BBX32, an Arabidopsis B-Box Protein, Functions in Light Signaling by Suppressing HY5-Regulated Gene Expression and Interacting with STH2/BBX21

Hans E. Holtan, Simona Bandong, Colleen M. Marion, Luc Adam, Shiv Tiwari, Yu Shen, Julin N. Maloof, Don R. Maszle, Masa-aki Ohto, Sasha Preuss, Rob Meister, Marie Petracek, Peter P. Repetti, T. Lynne Reuber, Oliver J. Ratcliffe, and Rajnish Khanna*

Mendel Biotechnology, Inc., Hayward, California 94545 (H.E.H., S.B., C.M.M., L.A., S.T., Y.S., D.R.M., M.-a.O., P.P.R., T.L.R., O.J.R., R.K.); Department of Plant Biology, University of California, Davis, California 95616 (J.N.M.); and Monsanto Company, Chesterfield, Missouri 63017 (S.P., R.M., M.P.)

A B-box zinc finger protein, B-BOX32 (BBX32), was identified as playing a role in determining hypocotyl length during a large-scale functional genomics study in Arabidopsis (Arabidopsis thaliana). Further analysis revealed that seedlings overexpressing BBX32 display elongated hypocotyls in red, far-red, and blue light, along with reduced cotyledon expansion in red light. Through comparative analysis of mutant and overexpression line phenotypes, including global expression profiling and growth curve studies, we demonstrate that BBX32 interacts with SALT TOLERANCE HOMOLOG2/BBX21, another B-box protein previously shown to interact with HY5. Based on these data, we propose that BBX32 functions downstream of multiple photoreceptors as a modulator of light responses. As such, BBX32 potentially has a native role in mediating gene repression to maintain dark adaptation.

* Corresponding author; e-mail rkhanna@mendelbio.com.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Rajnish Khanna (rkhanna@mendelbio.com).

[W] The online version of this article contains Web-only data.

[OA] Open Access articles can be viewed online without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.111.177139

Copyright © 2011 American Society of Plant Biologists.
elongated hypocotyls in all wavelengths of light and exhibits an increased number of lateral roots, shows defects in secondary thickening in roots, and accumulates markedly lower levels of chlorophyll and anthocyanin (Oyama et al., 1997; Holm et al., 2002). A study of in vivo binding sites of HY5 using chromatin immunoprecipitation coupled with DNA chip analysis demonstrated that HY5 preferentially binds to promoter regions throughout the genome (Lee et al., 2007). These direct HY5 binding sites include promoters of genes involved in photosynthesis and pigment synthesis, like CAB, RbcS1A, F3H, and CHS (Ang et al., 1998; Chattopadhyay et al., 1998; Lee et al., 2007), as well as genes involved in circadian regulation, such as CCA1, LHY, TOC1, and ELF4 (Lee et al., 2007).

HY5 protein accumulates in light and is degraded in the dark (Osterlund et al., 2000; Pokhliko et al., 2011). HY5 binding sites are largely found in promoters of genes that are either induced or repressed by light. In 35S::HA:HY5 transgenic plants, HY5 was found to bind its target sites constitutively, in darkness and in different light conditions, revealing that binding of HY5 alone is not sufficient for regulating the transcriptional activities of these light-responsive genes (Lee et al., 2007). Rather, HY5 activity is dependent on the interaction of the transcription factor with transcriptional accessory proteins. Lee et al. (2007) suggested that HY5 functions high in a regulatory hierarchy, regulating a branch of the transcriptional cascade involved in light-mediated development. Recent studies have shown that HY5 plays a role at points of convergence between light and phytohormonal signaling pathways (Cluis et al., 2004; Vandenbussche et al., 2007; Chen et al., 2008). HY5 transcript was detectable in all organs of mature plants, and the effects of hy5 mutation on the activation of light-responsive promoters fused to a GUS reporter could be detected in the leaves, stems, and roots of older plants (Oyama et al., 1997; Chattopadhyay et al., 1998). Loss of function of HY5 HOMOLOG (HYH) results in weak impairment in the inhibition of hypocotyl elongation specifically in blue light, suggesting that HYH functions redundantly with HY5 under specific light conditions (Holm et al., 2002).

**CONSTITUTIVE PHOTOMORPHOGENIC/DEETIOLATED/FUSCA (COP/DET/FUS)** are a group of 11 loci that function to suppress the photomorphogenesis program in dark-grown seedlings (Chory et al., 1989; Deng et al., 1991; Sullivan et al., 2003; Wei and Deng, 2003, Yi and Deng, 2005). COP1 is a RING-finger-type ubiquitin E3 ligase involved in ubiquitin-mediated proteolysis of photomorphogenesis-promoting factors, such as HY5 (Osterlund et al., 2000; Saijo et al., 2003), HYH (Holm et al., 2002), a MYB transcription factor, LONG AFTER FAR-RED LIGHT1 (Seo et al., 2003), and a bHLH transcription factor, LONG HYPOCOTYL IN FAR-RED1 (Duek et al., 2004; Jang et al., 2005; Yang et al., 2005). Other proteins have been identified that physically interact with COP1, including COP1-INTERACTING PROTEINS (Matsui et al., 1995; Yamamoto et al., 1998; Yamamoto et al., 2001), and several proteins with a zinc-binding B-box motif, namely CONSTANS (CO; Liu et al., 2008), CO-LIKE3 (COL3; Datta et al., 2006), SALT TOLERANCE (STO), and STO HOMOLOG1 (STH1; Holm et al., 2001). CO acts as a positive regulator of the floral transition in response to inductive photoperiods, whereas COL3 has been shown to act as a positive regulator of red light signaling (Putterill et al., 1995; Datta et al., 2006).

