RESEARCH COMMUNICATION

Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism

Hong-Li Lian,¹ Sheng-Bo He,¹ Yan-Chun Zhang,¹ Dan-Meng Zhu,² Jing-Yi Zhang,¹ Kun-Peng Jia,¹ Shu-Xia Sun,³ Ling Li,¹ and Hong-Quan Yang^{1,4}

¹Plant Science Institute, School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, China;

²Peking-Yale Joint Center of Plant Molecular Genetics and Agrobiotechnology, College of Life Sciences, Peking University, Beijing 100871, China; ³Horticulture Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China

Cryptochromes (CRYs) are blue-light photoreceptors that mediate various light responses in plants and animals. The signaling mechanism by which CRYs regulate light responses involves their physical interactions with COP1. Here, we report that CRY1 interacts physically with SPA1 in a blue-light-dependent manner. SPA acts genetically downstream from CRYs to regulate light-controlled development. Blue-light activation of CRY1 attenuates the association of COP1 with SPA1 in both yeast and plant cells. These results indicate that the blue-light-triggered CRY1–SPA1 interaction may negatively regulate COP1, at least in part, by promoting the dissociation of COP1 from SPA1. This interaction and consequent dissociation define a dynamic photosensory signaling mechanism.

Supplemental material is available for this article.

Received December 22, 2010; revised version accepted February 28, 2011.

Light is the primary environmental cue that is essential for plant growth and development. In plants, the light signal is perceived by multiple photoreceptors, including the blue/ultraviolet-A (320- to 500-nm)-light-absorbing cryptochromes (CRY1 and CRY2) (Cashmore et al. 1999; Lin and Shalitin 2003; Li and Yang 2007) and phototropins (PHOT1 and PHOT2) (Briggs and Christie 2002), and the red (600- to 700-nm)- and far-red (700- to 750-nm)-light-absorbing phytochromes (phyA to phyE) (Quail 2002). Plants have evolved a large set of negative regulators of photomorphogenic development, such as COP1, the COP9 signalosome (CSN), the COP10–DDB1–DET1 complex (CDD), and SPA (SPA1 to SPA4) (Pepper et al. 1994; Chamovitz et al. 1996; Suzuki et al. 2002; Saijo et al. 2003; Seo et al. 2003; Yanagawa et al. 2004). COP1 is one of the best characterized of these negative regulators. The

COP1 protein contains a RING finger domain, a coiled-coil domain, and a WD40 domain that directly recognizes its substrate proteins (Holm et al. 2001). It has been demonstrated that the abundance of HY5, a bZIP transcription factor, is positively correlated with the extent of photomorphogenesis (Osterlund et al. 2000). COP1 directly interacts with HY5 and targets it for proteasome-mediated degradation (Ang et al. 1998; Osterlund et al. 2000). The light signaling pathways initiated by the CRY1, CRY2, phyA, and phyB photoreceptors can eventually converge on COP1 and might negatively regulate its activity through physical interactions of these photoreceptors with COP1 (Wang et al. 2001; Yang et al. 2001; Seo et al. 2004; Jang et al. 2010).

SPA1 was initially identified as a nuclear-localized repressor of phyA-mediated signaling (Hoecker et al. 1999). Studies have shown that the four SPA proteins act redundantly to repress photomorphogenesis, and the loss-of-function *spa* quadruple mutant shows a constitutively photomorphogenic phenotype (Laubinger et al. 2004), similar to the *cop1* mutant (Deng et al. 1991). It has been demonstrated that SPA1 interacts with COP1 through their coiled-coil regions (Hoecker and Quail 2001), and that the coiled-coil domain of SPA1 is able to enhance the E3 ligase activity of COP1 on LAF1 in vitro (Seo et al. 2003). Consistent with this observation, HY5 accumulation is shown to be enhanced in the *spa1* mutant (Saijo et al. 2003). Genetic and biochemical studies suggest that all four SPA proteins act together with COP1 to negatively regulate light signaling (Laubinger et al. 2004; Hoecker 2005; Zhu et al. 2008).

Although it has been shown that CRY1 signaling is mediated through its physical interaction with COP1 (Wang et al. 2001; Yang et al. 2001), it is not known whether additional components are involved in CRY1 signaling (Supplemental Fig. S1A) and whether CRY1 signaling involves a blue-light-dependent biochemical mechanism. Here we characterized both the biochemical and genetic interactions between CRY1 and SPA1. Both CRY1 and CRY2 interact physically with SPA1 in yeast cells in a blue-light-dependent manner and colocalize in nuclear bodies (NBs) in vivo, and CRY1 associates with SPA1 in plant cells specifically under blue light. Furthermore, the blue-light-induced CRY1–SPA1 interaction promotes the dissociation of COP1 from SPA1 in both yeast and plant cells. Our results demonstrate a dynamic mechanism of CRY1 photosensory signaling.

