Glutathione S-Transferase Interacting with Far-Red Insensitive 219 Is Involved in Phytochrome A-Mediated Signaling in Arabidopsis

Ing-Chien Chen², I-Ching Huang², Ming-Jung Liu, Zhi-Gong Wang, Shu-Shiang Chung, and Hsu-Liang Hsieh*
Institute of Plant Biology, College of Life Science, National Taiwan University, Taipei 106, Taiwan

Far-red (FR) insensitive 219 (FIN219) was previously shown to be involved in phytochrome A-mediated FR light signaling. To further understand its function and regulatory relation with other light-signaling components, a yeast two-hybrid approach was used to isolate FIN219-interacting partners. Here, we demonstrate that FIN219-interacting protein 1 (FIP1) interacts with FIN219 in vitro and in vivo and is composed of 217 amino acids that belong to the tau class of the large glutathione S-transferase gene family. FIP1 was further shown to have glutathione S-transferase activity. The gain of function and partial loss of function of FIP1 resulted in a hyposensitive hypocotyl phenotype under continuous FR (cFR) light and a delayed flowering phenotype under long-day conditions, which suggests that FIP1 may exist in a complex to function in the regulation of Arabidopsis (Arabidopsis thaliana) development. In addition, FIP1 mRNA was down-regulated in the suppressor of phytochrome A-105 1 mutant and differentially expressed in constitutive photomorphogenic 1-4 (cop1-4) and cop1-5 mutants under cFR. Intriguingly, FIP1 expression was up-regulated in the fin219 mutant under all light conditions, except cFR. Furthermore, promoter activity assays revealed that FIP1 expression was light dependent, mainly associated with vascular tissues, and developmentally regulated. Subcellular localization studies revealed that the β-glucuronidase-FIP1 fusion protein was localized in the nucleus and cytoplasm. Taken together, these data indicate that FIP1 may interact with FIN219 to regulate cell elongation and flowering in response to light.

Light has a profound effect on plant growth and development. It not only provides an energy source for plant photosynthesis, but also acts as an important signal to regulate gene expression and various aspects of plant development (Kendrick and Kronenberg, 1994). Plants are equipped with different photoreceptors to sense changes in light. At least four different photoreceptor classes are found in Arabidopsis (Arabidopsis thaliana): phytochromes for red (R) and far-red (FR) light, cryptochromes and phototropins for blue (B) and UV-A light, and an unknown photoreceptor for UV-B light. Phytochromes are the most extensively studied among these photoreceptors and exist in phytochrome R-absorbing (Pr) and phytochrome FR-absorbing (Pfr) forms.

Research into light signal transduction by molecular genetics, cell biology, and DNA microarray approaches has made great progress (Tepperman et al., 2001, 2004; Jiao et al., 2003; Liscum et al., 2003; Matsushita et al., 2003; Parks, 2003; Bauer et al., 2004). In particular, the phytochrome A (phyA)-mediated signaling pathway in continuous FR (cFR) light has been intensively studied. Many intermediate transducers have been isolated (Soh et al., 1998, 2000; Hoecker et al., 1999; Hudson et al., 1999; Bolle et al., 2000; Fairchild et al., 2000; Funkhauser and Chory, 2000; Hsieh et al., 2000; Ballesteros et al., 2001; Desnos et al., 2001; Dieterle et al., 2001; Zeidler et al., 2001; Wang and Deng, 2002), mostly localized in the nuclei (Ni et al., 1998; Hoecker et al., 1999; Hudson et al., 1999; Fairchild et al., 2000; Funkhauser and Chory, 2000; Soh et al., 2000; Ballesteros et al., 2001; Wang and Deng, 2002), some in the cytosol (Bolle et al., 2000; Hsieh et al., 2000), and several in both subcellular locations (Desnos et al., 2001; Zeidler et al., 2001). So phyA can transduce the light signal through these components or directly interact with transcription factors, such as phytochrome-interacting factor 3 (PIF3) to turn on gene expression (Martinez-Garcia et al., 2000). In addition, recent evidence indicated that phyA-mediated signaling is desensitized through ubiquitination of the downstream positive regulators LONG AFTER FAR-RED LIGHT 1 (LAF1) and LONG HYPOCOTYL 5 (HY5) by CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) (Hardtke et al., 2000; So et al., 2003), a key repressor of photomorphogenesis in Arabidopsis. Furthermore, SUPPRESSOR OF PHYTOCHROME A-105 1 (SPA1), a nuclear-localized suppressor of phyA activity, can modulate expression of both LAF1 and HY5 by interacting with COP1, thus desensitizing the phyA-mediated signaling pathway (Saijo et al., 2003; Seo et al., 2003).

Protein-protein interactions play a critical role in signal transduction. For light signaling, a yeast two-hybrid method has been used to search for interaction...
partners of the phytochrome photoreceptors. A group of basic helix-loop-helix transcription factors, PIFs, isolated by this approach in Arabidopsis, have been found to interact with the C-terminal domain of phyA or phyB and are involved in different aspects of phytochrome-mediated phenotypes, such as chlorophyll biosynthesis (Huq et al., 2004), chloroplast development (Monte et al., 2004), light-responsive gene expression (Ni et al., 1998), and hypocotyl elongation (Kim et al., 2003). Moreover, a family of PIF-like (PIL) proteins, such as PIL5 and PIL6, which belong to basic helix-loop-helix factors, also play a crucial role in seed germination and circadian-controlled R light signaling, respectively (Fujimori et al., 2004; Oh et al., 2004). In addition, phytochrome kinase substrate 1 (PKS1), isolated from a yeast two-hybrid screen with the C-terminal 160 amino acids of PHYA used as bait, interacted with Pr and Pfr forms of both PHYA and PHYB and acted in the cytosol as a substrate of both photoreceptor kinases. Another PHYA-interacting protein, nucleoside diphosphate kinase 2 (NDPK2), has a preference for binding the Pfr form and functions as a positive regulator in both phyA and phyB signaling. Recent studies indicate that NDPK2 appears to be involved in auxin-regulated processes, such as cotyledon development, by modulating auxin transport (Choi et al., 2005). Therefore, phytochromes, through interacting with different factors either in the cytosol or in the nucleus, are able to regulate a number of aspects of plant development.

