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 Ca^{2+} -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 ($\text{T}\alpha\beta\gamma\text{-GDP}$) bound by light-bleached rhodopsin (R^*) is noticeably smaller than that of rhodopsin itself (BSJ 2007). Thus, it was suggested that $\text{R}^*\text{-T}\alpha\beta\gamma\text{-GDP}$ complex is surrounded by somewhat sticky micro lipid environment, or $\text{T}\alpha\beta\gamma$ 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 $\text{T}\alpha\beta\gamma\text{-GDP}$ 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 $\text{R}^*\text{-T}\alpha\beta\gamma$ 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 Ca^{2+} -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 Ca^{2+} 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, $\text{P}\alpha$ and $\text{P}\beta$, and two identical inhibitory subunits, $\text{P}\gamma$. 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 $\text{P}\gamma$ subunits have been speculated to have identical inhibitory activity on two catalytic subunits, while it has been reported that the two $\text{P}\gamma$ s exhibit significantly different affinities to active transducin (Gat^*). Here, we report that the two $\text{P}\gamma$ s can be discriminated in terms of their susceptibility to a thiol-oriented fluorescent dye, and that the functional differentiation within two $\text{P}\gamma$ s was suggested. This dye labeled one of two $\text{P}\gamma$ s on a holo-PDE6. The $\text{P}\gamma$ seemed to have a lower affinity to Gat^* . In contrast, the dye did not label the other $\text{P}\gamma$ that has a high affinity to Gat^* , and seemed to be involved in G protein signaling. Considering the fact that $\text{P}\gamma$ has only one thiol, the two $\text{P}\gamma$ s seems to bind to the catalytic subunits in different ways, i.e. the thiol group on one $\text{P}\gamma$ s is exposed, whereas the one on the other $\text{P}\gamma$ is shielded. Although the physiological significance of the functional and structural differentiation within two $\text{P}\gamma$ 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 $\text{P}\gamma$ 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

Photoreaction pathway in zebrafish Cryptochrome-DASH determined by low temperature spectroscopy

see 2TA2-03

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Blue-light photoreceptors control many important biological processes. Cryptochromes, members of DNA-photolyase/Cryptochrome superfamily, regulate a variety of photomorphogenetic responses in plants as well as resetting of circadian rhythm in animals. Recently, a new member of Cryptochrome family, Cryptochrome-DASH (Cry-DASH), was found. Cry-DASH is widely distributed from bacteria to the plant and animal kingdoms except for the mammal. Despite the wide distribution, physiological function of Cry-DASH is still to be known. In this paper, photoreaction pathway of Cry-DASH from zebrafish (Zf), that is determined by both ambient- and low-temperature UV-visible absorption spectroscopy in aqueous solutions, is reported. Zf Cry-DASH binds two chromophores, a flavin adenine dinucleotide (FAD) and a N5,N10-methenyl-5,6,7,8-tetrahydrofolate non-covalently. At an ambient temperature, 295 K, the bound FAD exists in an oxidized form in the dark. Blue light converts the oxidized FAD to a fully reduced form via a neutral radical form. The origin of the neutral radical formation was studied by low temperature spectroscopy. There are two possible reactions to form the neutral radical. Electron transfer precedes proton transfer or electron and proton migrate simultaneously that is H-atom migration. Low-temperature spectroscopy detected a new intermediate showing a characteristic spectrum with that of flavin anion radicals indicating that electron transfer is a primary reaction. Based on these results, photoreaction pathway of CRY-DASH will be summarized and discussed.

2P-215 シロイヌナズナ青色光受容体・フォトトロピン2の光シグナル伝達に対するアルギニン427の関与

Involvement of Arg427 in the light-signal transduction of Arabidopsis phototropin2, a blue-light photoreceptor

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Phototropin (phot) is a blue light photoreceptor in plants that mediates phototropism, chloroplast relocation, stomata opening and leaf expansion. A phot molecule has two photoreceptive domains named LOV1 and LOV2 in the N-terminal half and the C-terminal half forms Ser/Thr kinase. Between the two LOV domains, LOV2 is primarily responsible for the kinase activation. Both the LOV domains bind non-covalently a flavin-mono-nucleotide (FMN) in an oxidized form as a chromophore. Blue light irradiation induces a conversion of the LOV domains in the dark-state (D450) to a flavin-cysteiny adduct state (S390) that reverts to D450 spontaneously. S390 is thought to be a signaling state that activates phot kinase through conformational changes in the protein moiety, in which involvements of some key amino acid residues, such as Glu489 or Phe470 nearby the isoalloxazine head of FMN, have been proposed. Instead, we focused on the amino acid residues interacting with the phosphate tail of FMN. In the present paper, we report the involvement of Arg427 in the LOV2 domain of Arabidopsis phototropin 2 (phot2) in the light-signal transduction. Arg427 resides next to the conserved cysteine responsible for the adduct formation and interacts with the phosphate tail. Introduction of Arg/Lys mutation brought a significant reduction of phenotypes such as phototropic curvature and strong-light avoidance response of chloroplasts. Interestingly, the mutated LOV2 domain does bind FMN, however, does not show the adduct formation upon light excitation. Computer simulation proposes an origin for the inability of adduct formation.

