HHS Public Access

Author manuscript

Science. Author manuscript; available in PMC 2018 October 11.

Published in final edited form as:

Science. 2016 October 21; 354(6310): 343–347. doi:10.1126/science.aaf9030.

Photoactivation and inactivation of *Arabidopsis* cryptochrome 2

Qin Wang[#1,2], **Zecheng Zuo**[#1,3], **Xu Wang**[#1,2], **Lianfeng Gu**[1], **Takeshi Yoshizumi**[4], **Zhaohe Yang**[1], **Liang Yang**[1,3], **Qing Liu**[1,2,3], **Wei Liu**[3], **Yun-Jeong Han**[5], **Jeong-Il Kim**[5], **Bin Liu**[6], **James A. Wohlschlegel**[7], **Minami Matsui**[4], **Yoshito Oka**[1,4,†], and **Chentao Lin**[1,2,†]

[1]Basic Forestry and Proteomics Research Center, Fujian Agriculture and Forestry University, Fuzhou 350002, China.

[2]Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA 90095, USA.

[3]College of Plant Science, Jilin University, Changchun 130062, China.

[4]Biomass Engineering Research Division, RIKEN, Kanagawa 230-0045, Japan.

[5]Department of Biotechnology and Kumho Life Science Laboratory, Chonnam National University, Gwangju 61186, Republic of Korea.

[6]Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 10081, China.

[7]Department of Biological Chemistry, University of California, Los Angeles, CA 90095, USA.

[#] These authors contributed equally to this work.

Abstract

Cryptochromes are blue-light receptors that regulate development and the circadian clock in plants and animals. We found that *Arabidopsis* cryptochrome 2 (CRY2) undergoes blue light–dependent homodimerization to become physiologically active. We identified BIC1 (blue-light inhibitor of cryptochromes 1) as an inhibitor of plant cryptochromes that binds to CRY2 to suppress the blue light–dependent dimerization, photobody formation, phosphorylation, degradation, and physiological activities of CRY2. We hypothesize that regulated dimerization governs homeostasis of the active cryptochromes in plants and other evolutionary lineages.

The *Arabidopsis* genome encodes two cryptochromes (CRYs), CRY1 and CRY2, which act as photoreceptors mediating blue-light inhibition of hypocotyl elongation and long-day (LD) stimulation of floral initiation (1–4). CRYs regulate light responses by interacting with CRY signaling partners, such as CIBs (cryptochromeinteracting basic helix-loop-helixes) and COP1/SPA (constitutive photomorphogenic 1/suppressor of PhyA-105), to regulate blue light-responsive gene expression changes and photophysiology responses (5–7). Homodimers are the physiologically active form of plant CRYs, but it has remained unclear how light affects CRY dimerization or photoactivation (8, 9). Photoactivated CRYs are also

[†]**Corresponding author.** yoshitooka@fafu.edu.cn (Y.O.); clin@mcdb.ucla. edu (C.L.).

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/354/6310/343/suppl/DC1

expected to undergo inactivation to maintain sustainable photosensitivity of the cell, which may be accomplished by thermal relaxation or other mechanisms (10).

We reasoned that identification of possible negative regulators of CRYs may help to elucidate the photoactivation and inactivation mechanisms of CRYs. We therefore screened for such genes in the *Arabidopsis* FOX (full-length cDNA overexpressing gene hunting system) library, which contains transgenic lines individually overexpressing about 10,000 independent full-length *Arabidopsis* cDNAs (11). We identified multiple FOX lines (*bic1D-1, bic1D-2*, and *bic1D-3*) that overexpress the same gene and exhibit similar phenotypes resembling that of the *cry1cry2* mutant (12), including blue light–insensitive hypocotyl growth, reduced blue-light stimulation of anthocyanin accumulation and gene expression, and delayed floral initiation in LD photoperiod (Fig. 1 and figs. S1 and S2). The corresponding FOX gene was identified and referred to as *BIC1* (*Blue-light Inhibitor of Cryptochromes 1*, At3G52740), which has an *Arabidopsis* homolog referred to as *BIC2* (At3G44450) (fig. S3). BIC1 and BIC2 appear to be nuclear proteins (fig. S4).

