theoretical energies are in good agreement with the experimental ones.

[1] Seliger et al., Proc. Natl. Acad. Sci. U.S.A. 47, 1129 (1961) [2] Morton et al., Biochem. 8, 1598 (1969) [3] White et al., Bioorg. Chem. 1, 92 (1971) [4] Jung et al., J. Am. Chem. Soc. 98, 3949 (1976) [5] Gandelman et al., J. Photochem. Photobio. B: Biol. 19, 187 (1993) [6] Ando et al., Jpn. J. Appl. Phys. 49, 117002 (2010) [7] Hiyama et al., Photochem. Photobio. (2012) In press

2PT188 シアノバクテリア由来のフィトクロム様光受容体「PixJ1」における短波長シフトした光反応の分子基盤

Molecular Basis for the Blue-shifted Photoconversion of PixJ1, Phytochrome-like Photoreceptor in Cyanobacterium, Studied by FTIR

Kazunori Zikihara^{1,2}, Shizue Yoshihara¹, Takahiro Kitano¹, Hitomi Katsura¹, Takayuki Kohchi³, Satoru Tokutomi¹ (¹Grad. Sch. Sci., Osaka Pref. Univ., ²Grad. Sch. Med., Osaka Univ., ³Grad. Sch. Bio., Kyoto Univ.)

Plant phytochrome binds a linear tetrapyrrole, phytychromobilin (PΦB), as a chromophore covalently to a conserved Cys residue (1st Cys) in the GAF domain forming a thioether bond and has two absorption forms, named red light-absorbing (Pr) and far-red light-absorbing (Pfr) forms, between which they are photoconvertible by red and far-red light, respectively. Recently, a phytochrome-like chromoprotein, PixJ1, was found in cyanobacterium *Synechocystis* sp. PCC 6803 that binds a tetrapyrrole chromophore to the 1st Cys in the GAF2 domain and shows a photoconversion between blue light-absorbing (Pb) and green light-absorbing (Pg) forms instead between Pr and Pfr. To uncover the molecular basis for the blue-shifted photoconversion, Fourier transform infrared (FTIR) spectra of the PixJ1-GAF2 domain were measured and analyzed in reference to that of the photosensory domain of Arabidopsis phytochromeB (PhyB-N651).

Both the PixJ1-GAF2 and the PhyB-N651 showed light-induced changes in the fingerprint region of the FTIR difference spectra that are reproducible upon successive photoconversions. Notably, Pg minus Pb and Pg minus Pb difference spectra of PixJ1 showed positive and negative peaks, respectively, in the S-H stretching region indicating an association and a dissociation of H in a S-H group, possibly of the 2nd Cys in PixJ1-GAF2. On the other hand, neither Pfr minus Pr nor Pfr minus Pr difference spectra of the PhyB-N651 showed them. The details of the molecular basis for the blue shift in PixJ1-GAF2 will be discussed.

2PT189 視物質の熱雑音発生メカニズム

Mechanism of thermal activation of visual pigments in the dark

Daiki Kawata¹, Kota Katayama¹, Hiroo Imai², Hideki Kandoni¹ (¹Department of Frontier Materials, Nagoya Institute of Technology, ²Primate Research Institute, Kyoto University)

Visual pigments possess an 11-*cis* retinal as the chromophore, and its photoisomerization to the all-*trans* form initiates our vision. Highly sensitive vision originates from efficient photoisomerization and little thermal isomerization. In fact, the *cis-trans* isomerization coordinate suggests high thermal activation barrier (>180 kJ/mol), which is supported by the tightly packed chromophore in the crystal structure of rhodopsin. However, the reported activation enthalpy of the dark noise is 80-110 kJ/mol, and mechanism of such low thermal activation has been a paradox in vision. We recently challenged this paradox using FTIR spectroscopy and H/D exchange. Thr118 is located in contact with the chromophore and isolated from hydrogen-bonded network connecting to the outside of rhodopsin. We reported that the O-H group is not deuterated in D₂O at ambient temperature [1]. Nevertheless, we found that H/D exchange occurs at 50-70 °C, suggesting that protein fluctuation drives the retinal binding pocket transiently open. We proposed a quantitative two-step model that can explain the observed low thermal activation in rhodopsin [2]. In this study, we extend this analysis to other visual pigments. Temperature-dependent H/D exchange measurements of the O-H group are in progress for 9-*cis* rhodopsin and monkey green-sensitive pigment, and molecular origin of dark noise in vision will be discussed.

[1] Nagata et al. *J. Phys. Chem. A* 106, 1969-1975 (2002).

[2] Lorentz-Fonfria et al. *J. Am. Chem. Soc.* 132, 5693-5703 (2010).

