Light-dependent, Dark-promoted Interaction between Arabidopsis Cryptochrome 1 and Phytochrome B Proteins**

JUNE 22, 2012 • VOLUME 287 • NUMBER 26 • 22165

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Plant photoreceptors transduce environmental light cues to downstream signaling pathways, regulating a wide array of processes during growth and development. Two major plant photoreceptors with critical roles in photomorphogenesis are phytochrome B (phyB), a red/far-red absorbing photoreceptor, and cryptochrome 1 (CRY1), a UV-A/blue photoreceptor. Despite substantial genetic evidence for cross-talk between phyB and CRY1 pathways, a direct interaction between these proteins has not been observed. Here, we report that Arabidopsis phyB interacts directly with CRY1 in a light-dependent interaction. Surprisingly, the interaction is light-dissociated; CRY1 interacts specifically with the dark/far-red (Pr) state of phyB, but not with the red light-activated (Pfr) or the chromophore unconjugated form of the enzyme. The interaction is also regulated by light activation of CRY1; phyB Pr interacts only with the unstimulated form of CRY1 but not with the photostimulated protein. Further studies reveal that a small domain extending from the photolyase homology region (PHR) of CRY1 regulates the specificity of the interaction with different conformational states of phyB. We hypothesize that in plants, the phyB/CRY1 interaction may mediate cross-talk between the red/far-red- and blue/UV-sensing pathways, enabling fine-tuning of light responses to different spectral inputs.

The phyB and CRY1 pathways of Arabidopsis act to control large aspects of plant photomorphogenesis, with critical roles in light regulation of de-etiolation, germination, shade avoidance, and other diverse processes (1–5). phyB is a red/far-red-sensing photoreceptor that converts between two distinct structural states, a “Pr” form that exists predominantly in dark and far-red light and a “Pfr” form that exists predominantly in red light (6). The Pfr form of phyB is thought to be the signaling state, and most known light-dependent interactions with phyB, including interactions with the PIF family of transcription factors (7), occur with this conformation of the protein, although at least one protein complex has been observed to show some specificity in binding to phytochrome Pr (8).

CRY1 is a UV-A/blue photoreceptor that has been shown to interact in a light-dependent manner with only one other protein, SPA1 (9, 10). The CRY1/SPA1 interaction requires blue light and was shown to prevent interaction of SPA1 with COP1 (9, 10). CRY2, a closely related cryptochrome, was also shown to interact with SPA1 in a light-dependent manner, via a different site (9, 11). Only one other light-dependent interaction of a plant cryptochrome has been reported, an interaction of CRY2 with the basic helix-loop-helix protein CIB1, which also occurs only with the blue light-stimulated state (12).

Despite many prior studies in plants that have provided genetic evidence for cross-talk between the cryptochrome and phytochrome pathways (13–22), it has not been clear how this is mediated because evidence for a direct, light-dependent interaction between these photoreceptors has been elusive. Phytochrome A (phyA), a photoreceptor that mediates far-red and very low light responses, was previously found to interact with CRY1, but this interaction was not demonstrated to be light-dependent (23). A direct interaction was also shown between phyB and cryptochrome 2 (CRY2), where phyB and CRY2 were found to interact in nuclear speckles formed by light, but co-immunoprecipitation studies did not demonstrate light dependence (24).

In this study, we sought to identify novel proteins that interact in a light-dependent manner with phyB. Although most light-dependent interactions with phytochrome occur with the Pfr form, we speculated that there could exist additional interactions specific to the Pr form. We thus carried out yeast two-hybrid screens with both the Pr and the Pfr forms of phyB, allowing identification of Arabidopsis proteins that specifically interact with these conformations. Here, we report a novel interaction between the phyB and CRY1 photoreceptor proteins that is specific to the Pr state of phyB and is disrupted by light stimulation of either phyB or CRY1. This light-dissociated interaction may represent a novel mechanism for cross-talk between blue and red/far-red signaling pathways.
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EXPERIMENTAL PROCEDURES