Independent transgenic lines overexpressing various BIC fusion proteins under control of either the constitutive promoters or the respective *BIC* promoters recapitulated the light-insensitive phenotypes of the BIC1-overexpressing FOX lines and the *cry1cry2* mutant (Fig. 1 and fig. S1). The *bic1* and *bic2* monogenic mutants showed no obvious phenotypic alterations, whereas the *bic1bic2* double mutant and the *BIC* RNA interference lines exhibited phenotypes mimicking that of the CRY-overexpressing plants (Fig. 1 and figs. S2, S5, and S6), which suggests that BIC1 and BIC2 act redundantly to inhibit the function of CRYs. Analyses of the genetic interactions between the *BIC* and *CRY* genes support this hypothesis (fig. S7): Neither *bic1bic2* mutation nor BIC overexpression altered the blue light–insensitive phenotypes of the *cry1cry2* mutant (fig. S7, D to F), whereas overexpression of BIC1 or BIC2 effectively suppressed the blue light–hypersensitive phenotype of plants overexpressing CRY2 (fig. S7G).

The *cry1cry2* mutation and BIC1 overexpression caused similar transcriptome changes in response to blue light (Fig. 2 and table S2), which suggests that BICs inhibit early photoreactions of CRYs. As reported previously (13–16), CRY1 and CRY2 underwent blue light–dependent phosphorylation and the phosphorylated CRY2 was degraded rapidly (Fig. 3, A to E, and fig. S8, upshifted bands). However, neither blue light–dependent phosphorylation of CRYs nor blue light–dependent degradation of CRY2 (15, 16) was detected in the plants overexpressing BIC1 or BIC2 (Fig. 3, A to E, and fig. S8); hence, BICs inhibit CRY phosphorylation. Consistent with those results, the *bic1bic2* mutant plants grown in blue or white light accumulated lower levels of CRY2 (Fig. 1, G to J), which seems physiologically hyperactive because the *bic1bic2* mutant is hypersensitive to blue light (Fig. 1, A to C) The BIC-overexpressing plants grown in blue or white light accumulated higher levels of CRY2 (Fig. 1, G to J), which appears mostly inactive because the BIC-overexpressing plants are insensitive to blue light (Fig. 1, A to C).

BICs also inhibit the blue light-induced formation of CRY2 photobodies (Fig. 3 and fig. S9), which is another early photoreaction of CRY2 (17–19). Figure 3 shows that CRY2-YFP (CRY2 fused to yellow fluorescent protein) formed photobodies within 60 s of blue-light

exposure in the nucleus of the wild-type *Arabidopsis* protoplasts, whereas no CRY2-YFP photobodies were detected in the protoplasts overexpressing BIC1 or BIC2 after blue-light illumination for up to 60 min (Fig. 3, F and H). In both darkness and light, BIC1 interacted with CRY2 in yeast or HEK293T (human embryonic kidney) cells via the conserved CRY-interacting domain of BIC1 and the photolyase homologous region of CRY2 (Fig. 4, B and F, and fig. S10). The results of the coimmunoprecipitation (co-IP) experiments indicate that blue light enhances BIC1-CRY2 interaction in plants. BIC1 coimmunoprecipitated CRY2 in seedlings exposed to blue light, but little BIC1-CRY2 complex was coprecipitated in the dark (Fig. 4A). This observation suggests that BIC1 might interact with photoexcited CRY2 to inhibit its activity.

Homodimers are the physiologically active form of plant CRYs (8, 9), but the effect of light on CRY dimerization has not been detected in previous studies (9, 20). This could be explained by, among other interpretations, light-independent CRY dimerization or masking effects of regulatory proteins, such as BICs, on the light-dependent CRY dimerizationx (9, 20). We reexamined the blue-light dependence of CRY2 dimerization using multiple approaches. In the first experiment, we coexpressed Flag-CRY2 and Myc-CRY2 in HEK293T cells (21–24) and tested the interaction between the two differentially tagged CRY2s by co-IP assay. In the absence of blue light, antibody to Flag coprecipitated little Myc-CRY2 from HEK293T cells expressing similar amounts of Flag-CRY2 and Myc-CRY2 (Fig. 4B and fig. S12A). In contrast, antibody to Flag coprecipitated increasing amounts of Myc-CRY2 from HEK293T cells exposed to blue light for 10 to 120 min, thereby demonstrating the light-dependent CRY2 homodimerization in the absence of BIC or other plant proteins (Fig. 4B and fig. S12A). In a control experiment, human CRYs (hCRY1 and hCRY2) exhibited light-independent dimerization (Fig. 4C and fig. S12D), which appears consistent with the light-independent activity of hCRYs in cultured HEK293T cells (25). The blue light–dependent CRY2 dimerization was also detected by the two-hybrid assay in yeast cells (fig. S11) and the bimolecular fluorescence complementation (BiFC) assay in *Arabidopsis* protoplasts (Fig. 3, G and I, and fig. S9). The BiFC assays revealed a more complex behavior of the intermolecular interaction of CRY2: The BiFC signal resulting from the interaction between nYFP-CRY2 (N terminus of YFP fused to CRY2) and cCFP-CRY2 (C terminus of cyan fluorescent protein fused to CRY2) was detected regardless of blue-light treatment, whereas the fluorescent photobodies resulting from the interaction between nYFP-CRY2 and cCFP-CRY2 were detected only after blue-light treatment (Fig. 3, G and I, and fig. S9).

