rafts. TPO stimulation induced clustering of Mpl on the cell surface, which was dependent on cholesterol. TPO stimulation also promoted the lateral diffusion of Mpl. These data suggest that clustering of Mpl reduced the affinity of Mpl to lipid rafts. Clustering of Mpl promoted phosphorylation of Mpl and JAK2, not vice versa. These findings identify a positive regulatory mechanism for upstream of TPO signaling. We are now studying the interaction between Mpl and GM1-contained lipid rafts by observing Mpl and GM1 simultaneously at the single molecule level.

1P252 1分子計測とモデル計算による ErbB 受容体へのリガンド結合のキネティクス解析

Ligand-binding Kinetics of ErbB Receptors Analyzed by Single-molecule Measurement and Model Simulation

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The ErbB family of receptor tyrosine kinases consists of four members, ErbB1 to ErbB4, that are involved in various cellular processes by transducing ligand signals to the cytoplasmic proteins. Each receptor binds to specific ligands and shows peculiar functions through homo- and hetero-dimerization. For example, cell fate of the MCF7 human breast adenocarcinoma cells is determined by the ligand-specific manner, i.e., when stimulated by epidermal growth factor (EGF) or heregulin (HRG), cells are proliferated or differentiated, respectively. EGF mainly associates with ErbB1, whereas HRG with ErbB3 and ErbB4.

For analyzing the receptor-ligand binding kinetics combined with receptor dimerization, we employed single-molecule measurement and mathematical modeling for the association of dye-conjugated ligand with the receptors on the plasma membrane of MCF7 cells. Pathways of ligand-receptor binding and receptor dimerization were almost the same for both EGF and HRG systems, but 1) the binding rate of EGF was larger than that of HRG, and 2) the oligomeric conformation of ErbB3 and ErbB4 was more frequent than that of ErbB1. Different from typical cells respond to ErbB1 signals, numbers of ErbB1 were small ($\sim 10^3$ /cell) in MCF7 cells suggesting negligible amount of receptor dimers in the absence of ligand (predimer). This is probably the reason of smaller oligomer fraction of ErbB1 after ligand stimulation. Based on the experimental results, molecular processes correspond to the characteristic receptor-ligand kinetics will be discussed.

1P253 インターロイキン 13 受容体 $\alpha 2$ (IL-13R $\alpha 2$) によるインターロイキン 13 (IL-13) シグナルの阻害機構

Inhibition mechanism of Interleukin-13 (IL-13) signal by extracellular region of Interleukin-13 receptor $\alpha 2$ chain (IL-13R $\alpha 2$)

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Interleukin-13 (IL-13) is a key cytokine critical to the development of T-cell-mediated humoral immune responses which are associated with allergy and asthma. IL-13 possesses two types of receptor: the heterodimer, composed of IL-13R $\alpha 1$ and IL-4R α , transducing the IL-13 signals; and the IL-13R $\alpha 2$, it has high affinity rather than IL-13R $\alpha 1$ against IL-13, acting as a non-signaling "decoy" receptor. In order to investigate the inhibition mechanism of IL-13 signal by IL-13R $\alpha 2$, extra cellular region of IL-13R $\alpha 2$ was expressed by silkworm-baculovirus expression system after fusing the DNA coding the extracellular region of human IL-13R $\alpha 2$ with the Fc derived from mouse IgG2a. The expressed fusion protein (IL-13R $\alpha 2$)₂-Fc was purified by a protein A column followed by an ion exchange column. The affinities of the ligand complexes, IL-13/IL-13R $\alpha 1$ and IL-13/IL-13R $\alpha 2$ to (IL-4R α)₂-Fc were investigated to explore the inactivation mechanism of the IL-13R $\alpha 1$ signal with IL-13R $\alpha 2$ by surface plasmon resonance analysis. IL-13/IL-13R $\alpha 1$ complex has an affinity ($K_D=1.20 \times 10^{-7}$ M) with (IL-4R α)₂-Fc, whereas the IL-13/IL-13R $\alpha 2$ complex had no affinity with (IL-4R α)₂-Fc. This observation suggests that IL-13R $\alpha 2$ does not inactivate the IL-13R $\alpha 1$ through formation of a ternary complex with IL-13 and IL-4R α , but removes the IL-13 with its higher affinity to IL-13 without forming a ternary complex.