Strains, Plasmids, and Reagents—For two-hybrid studies, strains AH109 (MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, lys2::GAL1-lacZ, GAL1-TATA-HIS3, GAL2-TATA-ADEN, URA3::MELI-lacZ, MEL1-TATA-lacZ, MELI) and Y187 (MATa, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, gal80Δ, met-, URA3::GAL1-lacZ, MEL1) were grown (Clontech). The Arabidopsis library was generated from homologous recombination in plasmid pGADT7rec (Clontech) from a mixture of genetic backgrounds using the Matchmaker library construction and screening kit (Clontech) and showed a titer of 1.8 × 10^7 independent clones. The construct phyB-N1Gal4BD in vector D153 was a gift from Peter Quail. The initial CRY1 hit was a full-length CRY1 protein in-frame with the Gal4 activation domain (i.e., GINAEWPL-WPGMSGSV, where the CRY1 sequence is indicated in boldface and the vector is in italics). CRY1 mutants were generated in pGADT7rec by homologous recombination in yeast. SPA1 was amplified from Arabidopsis cDNA and cloned into the two-hybrid bait vector pDBTrp (25). Phycocyanobilin (PCB) was extracted from Spirulina using a protocol provided by Peter Quail and Jim Tepperman, also described in Toettcher et al. (26). Light sources used were a custom-built blue LED array (461 nm), a red LED array (660 nm, obtained from Heelspur) and a green LED array (532 nm). Yeast requiring assembly of holo-phyB were grown 4 h with PCB to allow incorporation of the chromophore and to allow them to reach log phase. Yeast were then subjected to light treatments, during which time they were incubated an additional 1–2 h. Measurement of β-galactosidase reporter activity was made with 2-nitrophenyl β-d-galactopyranoside (Sigma) as a substrate. Yeast from 1 ml of culture were lysed in 200 µl of Y-PER (Thermo Scientific), and then activity was measured using a standard protocol (27).

Western Blot Analysis—Bait or prey constructs were grown to log phase (A_600 of ~0.8) in synthetic medium. Yeast (~18 optical density units) were lysed in 2% SDS by glass bead disruption (425–600-µm beads, Sigma) or using Y-PER (Thermo Scientific) and then boiled for 3 min in Laemmli sample buffer. Equal amounts of total protein were run on an SDS-PAGE gel and transferred to a nitrocellulose membrane using standard procedures. Western blotting was performed using an anti-Gal4AD monoclonal antibody (Santa Cruz Biotechnology). The secondary antibody used was an IRDye 800CW goat anti-rabbit IgG (LI-COR), and proteins were visualized using an Odyssey infrared imaging system (LI-COR). Alternatively, the secondary antibody was an HRP-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology), and the proteins were visualized after exposure to ECL2 substrate (Thermo Scientific) using a CCD camera.

Co-immunoprecipitation—Full-length phyB was generated using a TnT T7-coupled wheat germ lysate expression system (Promega), using phyB-HA in vector pCMX-PL2 (a gift from Meng Chen). Upon phyB expression, PCB was added to the mixture at a final concentration of 100 µM under a green safe light and incubated in the dark for 1 h. For preclearing, 200 µl of the solution was combined with protein A-agarose beads in 600 µl of immunoprecipitation buffer (PBS containing 0.01% Triton X-100, 0.1% β-mercaptoethanol, and 1 mM PMSF) and incubated for 1 h in the dark. For CRY1 expression, BL21-DE3 bacteria expressing pET30a CRY1(1–551) were induced with 0.5 mM isopropyl-1-thio-β-d-galactopyranoside overnight at 29 °C. Bacteria lysates were lysed using B-PER (Thermo Scientific) containing 1 mM PMSF and 0.1% β-mercaptoethanol, and then 1 ml of lysate was incubated with 200 µl of washed protein A-agarose beads and 75 µl of S-tag antibody (Abcam) for 2 h at 4 °C in the dark. The resulting bead-protein conjugates were washed twice in immunoprecipitation buffer, and then 65 µl of the washed bead-protein slurry was added to 260 µl of preclear phyB lysate and incubated for 2 h in the dark. Samples exposed to light underwent blue light irradiation (3 min, 72 µmol m^{-2} s^{-1}) at the start of the immunoprecipitation and just prior to and during washing. Samples were washed three times and eluted in SDS sample buffer by boiling 5 min. Proteins were run on an SDS-PAGE gel, transferred to nitrocellulose, and then blocked and incubated overnight at 4 °C with an anti-HA antibody (Covance). The secondary antibody was HRP-antimouse (Sigma). Blots were exposed to Pierce ECL2 substrate (Thermo Scientific) and imaged using a CCD camera.