Because photoexcited CRY2 is known to oligomerize into photobodies (18, 19, 23, 24), it is possible that in darkness nYFP-CRY2 and cCFP-CRY2 interact weakly in a manner sufficient to reconstitute the fluorescent BiFC signal but insufficient to enable oligomerization of CRY2 into photobodies. In response to blue light, nYFP-CRY2 and cCFP-CRY2 may interact with higher affinity to reconstitute not only the fluorescent BiFC signals but also fluorescent photobodies. To test this interpretation, we used co-IP assays to examine effects of blue light on CRY2 dimerization or oligomerization in plants coexpressing GFP-CRY2 (CRY2 fused to green fluorescent protein) and Myc-CRY2 (Fig. 4D). Antibody to GFP coprecipitated little Myc-CRY2 in etiolated seedlings, whereas the same antibody coprecipitated abundant Myc-CRY2 in etiolated seedlings exposed to blue

light for 5 to 10 min (Fig. 4D). Similarly, the blue light–specific CRY2 homodimerization was also detected in adult plants (fig. S12B). We conclude that the high-affinity CRY2 dimerization is a photoreaction in plant cells.

We next investigated the effects of BIC1 on blue light–dependent CRY2 dimerization using the multiple assays described above. We first examined dimerization of Flag-CRY2 and Myc-CRY2 in HEK293T cells coexpressing BIC1. Figure 4B shows that in the cells coexpressing BIC1, antibody to Flag coprecipitated only residual Myc-CRY2 even after blue-light treatment for up to 120 min, thereby demonstrating that BIC1 suppresses blue light–dependent CRY2 dimerization. The specificity of BIC1 inhibition of CRY2 dimerization is verified by the result that CIB1, which also interacts with photoexcited CRY2 (6, 26), did not inhibit blue light–dependent CRY2 dimerization (Fig. 4E). The BIC1 inhibition of blue light–dependent CRY2 dimerization was also detected by the trihybrid assay in yeast cells (fig. S11) and by the BiFC photobody assay in *Arabidopsis* cells (Fig. 3, G and I, and fig. S9). Because physiologically active CRY2 dimers or oligomers are expected to interact with their signaling partners, such as CIB1 and SPA1 (7), we used co-IP assays to test the effects of BIC1 on the blue light–dependent CRY2-CIB1 and CRY2-SPA1 interactions. As expected, coexpression of BIC1 suppressed the blue light–dependent CRY2-CIB1 interaction (Fig. 4F) and CRY2-SPA1 interaction (fig. S12C), which explains how inhibition of CRY dimerization by BIC1 suppresses CRY2-dependent photoresponses of plants.

Homodimerization appears to be a common mechanism of photoreceptors (27–29). Our study supports a hypothesis that plant CRYs exist as inactive monomers in the absence of light, whereas photoexcited CRYs form active homodimers or oligomers that interact with CRY-signaling proteins to trigger transcriptome changes responsible for photomorphogenesis; the BIC proteins interact with CRYs to prevent CRY homodimerization and thereby maintain the appropriate homeostasis of the active and inactive pools of CRYs and sustainability of cellular photosensitivity (fig. S12E). It would be interesting to examine whether photoinsensitive mammalian CRYs and photosensitive insect CRYs undergo circadian phase–dependent and light-dependent dimerization, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Q.W., Y.O., and C.L. are inventors on a patent application (U.S. Serial No. 62/360,862). Supported by NIH grants GM56265 (C.L.) and GM089778 and GM112763 (J.A.W.), Rural Development Administration of Republic of Korea grant PJ01104001 (J.-I.K.), Biomass Engineering Program of RIKEN (M.M.), the Special Postdoctoral Researcher Program of RIKEN (Y.O.), National Natural Science Foundation of China grants 31500991 (Q.W.), 31570674 (L.G.), 31422041 (B.L.), and 31371411 (Z.Z.), and Fujian 2011 Program (2015-75). We thank K.Shuai, H. Goto, and H. Shimada for technical assistance, and UCLA-FAFU (Fujian Agriculture and Forestry University) Joint Research Center on Plant Proteomics, Haixia Institute of Science and Technology, and Fujian Provincial Key Laboratory of Haixia Applied Plant Systems Biology for institutional support.