Both CO and COL3 proteins contain two N-terminal B-box motifs and a CCT (for CO, CO-LIKE, TOC) domain in the C terminus (Wenkel et al., 2006; Chang et al., 2008; Khanna et al., 2009). Several other B-box proteins have been implicated in light-regulated development. STH2/BBX21 and LZF1/STH3/BBX22 contain two B-box motifs in the N terminus but lack the CCT domain (Datta et al., 2007, 2008; Chang et al., 2008; Kumagai et al., 2008; Khanna et al., 2009). STH2/BBX21 and LZF1/STH3/BBX22 are positive regulators of red, far-red, and blue light signaling. They physically interact with HY5 through the B-box motifs, promote anthocyanin accumulation in concert with HY5, and LZF1/STH3/BBX22 was shown to be ubiquitinated by COP1 (Datta et al., 2007, 2008; Chang et al., 2008). Some other double B-box proteins, DBB1a/BBX18, DBB1b/BBX19, and STO/BBX24, have been shown to function negatively in light signaling (Indorf et al., 2007; Kumagai et al., 2008; Khanna et al., 2009). Here, we characterize a novel gene from the B-box family, B-BOX32 (BBX32; Khanna et al., 2009). BBX32 protein contains a single N-terminal B-box motif but lacks a CCT domain. We show that BBX32 gene expression is robustly induced by early light (within 1 h) treatment. Seedlings overexpressing BBX32 are hyposensitive to red, far-red, and blue light, similar to the hy5 mutant. We present evidence that BBX32 is a modulator of light signaling, functioning as a transcriptional accessory protein through its direct interaction with STH2, which is known to act with HY5.

**RESULTS**

BBX32 (AT3G21150; Khanna et al., 2009) was identified as a putative regulator of hypocotyl growth in a large-scale functional genomics project performed in Arabidopsis. Overexpression of BBX32 was observed to produce increases in hypocotyl length; as such, the gene was chosen for a detailed study in which the phenotypes of 35S::BBX32 lines were compared with the wild type and with those produced by a T-DNA insertion mutation (bbx32-1) within the gene (Fig. 1A).

**Seedlings Overexpressing BBX32 Are Hyposensitive to Red, Far-Red, and Blue Light**

Two representative independent overexpression lines, 35S::BBX32-10 and 35S::BBX32-12, were examined; both lines showed clear hyposensitivity to continuous red (Rc), far-red (FRc), and blue (Bc) light (Fig. 1). These seedlings had elongated hypocotyls in re-
BBX32 Modulates Light Signaling

BBX32 Modulates Light-Regulated Gene Expression

Based on the effects on hypocotyl and cotyledon development, we surmised that overexpression of BBX32 might result in altered light-regulated gene expression. In order to test this hypothesis, we per-
formed a microarray-based analysis of 35S::BBX32 and the bbx32-1 mutant seedlings and included the hy5-1 (Ler) background mutant for comparison. As expected, a large number of genes responded to the red light treatment in wild-type (control) seedlings, including known light-responsive genes such as ELIP1 and SIGE (Fig. 2A; Supplemental Table S1). BBX32 gene expression was robustly induced by the red light treatment in wild-type seedlings (Supplemental Table S1). The gene expression profile of 35S::BBX32 seedlings showed a marked reduction in responsiveness to light (Fig. 2A, profile 2). BBX32-overexpressing seedlings exhibited a greater change in the expression of early-light responsive genes in comparison with the hy5-1 mutant seedlings (Fig. 2A, profiles 2 and 4). There was no significant effect of the bbx32-1 mutation in seedlings treated with 1 h of red light (Fig. 2A, profile 6). However, a number of light-responsive genes were derepressed in the dark-grown bbx32-1 mutants (Fig. 2A, profile 5). These data suggested that the native function of BBX32 might be in helping to maintain dark-mediated repression of gene expression and that constitutive expression of BBX32 resulted in the suppression of light-regulated gene expression. There is precedent that the overexpression of an individual gene can result in hyposensitivity to red light due to reduced phyB protein levels in seedlings (Khanna et al., 2007). However, both phyA and phyB proteins were detectable in 35S::BBX32 seedlings at levels similar to those found in EV-Col (Supplemental Fig. S3), indicating that the alterations in light responsiveness in the overexpression line were not due to changes in phyA or phyB protein levels.

Comparison of Genes Targeted by BBX32 and HY5

The two ecotypes (EV-Col and Ler) showed differences in gene responsiveness to treatment with 1 h of red light. About 61% of the induced and 44% of the repressed genes in EV-Col showed a similar response in Ler (Fig. 2B). In dark-grown 35S::BBX32 and hy5-1 mutant seedlings, the expression of a small number of genes was either elevated or reduced by 2-fold or higher (Fig. 2C). There was no overlap between the induced gene sets, and there were only four genes commonly reduced in these two genotypes in the dark (Fig. 2C). The number of genes induced over 2-fold in response to red light in the 35S::BBX32 (63 genes) and hy5-1 mutant (61 genes) was small in comparison with the number of genes that were reduced by 2-fold or higher in these lines, 504 and 172 genes, respectively (Fig. 2C). While only about 20% of induced genes were common between 35S::BBX32 and hy5-1 mutant seedlings, over 73% of the genes that failed to respond to red light in hy5-1 mutants were also reduced in 35S::BBX32 seedlings (Fig. 2C). These data showed that a majority of genes targeted by HY5 for induction were reduced in 35S::BBX32 seedlings, despite the differences between the two ecotypes. However, a set of genes were reduced specifically in 35S::BBX32 seedlings: 75% of the genes reduced in the 35S::BBX32 seedlings were distinct from the hy5 set. In summary, the 35S::BBX32 seedlings showed alterations in response to the early light signal, with significant overlap to the response profile of the hy5-1 mutant. However, the 35S::BBX32 seedlings showed an alteration in gene expression distinct from the hy5-1 mutant, as there was
a discrete set of genes affected only by the overexpression of BBX32.

We subjected the array data to functional category analysis (Gene Ontology [GO] annotations; see “Materials and Methods”) to determine the functional pathways most affected in each of the genotypes. Similar functional categories were repressed in both hy5-1 and 35S::BBX32 seedlings, although the magnitude of the reduction was greater in the BBX32 over-expression lines (Fig. 3). In dark-grown hy5-1 mutants and 35S::BBX32 seedlings, the most affected functional categories included response to UV and pigment biosynthesis (Fig. 3, A and C; Supplemental Table S2), albeit there were some differences in individual gene responses within these categories. These differences could represent distinct features of the two genotypes and could also be due to ecotypic differences between the Ler and EV-Col backgrounds. The functional categories most affected after 1 h of red light treatment in both hy5-1 and 35S::BBX32 seedlings were similar, representing genes that are light responsive or are involved in light-regulated processes (Fig. 3, B and D; Supplemental Table S2). However, the order of significance of these functional categories between the two genotypes was different due to the differences in responsiveness of some of the genes. Overall, the 35S::BBX32 seedlings, like the hy5-1 mutant lines, showed a dampening of light-regulated pathways. The effect of the bbx3-2-1 mutation on gene expression was relatively small, with significant differences observed only in dark-grown seedlings. There was no significant effect on gene expression in the light-treated bbx3-2-1 mutant seedlings. On the other hand, a distinct set of functional categories were induced in etiolated bbx3-2-1 seedlings (Fig. 3E; Supplemental Table S2). The three most derepressed functional categories in the dark included genes involved in UV and light-responsive pathways followed by categories related to photosynthesis (Fig. 3E). The altered expression of light-related genes in dark-grown bbx3-2-1 seedlings is consistent with BBX32 playing a native role in dark-mediated gene repression.

