

A Glutathione S-Transferase Regulated by Light and Hormones Participates in the Modulation of Arabidopsis Seedling Development^{1[C][W][OA]}

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Glutathione S-transferases (GSTs) have been well documented to be involved in diverse aspects of biotic and abiotic stresses, especially detoxification processes. Whether they regulate plant development remains unclear. Here, we report on our isolation by reverse transcription-polymerase chain reaction of a plant GST, *AtGSTU17*, from Arabidopsis (*Arabidopsis thaliana*) and demonstrate that its expression is regulated by multiple photoreceptors, especially phytochrome A (phyA) under all light conditions. Further physiological studies indicated that *AtGSTU17* participates in various aspects of seedling development, including hypocotyl elongation, anthocyanin accumulation, and far-red light-mediated inhibition of greening with a requirement of functional phyA. The loss-of-function mutant of *AtGSTU17* (*atgstu17*) resulted in reduced biomass of seedlings and number of lateral roots in the presence of auxin, as well as insensitivity to abscisic acid (ABA)-mediated inhibition of root elongation, with similarity to different *phyA* mutant alleles. Moreover, the root phenotype conferred by *atgstu17* was reflected by histochemical β -glucuronidase staining of *AtGSTU17* promoter activity with the addition of auxin or ABA. Further microarray analysis of wild-type Columbia and *atgstu17* seedlings treated with far-red irradiation or ABA revealed that *AtGSTU17* might modulate hypocotyl elongation by positively regulating some light-signaling components and negatively regulating a group of auxin-responsive genes and modulate root development by negatively controlling an auxin transport protein in the presence of ABA. Therefore, our data reveal that *AtGSTU17* participates in light signaling and might modulate various aspects of Arabidopsis development by affecting glutathione pools via a coordinated regulation with phyA and phytohormones.

Glutathione S-transferases (GSTs; E.C. 2.5.1.18) are a superfamily of multifunctional, dimeric enzymes, best known for their role in enzymatic detoxification of endo- and xenobiotics (Moons, 2005). Plant GSTs are divided into six classes, namely, phi (F), tau (U), zeta (Z), theta (T), lambda (L), and dehydroascorbate reductases (Dixon et al., 2002b; Moons, 2005). The large numbers of F and U classes are plant specific and have major roles in herbicide detoxification (Edwards et al., 2000; Dixon et al., 2002b; DeRidder and Goldsbrough, 2006). Recent evidence indicates that GSTs are also involved in endogenous metabolism, including oxidative stress, flavonoid binding, and regulation of apoptosis (Marrs et al., 1995; Alfenito et al., 1998; Mueller et al., 2000; Kilili et al., 2004). Classes Z and T are plant

homologs of mammals and other organisms and function in primary metabolism such as isomerization of maleylacetoacetate and detoxification of hydroperoxides formed during oxidative stress (Dixon et al., 2000; Kampranis et al., 2000). Classes L and dehydroascorbate reductases are newly found in plants and function in redox homeostasis (Dixon et al., 2002a; Edwards et al., 2005). Thus, the functions of plant GSTs are diverse and might be due to the ability to conjugate glutathione (GSH) to various targets involved in biotic and abiotic stress.

In addition to having functions in various stresses, plant GSTs appear to be involved in plant growth and development (Gong et al., 2005; Moons, 2005). They have been shown to bind hormones such as auxin and cytokinin (Zettl et al., 1994; Gonneau et al., 1998) and can be induced by a wide variety of phytohormones, including ethylene, auxin, methyl jasmonate, salicylic acid, and abscisic acid (ABA; Wagner et al., 2002; Moons, 2003; Smith et al., 2003). That all these hormones regulate many aspects of plant development implies that plant GSTs may play vital roles in plant growth and development as well. However, evidence to substantiate this role has been limited.

Several plant GSTs have been shown to be induced by different qualities of light (Loyall et al., 2000; Tepperman et al., 2001; Chen et al., 2007). *PcGST1* isolated from parsley (*Petroselinum crispum*) cell cultures by fluorescent differential display was induced

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by UV-B light and involved in a UV light-dependent signaling pathway to chalcone synthase (Loyall et al., 2000). Tepperman et al. (2001) used a DNA microarray approach to examine the gene expression profiles induced rapidly by far-red (FR) light irradiation. The expression of one GST, AAD32887, increased rapidly but was inhibited by *phytochrome A* (*phyA*) mutation (Tepperman et al., 2001). Our previous studies have shown that AtGSTU20 (At1g78370/FIN219-interacting protein1 [FIP1]) can interact in vitro and in vivo with FIN219 (Chen et al., 2007), which functions in a *phyA*-mediated FR signaling pathway (Hsieh et al., 2000). Moreover, transgenic *Arabidopsis* (*Arabidopsis thaliana*) seedlings overexpressing or reducing *FIP1* expression exhibited a hyposensitive long-hypocotyl phenotype under continuous FR (cFR) light (Chen et al., 2007). These data indicate that some plant GSTs are regulated by light. However, the functional roles of these GSTs involved in light signaling remain to be elucidated.

To further understand the functional mechanisms of plant GSTs in light signaling pathways, we focused on several candidates affected by *phyA* or FIN219. Here, we report on functional studies of the GST AAD32887/At1g10370/AtGSTU17 previously detected by microarray assay (Tepperman et al., 2001) and down-regulated by *fin219* mutation in FR (H.-J. Chen and H.-L. Hsieh, unpublished data). Our data presented here using transgenic plants and molecular genetic approaches provide further insight into possible functions of AtGSTU17 involved in light signaling, especially *phyA*-mediated photomorphogenesis, and in the integration of various phytohormones to modulate GSH homeostasis in the regulation of *Arabidopsis* development.

RESULTS

Expression of AtGSTU17 Is Regulated by Multiple Photoreceptors

To further confirm the expression patterns of FR-regulated AtGSTU17 transcripts, we performed dark-light transition experiments. Wild-type and *phyA* mutant seedlings were grown in the dark for 2 d, then transferred to FR light for various times; AtGSTU17 expression was examined by RNA gel-blot analysis. AtGSTU17 was induced in 2-d-old wild-type seedlings transferred from the dark to FR light for 1 h, and the level peaked with 6 h FR light; the expression was gradually reduced to constant levels for the remaining FR irradiation periods (Fig. 1A, left section). However, in the *phyA* mutant seedlings, AtGSTU17 induction by 1 and 6 h FR irradiation was substantially reduced (Fig. 1A, right section), which indicates that AtGSTU17 is indeed induced rapidly by FR, and its expression depends on *PHYA*.

To further understand whether AtGSTU17 expression is regulated by other light photoreceptors and

different qualities of light, we performed dark-light transition experiments by growing various photoreceptor mutant seedlings in the dark for 3 d, then transferring them to different colors of light for 6 h; the expression of AtGSTU17 transcripts was examined by RNA gel-blot analysis with a gene-specific probe. AtGSTU17 transcripts in the *phyA* mutant were barely detected under all light conditions, including darkness (Fig. 1, B–F), which implies that AtGSTU17 expression depends strictly on functional *PHYA*. Moreover, AtGSTU17 expression was also reduced in *cry1*, *cry2*, and *cry1cry2* (*cry1/2*) double mutants in the dark (Fig. 1, B and F). In contrast, the level of AtGSTU17 transcripts in *fin219* remained comparable to that in Columbia (Col) and was slightly reduced in the *phyB* mutant as compared with its ecotype Landsberg *erecta* (*Ler*) in the dark (Fig. 1, B and F). In the transition of dark-grown seedlings to 6 h FR irradiation, the level of AtGSTU17 transcripts was decreased in *fin219*, *cry1*, *cry2*, and *cry1/2* mutants (Fig. 1, C and F) but substantially increased in the *phyB* mutant under the same conditions (Fig. 1, C and F). In the transition from dark to red light, the level of AtGSTU17 transcripts appeared to be reduced in *cry1*, *cry2*, and *cry1/2* mutants (Fig. 1, D and F) but remained largely the same in the *phyB* mutant as in the *Ler* ecotype, which implies that *PHYB* under red light may play a lesser role in the regulation of AtGSTU17 expression. As well, the AtGSTU17 transcript level was slightly lower in *fin219* than in Col (Fig. 1, D and F). In the transition from dark to blue light, the AtGSTU17 transcript level was decreased in *cry1*, *cry2*, and *cry1/2* double mutants, with a substantial reduction in *cry1* and *cry1/2* (Fig. 1, E and F). Intriguingly, the level was markedly higher in the *phyB* mutant than in the *Ler* ecotype, in which AtGSTU17 transcripts were barely detected. In addition, AtGSTU17 expression was not affected by *fin219* under the blue light transition (Fig. 1, E and F). Taken together, these expression data reveal that AtGSTU17 is differentially regulated by various photoreceptors and highly controlled by functional *phyA*.