REFERENCES AND NOTES

1. Ahmad M, Cashmore AR, Nature 366, 162–166 (1993). [PubMed: 8232555]
2. Guo H, Yang H, Mockler TC, Lin C, Science 279,1360–1363 (1998). [PubMed: 9478898]
3. Cashmore AR, Cell 114, 537–543 (2003). [PubMed: 13678578]
4. Sancar A, Chem. Rev 103, 2203–2237 (2003). [PubMed: 12797829]
5. Yang H-Q et al., Cell 103, 815–827 (2000). [PubMed: 11114337]
6. Liu H et al., Science 322, 1535–1539 (2008). [PubMed: 18988809]
7. Liu H, Liu B, Zhao C, Pepper M, Lin C, Trends Plant Sci. 16, 684–691 (2011). [PubMed: 21983106]
8. Rosenfeldt G, Viana RM, Mootz HD, von Arnim AG, Batschauer A, Mol. Plant 1, 4–14 (2008). [PubMed: 20031911]
9. Sang Y et al., Plant Cell 17, 1569–1584 (2005). [PubMed: 15805487]
10. Shcherbakova DM, Shemetov AA, Kaberniuk AA, Verkhusha VV, Annu. Rev. Biochem 84, 519–550 (2015). [PubMed: 25706899]
11. Ichikawa T et al., Plant J. 48, 974–985 (2006). [PubMed: 17227551]
12. Mockler TC, Guo H, Yang H, Duong H, Lin C, Development 126, 2073–2082 (1999). [PubMed: 10207133]
13. Shalitin D et al., Nature 417, 763–767 (2002). [PubMed: 12066190]
14. Shalitin D, Yu X, Maymon M, Mockler T, Lin C, Plant Cell 15, 2421–2429 (2003). [PubMed: 14523249]
15. Wang Q et al., Mol. Plant 8, 631–643 (2015). [PubMed: 25792146]
16. Yu X et al., Plant Cell 19, 3146–3156 (2007). [PubMed: 17965271]
17. Más P, Devlin PF, Panda S, Kay SA, Nature 408, 207–211 (2000). [PubMed: 11089975]
18. Yu X et al., Plant Cell 21, 118–130 (2009). [PubMed: 19141709]
19. Bugaj LJ, Choksi AT, Mesuda CK, Kane RS, Schaffer DV, Nat. Methods 10, 249–252 (2013) [PubMed: 23377377]
20. Yu X et al., Proc. Natl. Acad Sci. U.S.A 104, 7289–7294 (2007). [PubMed: 17438275]
21. Gao J et al., Proc. Natl. Acad. Sci. U.S.A 112, 9135–9140 (2015). [PubMed: 26106155]
22. Pedmale UV et al., Cell 164, 233–245 (2016). [PubMed: 26724867]
23. Taslimi A et al., Nat. Commun 5, 4925 (2014). [PubMed: 25233328]
24. Ozkan-Dagliyan I et al., J. Biol. Chem 288, 23244–23251 (2013). [PubMed: 23833191]
25. Griffin EA, Jr., Staknis D, Weitz CJ, Science 286, 768–771 (1999). [PubMed: 10531061]
26. Kennedy MJ et al., Nat. Methods 7, 973–975 (2010). [PubMed: 21037589]
27. Rizzini L et al., Science 332, 103–106 (2011). [PubMed: 21454788]
28. Heijde M, Ulm R, Proc. Natl. Acad. Sci. U.S.A 110, 1113–1118 (2013). [PubMed: 23277547]
29. Chen C-H, DeMay BS, Gladfelter AS, Dunlap JC, Loros JJ, Proc. Natl. Acad. Sci. U.S.A 107, 16715–16720 (2010). [PubMed: 20733070]

