

Crystal Structures of LOV1 Domains in *Arabidopsis* Phototropin 1 and 2

In 1882, Charles Darwin wrote that blue light was effective for inducing the tropic response of plants in his famous book "The Power of Movement in Plants." Since then, many researchers reported the blue-light-regulated movements of plants, such as whole body, stem, leaf, cell, or micro-organ. In the last decade, molecular biological studies revealed that all these blue light responses are mediated by a single species of blue-light receptor named phototropin (phot) [1]. phot was first found as a receptor for phototropic responses and has since been revealed to regulate a variety of physiological activities for maximizing the efficiency of photosynthesis, such as chloroplast relocation, stomata opening, and leaf expansion. Most higher plants have two isoforms named phototropin1 (phot1) and phototropin2 (phot2). phot1 senses blue light in a wide range of light intensity, whereas phot2 acts as a light sensor under high irradiance. For instance, in *Arabidopsis*, phot1 and phot2 share phototropism and photoaccumulation responses (migration to appropriate light condition) of chloroplasts depending on the blue light intensity, while photoavoidance response (escape from intense light) of chloroplast is mediated solely by phot2. In contrast, stomata opening is mediated redundantly by both.

phot molecules comprise about 900 - 1000 amino acid residues and have two photoreceptive domains called LOV (light, oxygen and voltage-sensing) 1 and

2 in their N-terminal halves that bind flavin mononucleotide (FMN) noncovalently as a chromophore (Fig. 1). LOV is a subfamily of the PAS (PER-ARNT-SIM) superfamily involved in protein-protein interactions in cellular signaling. The C-terminal half forms a serine/threonine kinase domain (Fig. 1) inducing blue-light-activated autophosphorylation, which is considered to be the primary step in the light-signal transduction by phot. Thus, phot is thought to be a light-regulated protein kinase, in which LOV2 acts as a major molecular switch [2]. LOV1 is thought to regulate the sensitivity to blue light [2] and to work as a dimerization site [3].

In the present study, we determined the crystal structures of LOV1 domains of *Arabidopsis* phot1 (P1L1: Gly180-Lys329, Fig. 1) and phot2 (P2L1: Phe117-Lys265) in the dark at resolutions of 2.1 and 2.0 Å, respectively [4]. The P1L1 crystals appeared as bundles of thin needles. The X-ray diffraction data of P1L1 was collected from a crystal carefully broken off from a bundle at beamline **BL44B2** [5]. P2L1 crystal appeared as considerably stacked thin plates. The diffraction data was collected at beamline **BL41XU** by irradiating X-ray microbeam to a small volume likely composed of a few plates in a polycrystal [5]. The crystal structure of either LOV1 appears as a dimer in a face-to-face association mode of their β -scaffolds, which is composed of three antiparallel β -strands (Fig. 2). Three types of

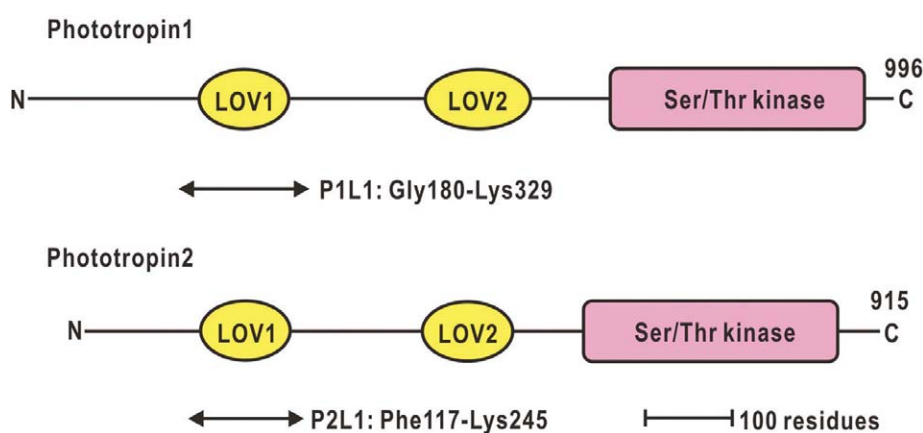


Fig. 1. Schematic of domain organization in *Arabidopsis* phot1 and phot2. P1L1 and P2L1 are the LOV1 domains targeted in this study and are indicated by solid lines.

interaction indispensable for stabilizing the dimer structures are contacts of side chains in the β -scaffolds, hydrophobic interactions induced by a short helix in the N-terminus of a subunit, and hydrogen bonds mediated by hydration water molecules confined in the dimer interface. In P1L1, the critical residue for dimerization is Cys261 that forms a disulfide bridge between subunits, and in P2L1, those are Thr217 and Met232 that may be involved in the difference in light sensitivity between phot1 and phot2. Putative models for the tertiary structures of full-length phot1 and phot2 based on the dimeric structures of their LOV1 domains are speculated (Fig. 3). In addition, the topologies in the homodimeric associations of the LOV1 domains also provide clues to understand the structural basis of the dimeric interactions of Per-ARNT-Sim protein modules in cellular signaling.