Results and Discussion

The C-terminal domain of CRY1 interacts with SPA1 in yeast cells

It has been shown that the C-terminal WD40 domain of COP1 mediates the interaction with CRY1 or CRY2 (Wang et al. 2001; Yang et al. 2001), and that SPA1 and COP1 share a highly structurally related coiled-coil region and a C-terminal WD40 domain (Hoecker et al. 1999; Hoecker and Quail 2001). To examine whether CRY1 might also interact with SPA1, a yeast two-hybrid assay was performed. The results indicate that the CRY1 C-terminal domain (CCT1) strongly interacts with SPA1, as indicated by the high β -galactosidase activity (Supplemental Fig. S2A–C). Furthermore, we observed that CCT1

[Keywords: photoreceptor; SPA1; COP1; cryptochrome 1 signaling]

⁴Corresponding author.

E-MAIL hqyang@sjtu.edu.cn; FAX (086) 21-3420-5877.

Article published online ahead of print. Article and publication date are online at <http://www.genesdev.org/cgi/doi/10.1101/gad.2025111>.

interacts with the C-terminal WD40-containing domain of SPA1 (SCT1, referring to as CT509) (Yang and Wang 2006; Liu et al. 2011) but hardly with the N-terminal domain (SNT1) or coiled-coil domain (SCC1) of SPA1 (Supplemental Fig. S2A–C; Seo et al. 2003). These data indicate that, like COP1 (Yang et al. 2001), SPA1 interacts with CRY1 through the C-terminal WD40 domain. We further truncated SPA2, SPA3, and SPA4 corresponding to SCT1 (Supplemental Fig. S3) and determined that CCT1 interacts with the C-terminal WD40-containing domain of SPA2, SPA3, and SPA4 proteins (SCT2, SCT3, and SCT4, respectively) (Supplemental Fig. S2A,B,D).

The full-length CRY1 and CRY2 interact with SPA1 in a blue-light-dependent manner in yeast cells

To determine whether the full-length CRY1 interacts with SPA1, we prepared bait constructs expressing a variety of SPA protein domains (Fig. 1A) and a prey construct expressing the full-length CRY1 (Fig. 1B). We then performed yeast two-hybrid assays in the dark and in blue light, respectively. Strikingly, we found that full-length CRY1 interacts with SPA1 under blue light, but not in the dark (Fig. 1C; Supplemental Fig. S4). CRY1 also interacts with SCT1 in a blue-light-dependent manner, but fails to interact with SNT1 (Fig. 1C). We further observed that blue-light irradiation clearly promoted the interaction of SCT2, SCT3, and SPA4 with CRY1, although SPA4 is able to interact with CRY1 in darkness (Fig. 1C). Likewise, we

found that full-length CRY2 interacts with SPA1 in a blue-light-dependent manner (Fig. 1C; Supplemental Fig. S4). It has been shown that, when fused to β -glucuronidase (GUS), CCT1 is constitutively active in both darkness and light and mediates a strong constitutive light response in *Arabidopsis* (Yang et al. 2000; Sang et al. 2005). We then tested whether GUS-CCT1 interacts with SCT1, SCT2, SCT3, and SPA4 under blue light and in the dark in yeast cells. The results demonstrated a clear constitutive interaction for each of these pairs independent of blue light (Fig. 1C).

Next, we investigated the blue-light fluence rate response of the CRY–SPA1 interaction. As shown in Figure 1D, CRY1–SPA1 and CRY2–SPA1 interactions increased in yeast cells exposed to increasing fluence rates of blue light. CRY1–SPA1 and CRY2–SPA1 interactions were barely detectable in yeast cells treated with the lowest fluence of blue light tested ($0.1 \mu\text{mol}/\text{m}^2/\text{sec}$ for 120 min). When the fluence rate was increased to the highest ($100 \mu\text{mol}/\text{m}^2/\text{sec}$ for 120 min), the levels of CRY1–SPA1 and CRY2–SPA1 interactions were increased by >14-fold and sixfold, respectively (Fig. 1D). We then demonstrated that CRY1–SPA1 and CRY2–SPA1 interactions increased in yeast cells with increasing irradiation time. As shown in Figure 1E, the levels of CRY1–SPA1 and CRY2–SPA1 interactions in yeast cells exposed to $20 \mu\text{mol}/\text{m}^2/\text{sec}$ blue light for 240 min were approximately seven times and three times greater, respectively, than those in yeast cells exposed to $20 \mu\text{mol}/\text{m}^2/\text{sec}$ blue light for 15 min.