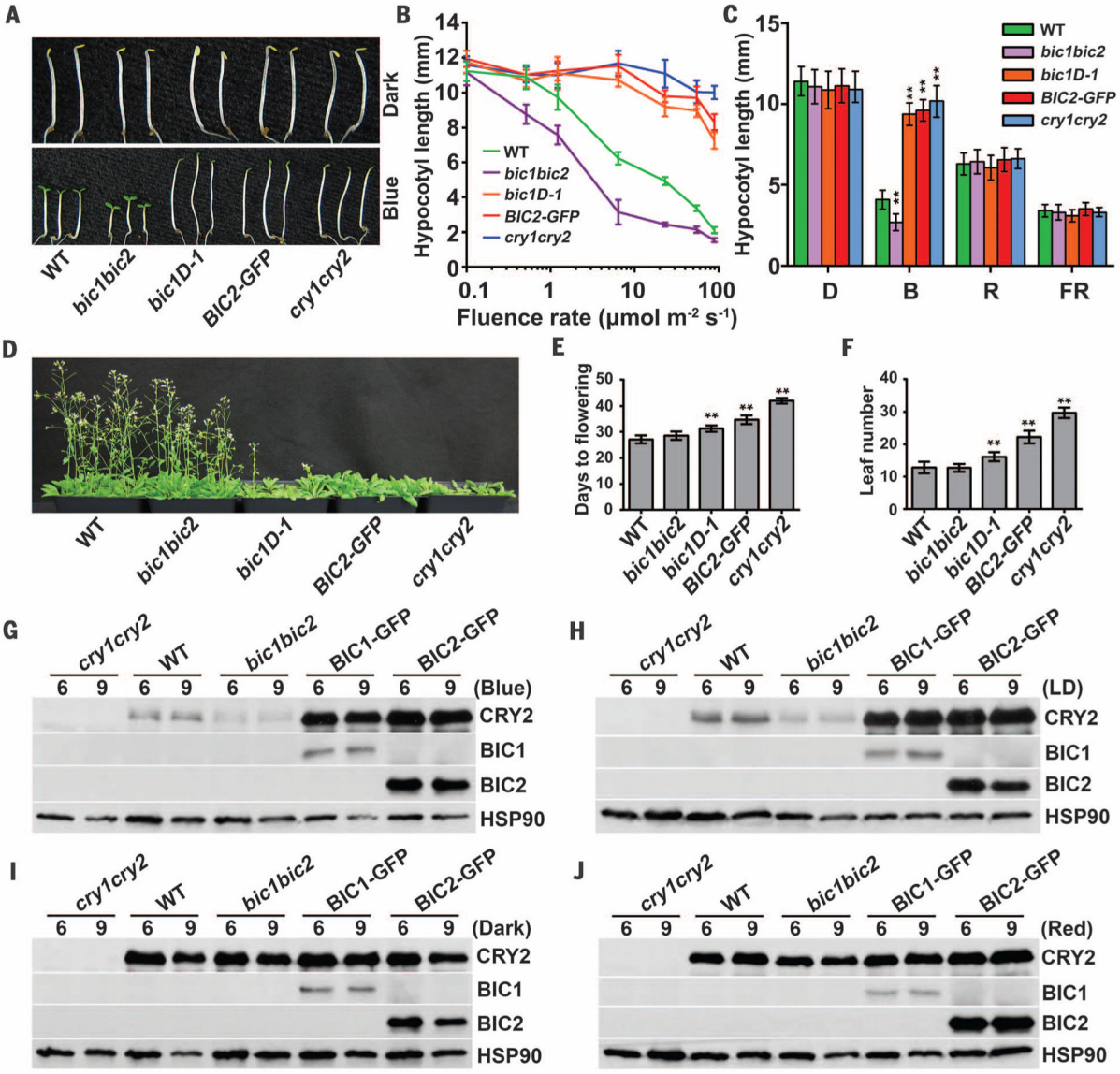

Fig. 1. BIC1 and BIC2 suppress the functions of *Arabidopsis* cryptochromes.

(**A**) Five-day-old seedlings overexpressing BIC1 (*bic1D-1*) or BIC2-GFP (*BIC2-GFP*) and *cry1cry2* or *bic1bic2* mutant seedlings grown in continuous blue light (4 μmol m^{-2} s^{-1}) or dark; WT, wild type. (**B**) Hypocotyl lengths of 5-day-old seedlings grown under continuous blue light with fluence rates of 0.1 to 100 μmol m^{-2} s^{-1}. (**C**) Hypocotyl lengths of indicated genotypes grown in the dark (D), under blue light (B; 10 μmol m^{-2} s^{-1}), under red light (R; 10 μmol m^{-2} s^{-1}), or under far-red light (FR; 5 μmol m^{-2} s^{-1}) for 5 days. (**D**) Plants of indicated genotypes grown in LD photoperiods (16 hours light, 8 hours dark) for 31 days. (**E** and **F**) Days to flowering (E) and number of rosette leaves at the time of flowering (F) of the indicated genotypes grown in LD. (**G** to **J**) Immunoblots of samples prepared from seedlings grown in continuous blue light (30 μ;mol m^{-2} s^{-1}) (G), LD photoperiods (H), darkness (I), or continuous red light (30 μmol m^{-2} s^{-1}) (J) for 6 or 9 days were probed with antibodies to

CRY2, GFP, or HSP90 (loading control). Data in (B), (C), (E), and (F) are means ± SD ($n > 20$); $^{**}P < 0.01$.