RESULTS

To identify novel interacting partners of phyB, we carried out a yeast two-hybrid screen using an Arabidopsis library. Phyto-
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FIGURE 1. CRY1 interacts directly with phyB in an interaction regulated by PCB and light. A, CRY1 and phyBNT were tested for interaction using yeast two-hybrid. Yeast were incubated in the dark in the presence or absence of PCB for 4 h and then assayed for β-galactosidase reporter activity. Bait-BD and AD-prey constructs tested are indicated below the graph. The control vectors contained bait (D153) or prey (pGADT7rec) vectors with no insert. The error bars represent S.D. B, co-immunoprecipitation of phyBNT and CRY1. A bacterial lysate containing overexpressed S-tagged CRY(1–551) was combined with in vitro translated HA-tagged phyBNT, and CRY1 was immunoprecipitated using an anti-S-tag antibody under blue light (1-s pulses every 3 min, 72 µmol m−2 s−1) or dark conditions. The immunoblot was probed with an anti-HA antibody (top panel) or with an S-tag antibody (bottom panel). The lanes labeled input contain phyBNT or CRY1 lysates run directly on the gel and not immunoprecipitated.

chromes require a bilin chromophore, phytochromobilin, to mediate light responses. Although this chromophore is not present in yeast, incubation of yeast with phycocyanobilin (PCB), a bilin from Spirulina, allows reconstitution of functional phyB holoenzyme (28). We thus carried out two-hybrid screening in the presence of 5 µM PCB to identify proteins that interact with holo-phyB. We used the amino terminus of phyB (residues 1–621, phyBNT) as bait because a full-length version of phyB bait was found to be self-activating and stimulated the two-hybrid reporter on its own (data not shown). The screen was carried out under red light or dark conditions and in the presence or absence of PCB. From the + PCB plates that were incubated in the dark, we identified full-length CRY1 as a novel, PCB-dependent interacting partner of phyB (Fig. 1A).

To confirm the two-hybrid results and examine the interaction of CRY1 with full-length phyB, we used an in vitro co-immunoprecipitation assay. We used an S-tagged truncated CRY1 (residues 1–551, CRY(551)) as it showed improved expression in bacteria when compared with full-length CRY1. Full-length HA-tagged apo-phyB was generated in vitro using a TnT T7-coupled expression system. Wheat germ lysates containing apo-phyB were first incubated with PCB in the dark to generate holo-phyB and then combined with antibody-conjugated CRY(551) and immunoprecipitated in the dark or under blue light conditions. As shown in Fig. 1B, full-length phyB co-immunoprecipitates with CRY(551), indicating that the full-length phyB protein also interacts. Samples exposed to blue light showed reduced levels of immunoprecipitated phyB-HA when compared with those incubated in the dark, indicating that the interaction is dependent on the light activation state of CRY1.

Our initial studies suggested that the interaction between phyB and CRY1 is dissociated by light. To further explore the light dependence of the interaction for each photoreceptor, we used the yeast two-hybrid assay. In the presence of chromophore, phyB converts from a Pr state in dark or far-red light to a Pfr state with red light illumination. The Pfr and Pr states are structurally distinct; the Pfr, but not Pr, form of phyB interacts with the PIF family of basic helix-loop-helix transcription factors to induce expression of downstream proteins (29–31). To determine whether CRY1 interacts specifically with the Pfr or Pr form of phyB, we examined the red light dependence of the interaction. After incubating cells for 4 h to allow PCB uptake and generate holo-phyBNT, samples were subjected to various light treatments for 2 h, and then reporter activity was determined (Fig. 2A). We observed a 7-fold difference between the red- and far-red-treated samples that was reversed by red/ far-red pulses, indicating that CRY1 binds specifically to the Pr form of phyBNT.