CRY1 interacts physically with SPA1 in a blue-light-dependent manner in plant cells

To test whether CRY interacts with SPA1 in vivo, we first transiently expressed CRY1 or CRY2 tagged with yellow fluorescent protein (YFP) and SPA1 tagged with cyan fluorescent protein (CFP), either individually or together, in onion epidermal cells. As anticipated, SPA1 and CRY1, and SPA1 and CRY2, were localized in the same NBs (Fig. 2). This finding was further confirmed in *Arabidopsis* protoplasts transiently coexpressing CFP-SPA1 and CRY1-YFP or CFP-SPA1 and CRY2-YFP (Supplemental Fig. S5).

Next, we investigated whether the CRY1–SPA1 interaction might be blue-light-dependent in vivo. We used transgenic *spa1* mutant plants expressing Myc-SPA1 (Myc-SPA1/*spa1*) (Yang and Wang 2006) and performed coimmunoprecipitation (co-IP) assays. As shown in Figure 3A, immunoprecipitation of the endogenous CRY1 from seedlings exposed to blue light also pulled down Myc-SPA1. However, Myc-SPA1 was not pulled down by immunoprecipitation of CRY1 from seedlings either adapted in darkness or exposed to red light or far-red light. Consistent with the yeast two-hybrid study (Fig. 1D,E), co-IP assays also demonstrated that the CRY1–SPA1 interaction was enhanced in seedlings exposed to either higher fluence rates of blue light (Fig. 3B) or blue light for increasing times (Fig. 3C). Noticeably, the CRY1–SPA1 interaction was detected in seedlings exposed to $10 \mu\text{mol}/\text{m}^2/\text{sec}$ blue light for as short as 0.5 min (Fig. 3C), indicating that blue-light-induced the CRY1–SPA1 interaction is a very rapid and sensitive process in planta and may define a critical early regulatory step in CRY1 signaling.

Figure 1. CRY interacts with SPA in a blue-light-dependent manner in yeast cells. (A) Yeast two-hybrid bait constructs. All proteins are fused with the LexA DNA-binding domain (LexA). (SNT1) N-terminal domain of SPA1; (SCT) C-terminal domain of SPA. (B) Yeast two-hybrid prey constructs. All proteins are fused with the B42 activation domain (AD). (C) Yeast two-hybrid analyses of the CRY–SPA interactions and the interactions of GUS–CCT1 with SPA under blue light (BL) and in darkness (DK). (D,E) Quantitative yeast two-hybrid analyses of the CRY–SPA1 interactions under the indicated fluence rates of blue light (D) and $20 \mu\text{mol}/\text{m}^2/\text{sec}$ blue light for the indicated exposure time (E). Data are mean $\pm$ SD ($n = 12$).

Figure 2. Colocalization of CRY1 and CRY2 with SPA1 in onion epidermal cells. Onion peels were cobombarded with the DNA constructs indicated. CRY1 and CRY2 localize together with SPA1 to the NBs in onion epidermal cells. (Dic) Differential interference contrast in light microscope mode. Bars, 10 μ m.

SPA acts genetically downstream from CRY to regulate light-controlled development

To examine whether *CRY1* and *SPA* might genetically interact, we generated a *spa1 spa2 spa3 spa4* quadruple mutant and a *cry1 spa1 spa2 spa3 spa4* quintuple mutant and analyzed their photomorphogenic phenotypes under blue light. The results showed that the *cry1 spa1 spa2 spa3 spa4* quintuple mutant exhibited an enhanced photomorphogenic phenotype similar to that of the *spa1 spa2 spa3 spa4* quadruple mutant (Fig. 4A,C). Consistent with these phenotypes, Western blotting analysis using an antibody prepared against HY5 indicated that the *cry1 spa1 spa2 spa3 spa4* quintuple mutant and *spa1 spa2 spa3 spa4* quadruple mutant seedlings accumulated similarly high levels of HY5 protein under blue light (Supplemental Fig. S6). It has been shown that CRY1 mediates blue-light-triggered stomatal development, and that SPA acts to repress stomatal development (Kang et al. 2009). We then analyzed stomatal development in these mutants and found that the stomatal phenotype of the *cry1 spa1 spa2 spa3 spa4* quintuple mutant under blue light resembled that observed for the *spa1 spa2 spa3 spa4* quadruple mutant, as shown by the stomata developing in clusters and the stomatal index (the ratio of stomata to stomata plus other epidermal cells) (Fig. 4E). We also generated *cry2 spa1 spa2 spa3* and *cry2 spa1 spa3 spa4* quadruple mutants and investigated their photomorphogenic phenotypes. The results showed that the phenotypes of the *cry2 spa1 spa2 spa3* and *cry2 spa1 spa3 spa4* quadruple mutants under blue light were similar to those of the *spa1 spa2 spa3* and *spa1 spa3 spa4* triple mutants, respectively (Fig. 4B,D). We then determined the flowering time phenotype under a long day photoperiod (16 h light/8 h dark) and found that the *cry2 spa1 spa2 spa3* and *cry2 spa1 spa3 spa4* quadruple mutants flowered essentially as early as the *spa1 spa2 spa3* and *spa1 spa3 spa4* triple mutants, respectively (Fig. 4F).