AtGSTU17 Regulates Hypocotyl Elongation Mainly in FR Light

To further understand the functions of AtGSTU17 involved in light signaling, we cloned it by reverse transcription (RT)-PCR on the basis of sequence information deposited in the National Center for Biotechnology Information database. The full-length complementary DNA (cDNA) encodes a 227 amino acid protein and shares 74% identity at the amino acid level with AtGSTU18 (At1g10360) within the same U class (Supplemental Fig. S1). The recombinant proteins generated from an *Escherichia coli* expression system showed enzymatic activities to the substrates GSH and 1-chloro-2,4-dinitrobenzene (CDNB), with K_m value 0.285 mM for GSH at 1.0 mM CDNB and 1.400 mM for CDNB at 1.0 mM GSH (Supplemental Fig. S1), which indicates that AtGSTU17 has higher affinity to both

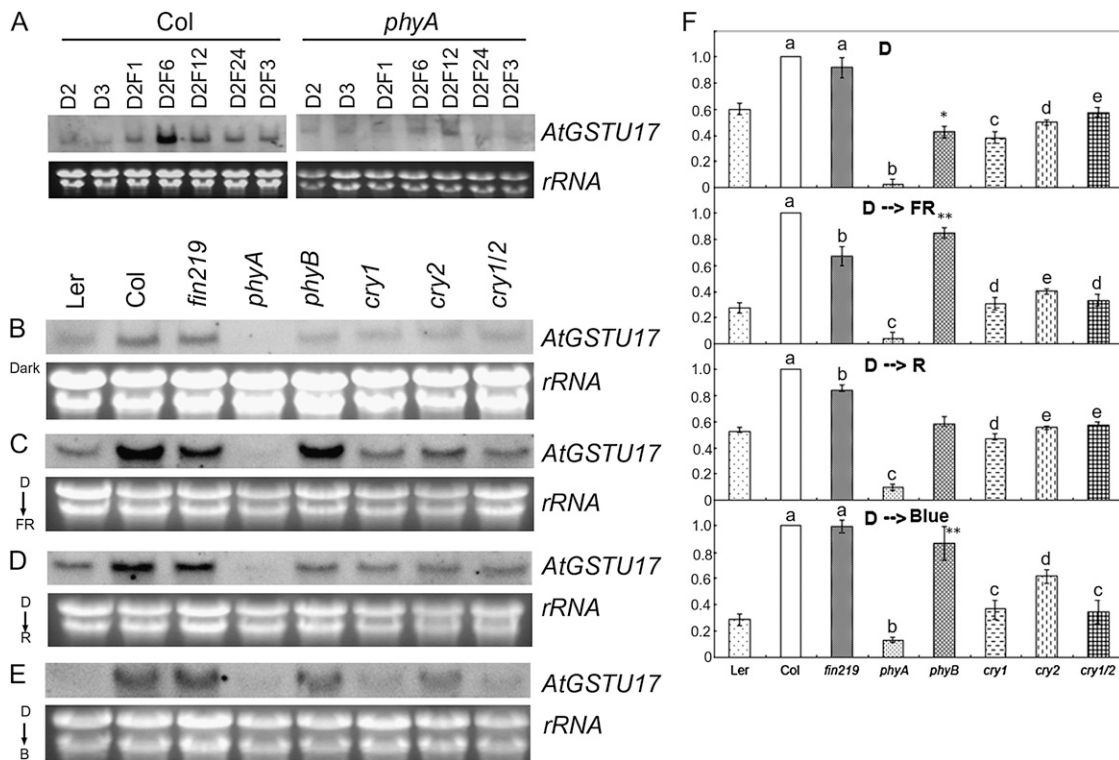


Figure 1. *AtGSTU17* expression is regulated by different photoreceptors. A, RNA gel-blot analysis of *AtGSTU17* expression in wild-type Col and the *phyA* mutant by dark FR light transition. Seedlings of Col and *phyA* were grown in the dark for 2 d (D2) or 3 d (D3) and then transferred to FR light for 1 h (D2F1), 6 h (D2F6), 12 h (D2F12), 24 h (D2F24) h, or 3 d (D2F3). B to E, RNA gel-blot analysis of *AtGSTU17* expression in various photoreceptor mutants under the transition from 3 d dark (B) to 6 h FR light (C), red light (D), and blue light (E). Twenty micrograms of total RNA isolated from treated seedlings were loaded onto each lane and used for RNA gel-blot analyses. The probe is the Dig-labeled 3' UTR of *AtGSTU17*. Ribosomal RNA (rRNA) levels in the ethidium bromide-stained gel were used for a loading control. All experiments were repeated twice independently. Light conditions: FR light ($1.43 \mu\text{mol m}^{-2} \text{s}^{-1}$), red light ($16.71 \mu\text{mol m}^{-2} \text{s}^{-1}$), and blue light ($3.75 \mu\text{mol m}^{-2} \text{s}^{-1}$). F, Quantitative representation of *AtGSTU17* expression levels shown in B to E. The level of *AtGSTU17* expression in Col was set at 1 under the respective conditions. Light conditions: D, dark; R, red; B, blue. Different letters represent statistically different means ($P < 0.05$). Asterisks indicate significant difference (** at $P < 0.01$, $n = 30$; * at $P < 0.05$, $n = 30$) compared to wild-type Ler.

substrates than do other plant GSTs reported in the literature (23). Thus, *AtGSTU17* has GST activities.

To further elucidate the functional regulation between *AtGSTU17* and *phyA*, we used two T-DNA-inserted null mutants, SALK_139615 and SALK_025503, named *atgstu17-1* and *atgstu17-2* from the Arabidopsis Biological Resource Center (ABRC; Supplemental Fig. S2), a new *phyA* allele in a Col background (Supplemental Fig. S4), the double mutant *gstu17-2phyA*, and *AtGSTU17*-overexpressed transgenic lines in Col (*GSTU17OE-2*) for phenotypic examination under various light conditions. The *atgstu17* mutants *atgstu17-1* and *atgstu17-2* exhibited a hypersensitive long-hypocotyl phenotype as compared with Col under low fluences of FR light (Figs. 2A and 3A) but not other light conditions (Figs. 2, B and C, and 3A). In contrast, *GSTU17OE-2* displayed a short-hypocotyl phenotype comparable to that of Col under all light conditions examined (Figs. 2 and 3A). The double-mutant *atgstu17phyA* showed slightly longer hypocotyls than did *phyA* in the low FR fluence (<5

$\mu\text{mol m}^{-2} \text{s}^{-1}$) and was much longer than *atgstu17* under FR light (Figs. 2A and 3A) but did not display a significant hypocotyl phenotype under other light conditions, including darkness (Figs. 2, B and C, and 3A). The phenotype examination indicated that *AtGSTU17* might have a function in the control of hypocotyl elongation in response to FR irradiation.

AtGSTU17-Regulated Photomorphogenic Responses Require Functional *phyA*

Because *AtGSTU17* is regulated by *phyA* and its loss-of-function or gain-of-function mutants show altered hypocotyl response to FR irradiation, we further examined whether changes in *AtGSTU17* expression affected *phyA*-regulated responses. *GSTU17OE-2* in the *phyA* mutant background (*GSTU17OE-2phyA*) showed a slightly shorter long-hypocotyl phenotype as compared with *phyA* but longer hypocotyls than *atgstu17-2* under FR light (Fig. 3A); in contrast, *GSTU17OE-2* in Col under the same condition exhib-