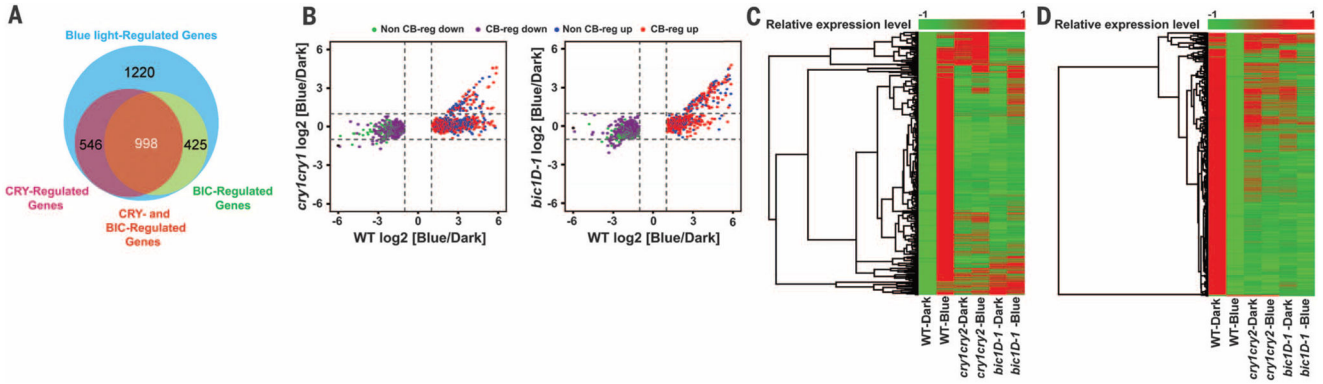

Fig. 2. CRY and BIC regulate similar transcriptome changes in response to blue light.

(**A**) Venn diagram depicting overlaps among blue light–regulated, CRY-regulated, and BIC-regulated genes determined by RNA sequencing. Five-day-old etiolated seedlings were exposed to blue light (20 μmol m^{-2} s^{-1}) or kept in the dark for 2 hours.The blue light–regulated genes are defined as those that showed a factor of >2 change of mRNA [fold change (FC) > 2, $P < 0.01$, false discovery rate <*** 0.01; see table S2 for details] between light- and dark-treated wild-type plants. The blue light–regulated genes that showed 200% reduction of FC or changed significance to $P>0.01$ in *cry1cry2* or *bic1D-1* are defined as CRY-regulated and BIC-regulated genes, respectively; those that showed similar change in both *cry1cry2* and *bic1D-1* are defined as CRY- and BIC-regulated ("CB-reg") genes. (**B**) Scatter-plot showing plots of log2 (FC) of wild-type (*x* axis) versus log2 (FC) of *cry1cry2* or *bic1D-1* (*y* axis). The dashed lines indicate log2 (FC) = 1 and −1. The colored dots (see key at top) indicate up-regulated or down-regulated genes not co-regulated by CRY and BIC1 ("Non CB-reg") and genes that are co-regulated by CRY and BIC1 ("CB-reg"). (**C** and **D**) Hierarchical clustering (GENE-E) of the change of expression of blue light–induced genes (C) and blue light-repressed genes (D) detected in the indicated genotypes. Scale bars at top indicate relative expression levels of mRNA.