We performed quantitative reverse transcription (qRT)-PCR experiments on independently grown seedlings to confirm the array-based results for a selected set of genes. For the qRT-PCR analysis, we included another independently transformed line, 35S::BBX32-487. BBX32 transcript levels were robustly induced by red light treatment in all wild-type (EV-Col, Ler, and sib-Col) control seedlings (Supplemental Fig. S1A). Light induction of BBX32 expression was slightly affected in the hy5-1 mutant; similarly, HY5 transcript levels were reduced in seedlings overexpressing BBX32 but were not affected in the bbx3-2-1 mutant (Supplemental Fig. S1B; Supplemental Table S3). We selected about 20 genes for qRT-PCR analysis, either because of their known response to light or based upon differences in expression observed on the array. As a control set, we also selected some that showed no differences on the array. For the complete list of selected genes, primer sequences used, and the qRT-PCR data, see Supplemental Tables S3 and S4. Overall, the results from the qRT-PCR analysis confirmed the array data for the expression of each of these genes in 35S::BBX32 and hy5-1 mutant seedlings (Supplemental Fig. S1, Supplemental Materials and Methods S1, and Supplemental References S1). These data suggested that BBX32 and HY5 proteins function antagonistically in affecting the expression of these genes, including in etiolated seedlings.

35S::BBX32 Seedlings Exhibit Increased Hypocotyl Growth Rates at Night Similar to the hy5 Mutant

Arabidopsis seedlings exhibit diurnal hypocotyl growth patterns. Both light and the circadian clock coordinately regulate hypocotyl growth rates during the dark and light cycles. Seedlings grown under short-day (8 h of light/16 h of dark) conditions exhibit reduced hypocotyl growth during the day and increased growth at the end of the night (Nozue et al., 2007). It was shown that hy5 mutants displayed reduced light sensitivity during the day and exhibited higher growth rates in the dark (Nozue et al., 2007). We examined the diurnal growth patterns of 35S::BBX32 seedlings to see if they responded like the hy5 mutants under diurnal conditions and included the bbx3-2-1 mutants for comparison. There was no detectable difference between the diurnal growth patterns of the bbx3-2-1 mutant and the sib-Col control seedlings (Fig. 4A). A biphasic pattern of growth was observed in Col seedlings in the dark, marked by increased growth at the start and at the end of the night (Fig. 4B). As expected, the hy5-215 mutants exhibited an increased rate of growth in the dark and maintained a biphasic pattern of growth during the night (Fig. 4B). Like the hy5-215 mutants, both 35S::BBX32 lines showed increased growth during the night, with rates higher than the hy5 mutants (Fig. 4, B and C). These data are also consistent with our observations that the 35S::BBX32 seedlings show differences in light responses when compared with the hy5 mutants. These results suggested that constitutive expression of BBX32 resulted in alterations in the clock-regulated control of hypocotyl growth during the night, which could be due to direct or indirect effects on clock function (“Discussion”).

BBX32 Interacts with STH2

HY5 is known to interact with other B-box proteins like STH2/BBX21 and LZF1/STH3/BBX22, which function positively in light signaling with HY5; null mutations in STH2/BBX21 and LZF1/STH3/BBX22 genes cause phenotypes similar to the hy5 mutants (Datta et al., 2007, 2008; Chang et al., 2008). BBX32 might either directly interact with HY5 and/or bind one or more of the other B-box proteins that interact with HY5. To test these possibilities, we performed in vitro immunoprecipitation assays and protoplast-
did not detect any evidence of direct interaction between BBX32 and HY5 in these assays (data not shown). Next, we used STH2:3XHA fusion protein as bait for immunoprecipitation with a rabbit polyclonal anti-HA (for hemagglutinin) antibody; after several

**Figure 3.** Functional category analysis of genes differentially expressed in 35S::BBX32, hy5, and bbx32-1 lines compared with control lines. Categorizing the biological processes that were significantly affected in the different genotypes and treatments was done by calculating the relative overrepresentation of annotations to different GO terms among the misexpressed genes in dark-grown (A, C, and E) or red light-treated (B and D) 35S::BBX32, hy5-1 mutant, and bbx32-1 mutant seedlings. Based on an analysis (see “Materials and Methods”) of GO terms annotated to Arabidopsis genes (Berardini et al., 2004; as of January 19, 2009), the categories listed above were overrepresented (P < 0.01) among significantly misexpressed genes. For each overrepresented GO category, we list the name of the category followed by the number of genes with significantly altered expression and the total number of all genes annotated to the term. The horizontal length of the bar indicates the ratio of the number of genes annotated to the term that have significant expression changes relative to the number of genes from that category that would have been expected to show such changes by chance (based on maximizing the hypergeometric density function), and the vertical length of the bar represents the magnitude of the average absolute fold change of the genes with significantly altered gene expression that were annotated to the given GO term. The percentage of induced and repressed genes annotated to each GO term is indicated by red and green, respectively. For list of genes overrepresented in each of the GO categories, see Supplemental Table S2.
washes, each pellet was divided into two equal parts and subjected to western blotting to detect the presence of bait and prey proteins, as described in “Materials and Methods.” We used purified 6XHIS:NF-YB2 protein as prey to serve as a negative control, and two other control reactions lacked either the bait STH2:3XHA or the prey MBP:BBX32. A monoclonal anti-HA antibody was able to detect the bait protein STH2:3XHA in each pellet, except in the control reaction lacking the bait (Fig. 5A, left panel). The anti-His monoclonal antibody did not detect any 6XHIS:HY5 prey protein in the negative control reaction, but it did detect the 6XHIS:HY5 fusion protein in the pellet with STH2:3XHA, confirming the previously reported interaction between STH2 and HY5 (Fig. 5A, right top panel; Datta et al., 2007). The anti-MBP monoclonal antibody detected a band only in the reaction containing MBP:BBX32 but not in the two controls lacking either the bait or the prey protein (Fig. 5A, right bottom panel). These data suggested that the MBP:BBX32 fusion was able to bind STH2:3XHA. To exclude the possibility of MBP interaction with the bait protein,
we used an MBP:PARAMYOSIN fusion protein as a negative control. The anti-HA monoclonal antibody detected STH2:3XHA (bait) in the immunoprecipitated pellets from the three separate reactions with or without a prey protein (Fig. 5B, left panel). In these pellets, the anti-MBP monoclonal antibody did not find any significant amount of MBP:PARAMYOSIN or a cross-reacting band in the reaction lacking the prey protein; however, abundant MBP:BBX32 fusion protein was present in the pellet, with STH2:3XHA in the corresponding reaction (Fig. 5B, right panel). Therefore, we concluded that BBX32 is able to bind STH2.