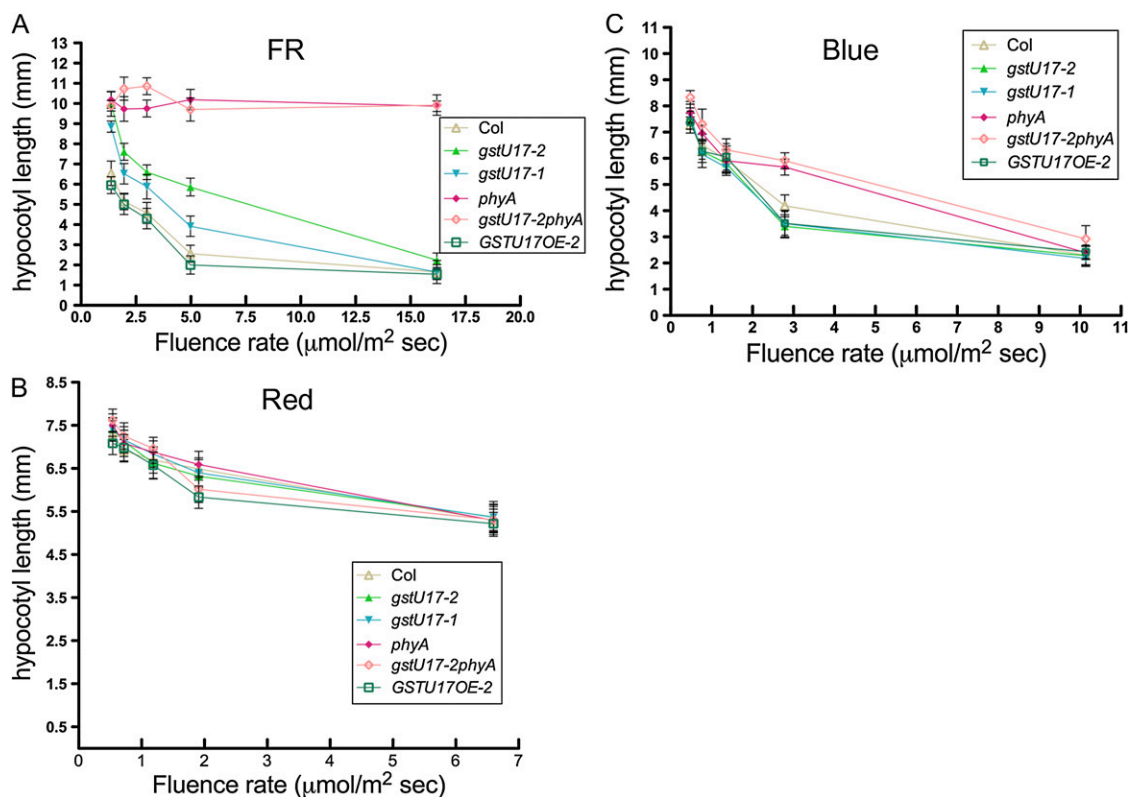


Figure 2. The *atgstU17* mutant displays a long-hypocotyl phenotype specifically in cFR light. Seedlings of wild-type Col, *atgstU17* mutants (*gstU17-1* and *gstU17-2*), *phyA*, the double mutant *gstU17-2phyA*, and *AtGSTU17* overexpression line (*GSTU17OE-2*) were grown in different fluence rates of FR (A), red (B), and blue (C) light for 3 d, then hypocotyl lengths were measured. Mean hypocotyl lengths were calculated from at least 40 seedlings. Error bar represents sd from two independent experiments.

ited a phenotype comparable to that of Col, which implies that *AtGSTU17* function requires functional *phyA* to regulate hypocotyl elongation in response to FR light. Moreover, *atgstU17* showed reduced anthocyanin accumulation and defects in FR-mediated blockage of greening regulated by *phyA* (Fig. 3, B and C). In contrast, *GSTU17OE-2* in Col showed opposite effects on anthocyanin levels and chlorophyll content (Fig. 3, B and C). The double-mutant *atgstU17-phyA* showed levels of anthocyanin and chlorophyll comparable to that of the respective single mutants *atgstU17* and *phyA* (Fig. 3, B and C). *GSTU17OE-2phyA* showed defects in chlorophyll content and anthocyanin accumulation that were substantially different from those of *GSTU17OE-2* in Col (Fig. 3, B and C), which suggests that the effects of *AtGSTU17* on FR blockage of greening and anthocyanin accumulation require functional *phyA*. In addition, *atgstU17* showed a delayed flowering phenotype similar to that of *phyA* (Fig. 3D). The double-mutant *atgstU17phyA* showed a flowering time similar to that of *atgstU17*. In contrast, *GSTU17OE-2* exhibited a flowering time similar to that of wild-type Col, but *GSTU17OE-2phyA* showed a delayed flowering phenotype under long-day conditions (Fig. 3D). Thus, *AtGSTU17* participates in the control of hypocotyl elongation, anthocyanin accumu-

lation, FR blockage of greening, and flowering in a *phyA*-dependent manner.

AtGSTU17 Mutants Show Defects in Auxin- and ABA-Regulated Root Development

GSTs are induced by stresses from biotic and abiotic factors, including herbicides, chemical toxins, osmotic stress, and plant hormones (Marrs, 1996; Smith et al., 2003; DeRidder and Goldsbrough, 2006). Our studies by RNA gel-blot analysis also revealed *AtGSTU17* transcripts up-regulated by ABA, 2,4-dichlorophenoxyacetic acid (2,4-D), and jasmonic acid (JA) treatment (Supplemental Fig. S3). Intriguingly, the induction of *AtGSTU17* expression by 2,4-D and JA was enhanced in white light (Supplemental Fig. S3), which implies that *AtGSTU17* function may involve interactions between light and phytohormones such as auxin and JA. In addition, *atgstU17* mutant seedlings showed less biomass and chlorophyll content than those of Col in the presence of auxin (Fig. 4A); however, *AtGSTU17* overexpression (*OE1* and *OE2*) resulted in an opposite phenotype, with increased biomass and chlorophyll content under the same condition (Fig. 4A), which implies that *AtGSTU17* is able to affect seedling development in response to auxin stimulation. Moreover,