2P-216 ロドプシンの細胞質側ループを組み込んだ古細菌ロドプシンの研究

Study on chimeric archaeal-rhodopsin containing cytoplasmic loops of bovine rhodopsin

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see 2TA2-04

2P-217 アーキロドプシンからのプロトン放出に関する分光研究

Spectroscopic study of proton release group of archaeorhodopsins

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2P-218 ロドプシンにおける相乗的な長距離相互作用による吸収波長制御機構

Molecular mechanism of long-range synergetic color tuning between multiple amino acid residues in rhodopsin

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see 2TA2-06

2P-219 発光キノコ *Mycena Chlorophos* の発光分子機構の解明

Molecular mechanism of bioluminescent *Mycena Chlorophos*

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Mycena Chlorophos belongs among luminescent fungi. The molecular mechanism of luminescent organisms can be generally divided into two reaction forms; the enzymatic luciferin-luciferase (L-L) and the non-enzymatic (NE) reactions, but little is known about the chemical nature of luminescence of *M.Chlorophos*. The purpose of this study is to assign the reaction forms of *M.Chlorophos* to which one of the two. The luminescence biological material extracted from *Lampteromyces japonicus*, a famous luminous fungus, was lampteroflavin derived from riboflavin. Performing HPLC analysis on a ODS-3 column for the sample extracted from *M.Chlorophos*, one peak was observed at almost the same elution time as that of riboflavin. HPLC analysis was applied to the same sample by the gradient method, while four peaks were detected. In order to check which fraction on the chromatogram contained luminescence related materials, we repeated 5 times the same extraction to scale up each sample volume of four peaks. Each extract sample was mixed with the quenched *M.Chlorophos*, placed in air for one day, to examine the light emission by the photon counter. Mixing experiment for one extract sample shows bioluminescence with a little bit stronger intensity than for other three specimens. This result means that bioluminescence of *M.Chlorophos* could not be necessarily attributed to the L-L nor NE reaction type. Thus, we need to study furthermore the bioluminescence mechanism of *M.Chlorophos* quantitatively using NMR and LC-MASS spectrometry.

2P-220 ピコ秒時間分解蛍光分光法による黄化葉の緑化途上における光合成系タンパク質複合体構築過程の観測

Assembly process of photosynthetic pigment-protein complexes during greening of etiolated leaves observed by picosecond time-resolved fluorescence spectroscopy

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Dark-grown higher plants have etiolated leaves, which lack chlorophyll (Chl) and most of the Chl-protein complexes, and instead accumulate the precursor pigment, protochlorophyllide (PChld). PChld's in etiolated leaves form quasi-crystalline aggregates called prolamellar body (PB). When etiolated leaves are illuminated, PChld is rapidly converted to Chl, Chl-protein complexes start to accumulate, and the structure of PB is transformed into the thylakoid membrane. Little is known about the assembly process of newly synthesized Chl and apo-protein in greening leaves so far. Characterizing the precursor states of the Chl-protein complexes during their structural formation is considered essential to understand the mechanism of their assembly process. Since the excitation-energy-transfer (EET) dynamics in a picosecond time region is highly sensitive to the state of the pigments in the Chl-protein complex, the measurement of the EET dynamics can be a powerful tool to characterize the state of the Chl-protein complexes during greening. In order to clarify the EET dynamics, we measured the picosecond time-resolved fluorescence of greening leaves, which was incubated for various periods after the initiation of greening. The results of the measurement at 77 K showed the emergence of the EET component 1 hour after the initiation of greening. The spectral analysis showed that this EET component reflects the energy flow from the pigments emitting around 670 nm to those around 685 nm. This EET process is different from that observed for the mature photosystems, and might reflect the accumulation of the precursor Chl-protein complex. Lowering temperature below 77 K will be effective to better reveal the state of pigment. The experiment at 4 K is now in progress and will be reported in the annual meeting.

2P-221 ラン藻 *Leptolyngbya boryana* における光非依存型プロトクロロフィリド還元酵素欠損株の細胞内色素分布の顕微分光法による解