These results suggest that *SPA* acts genetically downstream from *CRY1* and *CRY2* to regulate photomorphogenic development, stomatal development, and flowering time.

The COP1-SPA1 interaction is repressed by blue-light-activated CRY1 in both yeast and plant cells

The COP1-SPA1 association shows dynamic characteristics. In the dark, COP1 strongly interacts with SPA1, while light promotes their dissociation (Saijo et al. 2003). The demonstration that CRY1-SPA1 exhibits blue-light-induced interaction prompted us to examine whether CRY1 is directly involved in the regulation of the dynamic interaction of COP1 and SPA1 under blue light through yeast three-hybrid assays. We generated a construct expressing COP1 bait and the full-length CRY1 bridge protein or a mock CRY1 bridge (mCRY1) that does not express CRY1 and a construct expressing SPA1 prey (Fig. 5A). As shown in Figure 5B, the COP1-SPA1 interaction decreased in yeast cells expressing CRY1 but not mCRY1, even when exposed to a higher fluence rate of blue light. The level of COP1-SPA1 interaction in yeast cells exposed to the highest fluence of blue light tested (50 μ mol/m²/sec) decreased by ~80% compared with that in yeast cells exposed to the lowest fluence of blue light tested (0.5 μ mol/m²/sec).

It is possible that inhibition of COP1-SPA1 interaction by CRY1 is mediated through the CRY1-SPA1 and/or CRY1-COP1 interaction. To investigate these possibilities further, we first prepared a construct expressing the coiled-coil-containing domain of COP1 bait (CC1), which does not interact with CRY1 (Fig. 5A, Supplemental Fig. S7), and performed yeast three-hybrid assays under different

Figure 3. CRY1 interacts with SPA1 in a blue-light-dependent manner in vivo. (A–C) Co-IP using anti-CCT1 antiserum in the extracts from Myc-SPA1/*spa1* seedlings grown under continuous white light for 5 d and then transferred to darkness for another 3 d (A,B), or seedlings grown in continuous darkness for 6 d (C) before being exposed to the indicated light conditions for 10 h (A), the indicated fluence rates of blue light (B), or 10 μ mol/m²/sec blue light for the indicated exposure time (C). (D) Darkness; (B) blue light (30 μ mol/m²/sec); (R) red light (30 μ mol/m²/sec); (FR) far-red light (5 μ mol/m²/sec). The immunoprecipitates were probed with anti-CCT1 and anti-Myc antibodies, respectively.

Figure 4. SPA acts genetically downstream from CRY to regulate light-controlled development. (A) Wild-type (WT), *cry1*, *spa1 spa2 spa3 spa4* (*spa1234*), and *cry1 spa1 spa2 spa3 spa4* (*cry1spa1234*) mutant seedlings grown in blue light (30 $\mu\text{mol}/\text{m}^2/\text{sec}$) for 6 d. Bars, 2 mm. (B) Wild-type (WT), *cry2*, *spa1 spa2 spa3* (*spa123*), *cry2 spa1 spa2 spa3* (*cry2spa123*), *spa1 spa3 spa4* (*spa134*), and *cry2 spa1 spa3 spa4* (*cry2spa134*) mutant seedlings grown in blue light (3 $\mu\text{mol}/\text{m}^2/\text{sec}$) for 6 d. Bars, 2 mm. (C,D) Measurements of hypocotyl lengths of the genotypes of plants shown in A and B, respectively. Data are mean $\pm$ SD ($n = 30$). (E) Differential interference contrast (Dic) images of the abaxial cotyledon epidermis of 7-d-old seedlings with indicated genotypes grown in continuous blue light (10 $\mu\text{mol}/\text{m}^2/\text{sec}$). Stomatal index (percentages) are listed in each panel. Data are mean $\pm$ SD ($n = 10$). Bars, 20 μm . (F) The flowering phenotype of the plants with indicated genotypes in long days (LDs; 16 h light/8 h dark). Bars, 2 cm. Flowering time was measured as days to flowering and the number of leaves at bolting. Data are mean $\pm$ SD ($n \geq 30$).

fluence rates of blue light. As shown in Figure 5C, the CRY1–SPA1 interaction was inhibited in yeast cells expressing CRY1 but not mCRY1 as the fluence rate was increased $>5 \mu\text{mol}/\text{m}^2/\text{sec}$. These results indicated that the blue-light-induced CRY1–SPA1 interaction is able to promote the dissociation of COP1 from SPA1. We then generated a construct expressing the SCC1 bait, with which CRY1 does not interact (Supplemental Fig. S7), and performed yeast three-hybrid assays under different fluence rates of blue light. The results demonstrated that the SCC1–COP1 interaction decreased dramatically in yeast cells expressing CRY1 but not mCRY1 as the blue-light fluence rate increased (Fig. 5D), implying that the CRY1–COP1 interaction is also able to inhibit COP1 from associating with SPA1.