We performed protoplast-based protein-protein interaction assays to confirm the in vivo interaction potential between BBX32 and STH2. We used GAL4 DNA binding domain (GD) alone or GD fusions with HY5 and BBX32 as baits against HA(2X):STH2 prey proteins. The cMYC and HA tags were used to facilitate protein expression analysis (right panel). The diagram at top shows the structure of the reporter gene used (see “Materials and Methods”). Average values are plotted with SD from assays done in triplicate. Values were normalized to the 35S::luciferase reporter gene. The data are representative of three independent experiments.
HY5:cMyc(4X), STH2:cMyc(4X), and BBX32:cMyc(4X) to examine their interactions with HA(2X):STH2 or with chloramphenicol acetyl transferase (CAT) as the negative control (see “Materials and Methods”). Protein expression from these constructs was confirmed by western-blot analysis (Fig. 5C, right panel). There was no increase in GUS activity in negative control protoplasts cotransformed with CAT and GD alone, GD:HY5:cMyc(4X), or GD:BBX32:cMyc(4X) (Fig. 5C). Protoplasts containing CAT with GD:STH2:cMyc(4X) fusion had increased GUS activity (Fig. 5C), suggesting that GD:STH2:cMyc(4X) may have an inherent transcriptional activation potential when bound to DNA. Cotransfection of HA(2X):STH2 with GD:STH2:cMyc(4X) increased GUS activity further, possibly due to intramolecular interaction of STH2 (Fig. 5C). We found that HA(2X):STH2 did not interact with the negative control GD, but it did interact with both GD:HY5:cMyc(4X) and GD:BBX32:cMyc(4X) (Fig. 5C). These data confirmed that BBX32 has the capacity to interact with STH2 and are consistent with the possibility that this interaction leads to inactivation of a protein complex containing STH2 and HY5 in vivo.

Coexpression of 35S::BBX32 Reduces the Light Responsiveness of 35S::HY5 and 35S::STH2 Seedlings

HY5 function is necessary for optimal light-mediated inhibition of hypocotyl growth and for anthocyanin synthesis in seedlings (Ang et al., 1998). First, we examined hy5-1/bbx32-1 double mutant and 35S::BBX32/35S::HY5 coexpressing lines to study genetic interactions between BBX32 and HY5 (Supplemental Figs. S4–S6). Our data show that HY5 function is necessary for the light responsiveness of the hy5-1/bbx32-1 double mutant and that constitutive expression of BBX32 in the 35S::HY5 background reduces responsiveness to multiple wavelengths of light (Supplemental Fig. 5). Furthermore, the 35S::BBX32/35S::HY5 seedlings accumulated significantly lower anthocyanin levels compared with 35S::HY5 seedlings (Supplemental Fig. S4G). Collectively, these data suggested that BBX32 may function by suppressing HY5 activity. STH2 functions with HY5 in regulating hypocotyl growth (Datta et al., 2007). Since BBX32 directly binds STH2 (Fig. 5), we investigated the significance of this interaction on hypocotyl growth of sth2-1/bbx32-1 double mutants and lines coexpressing 35S::STH2/35S::BBX32. The sth2-1/bbx32-1 double mutant displayed long hypocotyls like the sib-Col; Fig. 6). Coexpression of 35S::STH2/35S::BBX32 reduced the effect of STH2 overexpression on hypocotyl length in the light (Fig. 6). These results are consistent with our hypothesis that BBX32 suppresses STH2 activity in HY5-dependent and independent pathways. Figure 7 depicts a simplified model of BBX32 function in suppressing the activities of positive cofactors like STH2 and HY5 involved in modulating light-regulated gene expression and growth.

**DISCUSSION**

BBX32 was identified as playing a role in light-regulated hypocotyl growth from a large-scale functional genomics screen. Seedlings with constitutive expression of BBX32 were hyposensitive to red, blue, and far-red light. These seedlings exhibited alterations in light-regulated development of hypocotyls and cotyledons, which are characteristic of changes in photomorphogenesis. In contrast, the bbx32-1 mutant seedlings displayed only weak, but partially opposite, phenotypes. There was only one mutant allele (bbx32-1) available for this study; therefore, we performed complementation analysis. The weak phenotype displayed by the bbx32-1 mutant seedlings was rescued by a prBBX32::BBX32 transgene (Supplemental Fig. S2), suggesting that the observed hyposensitivity to light was linked to this locus. Our microarray and qRT-PCR-based gene expression analyses of bbx32-1 mutant seedlings revealed altered expression of light-responsive genes in the dark. In particular, the expression of light-stimulated genes, ELIP, CHS, and SIEG, appeared to be derepressed in dark-grown bbx32-1 seedlings (Supplemental Fig. S1). This lack of gene repression in the dark-grown bbx32-1 mutant seedlings is consistent with the hypothesis that BBX32 acts with other proteins in a complex that functions as a modulator of light-regulated gene expression. Furthermore, a number of genes belonging to GO categories related to photosynthesis or carbon assimilation were induced in the bbx32-1 mutants grown in the dark (Fig. 3). Analysis of additional alleles and multiple mutant combinations with potential functional cofactors will help in confirming the role of the native BBX32 as an accessory protein in modifying gene expression in the dark. Our results with multiple independent transgenic 35S::BBX32 lines provide evidence that the constitutive expression of BBX32 reduces light responsiveness and promotes gene expression patterns that are characteristic of seedlings grown in darkness.