However, interacting partners of the intermediate components and their regulatory relations in light signaling remain to be elucidated. The far-red insensitive 219 (fin219) mutant was derived from the extragenic suppressor screening of the cop1-6 mutant in Arabidopsis and exhibited less sensitivity specifically to cFR. Its gene was cloned by a map-based method and the derived product shares 36% to 47% identity with a GH3 gene family of 19 members in the Arabidopsis genome (Hsieh et al., 2000). The GH3 gene was originally isolated from soybeans (Glycine max) in a rapid induction by auxin (Hagen et al., 1984). Recent studies found that JASMONATE INSENSITIVE 1 (JAR1), responsible for the jar1-1 mutation, has the same locus as FIN219 and belongs to the firefly (Photinus pyralis) luciferase family of adenylylate-forming enzymes (Staswick et al., 2002). Further results indicated that JAR1 is actually a jasmonic acid (JA)-amino synthetase and mediates the formation of JA conjugation with various amino acids. Interestingly, the JA-Ile conjugate can complement the jasmonate insensitivity of the jar1-1 mutant (Staswick and Tiryaki, 2004). In addition, FIN219 has been shown to be induced rapidly by auxin and localized constitutively in the cytosol without changes in subcellular location by light. Moreover, FIN219 was demonstrated to be a suppressor of COP1 (Hsieh et al., 2000), which suggests that FIN219/JAR1 is an important regulator in modulating the integration of phytohormone signaling through auxin, jasmonate, and light signaling. However, its physiological function in light signaling and plant development remains to be elucidated.

To further understand the function of FIN219, we show that FIN219 is indeed involved in phyA-mediated FR light signaling and report on the isolation of a FIN219-interacting partner from a yeast two-hybrid library obtained from the Arabidopsis Biological Resource Center (ABRC). This gene, encoding a glutathione S-transferase (GST; At1g78370/AtGSTU20), was demonstrated to interact with FIN219 in vitro and in vivo and thus was named FIN219-interacting protein 1 (FIP1). Transgenic studies revealed that FIP1 may exist in a complex to regulate hypocotyl elongation and flowering. Promoter activity assays indicated that FIP1 expression was highly associated with the vascular tissues of hypocotyls, cotyledons, leaves, and floral organs. Taken together, these data reveal that FIP1, through interaction with FIN219 in response to light, may play a crucial role in cell elongation and plant development.

RESULTS

The jar1-1 Allele Has a Hyposensitive Phenotype under cFR

The fin219 mutant in Arabidopsis has been shown to have a long hypocotyl phenotype under cFR (Hsieh et al., 2000). Recently, the JAR1 gene was reported to have the same locus as FIN219; however, its corresponding mutant alleles did not show a hyposensitive phenotype under cFR (Staswick et al., 2002). To understand the discrepancy between the fin219 and jar1-1 mutants under cFR, we investigated the phenotype of the jar1-1 mutant and found a longer hypocotyl phenotype under cFR than under other light conditions as compared with the wild-type and fin219 mutant (Fig. 1, A and B). jar1-1 consistently displayed an intermediate hypocotyl phenotype under cFR of less than 20 μmol m⁻² s⁻¹. Especially under low fluence rates of FR at 1.47 μmol m⁻² s⁻¹, the hypocotyl length was close to that of fin219 (Fig. 1C). In addition, a null allele (SALK_059774) of FIN219/JAR1 (Supplemental Fig. S1), obtained from the ABRC, exhibited a similar pattern to that of the jar1-1 allele (data not shown). Thus, the jar1 allele, similar to the fin219 mutant, exhibits a long hypocotyl phenotype under cFR.

Isolation of FIN219-Interacting Partners by the Yeast Two-Hybrid Method

FIN219 is involved in the phyA-mediated light-signaling pathway and is induced rapidly by auxin. It shares 36% to 47% identity at the amino acid level with GH3 members in Arabidopsis (Hsieh et al., 2000) and contains two coiled-coil domains, one in the N terminus and the other in the C terminus (Fig. 2A), which implies that FIN219 may interact with other proteins via these domains. To further understand the FIN219 function in light signaling and plant development, we used a yeast two-hybrid method to isolate FIN219-interacting partners. A FIN219 full-length cDNA was cloned into yeast GAL4 DNA-binding domain
(BD) vector pGBT9 (+2) and used as bait to screen a library CD4-10 obtained from the ABRC. Three clones recovered from medium plates lacking Trp, Leu, and His and were further checked by the use of a yeast mating approach. One of them, FIP1, showed potential interactions with the full-length, N-terminal, and C-terminal regions of FIN219 on the plates lacking Trp, Leu, and His (Fig. 2B). The interaction between FIP1 and FIN219 was further confirmed by in vitro pull-down assay (Fig. 2C, left). The results from pull-down assay with either glutathione (GSH) sepharose for the GST tag or nickel-nitrilotriacetic acid (Ni-NTA) for the His tag and the His-6-FIP1 fusion were expressed in *Escherichia coli*; purified recombinant proteins were used for pull-down assays (Fig. 2C, left). The GST-FIN219 full-length, N-terminal 300 amino acids and C-terminal 274 amino acid fusions and the His-6-FIP1 fusion were expressed in *Escherichia coli*; purified recombinant proteins were used for pull-down assays (Fig. 2C, left). The results from pull-down assay with either glutathione (GSH) sepharose for the GST tag or nickel-nitrilotriacetic acid agarose (Ni-NTA) for the His tag revealed that FIP1 can interact with full-length and C-terminal regions of FIN219 rather than the N terminus (Fig. 2C, right). Interestingly, when His-6-FIP1 and GST tags were mixed, only the GST tag was pulled down by GSH sepharose resins, but not His-6-FIP1 (Fig. 2C, first lane of the middle image); similarly, when His-6-FIP1 and GST tag mixtures were pulled down with Ni-NTA resins, the pulled-down His-6-FIP1 was not recognized by monoclonal anti-GST antibodies (Fig. 2C, first lane of the right image), which is consistent with the notion that GST members in the large GST gene family are quite diverse among different classes (Dixon et al., 2002b). The interaction between FIP1 and FIN219 was further demonstrated in Arabidopsis. Co-IP was carried out with either FIN219 polyclonal antibodies raised against the N-terminal 300 amino acids of FIN219 or c-myc antibodies to precipitate total proteins isolated from 3-d-old cFR-grown transgenic seedlings overexpressing FIP1 cDNA with a c-myc tag. Although FIN219 antibodies recognized two bands, the lower one is the correct one because it was compared with that of wild-type Columbia and a *fin219* null mutant, *fin219T* (SALK_059774; Supplemental Fig. S1). The cross-hybridized band was not immunoprecipitated by FIN219 antibodies in vivo, which indicates that the lower band recognized by antibodies was specific enough to show its interaction with FIP1 (Fig. 2D, a). Consistently, anti-c-myc antibodies were able to immunoprecipitate c-myc-FIP1 as well as FIN219 (Fig. 2D, a). In contrast, FIN219 antibodies can immunoprecipitate FIN219, but not c-myc-FIP1 from wild-type Columbia (Fig. 2D, b), whereas the *fin219* null mutant as a control showed negative results (Fig. 2D, c). Thus, FIP1 is indeed capable of interacting with the C-terminal region of FIN219.