To determine whether CRY1 is involved in the regulation of blue-light-induced dissociation of COP1 from SPA1 in vivo, we analyzed the association capacity of COP1 with SPA1 through co-IP assay using dark-grown Myc-SPA1/*spa1* and *cry1* mutant plants expressing Myc-SPA1 (Myc-SPA1/*cry1*) that were exposed to blue light for different times, respectively. The anti-COP1 antibody was prepared and its specificity was analyzed by Western blotting (Supplemental Fig. S8A). COP1 was shown to be coimmunoprecipitated with Myc-SPA1 in protein extracts

prepared from Myc-SPA1/*spa1* seedlings but not in those prepared from *spa1* mutant seedlings (Supplemental Fig. S8B). For unknown reasons, blue-light illumination reduced the amount of COP1 in Myc-SPA1/*spa1* and Myc-SPA1/*cry1* seedlings, but the COP1 levels were similar in these seedlings exposed to blue light for 0.5–4 h (Fig. 5E,F). We confirmed the equal loading by Western blotting using an antibody against CUL1 (Fig. 5E,F; Sang et al. 2005). The results demonstrated that, in the presence of CRY1, the COP1–SPA1 interaction was reduced progressively with prolonged exposure to blue light, with the amount of COP1 coimmunoprecipitated with Myc-SPA1 being reduced by $\sim 26\%$ and 42% on 2 and 4 h of blue-light irradiation compared with that on 0.5 h of irradiation, respectively (Fig. 5E). In contrast, in the *cry1* mutant background, blue-light-induced COP1–SPA1 dissociation was inhibited (Fig. 5F). These data indicate that blue-light-mediated dissociation of COP1 from SPA1 is achieved, at least in part, through the blue-light-induced interaction of CRY1 with SPA1.

Genetic and biochemical studies demonstrate that COP1 activity is regulated by multiple components, such as SPA, the CDD complex, and CSN (Chamovitz et al. 1996; Suzuki et al. 2002; Saijo et al. 2003; Seo et al. 2003; Yanagawa et al. 2004). We proposed previously that the outcome of the light activation of CRY1 through its physical interaction with COP1 is the disruption of the negative regulation of COP1 exerted on its substrates (Yang et al. 2001; Liu et al. 2008). Based on a previous study showing that SPA1 can enhance the COP1-mediated ubiquitination of LAF1 in vitro (Seo et al. 2003) and our results, we tentatively propose that the blue-light-triggered CRY1–SPA1 interaction might result in a change in SPA1 properties, as a result of which the COP1–SPA1 interaction is inhibited, COP1 activity is depressed, and the HY5 protein is relieved from the COP1–SPA1-mediated proteolysis and performs its role in regulating photomorphogenesis (Fig. 5G). Moreover, it is possible that, upon blue light irradiation, the CRY1–COP1 interaction might, on the one hand, suppress COP1 activity, and, on the other hand, also inhibit COP1 from associating with SPA1, further attenuating COP1 activity. In the dark, COP1 localizes to the nucleus, whereas, in the light, it localizes to the cytoplasm (von Arnim and Deng 1994). Thus, future studies should investigate whether regulation of COP1 by CRY1–COP1 and CRY1–SPA1 interactions involves regulation of the E3 ubiquitin ligase activity and the cellular localization properties of COP1.

We provide here new insights into the signaling mechanism of CRY1. The blue-light-induced CRY1–SPA1 interaction promotes the dissociation of COP1 from SPA1 to regulate COP1 activity negatively, a regulation that defines the dynamic nature of photosensory signaling induced by photoactivated CRY1 (Fig. 5G; Supplemental Fig. S1B). It has been established that COP1–SPA tetramer complexes exhibit different forms in response to various light conditions (Zhu et al. 2008). Our results indicate that the transformation among different forms of COP1–SPA complexes may result from the dynamic interactions and/or different association capacities of photoreceptors and SPA proteins. Given that CRY2 also interacts with COP1 (Wang et al. 2001) and SPA1 in a blue-light-dependent manner in yeast cells (Fig. 1C–E) and colocalizes in the same NBs with SPA1 in plant cells (Fig. 2; Supplemental Fig. S5), and that SPA acts genetically downstream from CRY2 to regulate photomorphogenic development and flowering time (Figs.