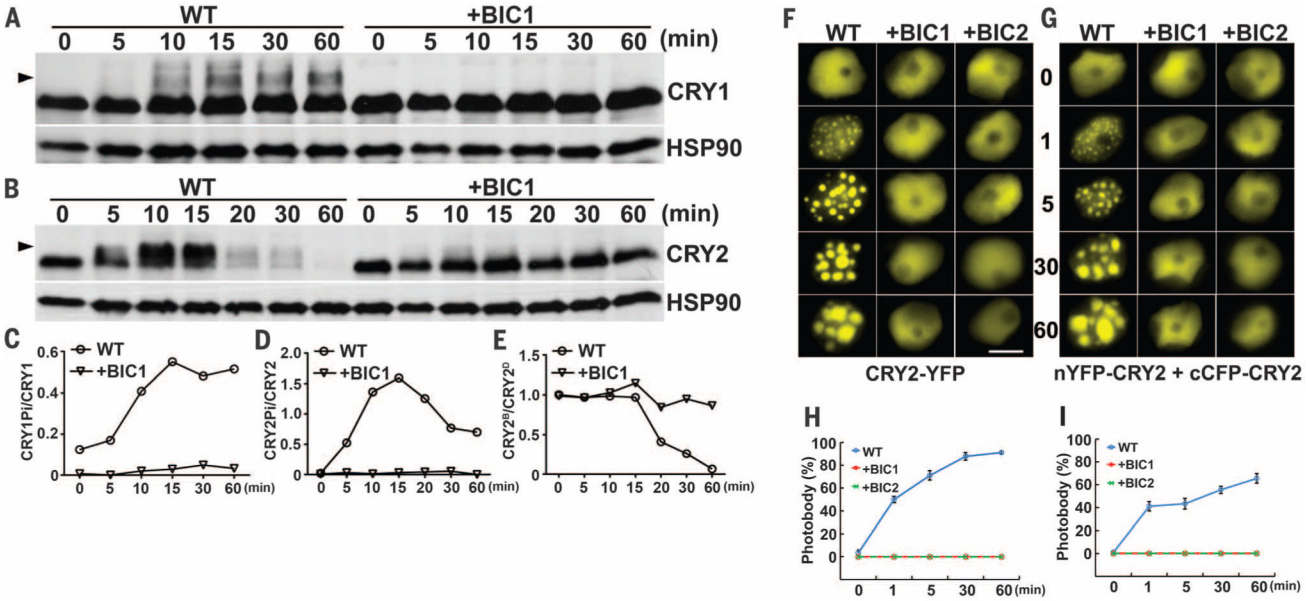

Fig. 3. BIC1 and BIC2 inhibit early photoreactions of cryptochromes.

(**A** and **B**) BIC1 inhibits blue light–dependent phosphorylation of CRY1 and CRY2 and blue light–dependent degradation of CRY2. Immunoblots of samples prepared from 7-day-old etiolated seedlings (WT, wild type; +BIC1, BIC1-overexpressing) exposed to blue light (30 μmol m^{-2} s^{-1}) for the indicated times were probed with antibodies to CRY1, CRY2, or HSP90 (loading control). Arrowheads indicate upshift bands of the phosphorylated CRYs. (**C** to **E**) Quantification of band intensities, showing CRY phosphorylation [(C), CRY1Pi/CRY1; (D), CRY2Pi/CRY2] or CRY2 degradation [(E), $CRY2^B/CRY2^D$]. (**F**) BIC inhibition of CRY2 photobodies. Protoplasts isolated from 4-week-old plants of indicated genotypes were transfected to express CRY2-YFP, exposed to blue light (20 μmol $m^{-2}s^{-1}$) for 0 to 60 min, fixed in 1% formaldehyde, and examined by a fluorescence microscope. Scale bar, 5 μm. (**G**) BiFC assay showing the blue light-independent (dispersed fluorescence) and blue light–dependent (photobodies) interactions between nYFP-CRY2 and cCFP-CRY2. Protoplasts transfected to express nYFP-CRY2 and cCFP-CRY2 were exposed to blue light and analyzed as in (F). (**H**) The CRY2-YFP photobody of the experiment shown in (F) was digitized and quantified; 30 nuclei per sample were counted, and the percentage of protoplasts containing photobodies was calculated (±SD; $n = 3$). (**I**) Same as (H) but for the nYFP-CRY2/cCFP-CRY2 photobody shown in (G).