**BBX32 Functions in HY5-Regulated Development**

The hy5 mutant was selected for comparison with 35S::BBX32 seedlings because, like hy5 mutants, these seedlings displayed hyposensitivity to multiple light wavelengths, suggesting that BBX32 functions antagonistically to HY5 in light-regulated development, downstream of multiple photoreceptors. We undertook three different approaches to study the possible relationship between BBX32 and HY5: first, we analyzed global gene expression in seedlings in response to early red light treatment; second, we compared the diurnal hypocotyl growth patterns of these seedlings grown under short days (8 h of light/16 h of dark); and third, we examined genetic relationships between BBX32 and HY5 using combinations of genetic mutants and transgenic lines. Our studies with hy5-1/bbx32-1 double mutants and the 35S::BBX32/35S::HY5 lines showed that the hy5 mutation was epistatic in the
double mutant and that the 35S::BBX32 function was dominant in seedlings coexpressing HY5 and BBX32 (Supplemental Figs. S4–S6). These results suggested that BBX32 acts in the same light signaling pathway as HY5. Seedlings of the hy5-1/bbx32-1 double mutant appeared to be slightly shorter than the hy5-1 single mutant under blue and white light (Supplemental Figs. S4–S6). This could be due to a possible repression of HYH activity by the native BBX32 protein present in the hy5-1 single mutant, whereas this repression will be absent in the hy5-1/bbx32-1 double mutant.

35S::BBX32 Seedlings Displayed Additional Changes in Light-Regulated Gene Expression When Compared with hy5 Mutants

In our microarray analysis, there were ecotypic differences between Lcer and EV-Col. Seventy-five percent of all the genes induced by light in Lcer were commonly induced in EV-Col (Fig. 2B). The 35S::BBX32 seedlings displayed a greater magnitude change in gene expression in response to red light than the hy5-1 mutants. Consistent with this observation, the 35S::BBX32 seedlings had longer hypocotyls than hy5-1 mutants under all wavelengths of light (Supplemental Fig. S4). These data support the hypothesis that BBX32 is an accessory protein to other transcription factors, in addition to HY5.

BBX32 Functions in a Regulatory Protein Complex to Modulate Changes in Gene Expression

Previous studies have shown that several B-box proteins, including STH2 and LZF1 (STH3), which both directly interact with HY5, play roles in light signaling (Datta et al., 2007, 2008; Chang et al., 2008; Kumagai et al., 2008). Despite extensive efforts, we did not detect any direct interaction between BBX32 and HY5, nor did we find an interaction of BBX32 with DNA. Our data suggest that it is likely that BBX32 acts...
BBX32 Modulates Light Signaling

Figure 7. Simplified model depicting a functional role of BBX32 in photomorphogenesis. The hypothetical model indicates that BBX32 interacts with STH2 and suppresses the activities of positive factors, STH2 and HY5, involved in photomorphogenesis that control multiple aspects of seedling deetiolation, including light-regulated gene expression and cell expansion.

as a member of a larger group of proteins modulating the response of the HY5 complex to light signal transduction. Consistent with the above prediction, STH2 was shown to function independently and with HY5 to regulate photomorphogenesis (Datta et al., 2007). It was suggested that STH2 may bind HY5 and possibly other cofactors to regulate transcription to promote light signaling (Datta et al., 2007). We selected STH2 to test whether BBX32 could bind other B-box proteins to form potentially inactive heterodimers. BBX32 interacted with STH2 in both in vitro immunoprecipitation assays and protoplast-based interaction assays (Fig. 5). These results show that BBX32 is capable of direct interactions with other B-box proteins to potentially modulate their activities. Our emerging understanding from this study as well as other work (Datta et al., 2007, 2008; Chang et al., 2008; Kumagai et al., 2008) suggests that B-box proteins as a whole may act to modulate the activity of other B-box proteins as well as the activity of specific transcription factors, allowing the plant to fine-tune a transcriptional response to specific endogenous or environmental inputs. STH2 and HY5 are thought to be targeted for protein degradation through COP1. Future experiments will reveal if BBX32 protein levels are also regulated by COP1.

Under diurnal short-day (8 h of light/16 h of dark) conditions, the 35S::BBX32 seedlings continued to grow during the night; only one of the two independent lines, 35S::BBX32-12, showed a weak biphasic pattern of growth, whereas the hy5-215 mutants showed two peaks of growth during the night, like the wild-type seedlings (Fig. 4). These data suggested that the growth pattern of 35S::BBX32 seedlings at night was less responsive to regulation by the circadian clock than that of the hy5-215 mutant. These results can be explained either by an effect of BBX32 on clock-regulated growth or by indirect effects on clock function due to a greater repression of light signaling in 35S::BBX32 seedlings. The diurnal growth rhythms displayed by the 35S::BBX32 seedlings were similar to the rhythms observed for seedlings overexpressing PIF4 and PIF5 (Fig. 4; Nozue et al., 2007). An alternative hypothesis is that BBX32 acts in conjunction with one or more of the PIF mutants to regulate hypocotyl growth. However, seedlings overexpressing PIF4 or PIF5 phenocopy phyB (Hu and Quail, 2002; Khanna et al., 2007), and PIF proteins have been shown to promote phyB polyubiquitination and degradation (Khanna et al., 2007; Leivar et al., 2008; Jang et al., 2010). On the other hand, seedlings overexpressing BBX32 phenocopy hy5 mutants and contain phyB protein levels similar to those of wild-type seedlings (Supplemental Fig. S3). BBX32 and PIFs may have overlapping and distinct roles in light signal transduction. Furthermore, the expression of some of the B-box genes (DDB1a, DDB1b, DDB3, STO, and STH1) was shown to be controlled by the circadian clock and to peak at distinct phases, and these genes were implicated in regulating hypocotyl growth (Kumagai et al., 2008). BBX32 may interact with one or more of these B-box proteins to regulate their activities in regulatory protein complexes with or without STH2 and HY5. BBX32 is likely to function as an accessory protein to a protein complex that modulates transcriptional responses downstream of multiple photoreceptors (Fig. 7) and to integrate external and internal cues to promote dark adaptation.

