state. All the spectra showed prominent bands in the CC/CN regions of PCB at 1300-1200 cm^{-1} , suggesting significant changes in the skeletal structure of PCB. The water OH bands were observed at 3657/3623 cm^{-1} upon photoconversion between Pg and Pr. This observation was interpreted to indicate the movement of a water molecule interacting with the D ring, consistent with the idea that rotation of this ring is involved in the reaction. Furthermore, observation of a negative COOH band at 1708 cm^{-1} by conversion of Pr to an intermediate at 200 K suggested that deprotonation of Asp291, which is hydrogen bonded with three nitrogen atoms of PCB, takes place in this process. Based on these results, the mechanism of photoreaction of AnPixJ is discussed.

3P270 シロイヌナズナフォトトロピン 1 LOV2-J α (N/A) 変異ペプチドは S390 の寿命が長くなる。 Arabidopsis phot1 LOV2-J α (N/A) mutant shows longer life time of adduct state, S390.

Sachiko Kasyojiya, Koji Okajima, Satoru Tokutomi, (Department of Biological Science, Graduate School of Science, Osaka Prefecture University)

Phototropin (phot), a blue light receptor in plant, mediates phototropism, stomata opening and chloroplast movement. Phot has two LOV (LOV1, LOV2) domains and a Ser/Thr kinase domain in the N-terminal and the C-terminal regions, respectively. LOV domain is composed of five β strands and a few α helices, and holds an FMN in a pocket as a chromophore. Upon blue light, covalent bond is formed between the FMN and a conserved Cys (S390 state) transiently. S390 returns to the ground state (D450 state) thermally with time constants from seconds to minutes. It is thought that the lifetime of S390 affects the sensitivity of phot functions to light intensity. However, little is known about the factor regulating the recovery rates. In this study, we focused on Arabidopsis phot1 Asn476 which interacts with Gln575, one of the key amino acid residues involved in S390 formation. Introduction of Gln575/Leu is reported to prolong the life time of S390. We prepared a LOV2-J α polypeptide with an Asn476/Ala (N/A) mutation and measured its UV-vis spectra. LOV2-J α (N/A) undergoes a cyclic photoreaction by blue light. S390 of the N/A mutant showed a blue-shifted absorption peak and a prolonged life time. We will discuss the molecular mechanisms regulating the recovery rate to S390 in LOV domains.

3P271 ゼブラフィッシュ クリプトクローム-DASH の円二色性分光測定 Circular Dichroism spectra of zebrafish Cryptochrome-DASH

Reo Fukazawa¹, Kazunori Zikihara^{1,2}, Tomoko Ishikawa², Takeshi Todo², Satoru Tokutomi¹, (¹Department of Biological Science, Graduate School of Science, Osaka Prefecture University; ²Department of Medical Genetics, Graduate School of Medicine, Osaka University)

Cryptochromes, members of 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. Zebrafish (Zf) Cry-DASH binds two chromophores, a flavin adenine dinucleotide (FAD) and a N5,N10-methenyl-5,6,7,8-tetrahydrofolate (MTHF), non-covalently.

Previously, we have reported the photoreaction pathway of Zf Cry-DASH determined by UV-visible absorption spectroscopy in aqueous solutions. 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.

In this study, circular dichroism (CD) spectra of Zf Cry-DASH were measured. In the dark, oxidized FAD and MTHF showed CD signals, indicating both the isoalloxazine ring of FAD and MTHF take an asymmetric conformation in the protein. Moreover, the neutral radical form of FAD exhibited a CD spectrum with negative and positive peaks. Furthermore, far-UV CD spectrum indicates the protein rich in α -helix. However, light-induced secondary structural change was not detectable by CD.

This is the first report on CD spectra of the Photolyase/Cryptochrome family proteins.

3P272 Molecular basis for the blue/green photoconversion of cyanobacteriochrome SyPixJ1 revealed by UV-VIS absorption and circular dichroism

Shizue Yoshihara, Kazunori Zikihara, Satoru Tokutomi, (Dept. Biological Science, Graduate School of Sciences, Osaka Prefecture University)

Plant phytochromes and some of bacteriophytochromes covalently bind a linear tetrapyrrole as a chromophore, and show red/ far-red reversible photoconversion.

