Two Coiled-Coil Proteins, WEB1 and PMI2, Suppress the Signaling Pathway of Chloroplast Accumulation Response that Is Mediated by Two Phototropin-Interacting Proteins, RPT2 and NCH1, in Seed Plants

Suetsugu, Noriyuki; Wada, Masamitsu

International Journal of Molecular Sciences (2017), 18(7)

URL: http://hdl.handle.net/2433/226673

© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. (CC BY 4.0).

Type: Journal Article

Textversion: publisher

Kyoto University
Brief Report

Two Coiled-Coil Proteins, WEB1 and PMI2, Suppress the Signaling Pathway of Chloroplast Accumulation Response that Is Mediated by Two Phototropin-Interacting Proteins, RPT2 and NCH1, in Seed Plants

Noriyuki Suetsugu 1,* and Masamitsu Wada 2

1 Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan
2 School of Science and Engineering, Tokyo Metropolitan University, Tokyo 192-0397, Japan; masamitsu.wada@gmail.com
* Correspondence: suetsugu@lif.kyoto-u.ac.jp; Tel.: +81-75-753-6390; Fax: +81-75-753-6120

Received: 10 May 2017; Accepted: 4 July 2017; Published: 8 July 2017

Abstract: Chloroplast movement is induced by blue light in a broad range of plant species. Weak light induces the chloroplast accumulation response and strong light induces the chloroplast avoidance response. Both responses are essential for efficient photosynthesis and are mediated by phototropin blue-light receptors. J-DOMAIN PROTEIN REQUIRED FOR CHLOROPLAST ACCUMULATION RESPONSE 1 (JAC1) and two coiled-coil domain proteins WEAK CHLOROPLAST MOVEMENT UNDER BLUE LIGHT 1 (WEB1) and PLASTID MOVEMENT IMPAIRED 2 (PMI2) are required for phototropin-mediated chloroplast movement. Genetic analysis suggests that JAC1 is essential for the accumulation response and WEB1/PMI2 inhibit the accumulation response through the suppression of JAC1 activity under the strong light. We recently identified two phototropin-interacting proteins, ROOT PHOTOTROPISM 2 (RPT2) and NPH3/RPT2-like (NRL) PROTEIN FOR CHLOROPLAST MOVEMENT 1 (NCH1) as the signaling components involved in chloroplast accumulation response. However, the relationship between RPT2/NCH1, JAC1, and WEB1/PMI2 remained to be determined. Here, we performed genetic analysis between RPT2/NCH1, JAC1, and WEB1/PMI2 to elucidate the signal transduction pathway.

Keywords: Arabidopsis; blue light; Marchantia; organelle movement; phototropin

1. Introduction

Phototropins (phot) are blue-light photoreceptor kinases that mediate phototropism, leaf flattening, stomatal opening, and chloroplast movement including low light-induced chloroplast accumulation response and strong light-induced chloroplast avoidance response (herein referred to as the accumulation and avoidance response, respectively). These responses contribute to optimal photosynthetic light utilization at the organ/tissue, cellular, and organelle level [1,2]. Most land plants have two or more phototropin genes and functional differences exist between them. There are two phototropins in Arabidopsis thaliana, phot1 and phot2. Phototropism, leaf flattening, stomatal opening, and the accumulation response are mediated by both phot1 and phot2; however, phot1 plays a greater role in these responses, especially at lower blue-light intensities [1,2]. In contrast, the avoidance response is mediated primarily by phot2 [1,2]. Clear functional divergence of phototropins in the accumulation and avoidance response is observed in the moss Physcomitrella patens and the fern Adiantum capillus-veneris [3,4]. However, in the liverwort Marchantia polymorpha, which is a basal land plant, a single phototropin mediates both the accumulation and avoidance responses [5].
Thus, phototropins can intrinsically mediate all phototropin-mediated responses and functional diversification of phototropins seems to have occurred during land plant evolution concomitant with phototropin gene duplication [6].