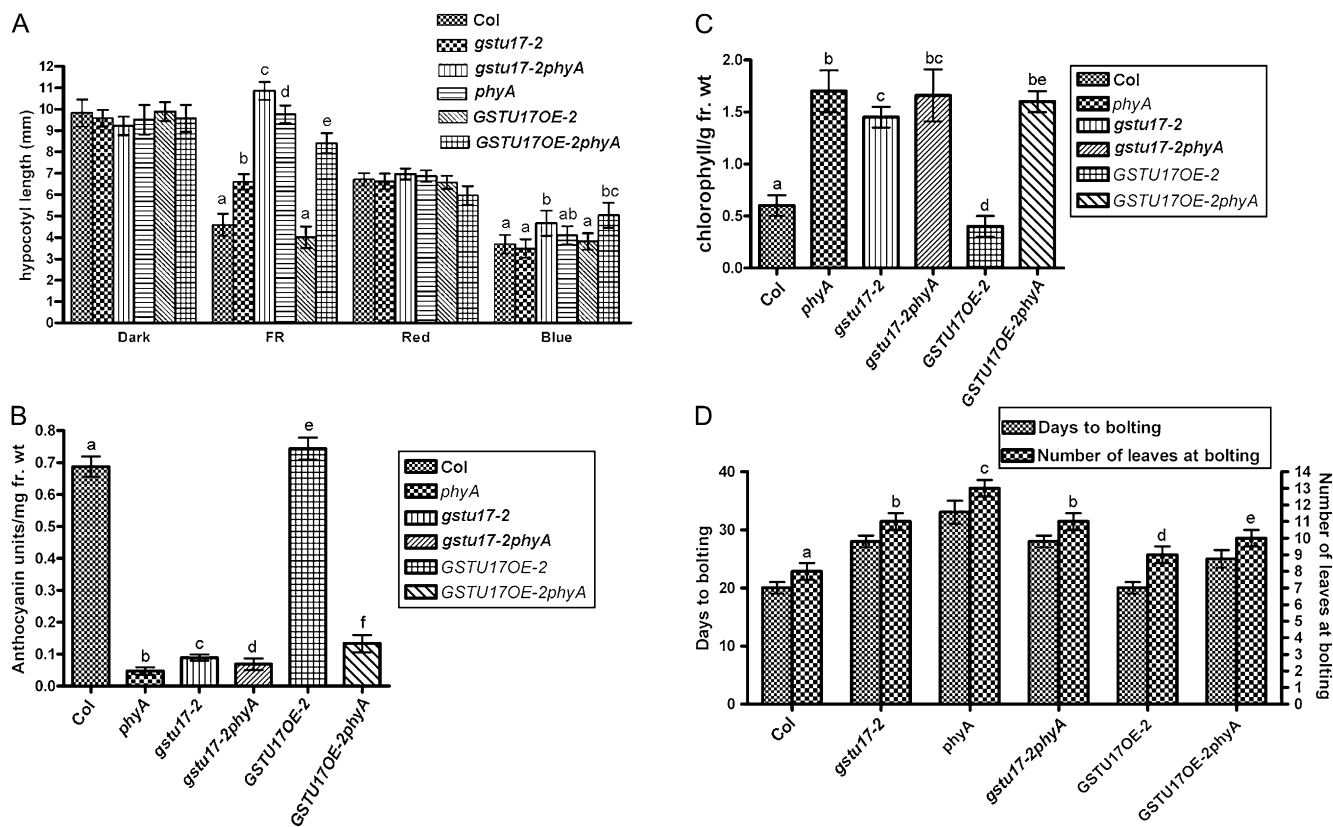


Figure 3. *AtGSTU17* shows a requirement for functional *phyA* in the regulation of various aspects of Arabidopsis development. A to C, Seedlings of Col, *atgstu17* mutant (*gstu17-2*), the double mutant *gstu17-2phyA*, *phyA*, *GSTU17OE-2*, and *GSTU17OE-2* in a *phyA* mutant background (*GSTU17OE-2phyA*) were grown in a low FR fluence rate ($2 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 3 d, then underwent measurement of hypocotyl lengths (A), anthocyanin accumulation (B), or chlorophyll content after 2 d exposure to white light (C). D, The same materials were grown in white light under long-day conditions until the inflorescent stem appeared. Days from seedlings sown in the soil to appearance of the first inflorescence and leaf number at bolting were recorded. All these experiments were replicated at least once. Error bar represents sd. Different letters represent statistically different means ($P < 0.05$).

AtGSTU17 affected the generation of lateral roots in the presence of auxin, with reduced number of lateral roots in *atgstu17* mutants and more lateral roots in *OE1* and *OE2*, but did not largely influence the length of primary roots (Fig. 4B).

Because *AtGSTU17* was highly induced at 1 h after ABA treatment (Supplemental Fig. S3A), we wondered about a phenotypic response on exposure to ABA. Surprisingly, *atgstu17* (*m2*) was less sensitive to ABA-mediated inhibition of root elongation as compared with Col (Fig. 4C). By contrast, *GSTU17OE-2* (*OE2*) displayed a root length comparable to that of Col. Intriguingly, the *phyA* mutant showed insensitivity to ABA-mediated inhibition of root elongation as well (Fig. 4C). To substantiate the insensitivity of *atgstu17* and *phyA* responses to ABA, the double-mutant *atgstU17phyA* and different *phyA* alleles, including *phyA-211*, *Salk_014575*, and *Salk_020360*, were included for further examination. *atgstu17phyA* (*m2phyA*) showed even longer primary roots and lateral roots than those in the respective single mutants (Fig. 4D, left section). As well, all the *phyA* alleles examined exhibited insensitivity to ABA inhibition of

root elongation (Fig. 4D, right section). Thus, *AtGSTU17* and *PHYA* might play a critical role in the control of root development regulated by auxin and ABA.

AtGSTU17 Mutants Affect GSH Homeostasis in Response to Auxin and FR Light Treatment

AtGSTU17 proteins showed enzymatic activity to GSH (Supplemental Fig. S1), which indicates that *AtGSTU17* may regulate GSH pools in Arabidopsis under light and various hormone conditions. The reduced (GSH) and oxidized forms (GSSG) of GSH, as well as its redox state, play critical roles in the control of plant development in response to changes in light and environmental signals (Loyall et al., 2000; Belmonte et al., 2005; Foyer and Noctor, 2009). To associate the GSH/GSSG ratio with the *atgstu17* mutant phenotype under cFR light and auxin treatment, we determined the GSH/GSSG ratio in seedlings of *atgstu17* (*m1* and *m2*) and *GSTU17OE* (*OE1* and *OE2*). The GSH/GSSG ratio was higher in the *atgstu17* mutant than in the wild-type Col in the absence of auxin; however, the GSH/GSSG ratio was decreased

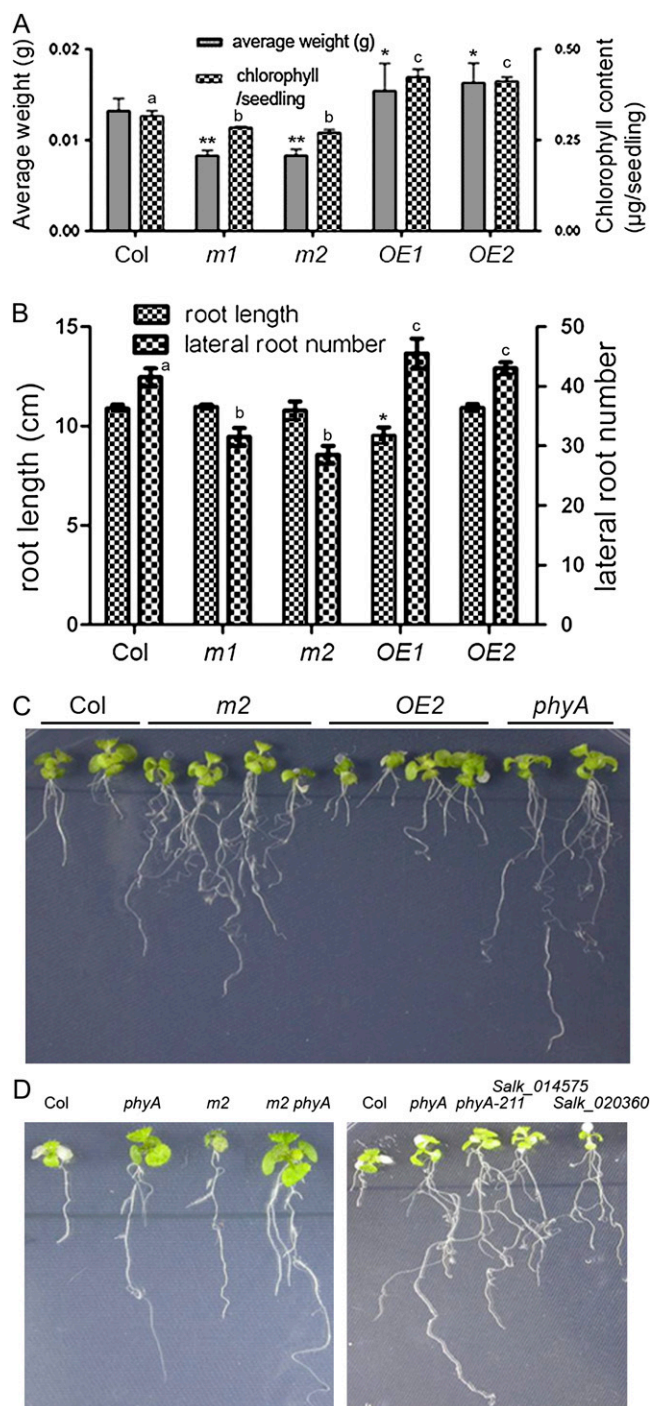


Figure 4. Loss- and gain-of-function mutants of *AtGSTU17* show changes in physiological responses and root development in response to auxin and ABA. **A**, Three-day-old seedlings of wild-type Col, *atgstu17* mutants (*m1* and *m2*), and *AtGSTU17* overexpression lines (*OE1* and *OE2*) grown in GM plates were transferred to vertical plates with 0.5 μM 2,4-D and incubated for another 10 d for measurement of biomass and chlorophyll content. Error bar represents SD. Different letters represent statistically different means ($P < 0.05$). Asterisks mark significantly different data at $P < 0.001$. **B** to **D**, Seedlings of 7-d-old Col, *m1*, *m2*, *OE1*, *OE2*, the double mutant *atgstu17phyA*, and different *phyA* alleles were transferred to vertical plates containing

in *atgstu17* in the presence of 0.5 μM 2,4-D but was still higher than that in Col under the same condition (Fig. 5A), which suggests that *AtGSTU17* may act as a key component to regulate a GSH pool for fine tuning the GSH/GSSG ratio in cells. In contrast, the GSH/GSSG ratio in *GSTU17OE* (*OE1* and *OE2*) was similar to that in Col in the control treatment and in the presence of auxin. Thus, *AtGSTU17* is able to affect GSH/GSSG homeostasis in response to the phytohormone auxin.

In addition, wild-type seedlings grown in cFR versus the dark control showed a significant reduction in GSH/GSSG ratio, which corresponds to a short-hypocotyl phenotype; however, *atgstu17* (*m1*) showed slightly increased GSH/GSSG ratio as compared to Col in cFR. *GSTU17OE* (*OE1* and *OE2*) showed a substantial reduction of GSH/GSSG ratio under the same condition (Fig. 5B). These data are consistent with a role of *AtGSTU17* induced by FR irradiation and leading to a decrease in GSH/GSSG ratio. By contrast, the GSH/GSSG ratio for *atgstu17* grown in the dark did not differ from that of Col, but *GSTU17OE* showed a reduction in GSH/GSSG ratio (Fig. 5B). Thus, *AtGSTU17* is highly regulated by the FR light photoreceptor *phyA* (Fig. 1) and may play a more prominent role in the regulation of GSH pools in FR light than in the dark.

