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Arabidopsis Blue Light Receptor Phototropin 1 Undergoes Blue Light-Induced Activation in Membrane Microdomains

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ABSTRACT

Phototropin (phot)-mediated signaling initiated by blue light (BL) plays a critical role in optimizing photosynthetic light capture at the plasma membrane (PM) in plants. However, the mechanisms underlying the regulation of phot activity at the PM in response to BL remain largely unclear. In this study, by single-particle tracking and stepwise photobleaching analysis of phot1-GFP proteins we demonstrated that in the dark phot1 proteins remain in an inactive state and mostly exist as monomers. Dimerization and the diffusion rate of phot1-GFP increased in a dose-dependent manner in response to BL. In contrast, BL did not affect the lateral diffusion of kinase-inactive phot1^{D806N}-GFP but did enhance its dimerization, suggesting that phot1 dimerization is independent of phosphorylation. Förster resonance energy transfer–fluorescence lifetime imaging microscopy analysis revealed that the interaction between phot1-GFP and a marker of sterol-rich lipid environments, AtRem1.3-mCherry, was enhanced with increased time of BL treatment. However, this BL-dependent interaction was not obvious in plants co-expressing phot1^{D806N}-GFP and AtRem1.3-mCherry, indicating that BL facilitates the translocation of functional phot1-GFP into AtRem1.3-labeled microdomains to activate phot-mediated signaling. Conversely, sterol depletion attenuated phot1-GFP dynamics, dimerization, and phosphorylation. Taken together, these results indicate that membrane microdomains act as organizing platforms essential for the proper function of activated phot1 at the PM.

Key words: phot1, VA-TIRFM, spatiotemporal dynamics, blue light signaling, membrane microdomains

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INTRODUCTION

Phototropins (phots) play key roles in phototropism, chloroplast movement, stomatal opening, leaf expansion, and solar tracking in response to blue light (BL) (Christie, 2007). *Arabidopsis thaliana* has two phots, phot1 and phot2, with overlapping but not completely redundant physiological functions (Liscum and Briggs, 1995; Sakai et al., 2001; Christie, 2007; Mo et al., 2015). Physiological processes mediated by phot1 and phot2 are therefore complex.

Studies aimed at understanding phot functions have so far focused on their individual signaling mechanisms, as well as their

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