Figure 5. CRY1 interferes with the COP1-SPA1 interaction under blue light in both yeast and plant cells. (A) Yeast three-hybrid constructs expressing both the bait and the bridge proteins. (BD) DNA-binding domain of GAL4; (P_{Met25}) inducible promoter driving the expression of the bridge protein; (CC1) coiled-coil domain of COP1; (SCC1) coiled-coil domain of SPA1; (mCRY1) mock CRY1 with a frameshift in CRY1 that does not express CRY1. (B–D) Quantitative yeast three-hybrid analyses showing the effects of CRY1 on the interactions of COP1-SPA1 (B), CC1-SPA1 (C), and SCC1-COP1 (D) under the indicated fluence rates of blue light. The interaction values (Miller units) in darkness were set to 100%, and the relative interaction under blue light was expressed as the percentage of the dark value. Data are mean $\pm$ SD ($n = 4$). (E,F) Co-IP using anti-Myc agarose beads in the extracts from Myc-SPA1/*spa1* seedlings (CRY1 present) (E) and Myc-SPA1/*cry1* seedlings (CRY1 absent) (F) grown in continuous darkness for 6 d before being exposed to 50 $\mu\text{mol/m}^2/\text{sec}$ blue light for the indicated time. The immunoprecipitates were probed with anti-COP1, anti-Myc, and anti-CUL1 antibodies, respectively. Relative band intensities were normalized for each panel and are shown below each lane. (G) Mode of CRY1 action. Photoactivation of CRY1 by blue-light irradiation may lead to a change in the conformation of SPA1 and COP1 through physical interactions, as a result of which COP1 dissociates from SPA1, its E3 ligase activity is attenuated, and HY5 is able to accumulate and promote photomorphogenesis.

4B,D,F), it is possible that CRY2 is also involved in blue-light-induced dissociation of SPA1 from COP1. Now we know that CRY1, CRY2, phyA, and phyB all interact with COP1 (Wang et al. 2001; Yang et al. 2001; Seo et al. 2004; Jang et al. 2010), and here we have established the blue-light-dependent interaction of CRY1 and SPA1. It will be worth investigating whether phyB and phyA interact with SPA1, and whether red- and far-red-light-promoted dissociation of COP1 from SPA1 (Saijo et al. 2003) involves direct phyB-SPA1 and phyA-SPA1 interactions.

Materials and methods

The plant materials and growth conditions, yeast two-hybrid assay, and subcellular colocalization study were as described previously (Yang et al. 2001; Sang et al. 2005; Liu et al. 2008). *Arabidopsis thaliana* Columbia accession (Col) was used as the wild type.

Construction of mutants

The *Arabidopsis* mutants used for this report were in the Col background, except for the *spa3* mutant, which was in the Wassilewskaja (Ws) background. *Spa1* (SALK_023840), *spa2* (SALK_083331), and *spa4* (SALK_082740)

mutants were obtained from the *Arabidopsis* Biological Resource Center, *spa3* (Flag_120T3) was from the Versailles Genetics and Plant Breeding Laboratory *Arabidopsis thaliana* Resource Center, and Myc-SPA1/*spa1-3*, *cry1-104*, and *cry2-1* mutants were generated previously (Bruggemann et al. 1996; Guo et al. 1998; Yang and Wang 2006). The *spa1 spa2 spa3*, *spa1 spa3 spa4*, *spa1 spa2 spa3 spa4*, *cry1 spa1 spa2 spa3 spa4*, *cry2 spa1 spa2 spa3*, *cry2 spa1 spa3 spa4*, and Myc-SPA1/*cry1* mutants were obtained by genetic crossing. The resulting mutants harboring a mutation in *SPA3* were backcrossed into the Col background four times to reduce the Ws background. All of the genotypes were confirmed by PCR and phenotypic analysis.

COP1 antibody preparation

Rabbits were vaccinated with recombinant His-tagged peptide corresponding to amino acids 1–212 of COP1 purified in *Escherichia coli* to generate anti-COP1 antiserum. The anti-COP1 antibody was further purified from the antiserum using protein A beads.

Co-IP study

Co-IP was performed by methods described previously (Shalitin et al. 2002; Zhu et al. 2008), with minor modifications. Myc-SPA1/*spa1* seedlings were harvested in dim green safe light and homogenized in lysis buffer (Zhu et al. 2008). After centrifugation, total protein concentration was determined by Bradford assay (Bio-Rad). Equal amounts of total protein (1–3 mg) in 1 mL of lysis buffer were incubated with 5 μL of anti-CCT1 antiserum (Sang et al. 2005) for 1 h at 4°C, then 20 μL of protein A-sepharose beads (bed volume, GE healthcare) was added to the mixture and incubated for another hour at 4°C. The immunoprecipitates were washed four times with wash buffer (Shalitin et al. 2002). For anti-Myc co-IP, equal amounts of total protein (1 mg, unless otherwise stated) in 1 mL of lysis buffer from Myc-SPA1/*spa1* and Myc-SPA1/*cry1* seedlings were incubated with 40 μL of anti-Myc agarose beads (bed volume; Sigma) for 2 h at 4°C. The immunoprecipitates were washed three times with wash buffer. Eluting and concentrating of the immunoprecipitates were performed as described previously (Zhu et al. 2008). The concentrates were resuspended with 2 $\times$ SDS sample buffer and boiled for 5 min, then subjected to Western blotting analysis. The immunoblots were quantified with ImageJ (<http://rsb.info.nih.gov/ij/>).