**FIP1 Encodes a Plant GST with Affinity on GSH and 1-Chloro-2,4-Dinitrobenzene Substrates**

Sequencing and BLAST searching revealed that the FIP1 clone isolated from yeast two-hybrid screening encodes a plant GST with 217 amino acids (AtGSTU20/At1g78370) and belongs to the tau class of a large GST gene family. Sequencing and BLAST searching revealed that the FIP1 clone isolated from yeast two-hybrid screening encodes a plant GST with 217 amino acids (AtGSTU20/At1g78370) and belongs to the tau class of a large GST gene family.
gene family composed of 53 members grouped into six classes in Arabidopsis (Dixon et al., 2002a; Wagner et al., 2002). The FIP1 gene consists of two exons and one intron and shares 67% identity at the amino acid level over the whole coding region with At1g78340 (AtGSTU22) within the same tau class (Fig. 3A). However, the similarity of FIP1 to other GSTs of different classes in the same gene family was below 25%. All plant GSTs display a conserved tertiary structure, although the primary amino acid sequences are quite
diverse, which indicates that binding of the ligand such as GSH was important for their activities and functions during evolution (Dixon et al., 2002b). To further understand whether FIP1 has GST activity implied from its primary amino acid sequence, we expressed it in *E. coli* and obtained the recombinant at about 95% purity for activity assay (Fig. 3C, inset). The result showed that FIP1 can use GSH and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates with $K_m$ 0.467 mM and 1.794 mM, respectively (Fig. 3, B and C), which is similar to the value reported in the literature (Singhal et al., 1991; Prapanthadara et al., 1996). Thus, FIP1 interacting with FIN219 does have GST activity.

**Transgenic Seedlings Overexpressing or Showing Reduced FIP1 Expression Exhibit a Hyposensitive Phenotype under cFR**

To determine whether *FIP1* is involved in the light-signaling pathway, we overexpressed the *FIP1* full-length cDNA as a 35S::*myc*-FIP1 construct into wild-type Columbia to examine any effect on the phenotype and its response to light signals. Seven of 14 T2 transgenic lines showed a hyposensitive phenotype under cFR whereby the hypocotyl length is intermediate to those of wild-type Columbia and the fin219 mutant (Fig. 4, A and B). Under other light conditions, no obvious hypocotyl phenotype was observed (Fig. 4B). In addition, we introduced another translational fusion construct, 35S::pRTL2-GUS-FIP1, into wild-type Columbia. Twelve of 30 T2 transgenic lines displayed the same phenotype as those containing the construct 35S::*myc*-FIP1, showing longer hypocotyls than those of the wild type under cFR (data not shown). To further confirm whether the longer hypocotyl phenotype of transgenic seedlings was indeed caused by the overexpression of *FIP1*, RNA gel-blot analysis revealed that transgenic seedlings grown in the same condition as the phenotype described above showed highly expressed *FIP1* mRNA (Fig. 4C, middle image), which confirms that *FIP1* overexpression resulted in a less sensitive hypocotyl phenotype to cFR. Moreover, transgene expression did not affect endogenous *FIP1* expression detected by the 3′-untranslated region (UTR) of *FIP1* as a riboprobe (Fig. 4C, top). In addition, we generated transgenic plants harboring an antisense construct of *FIP1* and obtained one T-DNA insertion line from the ABRC (SALK_080514). This T-DNA insertion line contains one T-DNA inserted in the promoter region of *FIP1*, which was confirmed by a *FIP1* gene-specific primer and T-DNA border sequences. Homozygous *FIP1* antisense transgenic seedlings and T-DNA-inserted seedlings underwent phenotypic investigation under various light conditions. Unexpectedly, all these transgenic seedlings displayed a long hypocotyl phenotype under cFR, with no obvious phenotype under other light conditions (Fig. 4, A and B). RNA gel-blot analysis further indicated that *FIP1* transcripts were slightly reduced in amount, by 20% to 30%, in transgenic seedlings of the *FIP1* antisense lines *FIP1AS*-171 and *FIP1AS*-421, as well as the T-DNA inserted line. When the same blot was reprobed by full-length *FIP1* cDNA, the signal was barely detected (Fig. 4C) and became visible under longer exposure (data not shown), which indicates that *FIP1* transcripts were low in amount under normal conditions in terms of sensitivity detectable by RNA gel-blot analysis. Given that transgenic seedlings overexpressing or showing decreased *FIP1* expression exhibit a similar long hypocotyl phenotype under cFR, *FIP1* might exist in a complex to perform its function, such as regulation of cell elongation. A similar case is also...
Overexpression of EBS results in phenotypic effects similar to those of recessive ebs mutations, namely, early flowering, dwarf phenotype, and reduced fertility (Pineiro et al., 2003). Therefore, pursuing further physiological functions of the potential complex containing FIP1 will be interesting.

**FIP1 Expression Is Regulated by Light-Signaling Transducers**

Because transgenic plants overexpressing or reducing FIP1 expression exhibit a hypo- or long-hypocotyl phenotype in cFR (Fig. 4, A and B) and FIP1 has been shown to interact with FIN219 in vivo under cFR (Fig. 2), a positive regulator involved in the phyA-mediated FR light-signaling pathway, we tested whether various photoreceptors and light-signaling components regulate FIP1 expression. FIP1 expression was examined by reverse transcription (RT)-PCR with gene-specific primers in various mutants grown in cFR for 4 d. FIP1 mRNA expression was comparably expressed among the wild type, phyA mutant, and fin219 mutant. In contrast, its transcripts were significantly decreased in spa1, a suppressor of phyA, and in the weak allele cop1-4, which produced N-terminal 282 amino acids of COP1, but in a wild-type level in the null allele cop1-5 (Fig. 5, A and B), which implies that FIP1 was positively regulated by SPA1 and negatively controlled by COP1. Recent evidence shows that SPA1 and COP1 work together to repress photomorphogenesis (Yang and Wang, 2006). It will be interesting to see how SPA1 and COP1 regulate FIP1 expression at the protein level. To further understand the functional features of FIP1 expression regulated by light, we then tested FIP1 mRNA levels by RT-PCR with gene-specific primers in the wild type and the fin219 mutant under different light conditions. Intriguingly, FIP1 expression in the wild type was greatly increased in cFR as compared to other light conditions (Fig. 5C). Moreover, its expression was up-regulated in the fin219 mutant under all light conditions, except for cFR (Fig. 5C), under which FIP1 expression was comparable to that of the wild type. This result suggests that FIN219 may negatively control FIP1 expression under most light conditions.

