Using high speed AFM (AFM), we here attempted to visualize the dynamic movement of rhodopsin in disk membrane to reveal molecular mechanism of signal transduction. This microscopy allows capturing images of single proteins at 30-60 ms/frame in fluid [1,2]. Disk membranes purified from bovine rod outer segment were adsorbed on a mica surface in buffer solution. High-speed AFM movies showed dynamic diffusion of numerous rhodopsin molecules in the membranes. Also, a few of them formed paracrystalline arrays of dimers in disk membranes similar as reported previously [3].

[1] T. Ando et al. *Pflügers Archiv. Eur. J. Physiol.* 456: 211-225 (2008).

[2] H. Yamashita et al. *J. Struct. Biol.* in press.

[3] D. Fotiadis et al. *Nature* 421: 127-128 (2003)

2P-209 イカロドプシンとウシオプシンの分子動力学シミュレーション Molecular Dynamics Simulation of Squid Rhodopsin and Bovine Opsin

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The visual rhodopsin is the photoreceptor in the family A of G-protein-coupled receptors (GPCRs). In particular bovine rhodopsin is one of the best studied GPCRs and the first membrane protein of which three dimensional structure was solved by X-ray crystallography. In 2008 the three dimensional structures of squid rhodopsin [1] and bovine opsin in its G-protein-interacting conformation [2] were solved. In these structures the helices 5 and 6 (H5 and H6) extend into the cytoplasmic side compared with the inactive form of bovine rhodopsin. The elongation of these helices might play a significant role in the activation although the mechanism is still unknown.

An additional helix (H9), which bovine rhodopsin does not possess, was found in squid rhodopsin and it is plausible that the interaction with this helix stabilizes the extended helical conformation of the H5 and H6 [1]. While in bovine rhodopsin the H5 and H6 seem to be elongated by interacting with G-protein. The correlation between the G-protein-coupling selectivity and the deformation in this region is speculated [3]. In this study, we apply molecular dynamics simulations to the crystal structures of squid rhodopsin and bovine opsin. We will discuss the stability of elongated helices and the G-protein-coupling selectivity in squid rhodopsin (Gq) and bovine opsin (Gt).

[1] M. Murakami, T. Kouyama *Nature* 453 (2008) 363; T. Shimamura et al. *J. Biol. Chem.* 283 (2008) 17753. [2] J. H. Park, et al. *Nature* 454 (2008) 183; P. Scheerer et al. *Nature* 455 (2008) 497. [3] M. Suwa, Seibutu Buturi 48 (2008) 335 (in Japanese).

2P-210 S-modulin 依存的なロドプシンのリン酸化抑制にはマイクロドメインが重要である Microdomain in Rod Photoreceptor Disk Membrane is Crucial for Ca²⁺-dependent Suppression of Rhodopsin Kinase by S-modulin

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Our single molecule tracking experiments have shown that the lateral diffusion rate of transducin (T α β γ -GDP) bound by light-bleached rhodopsin (R*) is noticeably smaller than that of rhodopsin itself (BSJ 2007). Thus, it was suggested that R*-T α β γ -GDP complex is surrounded by somewhat sticky micro lipid environment, or T α β γ makes complex with dimerized or oligomerized rhodopsin. In either case, the diffusion of the complex would be distracted in highly crowded disk membrane. Our recent studies have suggested that the dimerized rhodopsin has a propensity to recruit lipid microdomain around them, and T α β γ -GDP stabilizes the dimer of rhodopsin. Although the lifetime of the dimer and surrounding microdomain would be very short in the presence of GTP in physiological condition, the microdomain around the R*-T α β γ complex might have some functional significance in phototransduction in vertebrate rod photoreceptor. Here, we examined the effect of microdomain-disrupting agent, methyl- β -cyclodextrin, to the S-modulin-mediated Ca²⁺-dependent suppression of rhodopsin phosphorylation using reconstitution system (3% bleach by a flash light). In a cholesterol-depleted disk membrane, S-modulin required ~5 times higher concentration of Ca²⁺ than that required in a cholesterol-rich disk membrane to suppress GRK1. Functional significance of transducin-stabilized rhodopsin dimer and surrounding microdomain would be discussed in relationship with an efficient elongation of the lifetime of R* in highly sensitive rod photoreceptor disk membrane which is enriched in cholesterol.