AtGSTU17 Is Highly Expressed in the Initiation Site of Lateral Roots in Response to ABA

Further examination of the promoter activities of *AtGSTU17* by histochemical GUS staining revealed that *AtGSTU17* was mainly expressed in the maturation zone of roots and the basal region of hypocotyls (Fig. 6, A–C). In the presence of auxin, the staining pattern was extended to the meristematic zone with enhanced stains in the initiation sites of lateral roots (Fig. 6, D–F). By contrast, in the presence of ABA, GUS staining was detected in cotyledons, the elongation region (upper portion) and vascular tissue of hypocotyls, the profound initiation sites of lateral roots, and the elongation zone, as well as the meristematic zone of roots (Fig. 6, G–K). Thus, *AtGSTU17* expression patterns appear to be associated with phenotypic responses to both auxin and ABA.

AtGSTU17 Mutation Results in Misregulation of Light-Responsive Genes, Auxin-Induced Genes, and Transcription Factors

To reveal the molecular mechanisms underlying *atgstu17* insensitivity to FR and ABA responses, we performed microarray analysis using wild-type Col

0.01 μM 2,4-D (B) or 30 μM ABA (C and D) and incubated for 10 more days in white light for measurement of primary root length and number of lateral roots. Different letters represent statistically different means ($P < 0.05$). Asterisks indicate significantly different data at $P < 0.05$. $n = 10$.

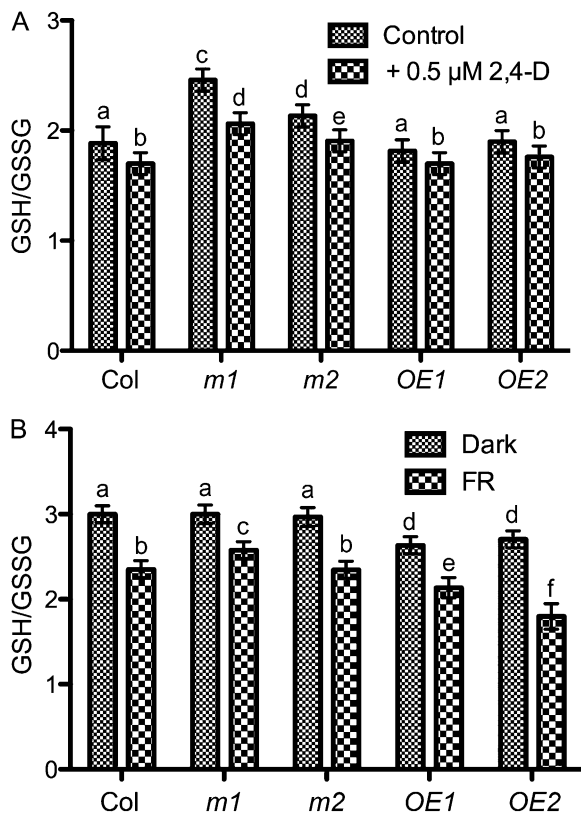


Figure 5. Ratio of GSH/GSSG in *AtGSTU17* mutant and overexpressed seedlings. A, Seedlings of wild type (Col), *atgstu17* mutants (*m1* and *m2*), and *AtGSTU17* overexpression lines (*OE1* and *OE2*) grown in GM plates for 3 d were transferred to vertical plates without (Control) or with 0.5 μM 2,4-D and incubated for another 10 d for measurement of total GSH and GSSG. Error bar represents SD based on two biological replicates. Different letters represent statistically different means ($P < 0.05$). B, Wild type (Col), *atgstu17* mutants (*m1* and *m2*), and *AtGSTU17* overexpression lines (*OE1* and *OE2*) were grown in GM plates under darkness or FR light for 3 d, then used for measurement of total GSH and GSSG. Error bar represents SD based on two biological replicates. Different letters represent statistically different means ($P < 0.05$).

and mutant seedlings grown under cFR or ABA treatment. The mutant *atgstu17* in cFR light showed altered expression of 1,332 genes, with at least 2-fold difference in expression as compared with Col; example genes included some light-regulated genes such as positive-regulator *FAR1-related Sequence* (*FRS8*; At1g80010), negative-regulator *Suppressor of PHYA-105* (*SPA1*; At2g46340) of photomorphogenesis, *Chalcone Isomerase*, and key regulators of flowering time, *AGAMOUS* (At4g18960) and *FLOWERING LOCUS C* (*FLC*; At5g10140). Surprisingly, the expression of phototropism-related genes such as *Non-phototropic Hypocotyl3* (*NPH3*; At3g08660) and *NPH1* (*PHOT1*; At3g45780) was affected in *atgstu17* under cFR (see Supplemental Table S1). As well, more than 20 auxin-responsive genes, including *IAA5* (At1g15580), *IAA6* (At1g52830), and *SAURs* (At3g03830; At4g13790; At4g38850) were

up-regulated in *atgstu17* under cFR light, which implies that *AtGSTU17* negatively regulates the expression of these auxin-responsive genes under cFR light. In addition, *atgstu17* showed altered positive or negative expression of more than 15 transcription factors (Supplemental Table S1). Moreover, *atgstu17* under ABA treatment showed altered levels of 1,850 genes, with at least 2-fold difference in expression from that in Col; examples included greatly reduced expression of florigen gene *FT* (At1g65480), *AGAMOUS* (At4g18960), and several light-regulated receptor kinases (Supplemental Table S2A). In contrast, *SPA1* was up-regulated in *atgstu17* under ABA treatment (Supplemental Table S2B). Some auxin-responsive genes, including auxin transport protein *PIN7* (At1g23080), were also affected in ABA-treated *atgstu17*. Intriguingly, 12 Myb-related transcription factors, one WRKY, and two bHLH transcription factors were down-regulated, and four Myb and three bZIP transcription factors were up-regulated in *atgstu17* under ABA treatment. As well, the negative regulator of flowering time, *FLC*, and MADS-box proteins *AGL3* and *AGL13* were up-regulated in *atgstu17* in the presence of ABA (Supplemental Table S2B). Intriguingly, 13% of the genes were the common genes affected by *atgstu17* mutation in both FR and ABA conditions account. Thus, *AtGSTU17* may function in upstream signaling networks to modulate various regulators, including transcription factors, in the control of diverse aspects of plant development via integration of FR light and ABA.

To validate the expression of the genes affected in *atgstu17* revealed by microarray analysis, several light-regulated genes, including *FRS8* (At1g80010), *RbcS1a*, *CHS*, and *COP1* like (At5g23730); auxin-responsive genes such as *IAA6* (At1g52830) and *IAA5* (At1g15580); and ABA-regulated genes, including *ABA_RP* (At5g13200), *DREB2B* (At3g11020), and *ESR* (At1g54040), were selected for confirmation of transcript levels under FR or ABA treatment. The genes examined by RT-PCR showed expression patterns consistent with those from microarray analysis (Fig. 7), which indicates that *AtGSTU17* can regulate a group of light-responsive genes positively and auxin-regulated genes negatively under FR light, as well as ABA-regulated genes, to modulate seedling development.