**FIP1 Was Mainly Expressed in Vascular Tissues and Developmentally Regulated**

To further explore the possible involvement of FIP1 in plant development, we examined its expression found in **EARLY BOLTING IN SHORT DAYS (EBS)**. Overexpression of EBS results in phenotypic effects similar to those of recessive ebs mutations, namely, early flowering, dwarf phenotype, and reduced fertility (Pineiro et al., 2003). Therefore, pursuing further physiological functions of the potential complex containing FIP1 will be interesting.
specific primers in the 3′-UTR of FIP1 were used to detect FIP1 expression in total RNA isolated from the seedlings. RT-PCR analyses were performed in the seedlings of different photoreceptor and light-signaling mutants grown under cFR (Fig. 6A) and various light conditions (Fig. 6C) for 4 d. Relative expression data of FIP1 are shown in Fig. 6B. One microgram of total RNA isolated from the seedlings underwent RT-PCR with gene-specific primers in the 3′-UTR of FIP1. UBQ, Ubiquitins used for internal control; WL, white light; D, dark; Col, wild-type Columbia. RT-PCR experiments were repeated twice with similar results. Images in A and C were taken under different sensitivity conditions of the fluorescent gel image system.

patterns and regulation by promoter fusion with the β-glucuronidase (GUS) reporter gene. Approximately 1-kb FIP1 promoter sequence was fused with GUS transcriptionally and introduced into wild-type Arabidopsis (ecotype Columbia). FIP1 activity indicated by GUS staining was primarily located in the vascular tissues of cotyledons in very early seedling development, such as at 2 d-white light (Fig. 6, A and B) and cFR (Fig. 6, M and N), then appeared at the shoot apex and the upper part of hypocotyls and in roots at 4- and 7-d white light (Fig. 6, D–G). FIP1 was highly expressed in the basal portion of trichomes on the surface of true leaves, veins, shoot apex, and whole hypocotyls (Fig. 6, J–L) of 7-d-old seedlings grown in white light. Thereafter, its expression was found only in the margins of leaves and in roots at 14-d white light (Fig. 6H). In addition, FIP1 expression was also seen in the vascular tissues of cotyledons of 4-d-old seedlings grown in darkness (Fig. 6I); however, it appeared only in restricted regions of vascular tissues near the hydathode of cotyledons under cFR (Fig. 6P). In continuous R light, its expression was highly restricted to the hydathode of cotyledons (Fig. 6Q); in B light, it was still expressed in the vascular tissues of the cotyledons (Fig. 6R). The site of free auxin production was recently reported to be in the hydathode of leaf tips, later in the lobes of leaves, then leaf margins and basal regions of trichomes, and finally in the central regions of the lamina (Aloni et al., 2003). Thus, the expression pattern of FIP1 seems to coincide with the sites of auxin production.

**FIP1 Was Highly Expressed in Flower Organs and Associated with Vascular Bundles**

Because GUS-staining patterns of the FIP1 promoter activity were primarily in the vascular tissues at the seedling stage and regulated by light and development (Fig. 6), we investigated FIP1 expression at the adult stage. Histochemical GUS staining revealed that FIP1 was highly expressed in the vascular tissues of flower organs, including sepals, petals, stamens, and carpels (Fig. 7, A, J, H, C, F, B, and E). For stamens, GUS staining was seen in the vascular bundles of the anther (Fig. 7F) and the upper part of the filament of the stamen (Fig. 7C). GUS staining was prominent in the stylar xylem underneath the stigma of carpels and in the two medial and lateral vascular bundles of the ovary (Fig. 7, B and E), as well as the internode right beneath the ovary (Fig. 7B). In addition, GUS staining was seen in both ends of younger siliques, especially at the basal region, but disappeared when siliques matured gradually (Fig. 7D). GUS staining was also found in the funiculus, which connects seeds and siliques (Fig. 7G). Moreover, heavy GUS staining was seen at the branch point of stems and young pedicels, as well as the basal region of the stem and the root and at the newly formed inflorescence, rather than the old one (Fig. 7, I and K). Thus, FIP1 was highly expressed in flower organs, especially vascular bundles.

**Transgenic Plants Overexpressing or Reducing FIP1 Expression Displayed a Delayed Flowering Phenotype under Long-Day Conditions**

To further elucidate the physiological functions of FIP1 implied from promoter activity assays showing that FIP1 is highly expressed in flower organs, we investigated the possible effect of the FIP1 gain of function or loss of function on Arabidopsis flowering under long-day conditions. Two different indices, days to flowering and leaf number at bolting, showed that transgenic plants showing overexpressed or underexpressed FIP1 grown to the adult stage displayed a delayed flowering phenotype compared to the wild type under long-day conditions (Table I). Also, the fin219 mutant exhibited slightly delayed flowering. Thus, gain of function or loss of function of FIP1 results in similar phenotypic effects, including long hypocotyls and late flowering, which points to the possibility that FIP1 may be a component of a complex that functions in various aspects of Arabidopsis development.
Figure 6. Histochemical GUS staining of FIP1 promoter activity at the seedling stage under different light conditions. A, Two-day-old seedlings grown in white light. B, Close-up view of the cotyledons in A. C, Close-up view of the roots in A. D, Four-day-old seedlings grown in white light. E, Close-up view of cotyledons in D. F, Close-up view of roots in D. G, Seven-day-old seedlings grown in white light. H, Fourteen-day-old seedlings grown in white light. I, Four-day-old seedlings grown in darkness. J to L, Close-up view of different portions in G. M, Two-day-old seedlings grown in FR light. N, Close-up view of cotyledons in M. O, Close-up view of roots in M. P, Four-day-old seedlings grown in FR light. Q, Four-day-old seedlings grown in R light. R, Four-day-old seedlings grown in B light. Scale bar: A, C, D, F, M, and O, 2.5 mm; B, E, and N, 1.25 mm; G and H, 5 mm; I, P, Q, and R, 1 mm.
GUS-FIP1 Fusion Protein Was Localized in Both the Cytoplasm and the Nucleus and Its Subcellular Location Was Not Changed by Light

