in the presence of substrate, which was similar to the case in (6-4) PHR [1], but the frequency change was opposite. Molecular mechanism of photoactivation and photorepair will be discussed on the basis of the spectral comparison between CPD and (6-4) PHR.

[1] Zhang et al. *Biochemistry* 50, 3591 (2011).

[2] Iwata et al. *Biochemistry* 49, 8882 (2010).

3Q1434 (6-4)光回復酵素における活性化と修復過程の分光解析

Spectroscopic study of light-dependent activation and DNA repair processes of

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Photolyases (PHRs) and cryptochromes (CRYs) have an FAD (flavin adenine dinucleotide) as the chromophore within homologous protein architectures, but their functions are entirely different. PHRs are the DNA repair enzyme that specifically revert UV-induced photoproducts, cyclobutane pyrimidine dimers (CPD) or (6-4) photoproduct, into normal bases to maintain genetic integrity. In contrast, CRYs function as photosensors or an element of biological clock. There are four different redox states for FAD; an oxidized form (FAD^{ox}), an anion radical form (FAD⁻), a neutral radical form (FADH[•]) and a fully reduced form (FADH⁻). The enzymatically active state of *Xenopus* (6-4) PHR is FADH⁻, and two photons are needed for the activation: FAD^{ox} is first converted to FADH[•] by light-induced one electron and one proton transfers and then to FADH⁻ by light-induced one electron transfer. We recently reported difference Fourier-transform infrared (FTIR) spectra between FAD^{ox} and FADH⁻ in *Xenopus* (6-4) PHR, together with those for the DNA repair process [1].

Here we studied the photoactivation and DNA repair mechanisms of *Xenopus* (6-4) PHR in more detail using UV-vis and FTIR spectroscopy. By optimizing the measuring conditions, we have obtained the difference FTIR spectra among four redox states. Identification of vibrational bands by use of mutants and isotope labelings is in progress, and molecular mechanism of (6-4) PHR will be discussed on the basis of the spectroscopic observations.

[1] Zhang et al, *Biochemistry* 50, 3591-3598 (2011).

3Q1446 緑/赤色光変換をするフィトクロム型光受容体の解析

Mechanism of green/red photoconversion of phytochrome-related photoreceptor

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Certain cyanobacteria modulate their light harvesting pigments in response to green and red light for photosynthesis. Recent studies have revealed that this phenomenon is regulated by a phytochrome-like photoreceptor that reversibly photoconverts between green-absorbing form (Pg) and red-light absorbing form (Pr) (1, 2). Here, we demonstrated that the Pg/Pr conversion is coupled with protonation and deprotonation of a linear tetrapyrrole chromophore, phycocyanobilin (PCB), which follows its C15-Z/C15-E isomerization. This mechanism is apparently distinct from that of phytochrome, where the chromophore is protonated in both Pr and far-red absorbing form (Pfr). References;

(1) Hirose Y. et al., 2008 *PNAS* 105, 9528;

(2) Hirose Y. et al., 2010 *PNAS* 107, 8854

3Q1458 機能が異なる二つの Cryptochrome-DASH タンパク質における分子特性の比較

Comparison of molecular properties between two functionally distinct Cryptochrome-DASH proteins

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Blue-light photoreceptors control many important biological functions. Cryptochromes, members of DNA-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 classified. Cry-DASH is widely distributed from bacteria to the plant and animal kingdoms except for the mammal.

In this study, we investigated the biophysical properties of the two Cry-DASH with distinct functions to see their molecular bases. Marine green alga *Ostreococcus tauri* (Ot) Cryptochrome/Photolyase-family protein (CPF2) has dual functions, cyclobutane pyrimidine dimer (CPD)-photolyase activity and the transcriptional-repressor activity in circadian clock. The other well-characterized DASH-type Cryptochrome in photoreaction, Zebrafish (Zf) Cry-DASH, has a weak CPD-photolyase activity but lack the transcriptional-repressor activity. Both recombinant Cry-DASH proteins expressed in *E. coli* bind two chromophores, a flavin adenine dinucleotide (FAD) and a N5,N10-methenyl-5, 6, 7, 8-tetrahydrofolate non-covalently. We compared their photochemistry and the secondary structure using UV-Vis absorption and far-UV Circular Dichroism (CD) spectroscopies. Based on these results, the origin of functional difference between Ot CPF2 and Zf CRY-DASH will be discussed.

3Q1510 Signaling mechanisms of phototropin, plant blue light receptor P69

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Phototropin (phot) is a blue light receptor in plant and involved in phototropism, chloroplast movement, etc. Phot has two LOV (Light-Oxygen-Voltage) domains (LOV1, LOV2) harboring an FMN in the N-terminal region. A kinase domain is connected with linker containing α -helix in the C-terminal region. In darkness, kinase activity is inhibited mainly by LOV2. Upon blue light, adduct is formed between FMN and Cys residue in LOV2 and then structural change is triggered within α -helix. Through these structural changes, the inhibition is canceled and kinase shows high activity. We produced polypeptide containing from LOV2 to kinase of *Arabidopsis* (At) phot1 (P1_L2K) from *E. coli*. P1_L2K showed a photocycle, structural changes within α -helix by peptide mapping and kinase activity using the N-terminal polypeptide of phot1 as an artificial substrate. According to predicted structural model of At phot1 LOV2, we found out some interactions between LOV2 and α -helix. We produced mutated P1_L2Ks and performed spectroscopy, peptide mapping and kinase assay. Lys/Ala mutant showed photocycle and structural changes, but abolished light activation of the kinase. Intra molecular signaling mechanisms of phot will be discussed.