Using blue and far-red light treatments, we examined whether the interaction is regulated by CRY1 photoactivation. To test light dependence, samples were exposed to periodic blue light pulses (1-s duration). Because a previous study of purified CRY1 had suggested that the signaling state extends for several minutes (τ1⁄2 ~ 5 min) (32), a rate that is consistent with the observed reversion rate of the CRY2/CIB1 interaction (τ1⁄2 ~ 5.5 min) (33), we exposed samples to light pulses delivered every 3 min, conditions that would maintain on average >90% of CRY1 protein in the light-stimulated form over this time period. Because blue light can also stimulate phyB (34), every pulse of blue light was followed immediately by a 30-s pulse far-red light, thus maintaining CRY1 in the light-activated state while converting any blue light-activated phyB to the Pr state. Cells treated in this manner yielded reduced levels of interaction similar to that obtained with red light (Fig. 2A), indicating that phyB does not interact with the light-stimulated form of CRY1.

To further probe the interaction of CRY1 with Pr phyB, we used a phyB mutant, Y276H. phyB Y276H (phyB(YH)) was initially identified as a constitutively active mutant that results in a light-independent dominant constitutive photomorphogenic (cop) phenotype in Arabidopsis (35). Tyr-276 is known to be of critical importance for phyB photochemistry, and the substitution of histidine at this residue has been proposed to “lock” phyB in the light-activated Pfr state (35). We tested interaction between phyBNT(YH) and CRY1 (Fig. 2B). As a control, we also examined interaction of phyBNT(YH) with PIF3, a protein that interacts with the Pfr conformation of phyB. Although phyBNT-
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**FIGURE 2. phyB/CRY1 interaction is disrupted by red or blue illumination.** A, yeast expressing phyBNT-BD and AD-CRY1 were incubated with PCB for 4 h in the presence of red light pulses (1-min pulses, 660 nm, 154 μmol m⁻² s⁻¹, every 30 min). Samples were then exposed to pulses of far-red (FR, single 3-min pulse, 414 μmol m⁻² s⁻¹), red (R, 1-min pulse every 30 min, 154 μmol m⁻² s⁻¹), red followed by far-red (R/FR, 1-min red pulse followed by 1-min far-red pulse every 30 min), or blue followed by far-red (B/FR, 1-s blue pulse (72 μmol m⁻² s⁻¹) followed by 30-s far-red pulse every 3 min) light for an additional 2 h before analysis of reporter levels. B, constitutively active phyBNT Y276H mutant interacts with PIF3 but not with CRY1. Yeast expressing phyBNT-BD with a Y276H mutation (phyBNT(YH)) and AD-CRY1 were grown for 4 h in the dark in the presence or absence of PCB and assayed for α-galactosidase activity. C, phyB(101–621) interacts with CRY1 but not PIF3. Yeast cultures expressing phyB(101–621)-BD and either AD-PIF3 or AD-CRY1 were incubated with PCB for 4 h in the dark. Samples were kept in the dark or exposed to a single pulse of red light (R) and then incubated an additional hour before analysis of reporter levels. D, phyB(101–621) interaction with CRY1 is light-dependent. Cultures expressing phyB(101–621)-BD and AD-CRY1 were incubated with PCB and subjected to light treatments as in A and then analyzed for reporter activity after 4 h. E, FAD chromophore binding is required for phyB interaction with CRY1. Yeast cultures expressing phyBNT-BD and AD-CRY1, vector controls, or phyBNT-BD and AD-CRY1 D390A were grown 4 h in the dark (± PCB) and assayed for α-galactosidase activity. The inset shows expression of CRY1 D390A in yeast. The error bars on all bar graphs represent S.D. (n = 3).

T(YH) interacted robustly with PIF3 in the dark, confirming the constitutive phenotype of this mutant, it did not interact with CRY1, providing additional support that CRY1 does not interact with the Pfr state of phyB (Fig. 2B).

We explored additional differences between the phyB/CRY1 and phyB/PIF interactions using a phyB truncation mutant. A previous study had shown that the N-terminal PI domain of phyB contained within the first 100 residues is essential for binding to PIF3 (36). A truncation mutant missing this region (phyB(101–621)) failed to interact with PIF3 in dark or red light but still interacted with CRY1 in the dark (Fig. 2C), indicating that CRY1 and PIF3 interact with phyB at distinct sites in the respective Pr and Pfr forms. Additional analysis showed that the interaction of CRY1 with phyB(101–621) shows similar light dependence as with phyBNT (Fig. 2D), with red or blue/ far-red light treatment dissociating the interaction.