In conclusion, this study provides evidence that BBX32 protein modulates light signaling by acting antagonistically to HY5. Constitutive expression of BBX32 caused hyposensitivity to red, far-red, and blue light. However, the 35S::BBX32 seedlings exhibited a higher magnitude of differences in light-regulated gene expression and hypocotyl growth than the hy5 mutant. By contrast, the single bbx32-1 gene mutation caused weak phenotypic effects and displayed derepression of light-responsive genes in the dark. These results suggest a native role for BBX32 in maintaining dark-mediated patterns of gene expression and hypocotyl elongation. We found that BBX32 is capable of interaction with STH2 and potentially with other B-box proteins. Therefore, it is probable that BBX32 belongs to a regulatory protein complex that acts to modulate light-regulated gene expression early in the hierarchy of light signal transduction.

MATERIALS AND METHODS

Generation of Overexpression Lines

BBX32, HY5, and STH2 overexpression lines were created in the Arabidopsis (Arabidopsis thaliana) accession Col. The BBX32 (AT3G21150; Khanna et al., 2009) gene was PCR amplified from genomic DNA isolated from Arabidopsis Col, containing the entire BBX32 open reading frame plus 63 bp of 5′-untranslated sequence and 39 bp of 3′-untranslated sequence. The STH2 (AT1G75540) clone was PCR amplified from an Arabidopsis Col cDNA library containing the open reading frame of STH2. The HY5 (AT5G111260) clone was
PCR amplified from an Arabidopsis Col cDNA library containing the entire HY5 open reading frame plus 58 bp of 5′-untranslated sequence and 73 bp of 3′-untranslated sequence. For Arabidopsis overexpression studies, both HY5 and the BBX32 clone were introduced into a standard binary vector harboring a kanamycin selection marker driven by a NOS promoter and the selected coding region downstream from the cauliflower mosaic virus (CaMV) 35S promoter. STH2 was introduced into a similar binary vector except that it contained a sulfonamide selection marker driven by the CaMV 35S promoter. For the creation of the BBX32/STH2 double overexpression lines, the STH2 construct described above was supertransformed (see below) into an established BBX32-overexpressing line (BBX32-10-6). Creation of BBX32/HY5 double-overexpressing lines and isolation of the hy5-1/bbx32-1 double mutant are described in Supplemental Materials and Methods S1. All lines were verified to contain the transgenes by PCR.

Arabidopsis plants were transformed by the floral dip method (Clough and Bent, 1998; Bechtold et al., 2003). For each of the genes, between 20 and 40 independent primary transformants were isolated on selection medium. Transformants were PCR genotyped to confirm that they harbored the correct transgene, and gene expression was verified by RT-PCR on RNA extracted from 7-d-old seedlings. For each of the genes, one or more representative lines that showed substantial levels of overexpression versus the wild-type was selected and used in subsequent experiments. For detailed studies on the effects on BBX32 overexpression, each experiment was performed on one or more of three independently transformed lines (35S::BBX32-10-6, 35S::BBX32-12-2, and 35S::BBX32-487). These three lines exhibited and maintained stable phenotypes over several generations. Based upon genomic Southern-blot analysis, 35S::BBX32-10-6 is predicted to harbor two separate insertions of the T-DNA, while 35S::BBX32-12-2 and 35S::BBX32-487 have multiple copies of the T-DNA (Supplemental Fig. S7).

Isolation of Mutant Alleles and Genetic Crosses

Database searches revealed a single mutant allele (Salk Institute Genomic Analysis Laboratory; Alonso et al., 2003) with a T-DNA insertional disruption of the BBX32 gene, bbx32-1 (SALK_095934), available from the Arabidopsis Biological Resource Center. The insertion site of the disruptive T-DNA is predicted to be downstream of the B-box domain, approximately 670 nucleotides from the start codon at residue 172 of the 225 amino acids constituting the BBX32 protein (Fig. 1A). We used gene-specific (5′-AATCTCCAGCC-TCTTTCTCCT-3′ and 5′-TTGGATTACCTATTCGTTTC-3′) and the other primer pair targeted sequences upstream of the T-DNA (BBX32-B [upstream]; see qRT-PCR section below; for primer sequences, see Supplemental Table S3). We found that the bbx32-1 mutant lacks any detectable BBX32 transcript spanning the predicted T-DNA insertion site; however, with primers targeted upstream of the insertion site, a possibly truncated BBX32 mRNA was detected at levels lower than those in control (sib-Col) seedlings (Supplemental Fig. S1A). Based on these results, it is unlikely that the bbx32-1 mutant expressed any significant quantity of functional full-length BBX32 protein.

Double and triple mutants between bbx32-1, phyA-211, and phyB-9 were selected from F2 populations, created by crossing bbx32-1 to a phyA-211/phyB-9 double mutant in the Col background (a gift from Dr. Peter H. Quail), by a combination of PCR and phenotypic characterization. Using a similar method, double and triple mutants between bbx32-1 and cry1 (cry1-3), available from the Arabidopsis Biological Resource Center; accession Ler) were identified from F2 segregating populations, PCR genotyped for bbx32-1, and screened in blue light to identify plants containing both mutations. Double mutants between bbx32-1 and sh2-1 (a gift from Dr. Magnus Holm) were made by crossing and PCR genotyping the F2 population for bbx32-1 and sh2-1.

Plant Growth Conditions

For plate-based assays, seeds were sterilized and grown on plates containing growth medium without Suc as described (Khanna et al., 2006). An E-30LED plant growth chamber (Percival) was used for red, far-red, and blue light treatments, and an AR75L chamber (Percival) was used for white light. Fluence rates were measured using a spectroradiometer (EPP2000-VIS-50; StellarNet). Hypocotyl and cotyledon area measurements were performed using digital images (Canon G9) analyzed with ImageJ (version 1.47) software (National Institutes of Health). Approximately 30 seedlings per genotype were used for hypocotyl measurements, and 10 seedlings (20 cotyledons) were used for cotyledon area measurements. Average values were plotted with se values. All experiments were performed with two or more biological replicates. For soil-grown plants, Arabidopsis seeds were chlorinated gas sterilized, cold stratified for 4 d at 4°C, plated on 80% Murashige and Skoog agar containing 1% Suc, and transferred to growth chambers. Seedlings were transplanted to soil at 7 d and grown under cool-white fluorescent light at 22°C in Sunshine mix soil.