To further understand the FIP1 function implied from its location at the subcellular level, FIP1 full-length cDNA was fused with a pRTL2-GUS vector to become a translational fusion construct and subjected to a subcellular localization study in onion (Allium cepa) epidermal cells by particle bombardment. As compared with controls, bombarded cells showed the GUS-FIP1 fusion protein localized in the cytoplasm and the nucleus (Fig. 8B). Moreover, under light, the fusion protein did not change its subcellular location (data not shown). Accordingly, FIN219 is also localized...
in the cytoplasm and remains there regardless of light conditions (Hsieh et al., 2000); interaction between FIN219 and FIP1 as shown in Figure 2 may occur in the cytoplasm to complete their physiological functions.

**DISCUSSION**

Here, we report that a FIN219-interacting protein, FIP1, isolated from a screening of a yeast two-hybrid library CD4-10 from the ABRC, interacted with the C terminus of FIN219 on pull-down assay and with the full length of FIN219 from FIP1-overexpressed plant extracts obtained from FR light-grown transgenic seedlings (Fig. 2). FIP1 may work together with FIN219 under cFR to regulate hypocotyl elongation of Arabidopsis seedlings. This observation was also implied by the result that the gain of function or partial loss of function of FIP1 resulted in similar phenotypic effects, such as hyposensitive hypocotyls under cFR and delayed flowering under long-day conditions (Fig. 4; Table I). A similar case was found in the EBS gene. Its gain-of-function and loss-of-function transgenic plants shared the same phenotypes, including early flowering, dwarf phenotype, and reduced fertility (Pineiro et al., 2003), probably resulting from a failure to form an accurate complex when the target protein is either overexpressed or abolished.

In addition, we found that c-myc-FIP1 fusion proteins isolated from FIP1-overexpressed transgenic seedlings grown under cFR existed in two bands in a native gel, one 70.2 kD and the other 96.2 kD (Supplemental Fig. S3). The 70.2-kD protein band is probably a homodimer of c-myc-FIP1 (a monomer is about 32 kD), whereas the 96.2-kD band is probably a heterodimer consisting of one c-myc-FIP1 and one FIN219 (64.5 kD), which is consistent with the co-IP result showing that FIP1 interacts with FIN219 under cFR (Fig. 2D). In addition, the 96.2-kD band is about 50% the amount of the 70.2-kD band, which implies that most of the FIP1 exists in homodimers and the rest is associated with FIN219. Thus, the heterodimer of FIP1 associated with FIN219 might be responsible for the control of hypocotyl elongation and flowering time.

The fin219 mutant was derived from a suppressor screen of the cop1-6 allele and shown to have a long hypocotyl phenotype specifically under cFR; its gene product belongs to a GH3-like gene family (Hsieh et al., 2000). jar1 was isolated as a jasmonate-resistant mutant and could be involved in a jasmonate-signaling pathway (Staswick et al., 1992). Recently, JAR1 was isolated at the same locus as FIN219, but all the jar1 alleles had no hypocotyl phenotype under cFR (Staswick et al., 2002). Here, we demonstrated that the jar1-1 allele showed a long hypocotyl phenotype under FR below 20 μmol m⁻² s⁻¹ (Fig. 1, A and C); in particular, its hypocotyl length was close to that of the fin219 mutant at approximately 1.47 μmol m⁻² s⁻¹ of FR (Fig. 1C). Moreover, the jar1-1 mutant, like the fin219 mutant, displayed a long hypocotyl phenotype only under cFR (Fig. 1B). That previous results of the jar1 alleles did not show any phenotype under FR is probably due to a single higher FR fluence rate used, resulting in no obvious phenotype. Also, a null mutant of the fin219/jar1 allele with a T-DNA inserted in the second exon displayed a long hypocotyl phenotype with a similar FR fluence-dependent pattern (Supplemental Fig. S1; data not shown). Moreover, FIN219-overexpressed transgenic seedlings exhibited a hypersensitive phenotype at FR below 10 μmol m⁻² s⁻¹ (Hsieh et al., 2000).
Thus, FIN219/JAR1 plays a role in FR light signaling to regulate cell elongation.

Although JAR1 has been shown to be a JA amino synthetase and may play a vital role in the homeostasis of jasmonate, leading to the regulation of plant metabolic, reproductive, and defensive processes (Staswick and Tiryaki, 2004), the function of FIN219/JAR1 in phyA-mediated FR signaling remains to be elucidated. Because FIN219/JAR1 is induced by auxin and methyl jasmonate (Hsieh et al., 2000; data not shown), FIN219/JAR1 plays a key role in integrating both hormone signaling and light signaling, especially phyA-mediated signal transduction. Here, we showed that FIP1 interacting with FIN219 was also involved in FR light signaling, which was further supported by transgenic studies showing that the gain of function or partial loss of function of FIP1 work together to repress photomorphogenesis (Saijo et al., 2003; Yang and Wang, 2006). Previous studies also indicated that FIN219 is a suppressor of COP1 on a genetic basis. Therefore, regulatory relationships among these players become complicated. Examining further how SPA1 and COP1 affect FIP1 at the protein level might be interesting. In addition, FIP1 transcripts were up-regulated in the fin219 mutant under white light, B light, R light, and darkness, whereas its transcripts were comparable in expression in the wild type and the fin219 mutant under FR light (Fig. 5), which implies that FIN219 may regulate the levels of FIP1 through different mechanisms dependent on light conditions. Moreover, we found that FIP1 expression was not induced by methyl jasmonate on histochemical staining of FIP1 promoter::GUS transgenic seedlings and by GUS activity assay for FIP1 promoter activity under cFR (data not shown). Therefore, FIP1 interacts with FIN219 under cFR without the effect of jasmonate.

