CPD PHRs. FAD is a common chromophore molecule of both PHRs. The oxidized form of FAD in PHR is activated by photoreduction, where two photons convert FAD to the reduced form, FADH⁻, and electron transfer from FADH⁻ to the photoproducts initiates the repair process.

Light-induced difference Fourier-transform infrared (FTIR) spectroscopy is a powerful method to study structure-function relationships in photoreceptive proteins. In this study, we applied FTIR spectroscopy to *Xenopus* (6-4) PHR to gain insights into the activation and repair processes induced by light. By use of a concentrated solution, we successfully obtained difference FTIR spectra that correspond to the photoactivation and photorepair processes of (6-4) PHR. Light-dependent protein activation is accompanied by IR spectral changes of FAD and the surrounding protein, while the peptide backbone and a protonated carboxylic acid are influenced by the presence of the (6-4) photoproduct. Difference FTIR spectra for the light-triggered DNA repair process show structural changes for the phosphate group and the (6-4) lesion. Combined with the results from X-ray crystallographic analyses [1], this work opens a new understanding of the molecular mechanism.

[1] Hitomi et al. PNAS 106, 6962 (2009).

3P-212 2種類のBLUFドメイン、AppAとPACの赤外分光解析 FTIR study of two BLUF domains, AppA and PAC

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see 3TA2-01

3P-213 バクテリオロドプシンとハロロドプシンを発現している大腸菌の比較生理学 Comparative physiology between bR and hR expressing *Escherichia coli*

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Halorhodopsin (hR) is a light-driven chloride ion pump found in an extremely halophilic archaeon, *Halobacterium salinarum*. hR is a membrane protein possessing the 7-transmembrane helix, and a retinal moiety is attached to the Lys residue in the middle of the 7th helix. Light is absorbed by the retinal, which promote the retinal twisting from all-*trans* form to 13-*cis* form. This conformation changes (and charge separation) further drive a chloride ion translocation from extracellular side to intercellular space. Resulting membrane potential difference drive the A-type ATP synthase to make ATP from ADP and P_i. However prolonged lightning cause the intercellular acidification to prohibit ATP synthesis and the amount of intercellular ATP concentration of hR expressing strain is decreasing unlike bacteriorhodopsin (bR) expressing strain. Thus, it is still unclear the real physiological function of hR. Recent genomic analyses revealed the wide and patchy distribution of archaeal type rhodopsins. Almost all found rhodopsins in the eukaryote or the eubacteria is putative proton pump (bR type) or sensor (sR or pR types), and anion pump (hR) is found only in extremely halophilic archaea with an only exception of the eubacterium *Salinibacter ruber*. We have cloned these hRs (from *Haloarcula vallismortis*, *Halorubrum* sp. aus-1, *Haloquadratum walsbyi*, *Natronomonas pharaonis*, *Haloterrigena* sp. arg-4 and *Salinibacter ruber*) genes and expressed in the *Escherichia coli*. Some physiological character of the hR expressing *E. coli* are investigated especially in the response against the light and compared with the bR expressing *E. coli*.

3P-214 細菌の光センサーSRIとトランスドューサーHtrIの複合体の性質 The properties of eubacterial sensory rhodopsin I in the complex with its cognate transducer protein

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Motile bacteria sense and respond to extracellular stimuli by changing their swimming mode to migrate toward more favorite habitats. This behavior, termed *taxis*, is achieved by a signaling system from membrane-embedded receptors (input) to flagella motor (output). Sensory rhodopsin I (SRI) is a 7-transmembranous dual photoreceptor both for positive and negative signaling with its transducer protein, HtrI, depending on light color. Recently, we characterized highly stable SRI from eubacterium *Salinibacter ruber* (SrSRI) [1]. Here we succeeded in the cloning and purification of SrSRI with its cognate transducer protein SrHtrI [2]. However the expression level was low, and the reconstitution into the liposomes was difficult because of high solubility of full-length HtrI, and they make difficult to analyze the structure and structural changes of SRI-HtrI complex in atomic level. Then, we prepared SRI with truncated HtrI (SrSRI-SrHtrI128). The complex gave us large amount of proteins and showed similar photochemical properties to full-length SrSRI-SrHtrI. In addition, it can be

reconstituted into phosphatidyl-glycerol lipids. We performed here fourier transform infrared (FTIR) spectroscopy, and its structural changes were compared with SrSRI alone. Furthermore, we intend to analyze the interaction mechanism between SrSRI and SrHtrI128 and the structural changes of the complex using water-soluble reagents, hydroxylamine and azide, and site-directed mutagenesis of the putative interaction surface.