Microarray Analysis

Seedlings of 35S::BBX32-10-6, bbx32-1, and hy5-1 mutants and their respective controls, EV-Col, sib-Col, and Ler, were grown on plates as described above. Seedlings were grown in the dark for 4 d, and some seedlings were treated with 1 h of monochromatic red light (10 μmol m⁻² s⁻¹). Three biological replicates, each consisting of approximately 300 seedlings, were harvested for two time points: dark (time 0) and after 1 h of red light treatment. Total RNA was isolated from liquid-nitrogen-pulverized tissue, and bioin-labeled target RNA was produced from 4 μg of total RNA using the MessageAmp II kit from Ambion (catalog no. 1751) according to the manufacturer’s instructions. Hybridizations were performed using the standard Affymetrix protocol, with 6.5 μg of labeled copy RNA for each array. To ensure that all samples were high quality, RNA quantity and quality were determined with the Nanodrop 1000 spectrophotometer and the QIAxcel capillary gel electrophoresis system. Arrays were processed and scanned with the Affymetrix GeneChip Workstation (Hybridization Oven 640, Fluidics Station 450, and Scanner 3000) using the GCOS version 1.4.0.86.3 software.

All transcript profiling experiments were performed using a custom full-genome Arabidopsis Affymetrix GeneChip (mboArth1a520184) microarray designed by Mendel Biotechnology. Based on the TAIR6 (www.arabidopsis.org) November 15, 2005, release of the Arabidopsis genome, this array unambiguously probe sets 29,544 unique loci. More details about this custom array can be found under Gene Expression Omnibus accession number GPL10037 at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/geo).

Microarray data were preprocessed in R (R Development Core Team, 2008), where the data were first background corrected using Robust Multi-chip Average background correction (Bolstad et al., 2003; Irizarry et al., 2003) and normalized with a quantile normalization. Probe sets were then summarized using a linear probe-level model (Bolstad, 2004), which provided both an estimate of absolute expression level and an estimated error. Both of these data sets were imported into the Rosetta Resolver system (Rosetta Biosoftware) for gene expression data analysis. All derived data sets, including scan profiles, average intensity experiments, and intensity ratios, were analyzed within the Experiment Definition context of the Resolver system. Significance estimates (P values) for intensity ratios defined in this way were subsequently adjusted by the method of Benjamini and Hochberg (1995), resulting in multistest-corrected P raw values. The Affymetrix data files (CEL), preprocessed profiles, and supplemental files described herein have been deposited at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/geo) under Gene Expression Omnibus accession number GSE2117.

The annotation of the Arabidopsis genome (Berardini et al., 2004) to categories in GO (Ashburner et al., 2000) has been used as the basis for overrepresentation analysis of various logical sets of genes. Overrepresenta-
tion analysis examines the intersection between a given gene list and genes annotated to all included GO terms and returns the likelihood of observing the resulting degree of overlap. In this study, we defined gene lists based on expression intensity ratios and used a combination of significance threshold (P < 0.01) and fold change, depending on genotype (greater than 1.5-fold for bbx32-1 and greater than 2.0-fold for 35S::BBX32 and hy5-1), as the selection criterion. The calculations for the overrepresentation analysis were performed with freely available software called enrichml (Lee et al., 2005), and the results were then postprocessed with custom Perl (www.perl.org) and R scripts to calculate and collate additional information about the overrepresented cate-
gories. The number of genes expected both to be annotated with a given GO term and to show a significant change in gene expression was calculated based on maximizing the hypergeometric density function given the size of the genome, the number of genes passing our significance parameters in a given ratio, and the total number of genes annotated to the GO category.
Hypocotyl Growth Curve

Hypocotyl growth curve experiments were performed during a diurnal photoperiod, essentially as described (Nozue et al., 2007). Images were collected using a PixiLINK PL-781 camera controlled by National Instruments LABView software. Data analysis was performed in R (R Development Core Team, 2008), as described (Nozue et al., 2007), except that Loess and spline smoothing were used instead of a running average.

Expression and Purification of Recombinant Proteins

Sequences encoding BBX32, STH2, HY5, and NF-YB2 cDNA were amplified by PCR (KOD DNA Polymerases; Novagen) from a Col cDNA library and cloned into vectors (BBX32 into PMal-c2E [New England Biolabs] and HY5 and NF-YB2 into pET45b [Novagen]) to produce fusion proteins (MBP:BBX32, 6XHis:HY5, and 6XHis:NF-YB2). The insertion of each clone was sequence validated on both strands.

Total protein extracts were prepared 16 h after autoinduction at 28°C using a quick-freeze extraction method in the presence of 1× Bugbuster, 7.5 kilonits ml−1 lysozyme, 25 units ml−1 Benzonase (Novagen), and protease inhibitors (Complete Mini Protease Inhibitor tablet; Roche). The MBP:BBX32 fusion protein was purified according to the manufacturer’s recommendation (New England Biolabs). The 6XHis-tagged fusion proteins were purified under native conditions on His-bind Quick 300 cartridges (Novagen). Protein samples were dialyzed for 6 to 12 h (D tubes; Novagen) against a solution of 20 mM HEPES, pH 7.9, 100 mM KCl, 0.5 mM EDTA, and glycerol added to a 30% final concentration prior to storage at −20°C. The enrichment of the fusion proteins was evaluated by SDS-PAGE and western blotting using anti-cMYC (Upstate Biotechnology) or anti-HA (Sigma–Aldrich), anti-6XHIS (Qiagen), or anti-MBP monoclonal antibodies.

BBX32 Modulates Light Signaling

Protoplast Transfection Assays

Isolation of protoplasts from Arabidopsis leaves and transfection have been described previously (Tiwari et al., 2006). Briefly, protoplasts were isolated from 3- to 4-week-old plants grown under long-day conditions (16 h of light and 8 h of dark). Reporter plasmid (10 μg) and effector plasmid (5 μg) were used in transfection assays. Transfected protoplasts were kept in the dark for at least 16 h at room temperature before the GUS activity was measured. All assays were performed in triplicate, and at least two independent transfections were carried out for each experiment. The transfected protoplasts were lysed in 2× sample loading buffer (Sigma S3401), and the expression of effector proteins was confirmed by western blotting using anti-cMYC (Upstate Biotechnology) or anti-HA (Sigma Aldrich) antibodies.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Array results are highly correlated with results from an independent qRT-PCR experiment.