FIP1 is a member of the plant tau class of the large GST gene family composed of 53 members involved in diverse functions, including detoxification, transport of flavonoid compounds, reduction of oxidative stress, prevention of Bax-induced cell death, and induction of chalcone synthase expression to deter UV damage. So far, no GST members have been reported to be involved in light signaling at the molecular level. Tepperman et al. (2001) used a DNA microarray approach to examine the gene expression profiles induced rapidly by FR irradiation. One of the up-regulated genes is GST (AAD32887) and its expression increased promptly; however, the induction was inhibited by the phyA mutation. Here, we show that FIP1 interacts with FIN219 under cFR and is also involved in phyA-mediated FR signaling, which indicates that some members of GST participate in light signaling to regulate cell elongation. FIP1 was highly expressed in the vascular bundles of male and female flower tissues (Fig. 7, A, F, B, and E) and vascular tissues of seedling stages under different light conditions (Fig. 6). Furthermore, FIP1 was substantially expressed at the branching points of stems and young pedicels (Fig. 7I). It was also highly expressed at the basal region of young siliques, but reduced in expression in mature siliques (Fig. 7D). All these data indicate that FIP1 responds to light signals and is developmentally regulated. Also, FIP1 did not bind to GSH sepharose and was not recognized by anti-GST monoclonal antibodies (Fig. 2C, lane 1, middle and right sections), which is consistent with the notion that plant GSTs differ from animal GSTs in terms of tertiary structure as well as different classes.

The fin219 mutant displayed a slightly delayed flowering phenotype under long-day conditions (Table I), and its late-flowering phenotype was even more prominent under short-day treatment (data not shown). FIN219/JAR1 is a jasmonate-conjugating enzyme (Staswick and Tiryaki, 2004) and may play a crucial role in regulating homeostasis of jasmonate levels that mediate various signaling, including flowering. FIN219 promoter activity studies revealed that FIN219 was also expressed in floral organs, trichomes, and funiculus (Supplemental Fig. S2), which indicates that FIP1 and FIN219/JAR1 share partially overlapped expression patterns. Moreover, FIP1 transcripts were up-regulated by the fin219 mutation under white light (Fig. 5C), possibly leading to lower levels of GSH that may trigger a delayed flowering phenotype (Cobbett, 2000; Ogawa et al., 2001), which is consistent with fin219 null mutants showing a late-flowering phenotype (data not shown). Alternatively, FIP1 acts as a ligand to interact with FIN219 and then binds to jasmonate, thus mobilizing the resulting complex to regulate floral organ development, which leads to control of flowering time. To further explore the involvement of both FIP1 and FIN219/JAR1 in flowering, obtaining the double mutant fip1fin219 and measuring GSH levels in the flowering stage may be worthwhile.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Throughout this article, the wild type is Arabidopsis (Arabidopsis thaliana) ecotype Columbia. B light photoreceptor mutants cry1 (cry1-304), cry2 (cry2-1), and cry2cry2 (cry2-304cry2-2) double mutants are in the Columbia ecotype as described (Mockler et al., 1999). All other mutants used in this study were described previously (Hsieh et al., 2000; Wang and Deng, 2002). The T-DNA insertion line SALK_080514 was obtained from the ABRC and T-DNA was inserted into the promoter region of FIP1, 498 nucleotides upstream from the translation start site.

Surface sterilization and cold treatment of the seeds and the seedling growth conditions were described previously (Hsieh et al., 2000). The light source used in this study was described previously (Peters et al., 1998) and light intensities used for FR, R, B, and white light were described in the figure legends. All transgenic lines used for phenotypic analysis and RNA gel-blot assays were the T3 homozygous generation.
Yeast Two-Hybrid Assay

A GAL4 yeast two-hybrid system was used for screens of FIN219-interacting partners and protein-protein interaction studies. For the pGB79 (+2)-FIN219 construct, a full-length FIN219 cDNA was derived from BamHI digestion of the recombinant plasmid pZPY122-FIN219 and then ligated into the BamHI site of the BD vector pGB79 (+2) to form a bait construct. An activation domain (AD) fusion of yeast two-hybrid library CD4-10 from the ABRC was used as prey. AD fusion recombinant plasmids were isolated by a standard method (Sambrook and Russell, 2001). The bait fusion plasmid pGB79 (+2)-FIN219 and the prey recombinant fusion plasmids were then co-transformed into the yeast host AH109 (CLONTECH) and screened for repeated four times and then the pellet and supernatant were boiled with SDS blotting.

To construct the GUS-FIP1 fusion protein, a full-length FIN219 cDNA fragment containing the N-terminal 300 amino acids of FIN219 was released directly from pZPY22-FIN219 and cloned into the expression vector pGEX-4T-1 (Amersham-Pharmacia). FIP1 was amplified from 1 mg/mL hygromycin for the c-myc-tagged construct and 100 mg/mL gentamycin for the GUS-tagged construct.

Recombinant Plasmids for Plant Transformation

To overexpress FIP1 in Arabidopsis ecotype Columbia and the fn219 mutant, a Ncol-BglII DNA fragment containing full-length FIP1 cDNA was obtained by PCR amplification with the following primer pairs: FIP1-L, 5'-TGGCATCTGTGGGACCACCCCTGATG-3'; and FIP1-R, 5'-CATACATCAGACACATCGGTACCATGAACA-3' (underline indicates the built-in EcoRI site). The fragment was then ligated into the binary transformation vector pCAMBIA1390-c-myc or pPZP221 with a PRTL2-GUS expression cassette. The resulting constructs pCAMBIA1390-c-myc-FIP1 and pPZP221-GUS-FIP1 were introduced into the Agrobacterium GV301 (Jefferson et al., 1987) and then transformed into Arabidopsis ecotype Columbia and the fn219 mutant by floral dipping (Clough and Bent, 1998). Transgenic plants containing transgenes were selected with use of 25 mg/mL hygromycin for the c-myc-tagged construct and 100 mg/mL gentamycin for the GUS-tagged construct.

GST Activity Assay

GST activity of FIP1 as purified recombinant proteins His-6-FIP1 was determined according to Habig et al. (1974) with use of the Microplate Spectrophotometer SPECTRAMAX PLUS (Molecular Devices). The reaction solution contained 100 mM phosphate buffer, pH 6.5, 1 mM GSH, 1 mM CDNB, and an appropriate amount of samples. We read the absorbance change for 3 min. The extinction coefficient for the CDNB-GSH product is 9.6 msec1 cm-1. We adjusted the concentration of CDNB or GSH to evaluate Kmax or Vmax. Lineweaver-Burk analysis was performed by plotting 1/V versus 1/[substrate]. Kmax and Vmax values were determined from the slope (Kmax/Vmax) and y intercept (1/Vmax) of the plot.