Our initial studies in yeast established that chromophore binding to phyB is essential for the phyB/CRY1 interaction, but did not reveal the importance of chromophore binding to CRY1 because the chromophore, FAD, is present in yeast. To examine the importance of FAD binding to CRY1, we used a mutant that disrupts FAD binding, D390A. This mutation was previously generated in the homologous region of CRY2 (D387A), where it resulted in a CRY2 protein that expressed well but did not bind FAD (12). We examined interaction of phyB with CRY1 (D390A). Although CRY1 D390A expressed well, it failed to interact with phyB (Fig. 2E), providing strong support that FAD conjugation to CRY1 is required for phyB interaction.

**Characterization of phyB/CRY1 Interaction Using Cryptochrome Truncations**—Cryptochromes contain conserved N-terminal domains that have homology to DNA photolyases and divergent C-terminal domains. Studies have indicated that the chromophore-binding N-terminal domains mediate light responsiveness, but that the C-terminal domains alone can act constitutively to mediate signaling responses in plant cells (37). To map the location of interaction of phyB on CRY1, we generated N-terminal and C-terminal truncations of CRY1 (Fig. 3A). The C termini of cryptochromes are thought to be disordered (38), and we were unable to observe expression or interaction of a C-terminal fragment (CRY1(505–681)) fused to the Gal4 activation domain. An N-terminal truncation construct containing only the PHR domain of CRY1 (residues 1–506, CRY(506)), which expressed much better than full-length CRY1 (Fig. 3A, inset), interacted well with phyB in the dark, as did a slightly longer truncation (CRY(551), amino acids 1–551), indicating that the PHR domain of CRY1 is sufficient for inter-
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FIGURE 3. Mapping of phyB/CRY1 interaction using CRY1 mutants. A, domain mapping of interaction using CRY1 truncations. CRY(551) and CRY(506), containing residues 1–551 or 1–506 of CRY1, respectively, were tested for interaction with phyB by yeast two-hybrid. Cultures were incubated in the presence or absence of PCB for 4 h in the dark and then assayed for β-galactosidase reporter activity. The inset shows expression of full-length CRY1 when compared with mutant constructs analyzed by Western blot. B, light dependence of phyB interaction with CRY1 truncation mutants. Yeast expressing phyBNT-BD and the indicated AD-CRY1 truncations were tested for interaction under different light conditions. Samples were given similar light treatments as in Fig. 2A, with exposure to far-red (FR), red (R), or blue followed by far-red (FR/FAR) light flashes for 2 h. C, constitutively active phyB Y276H interacts with CRY1(506) but not CRY1(551). Yeast expressing phyBNT-BD and the indicated AD-CRY1 truncations were grown for 4 h in the dark in the presence or absence of PCB and assayed for β-galactosidase activity. D, phyBNT does not interact with the PHR domain of CRY2. CRY1 or CRY2PHR (amino acids 1–498) was tested for interaction with phyBNT in the presence or absence of PCB. The inset shows comparative expression levels of CRY1 and CRY2PHR by Western blot. The error bars on the bar graphs represent S.D. (n = 3).

FIGURE 4. Constitutively active CRY1 L407F mutant interacts with SPA1, but not with phyB. A, yeast cultures expressing AD-CRY1 L407F were tested for interaction with BD-SPA1. Samples were incubated for 2 h in the dark or with blue light (B) pulses (1-s pulse every 3 min, 72 μmol m⁻² s⁻¹) and then assayed for β-galactosidase activity. B, testing of phyBNT-BD interaction with wild-type or L407F AD-CRY1. Samples were incubated for 4 h in the dark and then assayed for β-galactosidase activity. The inset shows expression of full-length CRY1 and L407F CRY1 by Western blot. The error bars on the bar graphs represent S.D. (n = 3).

Action (Fig. 3A). Interestingly, although the interaction of CRY(551) with phyB remained strictly dependent on PCB, CRY(506) showed considerable basal interaction with apo-phyB, suggesting that the C terminus of CRY1 plays a regulatory role preventing interaction with non-Pr conformations of phyB.

