70S ribosomes bind to each other through the 30S subunit by electron microscopic observation. However, the mechanism of 100S ribosome formation by RMF and HPF is not well understood. We tried to observe the structure of 100S ribosome by electron cryomicroscopy. Because the interaction between 70S ribosomes in the dimer structure is very weak, 100S ribosome dissociated into monomeric 70S ribosomes at the low protein concentration optimized for electron cryomicroscopic observation. Therefore, only few 100S ribosome particles were found in each electron micrograph. The problem was solved by applying a method called GraFix, which uses a density gradient centrifugation step with weak chemical fixation. About 5,000 particle images of 100S ribosome were collected, and then the three-dimensional structure was reconstructed by single particle image analysis. We were able to identify the protein responsible for the interaction between 70S ribosomes. We will report the structure of 100S ribosome and the mechanism of 100S ribosome formation.

3TA2-01 2種類のBLUFドメイン、AppAとPACの赤外分光解析

3P-212 FTIR study of two BLUF domains, AppA and PAC

Tatsuya Iwata (1), Akihide Watanabe (1), Mineo Iseki (2), Masakatsu Watanabe (2), Hideki Kandori (1) ((1) *Department of Frontier Materials, Nagoya Institute of Technology*; (2) *School of Advanced Sciences, The Graduate University for Advanced Studies*)

BLUF (Blue-Light Using FAD) domains regulate a variety of functions in bacteria and eukaryotes. Upon illumination, it is believed that ultrafast electron-coupled proton transfer takes place between FAD, and conserved Gln and Tyr, but these charges are restored in the red-shifted intermediate. Several models have been proposed (rotation of side chain, keto-enol tautomerism, etc.), where hydrogen-bonding network and its alteration are important, but it is not easy to experimentally monitor these changes.

Difference FTIR spectroscopy is a powerful tool to monitor protein structural changes for function, where spectral changes are normally detected at $<1800\text{ cm}^{-1}$. In contrast, we have shown that spectral acquisition at $4000\text{--}1800\text{ cm}^{-1}$ provides useful information of hydrogen-bonding donors (N-H, O-H and S-H stretching vibrations) in rhodopsins, photosynthetic reaction centers, and flavoproteins [1]. To gain insights into the molecular mechanism, here we measured the FTIR spectra of BLUF domains. We compared BLUF domains of AppA from *Rhodobacter sphaeroides* (AppA-BLUF) and F2 domain of α -subunit in PAC (photoactivated adenyllyl cyclase) from *Euglena gracilis* (PAC α F2). While the obtained spectra were similar at $<1800\text{ cm}^{-1}$, noticeable difference was observed at high frequency region with each other. Identification of the vibrational bands is in progress, and the activation mechanism in BLUF domains will be discussed.

[1] Kandori et al. *JPCB* 102, 7899 (1998); Iwata et al. *Biochemistry* 48, 1220 (2009); Iwata et al. *JACS* 124, 11840 (2002).

3TA2-02 フォトリポソンの温度依存的反応効率と温度センサーとしての機能の実験的証拠

Temperature-dependent reaction efficiency of phototropin and possible function of temperature sensor

Yusuke Nakasone (1), Kazunori Zikihara (2), Daisuke Matsuoka (3), Satoru Tokutomi (2), Tomomi Suzuki (1), Akira Nagatani (1), **Masahide Terazima** (1) ((1) *Kyoto University*; (2) *Osaka Prefecture University*; (3) *Kobe University*)

The phototropins (phot1 and phot2) are blue light sensor proteins in higher plants and regulate phototropism, stomatal opening and chloroplast relocation. So far, we have studied their reaction dynamics and detected that the photoexcitation of light receptive domain called LOV leads to the dissociation and unfolding of linker, which forms helical structure and binds to LOV domain at the dark state, to activate the kinase domain. In this study, we investigated the temperature effect on these reactions of phot1LOV2-linker sample by using the transient grating (TG) method and found that the initial state and consequently their reactions depended on the temperature strongly. With an increase of temperature, the dissociated form of the linker was increased at the dark state and the photoexcitation of this species did not result in unfolding of the linker. Since the dissociation of the linker from the LOV domain is an essential step for the kinase activation, we suggest that phototropin has a function of a temperature sensor. For examining this possibility, we performed in vivo analysis, that is, we compared the temperature effect on their functions of wild type of Arabidopsis to that of double mutant (both of phot1 and phot2 are knocked out). In the case of wild type, the degree of stomatal opening depends on the temperature clearly. Double mutant, however, did not show any temperature effects on their response. Additionally, the chloroplast accumulation was also suppressed by increasing of temperature in wild type. On the basis of these results, we can conclude that the phototropins play a role as a temperature sensor as well as a light sensor.