2P-211 カエル視細胞の cGMP 分解酵素 PDE6 の二つの γ サブユニットは機能分担を行っている

Functional differentiation within two γ subunits of cGMP-phosphodiesterase 6 (PDE6) in frog photoreceptor

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Phosphodiesterase 6 (PDE6) is the effector enzyme in vertebrate photoreceptors. PDE6 consists of two homologous catalytic subunits, P α and P β , and two identical inhibitory subunits, P γ . In spite of its functional importance, neither the regulatory mechanism nor the three-dimensional configuration of each subunit on PDE6 have been fully documented. The two P γ subunits have been speculated to have identical inhibitory activity on two catalytic subunits, while it has been reported that the two P γ s exhibit significantly different affinities to active transducin (Gat*). Here, we report that the two P γ s can be discriminated in terms of their susceptibility to a thiol-oriented fluorescent dye, and that the functional differentiation within two P γ s was suggested. This dye labeled one of two P γ s on a holo-PDE6. The P γ seemed to have a lower affinity to Gat*. In contrast, the dye did not label the other P γ that has a high affinity to Gat*, and seemed to be involved in G protein signaling. Considering the fact that P γ has only one thiol, the two P γ s seems to bind to the catalytic subunits in different ways, i.e. the thiol group on one P γ s is exposed, whereas the one on the other P γ is shielded. Although the physiological significance of the functional and structural differentiation within two P γ s has been yet obscure, we obtained fluorescent probe of PDE6 sufficient for single molecule tracking study of PDE6 in native disc membrane. In addition, anisotropic modification of P γ suggested unexpected complexity of the regulation mechanism of PDE6.

2P-212 組換えタンパク質を用いたオーレオクロームの解析 Analyses of aureochrome 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 and 2 (AUREO1 and AUREO2) are blue light receptors found in *Vaucheria* in 2007. It was suggested that AUREO1 and AUREO2 were responsible for light-mediated branching, an example of photomorphogenesis in stramenopiles. Each of aureochrome has one basic region/leucine zipper (bZIP) domain and one light-oxygen-voltage (LOV)-sensing domain. Though aureochromes were suggested to play roles as BL-regulated transcription factors, their molecular mechanisms are remains to be elucidated. We constructed an expression system of aureochromes in *E.coli* cells. Our recombinant AUREO1 showed an absorption spectrum and an emission spectrum typical of a flavoprotein, suggesting that it binds flavin mononucleotide via its LOV domain. When AUREO1 was illuminated at 450nm, the absorption around 360 and 450 nm were decreased, and was restored with the time-constant for 50% recovery ($t_{1/2}$) of 5 min at 25°C. About 0.1 mg recombinant AUREO1 was obtained from 1 liters of cultured *E.coli* cells. It was suggested that our expression system provides an opportunity to investigate the molecular mechanism of BL-regulated transcription factors.

2P-213 2種類の視物質キナーゼ (GRK1、GRK7) の活性の違いをもたらす領域の特定 Identification of a region responsible for higher phosphorylation activity of GRK7 than GRK1

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In vertebrate retina, there are two types of photoreceptor cells, rods and cones. The termination of a photoresponse is much faster in cones than in rods, which ensures higher time resolution of light detection by cones. To terminate a photoresponse, it is needed to inactivate light-activated visual pigment by phosphorylation. In many vertebrates including teleosts, GRK1 (G-protein coupled Receptor Kinase 1) and GRK7 are expressed as the visual pigment kinase in rods and cones, respectively. Recently, by using isolated rods and cones purified from carp (*Cyprinus carpio*) retina, we found that the total kinase activity is 100 times higher in cones than in rods. It has been suggested that this higher activity of visual pigment phosphorylation is one of the mechanisms of faster termination of a light response in cones. Our further study showed that this higher kinase activity is due to (1) 10 times higher content of cone GRK7 than rod GRK1, and (2) 10 times higher specific activity of a single GRK7 molecule than a single GRK1 molecule.

In this study, we tried to identify the site responsible for the higher specific activity of GRK7. GRKs are consisted of 3 domains: the N-terminal, the catalytic, and the C-terminal domains. By using chimera proteins of GRK1 and GRK7, we first tried to identify which domain(s) is necessary for the higher kinase activity. The result showed that the C-terminal domain of GRK7 is required for the higher activity. We further identified a 40 aa region in the middle of the C-terminal domain as the site responsible for the high GRK activity. The functional mechanism of this newly found region will be discussed.

2P-214 低温分光測定によるゼブラフィッシュクリプトクローム DASH 光反応経路の決定