We next probed the light dependence of the interactions of CRY(551) and CRY(506) with phyB. Both CRY(506) and CRY(551) retained red light dependence in their interaction with phyB, but CRY(506) showed significantly elevated reporter activity in red light (Fig. 3B), indicating that residues 507–551 of CRY1 are important for preventing binding to phyBPr. We further confirmed these results using the constitutively active phyB Y276H mutant, which we found to interact strongly with CRY(506) even in the absence of PCB, but which did not interact with CRY(551) (Fig. 3C). To probe the interaction of phyB with blue light-stimulated CRY1 truncations, we stimulated samples with pulses of blue light followed immediately by far-red light, thus activating CRY1 but maintaining phyB in the Pr state (Fig. 3B). Using the same light pulse conditions described in Fig. 2A (pulses every 3 min), we saw minimal interaction of either truncation construct with phyB, indicating that the light-stimulated form of CRY1 PHR, without any C-terminal extension, cannot bind phyB (Fig. 3B).

Significant sequence homology exists in the N-terminal domain of cryptochromes CRY1 and CRY2 (58.3% sequence identity over the first 492 residues), and previous studies have demonstrated an interaction between phyB and CRY2 (24). We thus examined the interaction of phyBNT with the N-terminal domain of CRY2 (CRY2PHR, amino acids 1–498). Although this protein expressed well, we were unable to observe interaction of CRY2PHR with phyBNT (Fig. 3D). These results suggest that CRY1 and CRY2 may interact with different regions of phyB, a surprising result given the considerable N-terminal sequence homology, but one that has some precedent. Recently, the protein SPA1 was found to interact with CRY1 and CRY2 at different sites, interacting with CRY1 through the C-terminal extension and with CRY2 through the PHR domain (9–11). Further experiments using chimeras between CRY1 and CRY2 will be useful for mapping of the regions important in conferring specificity of binding to phyB.

A Constitutively Active CRY1 Mutant Does Not Interact with phyB—Recently, a CRY1 mutant, L407F, was identified in a plant screen as a “gain-of-function” allele (40). The L407F mutation was shown to enhance CRY1 responses in the dark and increase its sensitivity to blue light, but the mutant was also proposed to have increased sensitivity to phyB signaling pathways (40). The mutation is in the PHR domain near the chromophore-binding site, and we speculated that this mutation may mimic photoexcited CRY1 and affect the binding of CRY1 to phyB. We tested interaction of CRY1 L407F with phyB (Fig. 4). As a control, we also tested for interaction with SPA1, a protein that was recently shown to interact with the C terminus of blue light-stimulated CRY1 (9, 10). Indeed CRY1 L407F interacts with SPA1 both in the light and in the dark (Fig. 4A), establishing that this mutant likely mimics the light-stimulated conformation of cryptochrome. In contrast, CRY1 L407F did not interact with phyB (Fig. 4B).
not interact with phyB (Fig. 4B), providing further support that phyB can only interact with the nonstimulated (dark) state of CRY1 and raising the possibility that the enhanced phyB signaling seen in the L407F mutants could be due to the altered phyB/CRY1 interaction.

**DISCUSSION**

Here, we show that a direct interaction occurs *in vitro* and in a yeast heterologous system between unstimulated CRY1 and phyB Pr. The functional consequences of this interaction in plants are unknown. *In vivo*, this interaction could serve to directly regulate activity or control binding to downstream effectors. For example, phytochrome and cryptochrome could form part of a larger scaffolding complex allowing local concentration of signaling molecules and cross-talk between multiple effectors (41, 42). Such a complex could also contain other photoreceptors; for example, both phyB and CRY1 have been found to bind to ADO1/ZTL/LKP1, a LOV domain-containing photosensory protein (43).

Previous genetic studies have speculated on interaction between cryptochrome and phytochrome pathways. One early study found that irradiating plant seedlings with blue, but not red, light caused subsequent illumination with red light to have a larger effect on hypocotyl growth (22). In studies of hypocotyl growth, *cry1* and *phyb* mutants were used to show synergistic interaction of CRY1 and phyB pathways under specific light conditions (14, 16, 44). phyB and cryptochromes were also found to synergistically regulate root greening in a process where the C terminus of phyB was found to be dispensable (20).