By regulating various signaling components, such as phototropin-interacting proteins, phototropins can regulate multiple, diverse responses. For example, BLUE LIGHT SIGNALING1 kinase is a direct phototropin substrate and specifically mediates stomatal opening [7]. In addition, the two phototropin-interacting bric á brac, tramtrack and broad complex/pox virus and zinc finger (BTB/POZ) domain proteins NONPHOTOTROPIC HYPOCOTYL 3 (NPH3) and ROOT PHOTOTROPISM 2 (RPT2), which belong to the NPH3/RPT2-like (NRL) protein family, mediate phototropism and leaf flattening [8–11]. Recently, we identified a phototropin-interacting NRL protein, NRL PROTEIN FOR CHLOROPLAST MOVEMENT 1 (NCH1), and found that NCH1 specifically mediates the accumulation response [12]. These results indicate that phototropism, leaf flattening, and the accumulation response are dependent on these NRL proteins, while stomatal opening and the avoidance response are independent of these NRL proteins. In M. polymorpha, the RPT2/NCH1 ortholog MpNCH1 specifically mediates the accumulation response, indicating that phototropin-regulated chloroplast movement is conserved in land plants [12].

Similar to NPH3 and RPT2, NCH1 is localized on the plasma membrane and interacts with phototropins [12], but the downstream function of NCH1 as well as other NRL proteins remained to be determined. Here, we performed genetic analysis of RPT2 and NCH1 using triple or quadruple mutant plants between rpt2nch1 and other mutants that were implicated in the signal transduction of chloroplast movement.

