Photoreaction pathway in zebrafish Cryptochrome-DASH determined by low temperature spectroscopy

see 2TA2-03

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Blue-light photoreceptors control many important biological processes. 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 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. In this paper, photoreaction pathway of Cry-DASH from zebrafish (Zf), that is determined by both ambient- and low-temperature UV-visible absorption spectroscopy in aqueous solutions, is reported. Zf Cry-DASH binds two chromophores, a flavin adenine dinucleotide (FAD) and a N5,N10-methenyl-5,6,7,8-tetrahydrofolate non-covalently. At an ambient temperature, 295 K, 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. The origin of the neutral radical formation was studied by low temperature spectroscopy. There are two possible reactions to form the neutral radical. Electron transfer precedes proton transfer or electron and proton migrate simultaneously that is H-atom migration. Low-temperature spectroscopy detected a new intermediate showing a characteristic spectrum with that of flavin anion radicals indicating that electron transfer is a primary reaction. Based on these results, photoreaction pathway of CRY-DASH will be summarized and discussed.

- 2P-218** ロドプシンにおける相乗的な長距離相互作用による吸収波長制御機構
Molecular mechanism of long-range synergetic color tuning between multiple amino acid residues in rhodopsin

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see 2TA2-06

- 2P-219** 発光キノコ *Mycena Chlorophos* の発光分子機構の解明
Molecular mechanism of bioluminescent *Mycena Chlorophos*

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Mycena Chlorophos belongs among luminescent fungi. The molecular mechanism of luminescent organisms can be generally divided into two reaction forms; the enzymatic luciferin-luciferase (L-L) and the non-enzymatic (NE) reactions, but little is known about the chemical nature of luminescence of *M.Chlorophos*. The purpose of this study is to assign the reaction forms of *M.Chlorophos* to which one of the two. The luminescence biological material extracted from *Lampteromyces japonicus*, a famous luminous fungus, was lampteroflavin derived from riboflavin. Performing HPLC analysis on a ODS-3 column for the sample extracted from *M.Chlorophos*, one peak was observed at almost the same elution time as that of riboflavin. HPLC analysis was applied to the same sample by the gradient method, while four peaks were detected. In order to check which fraction on the chromatogram contained luminescence related materials, we repeated 5 times the same extraction to scale up each sample volume of four peaks. Each extract sample was mixed with the quenched *M.Chlorophos*, placed in air for one day, to examine the light emission by the photon counter. Mixing experiment for one extract sample shows bioluminescence with a little bit stronger intensity than for other three specimens. This result means that bioluminescence of *M.Chlorophos* could not be necessarily attributed to the L-L nor NE reaction type. Thus, we need to study furthermore the bioluminescence mechanism of *M.Chlorophos* quantitatively using NMR and LC-MASS spectrometry.

- 2P-215** シロイヌナズナ青色光受容体・フォトトロピン2の光シグナル伝達に対するアルギニン427の関与

Involvement of Arg427 in the light-signal transduction of Arabidopsis phototropin2, a blue-light photoreceptor

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Phototropin (phot) is a blue light photoreceptor in plants that mediates phototropism, chloroplast relocation, stomata opening and leaf expansion. A phot molecule has two photoreceptive domains named LOV1 and LOV2 in the N-terminal half and the C-terminal half forms Ser/Thr kinase. Between the two LOV domains, LOV2 is primarily responsible for the kinase activation. Both the LOV domains bind non-covalently a flavin-mono-nucleotide (FMN) in an oxidized form as a chromophore. Blue light irradiation induces a conversion of the LOV domains in the dark-state (D450) to a flavin-cysteiny adduct state (S390) that reverts to D450 spontaneously. S390 is thought to be a signaling state that activates phot kinase through conformational changes in the protein moiety, in which involvements of some key amino acid residues, such as Glu489 or Phe470 nearby the isoalloxazine head of FMN, have been proposed. Instead, we focused on the amino acid residues interacting with the phosphate tail of FMN. In the present paper, we report the involvement of Arg427 in the LOV2 domain of Arabidopsis phototropin 2 (phot2) in the light-signal transduction. Arg427 resides next to the conserved cysteine responsible for the adduct formation and interacts with the phosphate tail. Introduction of Arg/Lys mutation brought a significant reduction of phenotypes such as phototropic curvature and strong-light avoidance response of chloroplasts. Interestingly, the mutated LOV2 domain does bind FMN, however, does not show the adduct formation upon light excitation. Computer simulation proposes an origin for the inability of adduct formation.

- 2P-220** ピコ秒時間分解蛍光分光法による黄化葉の緑化途上における光合成系タンパク質複合体構築過程の観測

Assembly process of photosynthetic pigment-protein complexes during greening of etiolated leaves observed by picosecond time-resolved fluorescence spectroscopy

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Dark-grown higher plants have etiolated leaves, which lack chlorophyll (Chl) and most of the Chl-protein complexes, and instead accumulate the precursor pigment, protochlorophyllide (PChld). PChld's in etiolated leaves form quasi-crystalline aggregates called prolamellar body (PB). When etiolated leaves are illuminated, PChld is rapidly converted to Chl, Chl-protein complexes start to accumulate, and the structure of PB is transformed into the thylakoid membrane. Little is known about the assembly process of newly synthesized Chl and apo-protein in greening leaves so far. Characterizing the precursor states of the Chl-protein complexes during their structural formation is considered essential to understand the mechanism of their assembly process. Since the excitation-energy-transfer (EET) dynamics in a picosecond time region is highly sensitive to the state of the pigments in the Chl-protein complex, the measurement of the EET dynamics can be a powerful tool to characterize the state of the Chl-protein complexes during greening. In order to clarify the EET dynamics, we measured the picosecond time-resolved fluorescence of greening leaves, which was incubated for various periods after the initiation of greening. The results of the measurement at 77 K showed the emergence of the EET component 1 hour after the initiation of greening. The spectral analysis showed that this EET component reflects the energy flow from the pigments emitting around 670 nm to those around 685 nm. This EET process is different from that observed for the mature photosystems, and might reflect the accumulation of the precursor Chl-protein complex. Lowering temperature below 77 K will be effective to better reveal the state of pigment. The experiment at 4 K is now in progress and will be reported in the annual meeting.

- 2P-216** ロドプシンの細胞質側ループを組み込んだ古細菌ロドプシンの研究

Study on chimeric archaeal-rhodopsin containing cytoplasmic loops of bovine rhodopsin

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see 2TA2-04

- 2P-217** アーキロドプシンからのプロトン放出に関する分光研究
Spectroscopic study of proton release group of archaeorhodopsins

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- 2P-221** ラン藻 *Leptolyngbya boryana* における光非依存型プロトクロロフィリド還元酵素欠損株の細胞内色素分布の顕微分光法による解