1P254 組換えタンパク質を用いた光制御型転写因子 AUREO1 の解析

Analyses of a light-regulated transcription factor, AUREO1, expressed in *E. coli*

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Blue light (BL) (350-500 nm) is an important environmental factor for many

plants to optimize their photosynthetic potential through responses such as phototropism. Aureochrome 1 (AUREO1) is a blue light receptor found in *Vaucheria* in 2007. AUREO1 has one basic region/leucine zipper (bZIP) domain and one light-oxygen-voltage (LOV)-sensing domain, and play roles as a BL-regulated transcription factor. We attempted to clarify the molecular mechanisms of AUREO1, and expressed truncated AUREO1 (A1-ZC), containing bZIP and LOV domains, in *E. coli*. A1-ZC showed absorption and emission spectra typical of a flavoprotein, and selectively bound to the target sequence (TGACGT) of AUREO1. Upon illumination of BL, the absorption around 360 and 450 nm were decreased, probably due to the formation of a cysteine photoadduct. A1-ZC is likely exists as a homodimer with disulfide linkage(s). The amount of A1-ZC dimer was decreased by substitution of Cys162 or Cys182 to Serine. Site-directed mutant C254S showed similar absorption and emission spectra with wild-type, but lost the ability of spectral change upon illumination. It was suggested that Cys254 in the LOV domain contributes the formation of photoadduct similar to phototropin.

1P255 ハロロドプシンの Cl⁻移動中における, シッフ塩基の pKa 変化

Shift in pKa of the protonated Schiff base (PSB) in halorhodopsin from *Natronomonas pharaonis* (pHR) during Cl⁻ translocation

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pHR is a light-driven inward-directed Cl⁻ pump. The crystal structure clearly shows that the primary Cl⁻ is proximal to the proton of PSB and is stabilized by electrostatic interactions with the positive charged PSB [Kouyama *et al.*, J. Mol. Biol., 396: 564-579 (2010)]. It is believed extensively that the PSB group to maintain a polar interaction with Cl⁻ plays a crucial role in its movement from extracellular to cytoplasm spaces. In the present study, we determined how pK_a of PSB altered during Cl⁻ movement, using azide (N₃⁻) to function as an acceptor for the proton of PSB. N₃⁻ caused a blue-shift of the absorption maximum in a concentration-dependent manner, indicating that N₃⁻ binds to pHR similar to Cl⁻. In the presence of N₃⁻, a typical M-intermediate (deprotonation of PSB) was observed in the photocycle of the N₃⁻-bound pHR, suggesting that pK_a of PSB is reduced after illumination, followed by the movement of proton from PSB to the acceptor, N₃⁻. The pH-titration curve of M formation with a range from 5 to 10 showed the bell-shape profile described by a Henderson-Hasselbalch equation with two pK_a values, 10.3 and 6.3, implying that pK_a of PSB shifted remarkably from 10.3 to 6.3 after illumination. Furthermore, photo-induced pK_a shift of PSB in pHR reconstituted with phospholipid on illumination was analyzed using SnO₂ electrode, resulting with the similar pH-titration profile of deprotonation from PSB. How this reduction in pK_a of PSB might be involved in Cl⁻ translocation mechanism is discussed here.

1P256 イカロドプシン光異性化反応産物の X 線結晶構造解析

X-ray crystallographic studies of the primary photoproducts of squid rhodopsin

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The signal transduction process in the visual photoreceptor cell is initiated by the photo-isomerization of the 11-cis retinal in rhodopsin. Previous structural analyses of squid rhodopsin showed that in the unphotolysed state, the retinal takes on a planar 11-cis configuration within the binding pocket. The plane of the retinal polyene chain is in perpendicular to the membrane normal, and the protonated Schiff base and the counterion Glu180 are separated by 5 Å. In this study, we have collected spectroscopic and diffraction data from frozen crystals under various illumination conditions. Spectroscopic data at 100K showed that a large amount of bathorhodopsin accumulated when the frozen crystal was illuminated at 473 nm, while the content of isorhodopsin, an artificial product with 9-cis retinal, was maximized under illumination at 570 nm. Analyses of diffraction data of crystals that were illuminated at these wavelengths at 100K provided the structural information of each isomeric state. The result showed that the formation of bathorhodopsin is accompanied by a large movement of the central moiety of the retinal polyene chain towards the cytoplasmic side with small movements of the beta-ionone ring and the Schiff base, i.e., the retinal takes on a right-handed screwed all-trans configuration. Conversely, the retinal in isorhodopsin takes on a planar 9-cis configuration, i.e., as compared to the 11-cis retinal in the unphotolysed state, a limited configurational change is observed around the C9=C10 bond.