2. Results and Discussion

J-domain protein required for chloroplast accumulation response 1 (jac1) and rpt2nch1 plants are both defective in the accumulation response [12,13]. JAC1 protein is a C-terminal J-domain protein similar to clathrin uncoating factor auxilin (Figure 1a) [13]. To analyze chloroplast movement in jac1 and rpt2nch1 in detail, we performed analysis of the light-induced changes in leaf transmittance, reflective of light-induced chloroplast movements (Figure 1b) [14]. In response to 3 µmol m$^{-2}$ s$^{-1}$ of blue light, which induces the accumulation response in wild type, a clear avoidance response is induced in rpt2nch1 but not in jac1 (Figure 1c,d) [12]. Therefore, RPT2/NCH1 could suppress the induction of the avoidance response to facilitate efficient induction of the accumulation response under low light conditions (Figure 2). Compared to wild type and jac1, a faster avoidance response was induced by 20 µmol m$^{-2}$ s$^{-1}$ of blue light in rpt2nch1 (Figure 1c,d; one-way ANOVA followed by Tukey–Kramer multiple comparison post hoc test, $p < 0.01$ for wild type or jac1 vs. rpt2nch1), although similar transmittance changes (defined as “amplitude” in Figure 1e) were observed for both rpt2nch1 and jac1 following 40 min of 20 µmol m$^{-2}$ s$^{-1}$ blue-light irradiation (Figure 1c,e; one-way ANOVA followed by Tukey–Kramer multiple comparison post hoc test, $p > 0.05$ for jac1 vs. rpt2nch1). Following subsequent application of 50 µmol m$^{-2}$ s$^{-1}$ blue light, only a slight additional avoidance response was observed in rpt2nch1 which contrasted with the stronger avoidance response induced in wild type (Figure 1c) [12]. This result could be attributed to prior movement of the majority of chloroplasts to the side walls in rpt2nch1 during the former irradiation period. Interestingly, avoidance responses of similar magnitudes were induced in jac1 in response to both 20 and 50 µmol m$^{-2}$ s$^{-1}$ of blue light and a decreased rate of avoidance response induction was not observed during strong light irradiation (Figure 1c). The changes observed in leaf transmittance for rpt2nch1jac1 were intermediate between those observed for rpt2nch1 and jac1.
Figure 1. (a) Protein structure of ROOT PHOTOTROPISM 2 (RPT2), NRL PROTEIN FOR CHLOROPLAST MOVEMENT 1 (NCH1), J-DOMAIN PROTEIN REQUIRED FOR CHLOROPLAST ACCUMULATION RESPONSE 1 (JAC1), WEAK CHLOROPLAST MOVEMENT UNDER BLUE LIGHT 1 (WEB1), and PLASTID MOVEMENT IMPAIRED 2 (PMI2). Blue boxes indicate the four conserved regions of NPH3/RPT2-like (NRL) proteins. The position of the BTB/POZ domain is indicated by a black bar. Red box is a J-domain. Green boxes indicate the coiled-coil domains; (b) Measurement of light-induced changes in leaf transmittance as a result of chloroplast photorelocation movements. The depicted trace represents typical data collected for wild type under the various light irradiation conditions (indicated by color boxes). There is a decrease in leaf transmittance in response to 3 μmol m⁻² s⁻¹ of blue light, indicating that the accumulation response is induced (downward arrow). Conversely, there is an increase in leaf transmittance in response to 20 and 50 μmol m⁻² s⁻¹ of blue light, indicating that the avoidance response is induced (upward arrows). Red lines mark the initial linear fragments of leaf transmittance rate change during the first 2–6 min of the irradiation period, indicating the velocity. A red parenthesis marks the difference between the transmittance level observed following 60 min of 3 μmol m⁻² s⁻¹ blue-light irradiation and the transmittance level observed a following further 40 min of 20 μmol m⁻² s⁻¹ blue-light irradiation, indicating the amplitude of the avoidance response caused by 20 μmol m⁻² s⁻¹ blue-light irradiation; (c–e) Distinct chloroplast movements observed between rpt2nch1 and jac1; (c) Light-induced changes in leaf transmittance of the indicated lines were measured using a custom-made plate reader system [14]. The samples were sequentially irradiated with 3, 20 and 50 μmol m⁻² s⁻¹ of continuous blue light. The beginning of each irradiation period is indicated by white, cyan and blue arrows, respectively. The light was extinguished after 150 min (black arrow); (d) The velocity of light-induced transmittance changes. (e) The amplitude of the avoidance response caused by 20 μmol m⁻² s⁻¹ blue-light irradiation. Data for wild type, rpt2nch1, jac1 and rpt2nch1jac1 from Suetsugu et al. (2016) [12] were used for comparison, because data for web1, rpt2nch1web1, pm12pmi15 and rpt2nch1pm12pmi15 were acquired in the same experiments using the same plate. Data are presented as means of three independent experiments and the error bars indicate standard errors. WT, wild type.
Recently, we showed that JAC1 mutation suppresses the defective avoidance response in weak chloroplast movement under blue light 1 (web1) and plastid movement impaired 2 (pmi2) [15]. WEB1 and PMI2 are related coiled-coil domain proteins that interact with each other (Figure 1a) [15]. Although the low light-induced accumulation response was normal in web1 and pmi2pmi15, both mutant plants exhibited attenuated avoidance response under the strong light conditions (Figure 1c to e) [15]. The jac1web1 and jac1pmi2pmi15 showed nearly the same phenotypes as jac1 single mutants [15]. Importantly, the weak avoidance response phenotype observed in web1 and pmi2pmi15 was completely suppressed in jac1web1 and jac1pmi2pmi15, respectively [15]. Therefore, we hypothesized that WEB1 and PMI2 suppress JAC1 function under strong light, preventing the induction of the JAC1-dependent accumulation response and leading to efficient induction of the avoidance response (Figure 2). The weak avoidance response phenotype observed in web1 and pmi2pmi15 was absent in rpt2nch1web1 and rpt2nch1pmi2pmi15, similar to jac1web1 and jac1pmi2pmi15 (Figure 1c–e). Mutation of JAC1 suppressed web1 and pmi2pmi15 phenotypes, because jac1web1 and jac1pmi2pmi15 phenotypes are indistinguishable from jac1 [15]. Although mutation of RPT2 and NCH1 largely suppressed the weak avoidance response phenotypes observed in web1 and pmi2pmi15, the velocity and amplitude of the avoidance response in these mutants did not match those in rpt2nch1 (Figure 1c–e; one-way ANOVA followed by Tukey–Kramer multiple comparison post hoc test; p < 0.01 for rpt2nch1web1 or rpt2nch1pmi2pmi15 vs. rpt2nch1 in velocity and p < 0.05 for rpt2nch1web1 or rpt2nch1pmi2pmi15 vs. rpt2nch1 in amplitude). The phenotypes of rpt2nch1web1 and rpt2nch1pmi2pmi15 were very similar to rpt2nch1jac1 in that no detectable chloroplast movement was observed under 3 μmol m−2 s−1 of blue light and their avoidance response phenotypes were similar to jac1 (Figure 1c; one-way ANOVA followed by Tukey–Kramer multiple comparison post hoc test; p > 0.05 for rpt2nch1web1 or rpt2nch1pmi2pmi15 vs. rpt2nch1jac1). Collectively, our results indicate that RPT2 and NCH1 are essential for the accumulation response and regulate JAC1-dependent and -independent pathways and that WEB1/PMI2 represses the signaling pathway for the accumulation response under the strong light conditions (Figure 2). However, how WEB1 and PMI2 suppress the accumulation response pathway remained to be determined. Interaction of WEB1 and/or PMI2 with JAC1 has never been detected [15]. At the least, the amounts of phototropins were normal in web1 and pmi2pmi15 mutant plants [15]. Further analysis of the relationship between WEB1/PMI2, RPT2/NCH1 and JAC1 is required.