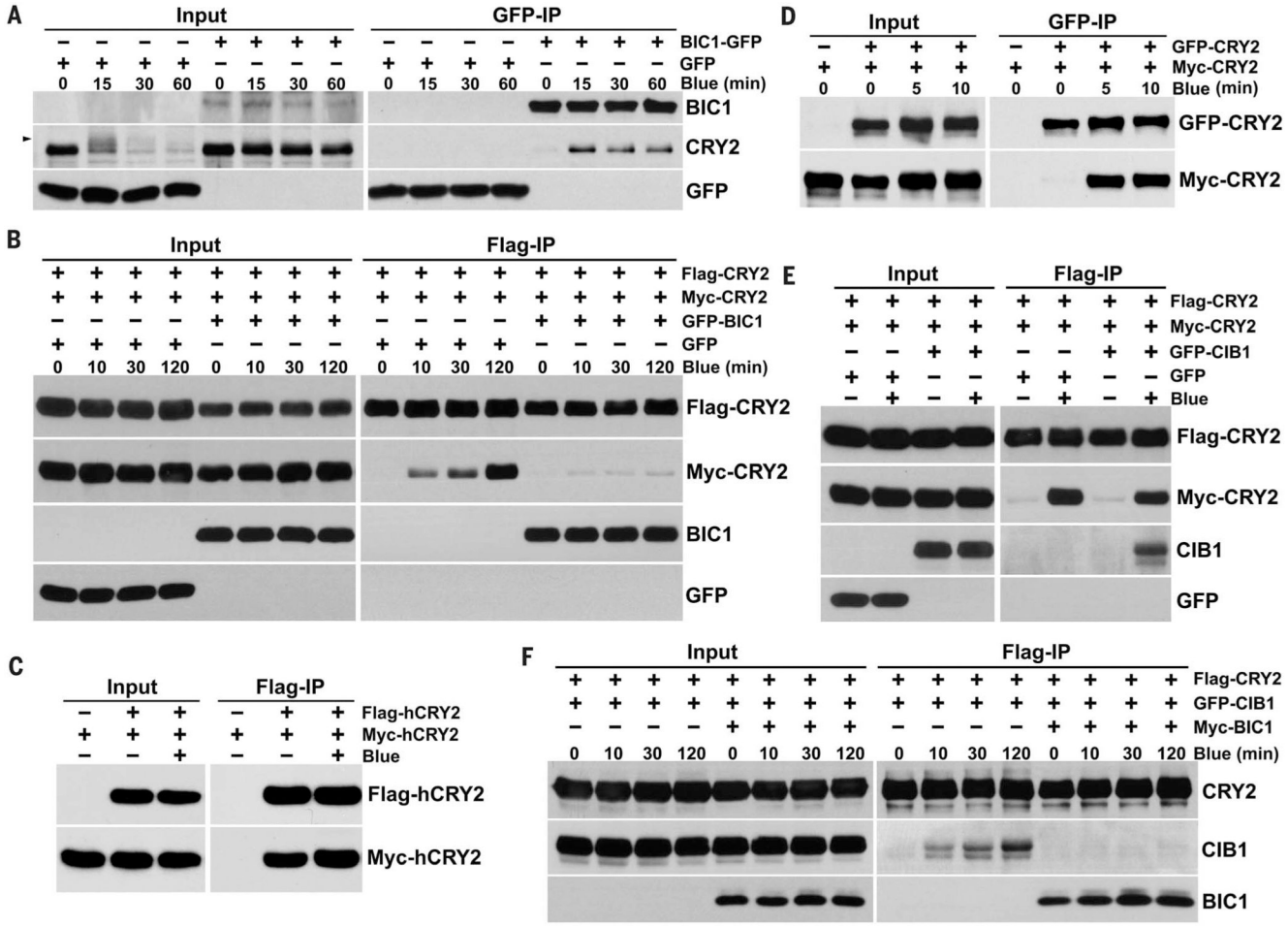

Fig. 4. BIC1 interacts with CRY2 to inhibit blue light–dependent CRY2 dimerization and CRY2-CIB1 interaction.

(**A**) Seven-day-old etiolated seedlings expressing *35S::BIC1-GFP* or *35S::GFP* (control) were exposed to blue light (30 μmol m^{-2} s^{-1}) for 0 to 120 min and immunoprecipitated by GFP-trap beads. The IP (BIC1) and co-IP signals (CRY2) were detected by immunoblots probed with antibodies to GFP and CRY2, respectively. (**B**) HEK293T cells were cotransfected to express the indicated proteins, exposed to blue light (180 μmol m^{-2} s^{-1}) for 0 to 120 min, and immunoprecipitated by antibody to Flag (Flag-IP).The IP signal (Flag-CRY2) or the co-IP signals (Myc-CRY2 and BIC1) were detected by immunoblots probed with antibodies to Flag or to Myc and GFP, respectively. (**C**) Co-IP assays showing light-independent dimerization of human CRY2 (hCRY2) in HEK293T cells. (**D**) Seven-day-old etiolated transgenic seedlings coexpressing the indicated proteins were exposed to blue light (30 μmol m^{-2} s^{-1}) for 0 to 10 min and immunoprecipitated by the GFP-trap beads. The IP signal (GFP-CRY2) and the co-IP signal (Myc-CRY2) were detected by immunoblots probed with antibodies to GFP or Myc, respectively. (**E**) HEK293T cells transfected to express the indicated fusion proteins were kept in the dark (–Blue) or exposed to blue light (180 μmol m^{-2} s^{-1}) for 2 hours (+Blue) and analyzed by co-IP assay as in (B). (**F**) HEK293T cells coexpressing the indicated proteins were exposed to blue light (180 μmol m

$^{-2}$ s^{-1}) for 0 to 120 min. The IP signal (CRY2) or the co-IP signals (CIB1 and BIC1) were detected by immunoblots probed with antibodies to Flag or to GFP and Myc, respectively.