In a previously mentioned study with the L407F CRY1 mutant, plants showed a substantial increase in the rate of red light-dependent germination, which was completely reversed by far-red light (40). As the seed germination pathway is thought to be controlled by phytochromes but not cryptochromes (and by phyB in response to red light), the authors speculated that CRY1 L407F increases the sensitivity of phyB signaling in this pathway. This conclusion, along with our results that CRY1 L407F fails to bind phyB in the dark, suggests the possibility that CRY1 could negatively regulate phyB activity. Although these and other possibilities have yet to be fully explored, it seems possible that direct phytochrome-cryptochrome interactions may play a significant role in mediating cross-talk between red/far-red- and blue light-regulated pathways in plants.

In plants, CRY1 and phyB colocalize in the cytosol in the dark, and thus, it is possible that such an interaction may occur in the cytosol. Upon light stimulation, Pfr phyB moves into the nucleus (45, 46), although the Pr and Pfr forms of phyB contain overlapping absorbance spectra, and some phyB remains in the cytosol even in the light (47). Although CRY1 remains mostly in the cytosol in light, a small amount of CRY1 is nuclear-localized (48), and CRY1 appears to have separable functions in the nucleus and cytoplasm (49). Thus, the possibility also exists for formation of a CRY1-phyB complex in the nucleus (upon phyB reversion to the Pr state). Follow-up studies will be needed in plants to investigate these possibilities.

Our work provides the first demonstration of a direct light-dependent interaction between phytochromes and cryptochromes, as previously reported interactions between phyB/CRY2 and phyA/CRY1 were not shown to be directly dependent on the photostimulated state of the proteins. The phyB interaction also represents the first interaction of any *Arabidopsis* cryptochrome that is specific to the dark state as all previously identified light-dependent interactions occur with the light-stimulated conformation. The specific light- and ligand-dependent requirements of this interaction may have hindered its discovery as an interaction would only be detected under dark or far-red light conditions and in the presence of chromophore. Live cell imaging of the interaction would also be difficult as imaging using typical fluorescent reporters such as CFP, GFP, YFP, or RFP would dissociate the interaction.

We found that the truncated CRY1 PHR domain interacts preferentially with phyB Pr, but that this domain also interacts to some degree with both apo-phyB and phyB Pfr. This result suggests that the C terminus of CRY1 regulates interaction with phyB by preventing interaction with the apo and Pfr forms. We propose that in the apo or Pfr states, phyB is prevented from interacting with CRY1 by the C-terminal extension, but that conversion of phyB to the Pr state removes the steric conflict and allows binding (Fig. 5). Our studies with the CRY1 PHR domain (amino acids 507–551) of critical importance in conferring light specificity to the interaction, prevent binding to apo-phyB and phyB Pfr. On the other hand, although this short extension is essential for preventing interaction with non-Pr phyB, it does not play a role in conferring light specificity to CRY1; the truncated CRY1 PHR domain alone, without this extension, shows tight blue light dependence in interaction with phyB Pr.

Although the function of the CRY1/phyB interaction in plants remains to be elucidated, our work in yeast shows that these proteins can mediate tight light-dependent control of protein transcription in a heterologous system. A number of recent studies have explored using plant photoreceptor proteins to mediate light-dependent protein interactions with pre-
cise spatial and temporal resolution for control of cellular processes. For instance, an interaction between phyB and PIF3 was used for precise, light-inducible control of transcription in yeast (50), an interaction between phyB and PIF6 was used to inductively activate small GTPases using light (51), an interaction with CRY2 and CIB1 was used to generate a light-activated Cre recombinase (33), and an interaction between an engineered phototropin LOV2 domain and a PDZ domain was used to control cell polarity (39). As phyB and CRY1 interact in dark or far-red light and dissociate in red and blue light, they may well complement other optogenetic modules such as the phyB/PIF6 system (51), which respond in the opposite manner.

Acknowledgments—We thank Peter Quail for providing the DI53-phyBNT construct. We also thank Meng Chen and Rafaelo Galvao for providing the full-length phyB-HA construct for in vitro expression of phyB and for helpful discussions.

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