**Figure 2.** Working model of chloroplast photorelocation movements. The photoreceptors phot1 and phot2 mediate the accumulation response under a low light condition through RPT2 and NCH1. RPT2 and NCH1 might regulate both JAC1-dependent and -independent (X) pathways. The signaling pathway by RPT2/NCH1 and JAC1 suppresses that of the avoidance response under a low light condition. Under the high light condition, the WEB1/PMI2 complex suppresses the signaling pathway for the accumulation response that is regulated by RPT2/NCH1 and JAC1 through an unknown mechanism, resulting in the efficient induction of the avoidance response mediated by phot2. **Gray** arrows indicate the suppressed signaling pathways. **Black** arrows indicate the activated signaling pathways.
RPT2 and NCH1 are localized on the plasma membrane and interact with phototropins, indicating that RPT2 and NCH1 are the initial downstream signaling components involved in the phototropin-mediated accumulation response (Figure 2) [12]. Notably, RPT2 and NCH1 are conserved in land plants, but JAC1, WEB1 and PMI2 orthologs are found only in seed plants [16]. Thus, to maximize light utilization through chloroplast movement, land plants have evolved a sophisticated mechanism of controlling chloroplast movement by increasing the molecular components involved in blue-light signaling.

3. Materials and Methods

3.1. Arabidopsis Lines and the Growth Condition

The wild-type and mutant lines are a Columbia gl1 background. Seeds were sown on 0.8% agar medium containing 1/3 strength Murashige & Skoog salt and 1% sucrose, and grown under white light at ca. ~100 µmol m$^{-2}$ s$^{-1}$ (16 h)/dark (8 h) cycle at 23 °C in an incubator. rpt2-4nch1-1 [12], jac1-1 [13], web1-2 [15] and pmi2-2pmi15-1 [15] were described previously. For PMI2 mutant plants, pmi2pmi15 was used, because PMI15 is closely related to PMI2 and pmi15 exhibits a very weak defect in chloroplast movement [17]. rpt2-4, nch1-1, pmi2-2 and pmi15-1 are T-DNA knockout lines [12,17]. jac1-1 carries a missense mutation [13] and web1-2 carries a deletion of one nucleotide [15]. Western blot analysis showed that JAC1 and WEB1 proteins were not detected in jac1-1 and web1-2, respectively [13,15]. Double, triple and quadruple mutants were generated by genetic crossing.

3.2. Analyses of Chloroplast Photorelocation Movements

Chloroplast photorelocation movements were analyzed by the measurement of light-induced changes in leaf transmittance as described previously [14]. Third leaves that were detached from 16-day-old seedlings were placed on 1% (w/v) gellan gum in a 96-well plate and then dark-adapted at least for 1 h before transmittance measurement.

3.3. Statistical Analysis

Statistical analyses were performed by one-way ANOVA followed by Tukey–Kramer multiple comparison post hoc test.

Acknowledgments: This work was supported in part by the Grant-in-Aid for Scientific Research Grants (26840097 and 15KK0254 to Noriyuki Suetsugu; 20227001, 23120523, 25120721, and 25251033 to Masamitsu Wada).