Yeast three-hybrid assay

A yeast three-hybrid assay was performed according to the manufacturer's instructions except that the yeast strain SFY526 was used. Transformed colonies were selected on synthetic complete medium lacking Leu and Trp. Three independent clones with four respective replicates were used in each performance. Cell cultures were placed under indicated light conditions and incubated at 30°C until the OD600 was between 0.5 and 0.8 with the conditional expression of the bridge proteins. Calculations of relative β -galactosidase activities were performed as described previously (Yang et al. 2001). At least three independent experiments were performed, and the result of one representative is shown.

Acknowledgments

We thank W.R. Briggs for critically reading our manuscript, C. Lin for discussions and communication of data, H.Y. Wang for Myc-SPA1 seeds, Y.J. Wu and A.R. Cashmore for *hy5* seeds, and X.W. Deng for helpful comments. This work was supported by the National Natural Science

Lian et al.

Foundation of China [30830012 to H.-Q.Y.], a National Special Grant for Transgenic Crops [2009ZX08009-081B to H.-Q.Y.], the Science and Technology Commission of Shanghai Municipality [10XD1402300 to H.-Q.Y.], and the Shanghai Leading Academic Discipline Project [B209].

References

- Ang LH, Chattopadhyay S, Wei N, Oyama T, Okada K, Batschauer A, Deng XW. 1998. Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of *Arabidopsis* development. *Mol Cell* **1**: 213–222.
- Briggs WR, Christie JM. 2002. Phototropins 1 and 2: versatile plant blue-light receptors. *Trends Plant Sci* **7**: 204–210.
- Bruggemann E, Handwerger K, Essex C, Storz G. 1996. Analysis of fast neutron-generated mutants at the *Arabidopsis thaliana* HY4 locus. *Plant J* **10**: 755–760.
- Cashmore AR, Jarillo JA, Wu YJ, Liu D. 1999. Cryptochromes: blue light receptors for plants and animals. *Science* **284**: 760–765.
- Chamovitz DA, Wei N, Osterlund MT, von Arnim AG, Staub JM, Matsui M, Deng XW. 1996. The COP9 complex, a novel multisubunit nuclear regulator involved in light control of a plant developmental switch. *Cell* **86**: 115–121.
- Deng XW, Caspar T, Quail PH. 1991. *cop1*: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. *Genes Dev* **5**: 1172–1182.
- Guo H, Yang H, Mockler TC, Lin C. 1998. Regulation of flowering time by *Arabidopsis* photoreceptors. *Science* **279**: 1360–1363.
- Hoecker U. 2005. Regulated proteolysis in light signaling. *Curr Opin Plant Biol* **8**: 469–476.
- Hoecker U, Quail PH. 2001. The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in *Arabidopsis*. *J Biol Chem* **276**: 38173–38178.
- Hoecker U, Tepperman JM, Quail PH. 1999. SPA1, a WD-repeat protein specific to phytochrome A signal transduction. *Science* **284**: 496–499.
- Holm M, Hardtke CS, Gaudet R, Deng XW. 2001. Identification of a structural motif that confers specific interaction with the WD40 repeat domain of *Arabidopsis* COP1. *EMBO J* **20**: 118–127.
- Jang IC, Henriques R, Seo HS, Nagatani A, Chua NH. 2010. *Arabidopsis* PHYTOCHROME INTERACTING FACTOR proteins promote phytochrome B polyubiquitination by COP1 E3 ligase in the nucleus. *Plant Cell* **22**: 2370–2383.
- Kang CY, Lian HL, Wang FF, Huang JR, Yang HQ. 2009. Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in *Arabidopsis*. *Plant Cell* **21**: 2624–2641.
- Laubinger S, Fittinghoff K, Hoecker U. 2004. The SPA quartet: a family of WD-repeat proteins with a central role in suppression of photomorphogenesis in *Arabidopsis*. *Plant Cell* **16**: 2293–2306.
- Li QH, Yang HQ. 2007. Cryptochrome signaling in plants. *Photochem Photobiol* **83**: 94–101.
- Lin C, Shalitin D. 2003. Cryptochrome structure and signal transduction. *Annu Rev Plant Biol* **54**: 469–496.
- Liu LJ, Zhang YC, Li QH, Sang Y, Mao J, Lian HL, Wang L, Yang HQ. 2008. COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in *Arabidopsis*. *Plant Cell* **20**: 292–306.
