Blue light-induced conformational changes in *Chlamydomonas* phototropin

In 1880, Charles Darwin, together with his son Francis, wrote a book 'The power of movements in plants' in which they reported observations on various types of tropic responses of plants. One of the responses that received a great deal of attention is the phototropic response where plants display a curvature response to the direction of light, which is known as phototropism. One hundred ten years later, phototropin1 (phot1) was discovered in *Arabidopsis thaliana* (At) as a blue-light (BL) receptor protein that causes the phototropism in plants. Since then, phototopins (phots) have been identified in many organisms from *Chlamydomonas* to higher plants based on the sequence homology to phot1. In particular, the phototropin (phot) found in *Chlamydomonas reinhardtii* (Cr) is used as a model to study the molecular architecture and mechanism for the initial step of phototropism. In higher plants, phots are known to act as receptors for the phototropic response as well as mediate chloroplast relocation, stomata opening, leaf flattening, and leaf photomorphogenesis. All of these responses serve to optimize the efficiency of photosynthetic activities.

Phot is a blue-light-regulated protein kinase, and comprises approximately 700-1000 amino acid residues and two flavin mononucleotide (FMN) molecules. Phot folds into three functional domains, two light-oxygen-voltage sensing domains (designated as LOV1 and LOV2) in the N-terminal half and one Ser/Thr kinase domain (STK) in the C-terminal half (Fig. 1). Each LOV non-covalently binds one FMN to receive blue-light. Upon BL excitation, LOV undergoes a cyclic photoreaction with a small conformational change in the protein moiety. Currently, the mechanism to activate the STK is being investigated in order to understand the mechanism to convert physical light stimuli into biochemical cellular signals through signaling cascades initiated by the photo-induced phosphorylation of STK.

We have been studying the molecular structure of Cr phot to understand the molecular mechanism underlying the BL-dependent activation of the kinase [1-3]. Currently, we are studying the domain organization and BL-dependent conformational changes of Cr phot [4] using small-angle X-ray scattering (SAXS) conducted at beamline **BL45XU** and a PILATUS3 detector (DECTRIS, Switzerland). The X-ray wavelength was 0.9000 Å, and the camera distance was approximately 2000 mm. Each SAXS pattern was collected as a set of time-resolved 18 frames during an exposure of 60 s. For each

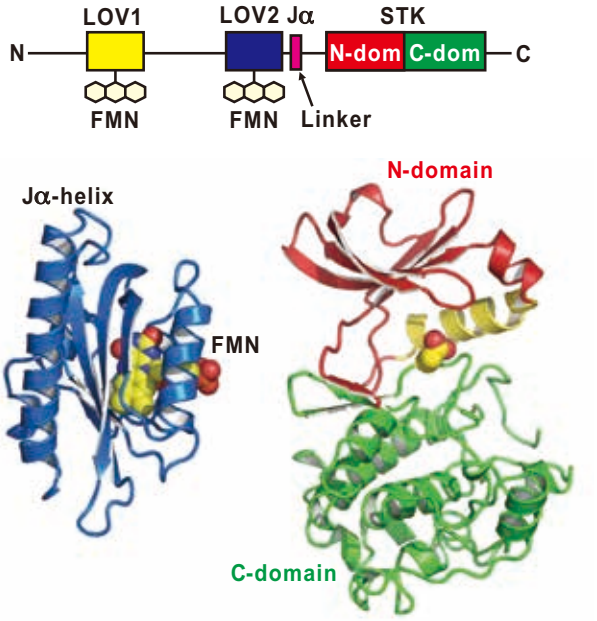

Fig. 1. Schematics of the domain structures of phot, and the homology models of LOV2 and STK.

sample, SAXS profiles were measured sequentially in the dark, under BL with the fluence rate of 450 $\mu mol \cdot m^{-2} s^{-1}$ after pre-irradiation for 5 min, and again in the dark after adapting to the dark for more than 5 min. The SAXS profiles after the subtraction of the background scattering and normalization regarding the concentration were subjected to analyses using Guinier's plot, the distance distribution function, and the *ab initio* construction of low-resolution molecular models.

SAXS profiles display BL-dependent changes. Figure 2 shows a typically example. Owing to the almost monodispersive characteristics under both dark and light conditions, the apparent molecular weight is estimated to be 70,000, which is as large as that determined by size exclusion chromatography. Thus, Cr phot exists in the monomeric form in solution. The radius of gyration (R_g) values in infinitely diluted conditions are 42.7 Å in the dark and 43.4 Å under BL irradiation. The maximum dimension estimated from the $P(r)$ function in the dark is 157 Å, which is smaller than the 167 Å under BL. These changes with regard to the molecular dimensions indicate a BL-induced extension of molecular size.

The molecular model in the dark appears as an elongated shape with dimensions of approximately 140×40×40 Å^3. The main body is very similar to that of the LOV2-kinase fragment from Arabidopsis phot2 with respect to its size and shape. Moreover, the crystal structure of LOV1 fits well with the tip of the molecular model. The molecular model under BL is similar to that in the dark but the LOV1 part under BL a different relative position. The differences in the molecular models suggest that the positions and orientations of the LOV2-LOV1 region would be light dependent (Fig. 3 right). The present SAXS studies are the first report on the whole structure of Cr phot and the position of the LOV1 relative to LOV2-STK.