Supplemental Figure S2. Complementation analysis of the bbx32-1 mutant with a pRBBX32−3BBX32 transgene.

Supplemental Figure S3. Western-blot analysis of phyA and phyB protein levels in seedlings overexpressing BBX32.

Supplemental Figure S4. Hypocotyl length responsiveness of seedlings correlates to BBX32 expression in various genetic backgrounds.

Supplemental Figure S5. Hypocotyl length responsiveness to red light compared with BBX32 and HY5 gene expression in seedlings.

Supplemental Figure S6. Hypocotyl length responsiveness of wild-type and mutant siblings in the Ler/Col background.

Supplemental Figure S7. Genomic Southern hybridization using pRB55 sequence as a probe.

Supplemental Table S1. Genes responding significantly differentially in each of the experimental lines compared with respective control seedlings.

Supplemental Table S2. Overrepresented GO categories.

Supplemental Table S3. Primers used for qRT-PCR.

Supplemental Table S4. Results of qRT-PCR.

Supplemental Materials and Methods S1. Materials and Methods for Supplemental Figures S1 to S7.

Supplemental References S1. References for Supplemental Figures S1 to S7 and Supplemental Materials and Methods S1.

ACKNOWLEDGMENTS

We thank Alemu Belachew, Larry Biando, Bonnie Brayton, Han-Chang Chang, Yanhi Hou, Megan McPartland, Marjorie Lundgren, and Emily Queen for excellent technical assistance. We thank Robert A. Creelman and Graham Hymus for critical discussions. We thank Dr. Peter Quail for the gift of phy mutants and antibodies against phyA and phyB and Dr. Magnus Holm for the gift of sht2-1 mutant.

Received April 4, 2011; accepted May 30, 2011; published June 1, 2011.
blue light receptor, cry1, cry2, phot1, and phot2, by using combinatorial multiple mutants in Arabidopsis. Proc Natl Acad Sci USA 101: 2223–2228

Osterlund MT, Hardtke CS, Wei N, Deng XW (2000) Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature 405: 462–466

Oyama T, Shimura Y, Okada K (1997) The Arabidopsis HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. Genes Dev 11: 2983–2995

Pokhilko A, Ramos JA, Holtan H, Maszle DR, Khanna R, Millar AJ (2002b) Photosensory perception and signalling in plant cells: blue light receptor, cry1, cry2, phot1, and phot2, by using combination during uml multiple mutants in Arabidopsis. Proc Natl Acad Sci USA 101: 1397–1402

Quail PH (2004) Expression profiling of the 26S proteasome in the life of plants. Nat Rev Genet 4: 17: 180–188

R Development Core Team (2008). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Rizzini L, Favory JJ, Cloix C, Faggionato D, O’Hara A, Kaiserli E, R Development Core Team (2011) Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Proc Natl Acad Sci USA 101: 1397–1402

Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, Rubio V, Ma L, Hoyer U, Putterill J, Robson E, Nagy F, Jenkins GI, et al (2011) Perception of UV-B by the Arabidopsis UV88 protein. Science 332: 103–106

Salic R, Sibarita JB, Bechthold A, Bentsink M, Boller T, et al (2005) LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. Nature 435: 995–999

Sullivan JA, Shirasu K, Deng XW (2003) The diverse roles of ubiquitin and the 26S proteasome in the life of plants. Nat Rev Genet 4: 948–958

Tepperman JM, Hudson ME, Khanna R, Zhu T, Chang SH, Wang X, Quail PH (2004) Expression profiling of phyB mutant demonstrates substantial contribution of other phytochromes to red-light-regulated gene expression during seedling de-etiolation. Plant J 38: 725–739

Tepperman JM, Hwang YS, Quail PH (2006) phyA dominates in transduction of red-light signals to rapidly responding genes at the initiation of Arabidopsis seedling de-etiolation. Plant J 48: 728–742

Tepperman JM, Zhu T, Chang HS, Wang X, Quail PH (2001) Multiple transcription-factor genes are early targets of phytochrome A signaling. Proc Natl Acad Sci USA 98: 9437–9442

Tiwari SB, Wang S, Hagen G, Guilfoyle TJ (2006) Transfection assays with protoplasts containing integrated reporter genes. Methods Mol Biol 325: 237–244

Tiwari SB, Wang X-J, Hagen G, Guilfoyle TJ (2001) AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. Plant Cell 13: 2809–2822

Ulm R, Baumann A, Oravecz A, Máté Z, Adam É, Oakeley EJ, Schäfer E, Nagy F (2004) Genome-wide analysis of gene expression reveals functions of the bZIP transcription factor HY5 in the UV-B response of Arabidopsis. Proc Natl Acad Sci USA 101: 1397–1402

Vandenbussche F, Habricot Y, Condiff AS, Maldiney R, Van der Straeten D, Ahmad M (2007) HY5 is a point of convergence between cryptochrome and cytokinin signalling pathways in Arabidopsis thaliana. Plant J 49: 428–441

Wang Z-Y, Tobin EM (1998) Constitutive expression of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) gene disrupts circadian rhythms and suppresses its own expression. Cell 93: 1207–1217

Wei N, Deng XW (2003) The COP1 signalosome. Annu Rev Cell Dev Biol 19: 261–286

Wenkel S, Turck F, Singer K, Gissot L, Le Gourrierre C, Samach A, Coupland G (2006) CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of Arabidopsis. Plant Cell 18: 2971–2984

Whitelam GC, Halliday K (2007). Light and Plant Development. Blackwell, Oxford

Yamamoto YY, Deng XW, Matsui M (2001) CIP1, a new COP1 target, is a nucleus-localized positive regulator of Arabidopsis photomorphogenesis. Plant Physiol 126: 1397–1402

Yamamoto YY, Matsui M, Ang L-H, Deng X-W (1998) Role of COP1 interactive protein in mediating light-regulated gene expression in Arabidopsis. Plant Cell 10: 1083–1094

Yang J, Lin R, Sullivan J, Hoyer U, Liu B, Xu L, Deng XW, Wang H (2005) Light regulates COP1-mediated degradation of FR1, a transcription factor essential for light signaling in Arabidopsis. Plant Cell 17: 804–821

Yi C, Deng XW (2005) COP1: from plant photomorphogenesis to mamalian tumorigenesis. Trends Cell Biol 15: 618–625