DISCUSSION

GSTs belong to a large gene family of 53 members in *Arabidopsis* and have diverse functions such as detoxification, metabolism, and defense in plants (Dixon et al., 2002b). Here we report that *AtGSTU17*, a member of the U class in *Arabidopsis*, is mainly involved in FR light signaling and is regulated by various photoreceptors, especially *phyA*, under all light conditions examined. Moreover, its function appears to require functional *phyA* in hypocotyl elongation, FR-mediated inhibition of greening (Barnes et al., 1996), antho-

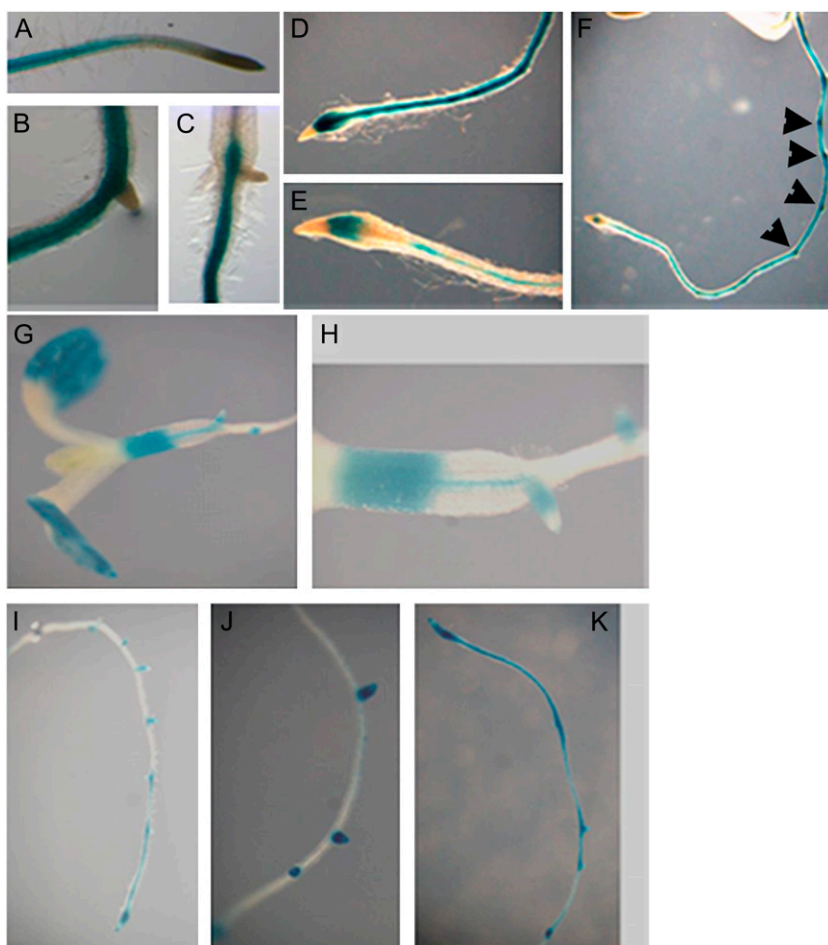


Figure 6. Histochemical GUS staining of *AtGSTU17* promoter activities. Transgenic seedlings harboring the construct of *AtGSTU17* promoter fused with GUS were grown in GM plates without 2,4-D (A–C) or with 1 μM 2,4-D (D–F) or 30 μM ABA (G–K) under white light for 1 d and then subjected to GUS staining. Arrowheads indicate the initiation sites of lateral roots.

cyanin accumulation, and flowering time (Fig. 3). As well, the double mutant *atgstu17phyA* showed defects in physiological responses, including slightly longer hypocotyls in low FR, reduced anthocyanin levels, and insensitivity in FR-mediated inhibition of greening, which suggests that *AtGSTU17* might act in a parallel manner to or interact with *phyA* to modulate some *phyA*-mediated responses. Furthermore, microarray analysis revealed *atgstu17* with up-regulated expression of at least 20 auxin-responsive genes and down-regulated expression of the FR signaling component *FRS8* under FR conditions, which may contribute to combined effects on the long-hypocotyl phenotype of *atgstu17* in FR. In addition, a suppressor of *phyA-1051* (*SPA1*; Hoecker et al., 1998) was up-regulated in *atgstu17* under cFR light, which implies that *phyA*, *AtGSTU17*, and *SPA1* might work in a feedback inhibition manner to maintain a dynamic balance for the regulation of photomorphogenesis.

In addition to its expression being regulated by *PHYA*, *AtGSTU17* was affected positively by the blue-light photoreceptors *cry1* and *cry2* under all light conditions, especially blue light, in which the *cry1* mutant showed greatly reduced *AtGSTU17* mRNA levels (Fig. 1E). Thus, various photoreceptors, includ-

ing *phyB*, may have specific effects on *AtGSTU17* functions in addition to their combined regulation. The physiological significance of *AtGSTU17* regulation by different photoreceptors might be related to the adaptation of certain stress conditions, as was revealed by Xu et al. (2009), who showed that wheat (*Triticum aestivum*) cryptochromes are involved in osmotic stress responses (Xu et al., 2009).

Plant GSTs are involved in a number of biotic and abiotic responses, which might be related to GSTs being able to conjugate GSH to different targets, including phytohormones, that regulate various aspects of plant development and thus may affect homeostasis of phytohormones such as auxin (Zettl et al., 1994) or cytokinin (Gonneau et al., 1998) and GSH within the cells or tissues. Changes in GSH levels have been implicated as affecting plant flowering (Ogawa et al., 2001), root elongation (Vernoux et al., 2000; Espunya et al., 2006), chloroplast transcription (Baena-González et al., 2001), and responses to pathogen infection (Gullner et al., 1999). The *ROOT MERISTEMLESS1* (*RML1*) gene encoding the first enzyme of GSH biosynthesis regulates the establishment of an active postembryonic meristem in the root apex. The *rml1* mutant is deficient in γ -glutamylcysteine synthetase

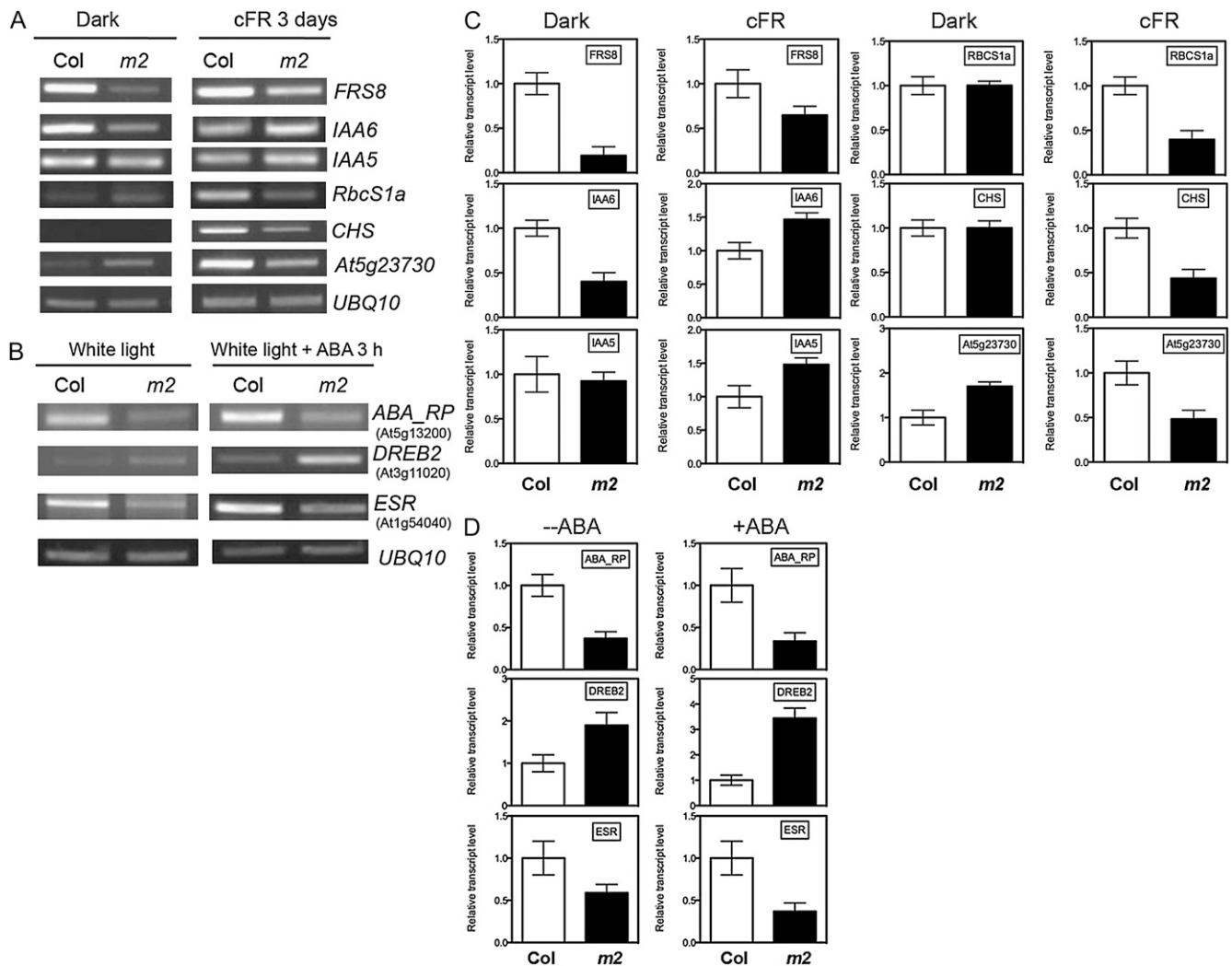


Figure 7. Validation of the expression of some light-, auxin-, and ABA-responsive genes derived from microarray analysis. A and B, Seedlings of Col and *atgstu17* were grown in the dark and cFR for 3 d (A) or white light with or without ABA (B) for 3 h and then underwent RT-PCR analysis with gene-specific primers for the genes shown in the figures. *UBQ10*, *ubiquitin10* gene used for the internal control. C and D, Quantitative representation of the expression of the genes shown in A and B, respectively. The level of the indicated gene in Col was set at 1 under the respective conditions.