SyPixJ1 is a phytochrome-like photoreceptor necessary for positive phototaxis in *Synechocystis* sp. PCC 6803. We have already reported that SyPixJ1 protein covalently bound a linear tetrapyrrole and showed a novel blue (Pb form, $\lambda_{\text{max}} = 435 \text{ nm}$) / green (Pg form, $\lambda_{\text{max}} = 535 \text{ nm}$) reversible photoconversion. The chromophore-binding GAF polypeptide prepared using the red absorbing phycocyanobilin (PCB) synthesizing *E. coli* revealed almost identical photoconversion between Pb and Pg to that of the full-length SyPixJ1, indicating that a PCB is responsible for the blue/ green photoconversion. In the previous report, we proposed that PCB is attached to the protein moiety in a twisted conformation that disrupts the π -electron conjugation over the four pyrrole rings, possibly through a second covalent linkage with the protein pocket. In the present study, we introduced mutations at cysteine residues conserved in the GAF domains of blue/ green convertible cyanobacteriochromes that are a candidate for the second linkage. Cys564Asp mutant exhibited a light-stable green absorbing spectrum with an absorption maximum at 555 nm. Based on the data from absorption and CD spectroscopic analyses of the wild type SyPixJ1 GAF and the mutant, we will discuss the molecular basis for the shift of the PCB absorption from the red to the blue/ green absorbing regions.

3P273 プロテオロドプシンが生理的な条件下でプロトンポンプとしてはたらくことの直接的な証明 3A1235 Proteorhodopsin functions as a light-driven proton pump in native cell membranes

Akira Kawanabe^{1,2}, Susumu Yoshizawa³, Kazuhiro Kogure³, Hideki Kandori², (¹Graduate School of Medicine, Osaka University; ²Department of Frontier Materials, Graduate School of Engineering, Nagoya Institute of Technology; ³Atmosphere and Ocean Research Institute, The University of Tokyo)

Bacteriorhodopsin (BR) is a light-driven proton pump in halophilic Archaea, and BR-like proton pumps have been widely discovered not only in Archaea, but also in Bacteria and Eucaryota. More than thousands of proteorhodopsin (PR) genes have been found in marine bacteria¹. PR pumps protons when expressed heterologously in *E. coli*², suggesting that light energy passing through PR may be substantial in marine environment. However, there has been no confirmation of the proton pump function in native marine bacterial cells, and thus a role as a sensor has drawn more attentions recently³.

Here, we report the direct evidence that PR pumps protons in native cell membranes. We cultured 8 different Flavobacterial cells containing PR gene. Upon illumination, all cells suspensions showed marked pH drop that was abolished by the addition of protonophore. Although the native cells contain huge amounts of carotenoids, we were able to obtain the light-induced difference spectra between PR and retinal oxime in the presence of hydroxylamine. The action spectrum of the proton pump activity coincides well with the photobleaching difference spectrum, indicating that PR is responsible for light-induced acidification.

In conclusion, we first show that PR functions as a proton pump in native cell membranes of marine bacteria. Our finding requires reexamination of solar energy consumption in marine environments.

[1] O. Beja et al.(2000) *Science* 289,1902. [2] A. Martinez et al.(2007) *PNAS* 104,5590. [3] J. A. Fuhrman et al.(2008) *Nat. Rev. Microbiol.* 6, 488.

3P274 A Long-lived photoproduct, P370, formed from salinarum phoborhodopsin at room temperature

Gang Dai¹, Jun Tamogami², Naoki Kamo², Makoto Demura³, Tatsuo Iwasa¹, (¹Division of Science for Composite Functions, Muroran Institute of Technology, Muroran, Japan; ²College of Pharmaceutical Science, Matsuyama University, Matsuyama, Japan; ³Faculty of Advanced Life Science, Hokkaido University, Sapporo, Japan)

Phoborhodopsin (spR, also called *Halobacterium salinarum* sensory rhodopsin II, or HsSRII), one of the retinal proteins in the membrane of *Halobacterium salinarum*, works as a phototransducer for the negative phototaxis. Like other retinal proteins, spR has a seven-helix structure with a retinal molecule binding to a lysine residue in the seventh helix. Absorption of light initiates a cyclic photoreaction (photocycle), which includes photoisomerization of the retinal and transient deprotonation of the retinal Schiff base, followed by recovery of the initial state. In our previous work, we found a long-lived photoproduct, which decayed to the original state in the dark with a half time of 190 min. (Gang Dai et al., [2010] *Photochem. Photobiol.*, 86, 571 - 579) This photoproduct was named P370, because of the difference maximum was at 370 nm. In the present work, we characterized P370 in more details and found that the formation of P370 was greatly enhanced by one amino acid replacement in the retinal binding pocket and that P370 is a thermal product of O. Based on our results, the molecular mechanism of the formation of P370 was discussed.