3TA2-03 赤感受性錐体視物質における塩化物イオン依存的な吸収波長シフトに関わるアミノ酸残基

Amino acid residues responsible for Cl⁻-dependent shift of absorption maximum in red-sensitive cone pigments

Takahiro Yamashita (1), Shuhei Nakamura (1), Kei Tsutsui (1), Takefumi Morizumi (1), Yoshinori Shichida (1) ((1) *Department of Biophysics, Graduate School of Science, Kyoto University*)

Vertebrate visual pigments share the structural features containing the protein moiety with seven transmembrane domains and the chromophore 11-*cis* retinal, and are classified into five groups based on their amino acid sequences; four groups of cone pigments and a single group of rhodopsin. The classification of four groups of cone pigments corresponds to the difference of their absorption maxima, which is the molecular basis of color discrimination of animals. It has been known that the difference of the absorption maxima of cone pigments results from the variety of the amino acid residues around the chromophore. Especially, many members in long wavelength-sensitive group (L-group) have a unique molecular mechanism to shift their absorption maxima. That is, Cl⁻ binding to the protein moiety causes their largely red-shifted absorption maxima. Although histidine residue in the second extracellular loop has been reported to be involved in Cl⁻ binding, there is limited information to account for the unique spectral tuning mechanism in L-group. In this study, we searched for other residues which are responsible for Cl⁻-dependent red-shift. Mouse green-sensitive cone pigment belongs to this group but shows almost no Cl⁻-dependent red-shift. Therefore we compared this pigment with other members in L-group which show Cl⁻-dependent red-shift. We successfully identified two important residues involved in Cl⁻-dependent red-shift and constructed Cl⁻-induced red-shifted mutant of mouse green pigment.

3TA2-04 オールトランスレチナルを発色団とするオプシン、ペロプシンの分子機能解析

Functional analysis of all-*trans*-retinal-containing opsin peropsin

Sari Fujita (1), Takahiro Yamashita (1), Yasushi Imamoto (1), Yoshinori Shichida (1) ((1) *Department of Biophysics, Graduate School of Science, Kyoto University*)

The opsins play important roles in animal photoreceptions such as vision and entrainment of circadian rhythm. They are divided into two groups from their chromophore in the dark state: one group has 11-*cis* form of retinal, and the other has its all-*trans* form. Rhodopsins in vertebrate and invertebrate visual cells contain 11-*cis*-retinal in the dark state and function as G protein-coupled receptors (GPCRs). In these opsins, photoisomerization of 11-*cis* form into all-*trans* form corresponds to the conversion of inverse agonist into agonist and results in emergence of G protein activation activities. In contrast, molluscan retinochrome and vertebrate RGR contain all-*trans*-retinal in the dark state and photoconvert it into 11-*cis* form. They have been thought to function as a supplier of 11-*cis*-retinal and not to couple to G proteins.

Peropsin, first identified in retinal pigment epithelium of mammals, binds to all-*trans*-retinal in the dark state and has the conserved sequence motifs important for G protein coupling. However, it is unknown whether peropsin functions as a supplier of 11-*cis*-retinal or a GPCR. If peropsin couples to G protein, it is of interest to analyze its active state by the comparison with that of 11-*cis*-retinal-containing opsins. Previously, we observed photobleaching process of amphioxus peropsin, and found the formation of a stable intermediate which has absorption maximum in visible region. In this study, we examined the possibility that peropsin couples to G protein.

3TA2-05 脂質膜におけるアレステンのロドプシンへの結合の解析

Analysis of arrestin binding to rhodopsin in lipid bilayer.

Hisao Tsukamoto (1), Abhinav Sinha (2), David Farrens (2) ((1) *Osaka City University*; (2) *Oregon Health & Science University*)

Rhodopsin is a light-sensitive G protein-coupled receptor (GPCR). When rhodopsin receives a light signal, its chromophore 11-*cis*-retinal is isomerized to an all-*trans* form, which then induces the formation of a photoproduct called meta-II that can bind to and activate heterotrimeric G proteins. Signaling by activated rhodopsin is quenched by phosphorylation of the C-terminal tail of rhodopsin by rhodopsin kinase followed by binding of a protein called arrestin. This termination mechanism appears to be used in other GPCRs as well. Thus, arrestin binding is an important step in signal transduction via GPCRs including rhodopsin. It was previously reported that phospholipids play important roles in arrestin binding to rhodopsin in detergent micelles [1]. In particular, phospholipids having acidic head groups are required for arrestin binding to rhodopsin in detergent, but the role of acidic phospholipids in lipid bilayer is not yet clear. In the present study, we are trying to determine the role of phospholipids for arrestin binding to rhodopsin in lipid bilayer. To do this, we made various lipid bilayers containing rhodopsin using several kinds of phospholipids and analyzed arresting binding to rhodopsin based on "extra meta-II" formation, which means a shift of equilibrium between meta-II and its precursor meta-I toward meta-II caused by arrestin binding. Our data suggested that acidic phospholipids are important for arrestin binding to rhodopsin in lipid bilayer, too.

[1] Sommer ME et al., (2006) *J. Biol. Chem.* 281, 9407.

3TA2-06 PYPのN末端領域の構造変化についての理論研究