activity and results in almost no GSH, thus leading to a very short root phenotype (Vernoux et al., 2000). GSH depletion by the inhibitor of its synthesis, buthionine sulfoximine, specifically reduces primary root growth and results in loss of the auxin efflux carriers PIN1, PIN2, and PIN7 from the root tips of primary roots, thus affecting auxin transport (Wiśniewska et al., 2006; Koprivova et al., 2010). Bashandy et al. (2010) reported that mutations of two key thiol reduction pathways, thioredoxin and GSH (the *ntra ntrb cad2* mutant), resulted in a loss of auxin-regulated phenotypes such as apical dominance, vasculature, and secondary root production, and auxin transport and auxin levels, which implies that the GSH pool within cells is linked to auxin homeostasis. Moreover, alterations of the GSH redox state can improve apical meristem struc-

ture and somatic embryo quality in white spruce (*Picea glauca*; Belmonte et al., 2005). *AtGSTU17*, encoding a U class member of GST, has GST activity (Supplemental Fig. S1). It can be induced by ABA (Fig. 6, G–K; Supplemental Fig. S3), and the loss-of-function mutant exhibits a less-sensitive root phenotype to ABA-mediated inhibition of root elongation than does the wild type (Fig. 4, C and D). This phenotype might be due to high levels of GSH accumulating in the roots of the loss-of-function *atgstu17* mutant as compared with the wild type under ABA treatment (Supplemental Fig. S5), which is consistent with high levels of endogenous GSH enhancing cell division in the root meristematic region leading to root elongation (Vernoux et al., 2000). Because promoter activity assay revealed *AtGSTU17* expression in roots during seedling development (Fig.

6, A–C), *AtGSTU17* expression was even enhanced in the meristematic zone and the initiation sites of lateral roots under auxin and ABA treatments (Fig. 6, D–F and I–K), which is consistent with high levels of GSH in *atgstu17* seedlings treated with auxin or ABA (Supplemental Fig. S5). Intriguingly, microarray data revealed that *atgstu17* showed substantially increased levels of *PIN7* transcript (Wiśniewska et al., 2006; Supplemental Table S2), thus leading to more auxin flow toward the initiation sites of lateral roots under ABA treatment, which may lead to more lateral roots in the *atgstu17* mutant in the presence of ABA (Fig. 4, C and D). This speculation is consistent with very recent reports indicating that the GSH level is linked to PIN proteins such as PIN7, and accordingly auxin transport (Bashandy et al., 2010; Koprivova et al., 2010). Surprisingly, the *phyA* mutant also showed an insensitive long-root phenotype in response to ABA-mediated inhibition of root elongation. Other *phyA* alleles, including *phyA-211* and SALK_014575 (T-DNA insertion line) and SALK_020360, were also less sensitive to ABA inhibition of root elongation (Fig. 4, C and D). Moreover, phytochrome mutants were reported to differentially affect lateral root production and showed up-regulated expression of *PIN3* and *PIN7* transcripts (Devlin et al., 2003; Salisbury et al., 2007). Intriguingly, Sung et al. (2007) reported that a genetic screen identified an arsenic-tolerant double mutant with a single T-DNA insertion in the *PHYA* gene, which exhibited elevated levels of the thiols Cys, γ -glutamylcysteine, and GSH. This finding indicates that *PHYA* negatively regulates thiol synthesis (Sung et al., 2007), which is consistent with our data showing that *AtGSTU17* transcripts are highly down-regulated in the *phyA* mutant (Fig. 1), corresponding to increased levels of GSH in the *atgstu17* mutants. Thus, *phyA* might regulate root development by integrating with *AtGSTU17* to fine tune GSH homeostasis in response to ABA.

As well, *atgstu17* exhibited a long-hypocotyl phenotype under cFR and showed a slight increase of GSH/GSSG ratio compared to wild-type Col; furthermore, *AtGSTU17* overexpression resulted in a significant reduction of GSH/GSSG ratio (Fig. 5B), which implies that *AtGSTU17* can contribute to the regulation of the GSH/GSSG ratio; a high GSH/GSSG ratio is essential for cell division (Belmonte et al., 2005). However, *AtGSTU17* in the dark may have a redundant effect with other GST members on GSH/GSSG ratio for hypocotyl elongation, which led to *atgstu17* having a GSH/GSSG ratio similar to that of Col (Fig. 5B). In addition to the FR effect on GSH/GSSG ratio, application of exogenous auxin could decrease the GSH/GSSG ratio in the wild-type Col (Fig. 5A), which is consistent with data showing that *AtGSTU17* is induced by auxin and has an enzymatic activity to the substrate GSH (Supplemental Figs. S1 and S3). In contrast, the *atgstu17* mutant showed an increase of GSH/GSSG ratio in the absence or presence of auxin (Fig. 5A), which resulted in a significant decrease of biomass and chlorophyll content (Fig. 4A). This finding is probably due to excess oxidant status caused by higher concentration of auxin, such as $0.5 \mu\text{M}$ 2,4-D, which results in a redox imbalance toward oxidation. This speculation is supported by our observation of the *atgstu17* mutant with more anthocyanin accumulation under the same condition (data not shown).

In addition, the *atgstu17* mutant in the presence of ABA showed down-regulated levels of 12 *MYB* genes (Supplemental Table S2), which mainly participate in the regulation of the architecture of Arabidopsis inflorescence (Kirik et al., 1998), floral development (Baumann et al., 2007; Zhou et al., 2008; Zhang et al., 2009), phosphate starvation response (Devaiah et al., 2009), epidermal cell outgrowth (Jakoby et al., 2008), cell wall biosynthesis (Zhong et al., 2008), ABA signaling, and drought stress (Urao et al., 1996; Abe et al.,

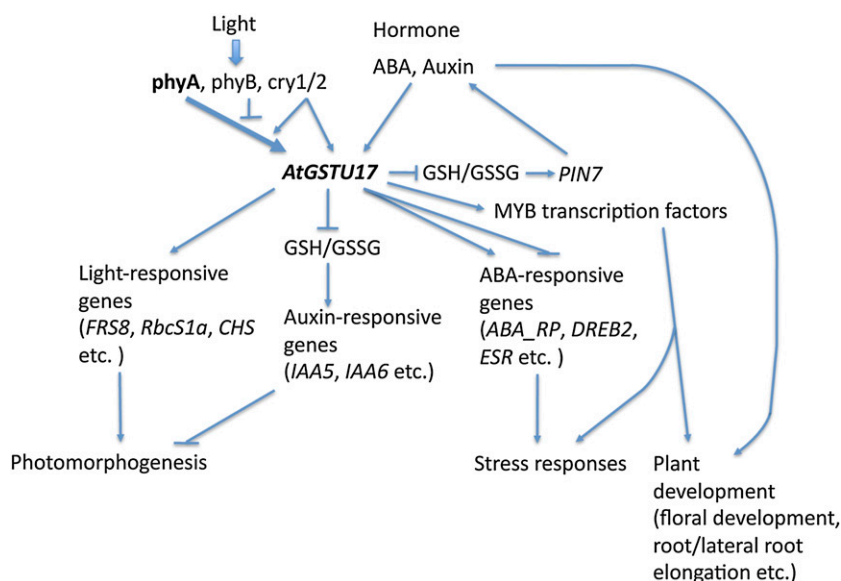


Figure 8. A model illustrates the possible functions of *AtGSTU17* in Arabidopsis. Arabidopsis *AtGSTU17* is differentially regulated by multiple photoreceptors, especially *phyA* under various light conditions, and by different phytohormones, including ABA and auxin. *AtGSTU17* can fine tune GSH homeostasis and GSH/GSSG ratio to regulate the expression of PIN proteins such as PIN7 and auxin-responsive genes, as well as light-responsive genes, ABA-responsive genes, and a number of transcription factors, including MYBs for the modulation of photomorphogenesis, Arabidopsis development such as hypocotyl and root elongation, and stress responses. Bold arrow, highly positive effect; regular arrow, positive effect; inverted T, negative effect. [See online article for color version of this figure.]

