2P352 明暗刺激に伴う、ホヤ幼生の神経、筋肉の Ca^{2+} イメージング
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Ca²⁺ imaging of the muscle and neural cells in ascidian larva upon the step-down of light
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Previously we have shown that the step-down of light triggers the swimming of ascidian larvae (Nakagawa *et al.* 1999). This indicated that the photoreceptors sensing the darkness transmit the signals through a specific neural pathway to the tail muscle cells and evoke the swimming behavior. We have been interested in the visual pathway and how the information is processed in the brain. The ascidian larva has extremely simple neural network. The number of neurons composing the central nervous system is only about 100 although basic architecture is similar to that of vertebrates. Since Ca²⁺ imaging method is suitable for monitoring the activity of neurons and muscles, we have measured Ca²⁺ imaging of those cells of the ascidian larva by using a calcium indicator protein, cameleon, that was expressed in the specific cells by a proper promoter, as presented in the last BPJ annual meeting. However, the presentation showed Ca²⁺ change derived from the spontaneous activity because a light stimulus disturbs the optical imaging. To overcome the problem, we have developed an apparatus providing light stimuli only on the larval photoreceptor cells with no effect on the image. Using it, we could record Ca²⁺ transient of muscle cells following the step-down of light. In the presentation, we will also show the neural activity upon light reduction stimuli.

Nakagawa M, Miyamoto T, Ohkuma M, Tsuda M (1999): Action spectrum for the photophobic response of *Ciona intestinalis* larvae implicates retinal protein. *Photochem Photobiol* 70:359-362

2P354 シロイヌナズナ・フォトリロビン2のLOV1ドメインにおける二量体形成サイト
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Dimerization site in LOV1 domain of Arabidopsis phototropin2
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Phototropin (phot) acts as a blue light sensor for tropic response, chloroplast relocation and stomata opening in plants. The protein owns two photoreceptive domains named LOV (Light-Oxygen-Voltage sensing)1 and 2 in the N-terminal half and C-terminal half forms a Ser/Thr kinase. Arabidopsis has two isoforms of phot, phot1 and phot2. LOV domain binds a flavin mononucleotide (FMN) as a chromophore. After blue light excitation, the dark state of LOV domain (D450) forms a FMN-cysteiny adduct (S390) that is thought to be a signaling state. LOV1 and LOV2 share roles in the light regulation of the kinase. Furthermore, oligomeric structures are reported to differ between the two domains. We found that LOV2 domain of Arabidopsis phot2 exists in a monomeric form, whereas its LOV1 domain forms a dimer by size-exclusion column chromatography. In order to identify the dimerization site in the LOV1 domain of phot2, we tried to monomerize the phot2 LOV1 dimer by introducing a variety of amino acid point mutation into the predicted dimerization site, that may locate in the betaD and betaE in the b-scaffold. Finally, we found that T217Q in the betaD and M232V in the betaE mutations are effective to the monomerization. Accompanying the monomerization, two components in the dark recovery from S390 to D450 changed to a single one. We also tried to make two LOV2 monomers into a dimer by introducing counterpart. However, the trial was unsuccessful suggesting different association modes of dimerization between LOV1 and LOV2 of Arabidopsis phot2.

2P353 フォトリロビンLOV1及びLOV2ドメインにおける動的構造の比較研究
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Comparative study of the dynamical and structural properties of the LOV1 and LOV2 domains in phototropin
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Phototropin is a blue-light receptor for the phototropic response in plants. It has two LOV domains (LOV1 and LOV2) which are similar in amino-acid sequence and tertiary structure, and Ser/Thr kinase. Each LOV domain contains a FMN as the chromophore and produces a covalent adduct between the FMN and a neighboring cysteine residue upon illumination. However the LOV2 domain alone plays a role in regulating the kinase activity. In this study, to explore the origin of such a functional difference between the LOV1 and LOV2 domains, we performed molecular dynamics simulation for both the dark and light states of the two domains. First, we examined the occupancy of hydrogen bonds (HBs) between two residues constituting the central β -sheet of the protein. As a result, the HB occupancy in the LOV2 light state was shown to be higher than in its dark state, which means that the interstrand interactions in the β -sheet became stronger on going from the dark to light state. In contrast, such changes were not observed for the dark $\rightarrow$ light transition of the LOV1 domain. Additionally, the fluctuation of the loop connecting the H β and I β strands in the LOV2 domain became enhanced accompanied by the dark $\rightarrow$ light transition, while the LOV1 domain exhibited no distinct change. The characteristic structural and dynamical changes found in the LOV2 domain may contribute to inducing the structural change of the J α -helix, which is a putative switch for the activation of the Ser/Thr kinase.

2P355 LOVドメインにおけるフラビンのラマンおよびIRスペクトルとその振動解析
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2aD11 Vibrational Analysis of the FMN chromophore in LOV Domains
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Molecular switches are integral to any sensory system, converting input stimuli into signals that propagate to downstream components. PAS (Per-ARNT-Sim) domains are used through all kingdoms of life to combine these detection and transduction roles required of a switch. This is achieved by regulating protein-protein interactions with bound organic cofactors, providing a way to sense environmental stimuli including light. One class of PAS photosensors is the light-oxygen-voltage (LOV) domains, which bind flavin chromophores. In plants, LOV domains are critical to the function of phototropins, a group of light-activated kinases that regulate phototropism, stomatal opening, chloroplast relocation, and leaf opening. Photoactivation converts a noncovalent protein-FMN complex (D450) into a covalent one (S390) by forming a cysteinyl-flavin bond, which is stable for tens of seconds before returning to the ground state. To understand the photochemistry and structural dynamics underlying the signaling state formation of LOV domains, we have performed Raman spectroscopic investigations LOV domain of FKF1. The Raman spectra D450 as well as S390 were obtained with 647.1 nm excitation, and we found that the formation of S390 significantly changes the spectra. Furthermore, we also carried out normal coordinate calculations based on the density functional theory to assign the observed Raman and reported IR spectra (1) of LOV domains.
(1) Iwata et al. (2006) *Biochemistry* 45, 15384.