- Liu B, Zuo Z, Liu H, Liu X, Lin C. 2011. *Arabidopsis* cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. *Genes Dev* (this issue). doi: 10.1101/gad.2025011.
- Osterlund MT, Hardtke CS, Wei N, Deng XW. 2000. Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* **405**: 462–466.
- Pepper A, Delaney T, Washburn T, Poole D, Chory J. 1994. DET1, a negative regulator of light-mediated development and gene expression in *Arabidopsis*, encodes a novel nuclear-localized protein. *Cell* **78**: 109–116.
- Quail PH. 2002. Phytochrome photosensory signalling networks. *Nat Rev Mol Cell Biol* **3**: 85–93.
- Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, Rubio V, Ma L, Hoecker U, Deng XW. 2003. The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes Dev* **17**: 2642–2647.
- Sang Y, Li QH, Rubio V, Zhang YC, Mao J, Deng XW, Yang HQ. 2005. N-terminal domain-mediated homodimerization is required for photoreceptor activity of *Arabidopsis* CRYPTOCHROME 1. *Plant Cell* **17**: 1569–1584.
- Seo HS, Yang JY, Ishikawa M, Bolle C, Ballesteros ML, Chua NH. 2003. LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. *Nature* **423**: 995–999.
- Seo HS, Watanabe E, Tokutomi S, Nagatani A, Chua NH. 2004. Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. *Genes Dev* **18**: 617–622.
- Shalitin D, Yang H, Mockler TC, Maymon M, Guo H, Whitelam GC, Lin C. 2002. Regulation of *Arabidopsis* cryptochrome 2 by blue-light-dependent phosphorylation. *Nature* **417**: 763–767.
- Suzuki G, Yanagawa Y, Kwok SF, Matsui M, Deng XW. 2002. *Arabidopsis* COP10 is a ubiquitin-conjugating enzyme variant that acts together with COP1 and the COP9 signalosome in repressing photomorphogenesis. *Genes Dev* **16**: 554–559.
- von Arnim AG, Deng XW. 1994. Light inactivation of *Arabidopsis* photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. *Cell* **79**: 1035–1045.
- Wang H, Ma LG, Li JM, Zhao HY, Deng XW. 2001. Direct interaction of *Arabidopsis* cryptochromes with COP1 in light control development. *Science* **294**: 154–158.
- Yanagawa Y, Sullivan JA, Komatsu S, Gusmaroli G, Suzuki G, Yin J, Ishibashi T, Saijo Y, Rubio V, Kimura S, et al. 2004. *Arabidopsis* COP10 forms a complex with DDB1 and DET1 in vivo and enhances the activity of ubiquitin conjugating enzymes. *Genes Dev* **18**: 2172–2181.
- Yang J, Wang H. 2006. The central coiled-coil domain and carboxyl-terminal WD-repeat domain of *Arabidopsis* SPA1 are responsible for mediating repression of light signaling. *Plant J* **47**: 564–576.
- Yang HQ, Wu YJ, Tang RH, Liu D, Liu Y, Cashmore AR. 2000. The C termini of *Arabidopsis* cryptochromes mediate a constitutive light response. *Cell* **103**: 815–827.
- Yang HQ, Tang RH, Cashmore AR. 2001. The signaling mechanism of *Arabidopsis* CRY1 involves direct interaction with COP1. *Plant Cell* **13**: 2573–2587.
- Zhu D, Maier A, Lee JH, Laubinger S, Saijo Y, Wang H, Qu LJ, Hoecker U, Deng XW. 2008. Biochemical characterization of *Arabidopsis* complexes containing CONSTITUTIVELY PHOTOMORPHOGENIC1 and SUPPRESSOR OF PHYA proteins in light control of plant development. *Plant Cell* **20**: 2307–2323.

Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism

Hong-Li Lian, Sheng-Bo He, Yan-Chun Zhang, et al.

Genes Dev. 2011, **25**: originally published online April 21, 2011
Access the most recent version at doi:[10.1101/gad.2025111](https://doi.org/10.1101/gad.2025111)

Supplemental Material

<http://genesdev.cshlp.org/content/suppl/2011/04/14/gad.2025111.DC1>

Related Content

Light-regulated interactions with SPA proteins underlie cryptochrome-mediated gene expression
Christian Fankhauser and Roman Ulm
[Genes Dev. May , 2011 25: 1004-1009](#)

References

This article cites 33 articles, 18 of which can be accessed free at:
<http://genesdev.cshlp.org/content/25/10/1023.full.html#ref-list-1>

Articles cited in:
<http://genesdev.cshlp.org/content/25/10/1023.full.html#related-urls>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).