are studied by spectroscopic methods, and modification of hydrogen-bonding network will be discussed in relation to the functional conversion.

[1] Sasaki et al. *Science* 269, 73 (1995). [2] Shibata and Kandori, *Biochemistry* 44, 7406 (2005). [3] Shibata et al. *Biochemistry* 44, 12279 (2005). [4] Kandori et al. *PNAS* 97, 4643 (2000). [5] Kandori et al. *PNAS* 98, 1571 (2001).

2P284 1A1535 光エネルギー変換タンパク質から光情報変換タンパク質への分子進化
Molecular evolution from the light-energy converting protein to the light sensing protein

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Organisms utilize light as not only energy sources but also signals. Microbial rhodopsins are photochemically reactive membrane-embedded proteins with seven transmembrane helices that form a pocket for the chromophore retinal. They are widespread in the microbial world, in prokaryotes, and in eukaryotes. A striking characteristic of these proteins is their wide range of seemingly dissimilar functions. Some are light-driven transporters, such as the proton pump bacteriorhodopsin (BR). Others are light sensors, such as the phototaxis receptor sensory rhodopsins II (SRII). SRII relays the signal by protein-protein interaction to integral membrane transducer protein HtrII. Phylogenetic analysis strongly supports that SRII evolved from BR. In fact, we demonstrated that only three mutations of BR convert into a photo-sensory receptor like SRII [1,2]. Here we would like to demonstrate a new microbial rhodopsin named BSR II having SRII-like absorption maximum (485 nm) and BR-like fast photocycle [3], suggesting that the color of the BR is initially altered during evolutionary process. Interestingly, the BSR II is the first discovered microbial rhodopsin having 11-cis retinal (~23 %). Combined with other spectroscopic data of BSR II, we discuss the evolutionary aspects of the microbial rhodopsins. Refs. [1] Sudo Y, and Spudich JL. (2006) *Proc. Natl. Acad. Sci. USA* 103, 16129. [2] Sudo Y, et al. (2007) *J. Biol. Chem.* 282, 15550. [3] Sudo Y, in preparation.

2P285 Rc-PYP の相互作用蛋白質の溶液状態解析
Analysis of solution state of the interaction protein of Rc-PYP

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Photoactive Yellow Protein (PYP) is a blue light receptor firstly isolated from purple photosynthetic bacterium, *Halorhodospira halophila* (Hh-PYP), which has a *p*-coumaric acid as chromophore. The PYP photoreaction and structural information has been analyzed in detail, but there has been little progress in the analysis of signal-transduction mechanism, since the target protein of Hh-PYP has not been identified. PYP from *Rhodobacter capsulatus* (Rc-PYP) is the only protein among 14 PYP species of PYPs that the interaction protein is identified so far. Therefore, Rc-PYP is more suitable for the research of PYP signal-transductions. Rc-PYP shows two absorption maxima at around 370 and 440 nm, and shows reactivity to UV-A light. Rc-PYP's reaction with its interaction protein is light dependent. UV-A illuminated Rc-PYP binds its interaction protein, with a dissociation constant of about 20 nM. Interaction mechanism of these proteins is of interest for understanding the signal-transduction process of PYP and also PAS protein family. We firstly analyzed the solution structure of the interaction protein by use of X-ray small angle scattering (SAXS). The result of SAXS revealed that the interaction protein in solution forms a trimer. We are carrying out spectroscopic measurements of the interaction. We will discuss the details of the interaction mechanism.

2P286 PYP ドメインと Bph ドメインから成る光センサー型ヒスチジンキナーゼ Ppr の溶液構造
Solution structure of the light sensor histidine kinase of Ppr comprised of the PYP and Bph domains.

