleotide exchange reaction of Ras GTPase, we synthesized a new fluorescent GTP analogue, NBD-GTP that changes its fluorescent intensity along the formation of Ras-GTP, Ras-GDP-Pi and Ras-GDP states. Experimental data suggested that NBD-GTP bind to the nucleotide-free Ras with burst kinetics and subsequently the Ras-GTP transit to Ras-GDP-Pi state slowly. Second, to photo-regulate the GTPase activity of Ras, we designed the Ras mutants, which have an additional reactive cysteine residue at the functional region. In the preliminary experiment, the GTPase activity of Ras mutants modified with PAM was reversibly alternated upon ultra-violet and visible light irradiation.

3PT183 Gradient sensing limit in Dictyostelium discoideum cell

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