Author Contributions: Noriyuki Suetsugu conceived, designed and performed the experiments; Noriyuki Suetsugu and Masamitsu Wada analyzed the data, contributed reagents/materials/analysis tools and wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Christie, J.M. Phototropin blue-light receptors. Annu. Rev. Plant Biol. 2007, 58, 21–45. [CrossRef] [PubMed]
2. Suetsugu, N.; Wada, M. Evolution of three LOV blue light receptor families in green plants and photosynthetic stramenopiles: Phototropin, ZTL/FKF1/LKP2 and aureochrome. Plant Cell Physiol. 2013, 54, 8–23. [CrossRef] [PubMed]
3. Kasahara, M.; Kagawa, T.; Sato, Y.; Kiyosue, T.; Wada, M. Phototropins mediate blue and red light-induced chloroplast movements in Physcomitrella patens. Plant Physiol. 2004, 135, 1388–1397. [CrossRef] [PubMed]
4. Kagawa, T.; Kasahara, M.; Abe, T.; Yoshida, S.; Wada, M. Function analysis of phototropin2 using fern mutants deficient in blue light-induced chloroplast avoidance movement. Plant Cell Physiol. 2004, 45, 416–426. [CrossRef] [PubMed]
5. Komatsu, A.; Terai, M.; Ishizaki, K.; Suetsugu, N.; Tsunoi, H.; Nishihama, R.; Yamato, K.T.; Wada, M.; Kohchi, T. Phototropin encoded by a single-copy gene mediates chloroplast photorelocation movements in the liverwort Marchantia polymorpha. Plant Physiol. 2014, 166, 411–427. [CrossRef] [PubMed]
6. Li, F.W.; Rothfels, C.J.; Melkonian, M.; Villarreal, J.C.; Stevenson, D.W.; Graham, S.W.; Wong, G.K.S.; Mathews, S.; Pryer, K.M. The origin and evolution of phototropins. *Front. Plant Sci.* **2015**, *6*, 637. [CrossRef] [PubMed]

7. Takemiya, A.; Sugiyama, N.; Fujimoto, H.; Tsutsumi, T.; Yamauchi, S.; Hiyama, A.; Tada, Y.; Christie, J.M.; Shimazaki, K. Phosphorylation of BLUS1 kinase by phototropins is a primary step in stomatal opening. *Nat. Commun.* **2013**, *4*, 2094. [CrossRef] [PubMed]

8. Motchoulski, A.; Liscum, E. Arabidopsis NPH3: A NPH1 photoreceptor-interacting protein essential for phototropism. *Science* **1999**, *286*, 961–964. [CrossRef] [PubMed]

9. Sakai, T.; Wada, T.; Ishiguro, S.; Okada, K. RPT2: A signal transducer of the phototropic response in Arabidopsis. *Plant Cell* **2000**, *12*, 225–236. [CrossRef] [PubMed]

10. Inoue, S.; Kinoshita, T.; Takemiya, A.; Doi, M.; Shimazaki, K. Leaf positioning of Arabidopsis in response to blue light. *Mol. Plant* **2008**, *1*, 15–26. [CrossRef] [PubMed]

11. Harada, A.; Takemiya, A.; Inoue, S.; Sakai, T.; Shimazaki, K. Role of RPT2 in leaf positioning and flattening and a possible inhibition of phot2 signaling by phot1. *Plant Cell Physiol.* **2013**, *54*, 36–47. [CrossRef] [PubMed]

12. Suetsugu, N.; Takemiya, A.; Kong, S.G.; Higa, T.; Komatsu, A.; Shimazaki, K.; Kohchi, T.; Wada, M. RPT2/NCH1 subfamily of NPH3-like proteins is essential for the chloroplast accumulation response in land plants. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 10424–10429. [CrossRef] [PubMed]

13. Suetsugu, N.; Kagawa, T.; Wada, M. An auxilin-like J-domain protein, JAC1, regulates phototropin-mediated chloroplast movement in Arabidopsis. *Plant Physiol.* **2005**, *139*, 151–162. [CrossRef] [PubMed]

14. Wada, M.; Kong, S.G. Analysis of chloroplast movement and relocation in Arabidopsis. *Methods Mol. Biol.* **2011**, *774*, 87–102. [CrossRef]

15. Kodama, Y.; Suetsugu, N.; Kong, S.G.; Wada, M. Two interacting coiled-coil proteins, WEB1 and PMI2, maintain the chloroplast photorelocation movement velocity in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 19591–19596. [CrossRef] [PubMed]

16. Suetsugu, N.; Wada, M. Evolution of the cp-actin-based motility system of chloroplasts in green plants. *Front. Plant Sci.* **2016**, *7*, 561. [CrossRef] [PubMed]

17. Luesse, D.R.; DeBlasio, S.L.; Hangarter, R.P. Plastid movement impaired 2, a new gene involved in normal blue-light-induced chloroplast movements in Arabidopsis. *Plant Physiol.* **2006**, *141*, 1328–1337. [CrossRef] [PubMed]

© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).