RNA Gel-Blot and RT-PCR Analyses

Total RNA was isolated from 4-d-old seedlings of various light photoreceptor and signaling mutants grown under different light conditions as described previously (Hsieh et al., 1996). Twenty micrograms of total RNA were loaded onto the gel and blotted to the positive-charge nylon membrane (Roche). Full-length FIP1 cDNA was prepared by PCR for digoxigenin labeling as a probe according to the manufacturer’s procedure (Roche). Furthermore, a pair of gene-specific primers at the 3’-UTR of FIP1 was also used to investigate the specificity of FIP1 expression. The primer sequences were as follows: FIP1-5'-GGCTGAGTGATGAGGAAACT-3', FIP1-3'-ATACATCAGACACATCGGTAGC-5'. Hybridization and washing conditions were performed as usual (Sambrook and Russell, 2001). For RT-PCR analyses, 1 mg of DNasel-treated total RNA underwent RT at 42°C with ImProm-II reverse transcriptase (Promega). FIP1 was amplified from 1 mL of the 20 mg/mL of cDNA by PCR for 35 cycles (94°C, 30 s; 50°C, 30 s; 72°C, 30 s) using a Peltier thermal cycler (MJ Research) with FIP1 and FIP1 primers described above and ubiquitin 10 for 26 cycles (94°C, 30 s; 56°C, 30 s; 72°C, 30 s) with the following primers for UBQ1, 5'-GACCTTTGCCGAAAAGAATGCGGATGTT and UBQ2, 5'-CGACTTGGCATAGGAAGAGGATACAGG.

Histochemical GUS Staining of FIP1 Promoter Activity

A 1,038-bp promoter region of FIP1 was cloned into the BamHI site of the binary vector pCAMBIA1301 to establish a transcriptional fusion construct and then introduced into wild-type Columbia. The resulting transgenic plants were grown to homozygous lines and underwent histochemical GUS staining as described previously (Jefferson et al., 1987).

Transient Assay of Subcellular Localization by Particle Bombardment

To construct the GUS-FIP1 fusion protein, a BglII DNA fragment was released from the recombinant plasmid pRSET-B-FIP1 and then cloned into
Supplemental Data

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

Supplemental Figure S1. Expression of the FIN219/FAR1 null mutant in Arabidopsis.

Supplemental Figure S2. Histochemical GUS staining of FIN219 promoter activity under white light conditions.

Supplemental Figure S3. Size determination of FIP1 in FIP1-overexpressed transgenic seedlings grown in FR light.

ACKNOWLEDGMENTS

We are grateful to the ABRC (Ohio State University, Columbus) for the yeast two-hybrid library CD4-10. We also appreciate Ashok Kumar H.G. for his interest in the project. We are thankful to W.A. previous reviewers for their helpful comments on the manuscript.

Received December 5, 2006; accepted January 1, 2007; published January 12, 2007.

LITERATURE CITED

Aloni R, Schwalm K, Langhans M, Ullrich CI (2003) Functional divergence in the N-terminal domain of phytochrome B are functional in the nucleus. Plant Cell Physiol 46:1246–1254

Bauer D, Viczian A, Kircher S, Nobis T, Nitschke R, Kunkel T, Panigrahi J, Kim J-I, Hong S-W, Shin B, Choi G, Blakeslee JJ, Murphy AS, Seo K, Fairchild C, Schumaker MA, Quail PH (2000) Direct targeting of light signals through cryptochromes and phototropins: so what’s the blues is all about. Plant Physiol 123:1429–1436

Bolte S, Conz C, Chua N-H (2000) PAT1, a new member of the GRAS family, is involved in phototropism A signal transduction. Genes Dev 14:1343–1345

Boller T, Schreiber S, Frigerio M, Jofre F, Smalle J, Leyser O, Schaller B, Schell J, Reuss K, Nguyen DT, Petrasek P, Greenblatt J, Boudonck K, Riesmeier J, Stiekema W, Jicke S, Fritschy S, Walter N, Driever W, Valverde F, Paszkowski J, Beppu T, Asami T, Chua N-H, Fukaki H, Busch K, Truernitz E, Thomas G (2002) Histochemical GUS staining of FIN219 promoter activity under white light conditions. Plant Cell Physiol 43:753–743

Cobbett CS (2000) Phytochrome and their roles in heavy metal detoxification. Plant Physiol 123:825–832

Desnos T, Puente P, Whitelam GC, Harberd NP (2001) FYH1: a phytochrome A-specific signal transducer. Genes Dev 15:2629–2690

Dieterle M, Zhou Y-C, Schafer E, Funk M, Kretsch T (2001) EID1, an F-box protein involved in phytochrome A-specific light signaling. Genes Dev 15:939–944

Dixon DP, Davis BG, Edwards R (2002a) Functional divergence in the glutathione transferase superfamilies in plants—identification of two classes with putative functions in redox homeostasis in Arabidopsis thaliana. J Biol Chem 277:30859–30869

Dixon DP, Lathrop A, Edwards R (2002b) Plant glutathione transferases. Genome Biol 3:reviews3004.1–3004.10

Fairchild C, Schumaker MA, Quail PH (2000) HFR1 encodes an atypical bHLH protein that acts in phytochrome A signal transduction. Genes Dev 14:2377–2391

Fankhauser C, Chory J (2000) RSI1, an Arabidopsis locus implicated in phytochrome A signaling. Plant Physiol 124:39–45

Fields S, Song O (1989) A novel genetic system to detect protein-protein interactions. Nature 340:245–246

Fujimori T, Yamashino T, Kato T, Mizuno T (2004) Circadian-controlled basic/helix-loop-helix factor, PIF6, implicated in light-signaling transduction in Arabidopsis thaliana. Plant Cell Physiol 45:1078–1086

Habich WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferase: the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Hagen G, Kleinschmidt A, Guilloyle T (1984) Auxin-regulated gene expression in intact soybean hypocotyls and excised hypocotyl sections. Planta 162:147–153

Hardtke CS, Gohda K, Osterlund MT, Oyama T, Okada K, Deng XW (2000) HY5 stability and activity in Arabidopsis is regulated by phosphorylation in its COP1-binding domain. EMBO J 19:497–5006

Hoecker U, Tepperman JM, Quail PH (1999) SPA1, a WD-repeat protein specific to phytochrome A signal transduction. Science 284:496–499

Hsieh HL, Okamoto H, Wang M, Ang LH, Matsui M, Goodman H, Deng XW (2000) FIN219, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of Arabidopsis development. Genes Dev 14:1958–1970