2PT190 クモにおけるピンぼけ像を用いた新規距離知覚メカニズム

1YS1015 A novel depth perception mechanism based on image defocus in a spider

Takashi Nagata¹, Mitsumasa Koyanagi², Hisao Tsukamoto², Shinjiro Saeki³, Kunio Isono³, Yoshinori Shichida⁴, Fumio Tokunaga⁵, Michiyo Kinoshita¹, Kentaro Arikawa¹, Akihisa Terakita² (¹Dept. Evol. Stud. Biol. Sys., Soken-dai-Hayama, ²Grad. Sch. Sci., Osaka City Univ., ³Grad. Sch. Information Sci., Tohoku Univ., ⁴Grad. Sch. Sci., Kyoto Univ., ⁵Grad. Sch. Sci., Osaka Univ.)

A pair of eyes of jumping spiders has a unique retina with four-tiered photoreceptor layers, on each of which light of different wavelength is focused due to chromatic aberration of the lens. Because the absorption spectrum of visual pigments is in general adaptively tuned to maximize visual information, the visual pigment in each layer was thought to be sensitive to the wavelength of the light focused on the layer. However, interestingly, we previously found that all photoreceptors in both the deepest and second-deepest layers contain a green-sensitive pigment, although green light is only focused on the deepest layer, indicating that the second-deepest layer always receives defocused images. Given that defocused images contain distance information of the scene in optical theory, we hypothesized that jumping spiders perceive distance from the amount of defocus in the second-deepest layer. In the present study, to test the hypothesis, we conducted detailed behavioral analysis of distance perception of the spiders under monochromatic illumination by measuring distances of jumps toward preys. Under red illumination, which generates a larger amount of defocus in the second-deepest layer than green light, jumping spiders exhibited shorter jumps than under green illumination. This result was in good agreement with theoretical predictions of our model based on experimentally determined optical parameters of the lens, strongly supporting our hypothesis that jumping spiders perceive distance based on how much the retinal image is defocused.

2PT191 酵母におけるニトリクリプトクロム 4 の大量発現と特異的抗体を用いた精製

Overexpression of chicken cryptochrome4 in a budding yeast and its purification using a specific monoclonal antibody

Hiromasa Mitsui, Toshinori Maeda, Chiaki Yamaguchi, Yusuke Tsuji, Yoko Kubo, Keiko Okano, Toshiyuki Okano (Dept. Eng. and Biosci., Grad. Sch. Adv. Sci. and Eng., Waseda Univ.)

A wide variety of living organisms on the earth have developed several photoreceptive proteins for photosynthesis, visual perception, circadian photoentrainment, seasonal reproduction, and so on. Cryptochromes (CRYs), which are flavoproteins and are likely function as blue-light photoreceptor, have been found in bacteria, plants, and animals. Although CRYs are known to work as central components in the circadian oscillator in mammals, many CRYs in all kingdoms of life are considered to work as photosensors. The photochemistry and photocycle of CRYs have been studied in plants and flies, but little is known about the photoreception mechanism of vertebrate CRYs partly due to the lack of the expression and purification system of vertebrate CRY. We have tried to express vertebrate (chicken or zebrafish) CRYs in various host cells such as *Escherichia coli*, insect cell line, mammalian cell line, and yeast, and here we report a novel expression system for chicken CRY4 (cCRY4) using a budding yeast, *Saccharomyces cerevisiae*. In this system, cCRY4 was constitutively expressed and the expression level was approximately 500 µg per liter, which is comparable to a conventional system using *E. coli*. We also established a purification system of cCRY4 without any tag by immunoaffinity chromatography using a monoclonal antibody to C-terminal region of cCRY4. The purified cCRY4 was subjected to UV-visible absorption spectroscopy to analyze its photocycle.

2PT192 青色光により誘起される転写因子 AUREO1 の構造変化

Blue-light induced conformational change of a transcription factor, AUREO1

Osamu Hisatomi¹, Ken Takeuchi¹, Youichi Nakatani¹, Fumio Takahashi², Hironao Kataoka³ (¹Graduate School of Science, Osaka University, ²PRESTO, JST, ³Botanical Gardens, Tohoku University)

Aureochrome 1 (AUREO1) found in *Vaucheria* has one basic region/leucine zipper (bZIP) domain and one light-oxygen-voltage sensing (LOV) domain, and is believed to play a role as a blue light (BL)-regulated transcription factor¹. To clarify the molecular mechanisms of AUREO1, we prepared recombinant AUREO1 proteins, containing full coding sequence (FL), bZIP and LOV domains (ZL), and LOV domain. Recombinant AUREO1s showed absorption and emission spectra typical of flavoproteins, and selectively bound to the target sequence (TGACGT). The absorption around 450 nm decreased immediately after the BL illumination, and was recovered to its original level with a half-life of about 6 min at pH 7 in the dark. Dynamic light scattering (DLS)