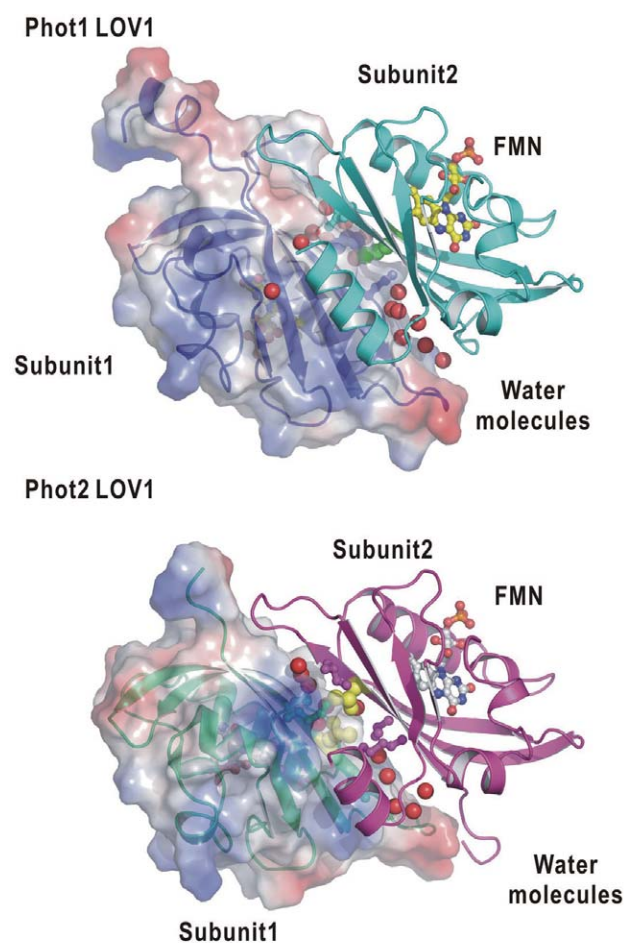


Fig. 2. Schematics of the crystal structures of P1L1 and P2L1 dimers. The structures of subunits are shown as ribbon models with cofactor FMN molecules shown as ball-and-stick models. The molecular surface of one of the subunits is also shown. Side chains of residues and hydration water molecules indispensable for the dimer association are shown in the space-filling models.

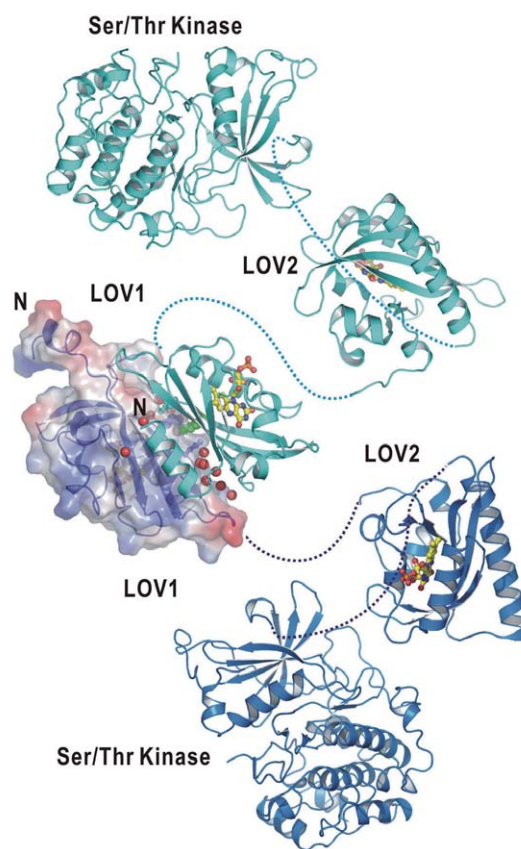


Fig. 3. A putative model for the quaternary structures of full-length phot1. The structures of LOV2 and the kinase domains are illustrated using the crystal structures of oat phot1 LOV2 (PDB accession code: 2V0U) and protein kinase A (1ATP), respectively. Protein kinase A has an amino acid sequence homologous to that of the phot serine/threonine kinase domain. The dashed lines are the linkers of the functional domains.

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