Hsieh HL, Tong CG, Thomas C, Roux SJ (1996) Light-regulated mRNA abundance of a gene encoding a calmodulin-regulated, chromatin-associated NTFase in pea. Plant Mol Biol 30:135–148

Hudson M, Ringli C, Boylan MT, Quail PH (1999) The FARI locus encodes a novel nuclear protein specific to phytochrome A signaling. Genes Dev 13:2017–2027

Huq E, Al-Sady B, Hudson M, Kim C, Apel K, Quail PH (2004) Phytochrome-interacting factor 1 is a critical bHLH regulator of chlorophyll biosynthesis. Science 305:1937–1941

Jefferson RA, Kavanagh TA, Bevan MW (1987) β-Glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901–3907

Jiao Y, Yang H, Ma L, Sun N, Yu H, Liu T, Gao Y, Gu H, Chen Z, Wada M, et al (2003) A genome-wide analysis of blue-light regulation of Arabidopsis transcription factor gene expression during seedling development. Plant Physiol 133:1480–1493

Kendrick RE, Kronenberg GHM (1994) Photomorphogenesis in Plants, Ed 2, Kluwer Academic Publishers, Dordrecht, The Netherlands

Kim J, Yi H, Choi G, Shin B, Song P-S, Choi G (2003) Functional characterization of phytochrome interacting factor 3 in phytochrome-mediated light signal transduction. Plant Cell 15:2399–2407

Liscum E, Hodgson DW, Campbell TJ (2003) Blue light signaling through the cryptochromes and phototropins: so what’s the blues is all about. Plant Physiol 133:1429–1436

Martinez-Garcia J, Huq E, Quail PH (2000) Direct targeting of light signals to a promoter element-bound transcription factor. Science 288:859–863

Matsushita T, Mochizuki N, Nagatani A (2003) Dimers of the N-terminal domain of phytochrome B are functional in the nucleus. Nature 424:571–574

Mockler TC, Guo H, Yang H, Duong H, Lin C (1999) Antagonistic actions of Arabidopsis cryptochromes and phytochrome B in the regulation of floral induction. Development 126:2073–2082

Monte E, Tepperman JM, Al-Sady B, Kazcorowski KA, Alonso JM, Ecker JR, Li X, Zhang Y, Quail PH (2004) The phytochrome-interacting transcription factor, PIF3, acts early, selectively, and positively in light-induced chloroplast development. Proc Natl Acad Sci USA 101:16091–16098

Ni M, Tepperman JM, Quail PH (1998) PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. Cell 95:657–667

Ogawa K, Tatsaka Y, Mino M, Tanaka Y, Iwabuchi M (2001) Association of glutathione with flowering in Arabidopsis thaliana. Plant Cell Physiol 42:524–530

Oh E, Kim J, Park E, Kim J-J, Kang C, Choi G (2004) PIF5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in Arabidopsis thaliana. Plant Cell 16:3045–3058

Parks BM (2003) The red side of photomorphogenesis. Plant Physiol 133:1437–1444

Peterls JL, Szell M, Kendrick RE (1998) The expression of light-regulated genes in the high-pigment-1 mutant of tomato. Plant Physiol 117:797–807

Pineiro M, Gomez-Mena C, Schafer R, Martinez-Zapater JM, Coupland G (2003) EARLY BOLTING IN SHORT DAYS is related to chromatin remodeling factors and regulates flowering in Arabidopsis by repressing FT. Plant Cell 15:1522–1562

Prapanthadara L-A, Koottathep S, Promtet N, Hemingway J, Ketterman AJ (1996) Purification and characterization of a major glutathione

FIP1 Involved in Phytochrome A-Mediated Signaling

Plant Physiol. Vol. 143, 2007 1201
S-transferase from the mosquito *Anopheles dirus* (species B). Insect Biochem Mol Biol 26: 277–285

Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, Rubio V, Ma L, Hoecker U, Deng XW (2003) The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. Genes Dev 17: 2642–2647

Sambrook J, Russell DW (2001) Molecular Cloning: A Laboratory Manual, Ed 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Seo HS, Yang J-Y, Ishikawa M, Ballesteros M, Chua N-H (2003) LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. Nature 423: 995–999

Singhal SS, Tiwari NK, Ahmad H, Srivastava SK, Awasthi YC (1991) Purification and characterization of glutathione S-transferase from sugarcane leaves. Phytochemistry 30: 1409–1414

Soh MS, Hong SH, Hanzawa H, Furuya M, Nam HG (1998) Genetic identification of FIN2, a far red light-specific signaling component of *Arabidopsis thaliana*. Plant J 16: 411–419

Soh MS, Kim Y-M, Han S-J, Song P-S (2000) REP1, a basic helix-loop-helix protein, is required for a branch pathway of phytochrome A signaling in *Arabidopsis*. Plant Cell 12: 2061–2073

Staswick PE, Su W, Howell SH (1992) Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proc Natl Acad Sci USA 89: 6837–6840

Staswick PE, Tiryaki I (2004) The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. Plant Cell 16: 2117–2127

Staswick PE, Tiryaki I, Rowe ML (2002) Jasmonate response locus FAR1 and several related *Arabidopsis* genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. Plant Cell 14: 1405–1415

Staub JM, Wei N, Deng X-W (1996) Evidence for FUS6 as a component of the nuclear-localized COP9 complex in *Arabidopsis*. Plant Cell 8: 2047–2056

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

von Arnim AG, Deng XW (1994) Light inactivation of *Arabidopsis* photomorphogenic repressor COP1 involves cell-specific regulation of its nucleocytoplasmic partitioning. Cell 79: 1035–1045

Wagner U, Edwards R, Dixon DP, Mauch F (2002) Probing the diversity of the *Arabidopsis* glutathione S-transferase gene family. Plant Mol Biol 49: 515–532

Wang H, Deng XW (2002) *Arabidopsis* FHY3 defines a key phytochrome A signaling component directly interacting with its homologous partner FAR1. EMBO J 21: 1339–1349

Yang J, Wang H (2006) The central coiled-coil domain and carboxyl-terminal WD-repeat domain of *Arabidopsis* SPA1 are responsible for mediating repression of light signaling. Plant J 47: 564–576

Zeidler M, Bolle C, Chua NH (2001) The phytochrome A specific signaling component PAT3 is a positive regulator of *Arabidopsis* photomorphogenesis. Plant Cell Physiol 42: 1193–1200