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Ppr (PYP-phytochrome-related protein) is found in the purple phototrophic bacterium, *Rhodospirillum rubrum*, as a light sensor for the regulation of a polyketide synthetase gene expression. Ppr consists of three domains, photoactive yellow protein (PYP) domain, bacteriophytochrome (Bph) domain, and histidine kinase (HK) domain from the N-terminus. The PYP domain entails a *p*-coumaric acid as a chromophore. The activated PYP domain upon light absorption is thought to modulate the HK domain to express the HK activity. However, it has not been clarified how the signal is transferred to the

HK domain. In order to clarify the signaling mechanism, the structural change in the Ppr fragment comprised of the PYP and Bph domains was examined. The intact Ppr was simultaneously expressed with TAL and pCL, resulting in the holo protein. The Ppr fragment can be obtained by the treatment with Factor Xa. The fragment exhibited a UV-Vis spectrum identical to that of the intact Ppr. The molecular weight of the fragment was about 68 kDa by the SDS-PAGE. The difference molecular weight between the intact Ppr and the fragment was almost identical to that of the HK domain. Although the CD spectra of the PYP domain did not change during the photoreaction, the dynamic light scattering measurements revealed that the molecular dimension of the fragment slightly decreases upon the light irradiation. From these observations, it can be proposed that the light signal is propagated through the domain rearrangement of the PYP and Bph domains.

2P287 光合成酸素発生反応におけるアンモニア阻害の分子機構
The molecular mechanism of ammonia inhibition of photosynthetic oxygen evolution

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Photosynthetic oxygen evolution in plants and cyanobacteria is performed at the Mn cluster in photosystem II (PSII). Ammonia, an analogue of water, has been proposed to inhibit oxygen evolution by replacing substrate water. Hence, studying the interaction of ammonia with the Mn cluster is expected to provide crucial information in understanding the molecular mechanism of oxygen evolution. In this study, we investigated the mechanism of ammonia inhibition by examining the pH dependence of O₂ evolution and detecting Fourier transform infrared (FTIR) difference spectra. PSII membranes depleted of the PsbP and PsbQ extrinsic proteins were used as samples to avoid the effect of the release of these proteins by NH₄Cl treatment. Upon treatment of 100 mM NH₄Cl, O₂ evolving activity showed ~40% inhibition irrespective of pH in the range of pH 5.5-8.0. This observation indicates that inhibition by NH₄Cl treatment is not attributed to NH₃, as has been proposed so far, but caused by NH₄⁺ cation. FTIR difference spectra upon S₁→S₂ transition measured at 10°C exhibited clear changes by NH₄Cl treatment in the asymmetric (1600-1450 cm⁻¹) and symmetric (1450-1300 cm⁻¹) COO⁻ stretching regions. The amplitude of the spectral changes as a function of the NH₄Cl concentration was correlated with the concentration dependence of the O₂ evolving activity. These results indicate that the interaction of NH₄⁺ with carboxylate groups that serve ligands to or a hydrogen bond network around the Mn cluster causes inhibition of O₂ evolution.

2P288 生合成的に Sr 置換された好熱性紅色硫黄細菌 *Thermochromatium tepidum* における色素蛋白質複合体の特性評価
Biosynthetic Replacement of Ca with Sr in pigment-protein complexes from thermophilic Purple Sulfur Bacterium *Thermochromatium tepidum*

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Thermochromatium tepidum (TTP) is a unique moderate thermophile which can grow at the highest temperature among purple bacteria. A recent study strongly indicated that an inorganic cofactor, Ca²⁺, is responsible for the thermal stability of light-harvesting (LH) 1 reaction center (RC) complexes of TTP. Based on the result, we discovered that Sr ion is an alternative cofactor structurally and functionally replaceable with Ca ion in this thermophilic organism. To access further details of the roles, thermal and spectroscopic properties were investigated for the pigment-protein complexes from Sr-TTP cells which were biosynthetically substituted Sr²⁺ for Ca²⁺.

The Sr-TTP cells grew anaerobically at 40 - 50 °C in a culture containing a small amount of SrCl₂ instead of CaCl₂. The photosynthetic growth was not observed when Ca/Sr ions were removed from the culture or replaced with other metal cations. An LH1-RC complex purified from the Sr-TTP cells exhibited its Qy maximum at 888 nm (B888), blue-shifted by 27 nm from the native position at 915 nm. Depletion of Sr²⁺ from B888 induced a further shift of the LH1 Qy peak and deterioration of the thermal stability. In contrast, redox-coupled soluble proteins from the Sr-TTP showed little change upon the biosynthetic Sr²⁺-substitution not only in the electronic and vibrational properties but the thermal stability. These results indicate that strategies for acquiring the thermal tolerance are largely different among the pigment-protein complexes in TTP.