From spectroscopic studies, the Jα helix connecting LOV2 and STK unfolds and dissociates from LOV2. This structural change likely plays a key role in the activation of STK. In the previous SAXS study on the LOV2-kinase fragment of At phot2, we observed BL induced a separation of the LOV2 from STK. Based on that observation, we proposed the photoactivation mechanism of STK where the light-activated LOV2 domain triggers conformational changes in the linker region, including Jα between LOV2 and KD, and the structure of which is in an intrinsically unfolded state. In addition, our biochemical study indicates that the amino acids around the N-terminus of LOV2 also play essential roles in the photoactivation of the kinase. In the crystal structure of LOV2-Jα of At phot1, Jα and the small helix are in contact. Therefore, the linker region and the N-terminal small helix would act as enhancers to transmit the small conformational changes in LOV2, modifying the conformation of STK in the BL-induced intramolecular signaling. Further details of the structure of Cr phot in the dark must be studied by crystal structure analysis in the near future.

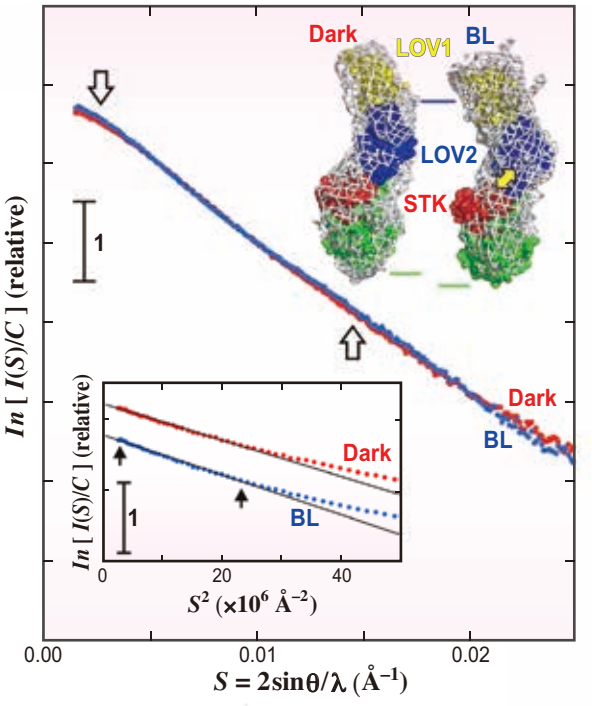

Fig. 2. SAXS profiles and their Guinier plots (inset) in the dark (red dots) and under BL irradiation (blue dots). Restored molecular models of CrPFul in the dark (red colored mesh) and under BL (blue colored mesh) are shown in the right panel. Low-resolution molecular models (mesh) of CrPFul in the dark and under BL irradiation. Homology modeled Ser/Thr kinase (N-lobe: red, C-lobe; green), LOV2 and the crystal structure of LOV1 (yellow) are fitted to the restored SAXS models.

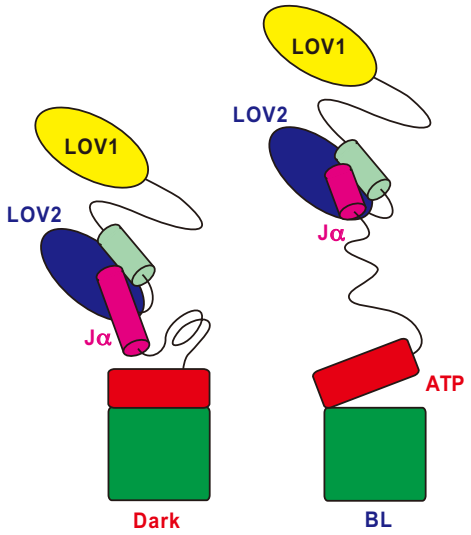

Fig. 3. Schematic illustration of the molecular organization of LOV1, LOV2 and STK as well as the Jα (pink) and N-terminal small helix (green) in a monomeric Cr phot molecule and its BL-induced changes.

Koji Okajima[a,b] and Masayoshi Nakasako[a,b,*]

[a] Department of Physics, Keio University
[b] SPring-8/RIKEN

*E-mail: nakasako@phys.keio.ac.jp

References

[1] M. Nakasako *et al.*: Biochemistry **43** (2004) 14881.
[2] M. Nakasako *et al.*: J. Mol. Biol. **381** (2008) 718.
[3] Y. Takayama *et al.*: Biochemistry **50** (2011) 1174.
[4] *K. Okajima, Y. Aihara, Y. Takayama, M. Nakajima, S. Kashojiya, T. Hikima, T. Oroguchi, A. Kobayashi, Y. Sekiguchi, M. Yamamoto, T. Suzuki, A. Nagatani, M. Nakasako and S. Tokutomi: J. Biol. Chem.* ***289*** *(2014) 413.*