2003). In conclusion, AtGSTU17 regulated by various photoreceptors, especially *phyA*, and different phytohormones, including auxin and ABA, may have complicated functions regulating gene expression and plant development such as hypocotyl and root elongation, as well as stress responses by fine tuning GSH homeostasis and redox status in response to changes in light and environmental signals (Fig. 8).

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Wild-type Col, *Ler*, *Arabidopsis* (*Arabidopsis thaliana*), and all mutants in the Col background, except for *phyB* in the *Ler* background, were used in this study. The T-DNA insertion lines *atgstu17-1* (SALK_139615) and *atgstu17-2* (SALK_025503) were obtained from ABRC. Wild-type Col, *Ler*, and mutant seeds were surface sterilized, sown onto the GM (Murashige and Skoog salts plus vitamins, 0.5 g/L MES buffer, pH 5.7, and 0.3% Suc) plates, and treated with various light wavelengths as described (Hsieh et al., 2000). Light fluence rates used in specific light qualities are in the figure legends. As well, homozygous *atgstu17-1* and *atgstu17-2* mutants were crossed to *phyA* to generate the homozygous double mutants *atgstu17-1phyA* and *atgstu17-2phyA*. For transgenic studies, at least T3 homozygous lines of seeds were grown for 3 d under different light conditions for phenotypic examination and RNA gel-blot analyses.

Generation of the Double Mutant *atgstu17phyA*

To generate the double mutant *atgstu17phyA*, T-DNA insertion lines of *AtGSTU17*, *atgstu17-1*, and *atgstu17-2* were crossed with the *phyA* mutant. The resulting F2 progenies were screened for the fast-greening phenotype in white light of the *phyA* mutation after 3 d FR irradiation, and then possible candidates were transferred to soil for seed harvesting. At the adult stage, genotyping for T-DNA insertion of *AtGSTU17* involved use of the genomic DNA as templates isolated from the leaves of the possible double mutants. The primers used for genotyping of homozygous *atgstu17* were the left border primer of T-DNA, Lba1: 5'-TGGTTCACGTAGTGGCCATCG-3', and two *AtGSTU17*-specific primers, CDS of GST30-F: 5'-GGAGGATCCAGAGAA-AATGGCAAGCA-3' and GST30-R: 5'-AAACCTAGTCCACCAACCAATTGGGC-3'. Respective gene expression levels and genotyping in the F3 generation further confirmed the putative double mutant *atgstu17phyA*.

Plasmid Constructs

To generate *AtGSTU17*-overexpressed transgenic lines, a 721-bp full-length cDNA was amplified by PCR with the primers GST30-F 5'-GGAGGATCCAGAGAAATGGCAAGCA-3' and GST30-R 5'-AAACCTAGTCCACCAACCAACCAATTGGGC-3' based on the sequence information deposited in the database of the National Center for Biotechnology Information. The resulting DNA fragment was cloned into the *Bam*H1/*Mfe*I site of the binary vector pCambia1390-cMyc. To establish the transgenic lines of the *AtGSTU17* promoter::GUS, a 1.7-kb *Bam*H1/*Eco*R1 fragment containing the *AtGSTU17* promoter region upstream of the 5' untranslated region (UTR) transcriptional start site was amplified from *Arabidopsis* genomic DNA by PCR and cloned into the binary vector pCambia1381Z.

Arabidopsis Transformation

The *AtGSTU17*-overexpressed or promoter::GUS construct was introduced into *Agrobacterium tumefaciens* (GV3101) by electroporation (Withers, 1995) and then transformed into wild-type Col and the *phyA* mutant by the floral-dip method (Clough and Bent, 1998). Transgenic seedlings were selected on 1% GM plates containing 25 µg/mL hygromycin. The resulting homozygous transgenic lines were used for phenotypic examination.

RNA Gel-Blot Analyses

Total RNA from 3-d-old seedlings under different light conditions, including darkness, or 3-d-old dark-grown seedlings transferred to various light

conditions was isolated as described (Hsieh et al., 1996). RNA samples were separated in a 1.2% denaturing agarose gel, transferred onto a positive-charged nylon membrane (Roche), and UV cross linked and then hybridized with probes. The riboprobe was synthesized by in vitro transcription according to Dig-labeling procedures (Roche). The gene-specific riboprobe was derived from a *Bam*H1-digested construct of the 3' UTR of *AtGSTU17*. All hybridization and washing conditions followed standard methods (Sambrook et al., 1989).

Measurement of Hypocotyl Length, Anthocyanin, Chlorophyll, and Flowering

The wild-type, *atgstu17* mutants, *phyA*, double mutant *atgstu17phyA*, and *AtGSTU17*-overexpressing lines *GSTU17OE* and *GSTU17OE-2phyA* were grown for 3 d under cFR or other light conditions, then hypocotyl lengths and anthocyanin accumulation were determined as described (Hsieh et al., 2000), or were grown for another 2 d of white light after FR light treatment for measurement of chlorophyll content as described (Hsieh et al., 2000). For investigation of flowering time, the seedlings were grown to the adult stage under long-day (16-h-day, 8-h-dark) conditions. Flowering time was recorded by use of two different indexes: days from seedlings sown in the soil to appearance of the first inflorescence, and leaf number at bolting. More than 10 plants for each condition were recorded.

Assays of Hormone-Mediated Inhibition of Root Elongation

Seeds of the wild type, *atgstu17* mutants, *GSTU17OE*, and *phyA* mutants were germinated on Murashige and Skoog medium for 7 d in white light, then transferred to medium plates containing 0.01 µM 2,4-D or 30 µM ABA for vertical growth of roots. The plates were incubated for 5 d under white light. The elongation lengths of new roots of seedlings were measured and recorded. For chlorophyll content and biomass of the wild type, *atgstu17* mutants, and *GSTU17OE* under 0.5 µM 2,4-D treatment were also recorded. All these physiological responses to auxin and ABA were examined in at least two biological replicates.

GST Activity Assay

GST activity of *AtGSTU17* as purified recombinant proteins His-6-GSTU17 was determined as described by Chen et al. (2007).

Analysis of GSH

About 200 mg of seedlings was ground in liquid nitrogen. Subsequently, 2 mL of 1 mM EDTA and 6% (v/v) metaphosphoric acid, pH 2.8, were added and mixed, then centrifuged at 15,000g for 20 min; the supernatant used for determination of total GSH and GSSG by a spectrophotometric assay was as described by Griffith (1980).

Histochemical GUS Staining of *AtGSTU17* Promoter Activity

Transgenic seedlings harboring the *pAtGSTU17::GUS* construct were selected on 1% Murashige and Skoog medium containing 25 mg/L hygromycin. The resulting transgenic plants were selected to homozygous lines and underwent histochemical GUS staining as described (Chen et al., 2007).

Microarray Experiment

Seedlings of the wild-type and *atgstu17* mutants were grown in the dark for 2 d, then transferred to FR for 6 h; or germinated on Murashige and Skoog medium for 7 d in white light, then transferred to medium plates containing 100 µM ABA for another 5 d under white light. Total RNA was extracted from treated plant materials as described (Hsieh et al., 1996). cDNA and cRNA synthesis and hybridization to 22 K Affymetrix Gene Chips (ATH1) were performed according to Affymetrix instructions. Genes with 2-fold expression difference between the wild-type and *atgstu17* mutants were selected for further analysis and validated by RT-PCR.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers AtGSTU17 (At1g10370), phyA (At1g09570), phyB (At2g18790), CRY1 (At4g08920), CRY2 (At1g04400), and FIN219 (At2g46370).

Supplemental Data

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

Supplemental Figure S1. AtGSTU17 alignment and enzymatic activity assays.

Supplemental Figure S2. Phenotypic examination of *AtGSTU17* T-DNA inserted lines in *Arabidopsis* Col.

Supplemental Figure S3. Induction of *AtGSTU17* transcripts by different phytohormones.

Supplemental Figure S4. Characterization of the *phyA* mutant.

Supplemental Figure S5. Measurement of GSH levels in *AtGSTU17* mutant lines and the overexpression line under auxin and ABA treatments.

Supplemental Table S1. A list of selected genes misregulated in *atgstu17* under FR light.

Supplemental Table S2. A list of selected genes affected in *atgstu17* in the presence of ABA.

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