3P-215 光センサータンパク質 AnPixJ のシリカ多孔体中への吸着 Adsorption of light-sensor protein AnPixJ to the silica nanoporous materials

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AnPixJ is a novel cyanobacteriochrome of a cyanobacterium *Anabaena* (Nostoc) sp. PCC 7120. Upon the irradiation of red- light and green-light, it shows a reversible photoconversion between a green-absorbing ($\lambda_{\text{max}}=543$ nm) and red-absorbing ($\lambda_{\text{max}}=648$ nm) forms. His-tagged chromophore-binding GAF domain of AnPixJ protein (AnPixJ-GAF) with a molecular weight of about 20 kDa was purified by using Ni affinity column. We introduced this water-soluble protein into SiO₂ mesoporous materials and tested its photoconversion. AnPixJ introduced into a silica mesoporous material showed photoconversion activity. We studied the effects of pore diameter on the extent of the adsorption and photoconversion activity.

3P-216 タコロドプシンの結晶化 Crystallization of octopus rhodopsin

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Octopus rhodopsin is a member of the family of G-protein-coupled receptors (GPCRs), which form a large superfamily of seven transmembrane helix proteins that mediate responses to stimuli—light, proteins, peptides, small molecules, hormones, and ions. Although several crystal structures of inactive form of GPCR were solved, little is known about the structure of an activated GPCR. In contrast to vertebrate rhodopsin, the retinal chromophore in octopus rhodopsin remains bound to the protein moiety under prolonged illumination. Its acid metarhodopsin is thermally stable and red light can drive all the pigment back to the initial state. Thus, octopus rhodopsin is an ideal model to elucidate the mechanism of GPCR activation. In this study, we have developed a simple procedure of purification of octopus rhodopsin, which involves only 4 times of centrifugation. After microvillar membranes containing uncleaved rhodopsin were prepared using a special buffer [10mM MES (pH6.3), 50mM EDTA, 1mM DTT], octopus rhodopsin was selectively extracted by nonyl-glucoside (NG) at pH 6.3. This is a great step toward our final goal (namely crystallization of the rhodopsin-lipid complex). So far, no crystal of uncleaved rhodopsin was obtained. Conversely, truncated rhodopsin, the C-terminus of which was cut by V8 protease treatment, was found to crystallize into stick-like crystals. Now, the optimal crystal condition for high resolution structural analysis of octopus rhodopsin is in progress.

3P-217 Natronomonas pharaonis 由来光駆動塩素イオンポンプハロロドプシンの結晶構造解析 Crystallographic studies of the light-driven chloride pump halorhodopsin from *Natronomonas pharaonis*

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The light-driven chloride pump halorhodopsin (pHR) from *Natronomonas pharaonis* was crystallized into a monoclinic crystal (the space group C2). Diffraction data from the C2 crystal showed that one pHR trimer is contained in the asymmetric unit and that the carotenoid bacterioruberin binds to crevices between adjacent pHR subunits within the trimer. This binding mode of bacterioruberin is similar to that observed in the trimeric structure of archaerhodopsin-2 (aR-2), a light-driven proton pump of *Halorubrum* sp. aus-2. Although the trimeric structure of pHR is very similar to that of aR-2, it is different largely from that observed in a bacterioruberin-free crystal of halorhodopsin (sHR) from *Halobacterium salinarum*. In the active site of pHR, a chloride ion interacts with the retinal Schiff base, Thr126 OH, Ser130 OH and one water molecule. This binding mode, which is slightly different from that seen in sHR, explains a higher binding constant of chloride ion in pHR than in sHR. Using the C2 crystal, we are now trying to analyze light-induced structural changes of pHR.