An Arabidopsis GH3 gene, Encoding an Auxin-Conjugating Enzyme, Mediates Phytochrome B-Regulated Light Signals in Hypocotyl Growth

Jung-Eun Park, Pil Joon Seo, An-Kyo Lee, Jae-Hoon Jung, Youn-Sung Kim and Chung-Mo Park *

Department of Chemistry, Seoul National University, Sillim-dong, Gwanak-gu, Seoul 151-742, Korea

An Arabidopsis GH3 gene WES1 encodes an auxin-conjugating enzyme that plays a role in stress responses by modulating endogenous levels of active auxin through a negative feedback regulation. Here, we report a photomorphogenetic role for WES1 in hypocotyl growth. Hypocotyls of the WES1-overexpressing wes1-D and the knockout wes1 mutants were similar to control hypocotyls in darkness. However, the wes1-D hypocotyls were significantly shorter but the wes1 hypocotyls were longer than control hypocotyls under red light. Accordingly, WES1 transcription was up-regulated in a phytochrome B mutant. These results provide support for WES1 regulating hypocotyl growth by mediating phytochrome B-perceived light signals.

Keywords: Auxin — GH3 — Hypocotyl growth — Light signalling — Phytochrome B.

Abbreviations: B, blue light; EOD, end-of-day; FR, far-red light; HR, hypersensitive reaction; PR, pathogenesis-related; R, red light; RT-PCR, reverse transcription-PCR; SA, salicylic acid; SAUR, small auxin-up RNA.

There are three major classes of genes that are rapidly induced by exogenous auxin: those encoding Aux/IAAs, small auxin-up RNAs (SAURs) and a group of GH3 proteins (Hagen and Guilfoyle 2002). A few Aux/IAAs have been demonstrated to regulate photomorphogenesis through light-auxin interactions (Woodward and Bartel 2005). Physiological roles for the SAUR genes recently have begun to emerge. A SAUR gene, named abolished apical hook maintenance 1 (AAM1), is required for shoot apical hook maintenance (Park et al. 2007a). Arabidopsis mutants overexpressing GH3 genes, such as dfil-D (Nakazawa et al. 2001), dfil2-D (Takase et al. 2003), ydk1-D (Takase et al. 2004) and wes1-D (Park et al. 2007b), exhibit dwarfed growth. Notably, several GH3 enzymes, including DFL1 and WES1, conjugate amino acids to IAA (Staswick et al. 2002, Staswick et al. 2005), suggesting that they may help to maintain endogenous auxin levels by conjugating excess auxins to amino acids.

The WES1 gene (At4g27260), which was originally designated AtGH3 (Tanaka et al. 2002) or GH3.5 (Staswick et al. 2005), is unique among the GH3 genes in that it encodes an enzyme adenylating both IAA and salicylic acid (SA) (Staswick et al. 2002). It is also induced by SA and ABA, as well as by auxin, suggesting a role for WES1 in environmental stress responses (Park et al. 2007b). Indeed, the WES1-overexpressing wes1-D mutant exhibits auxin-deficient phenotypes but is resistant to environmental stresses. Accordingly, diverse stress-responsive genes, including pathogenesis-related (PR) and C-repeat/dehydration responsive element-binding factor (CBF), are up-regulated in the mutant, linking auxin with stress responses (Park et al. 2007b). The notion is further supported by genome-wide analysis, in which genes encoding auxin-dampening enzymes, including WES1, are activated in infected plants (Dowd et al. 2004). The GH3-mediated auxin homeostasis may underlie symptoms frequently observed in infected or stressed plants (Bohnert et al. 1995, Heil and Baldwin 2002).

Another molecular feature of the GH3 genes is that they are regulated by light (Hagen and Guilfoyle 2002, Tanaka et al. 2002). A DNA microarray analysis has shown that a few GH3 genes are affected by far-red (FR) light and regulated primarily by phytochrome A (Tepperman et al. 2001). In contrast, a study using a promoter trap line has shown that WES1 is induced by end-of-day (EOD) FR light, suggesting that WES1 may be regulated by phytochrome B (Tanaka et al. 2002). However, the role of WES1 in photomorphogenesis has not been clearly defined because the molecular nature of the reporter gene induction was unclear and light responses of hypocotyl growth were unaltered in the line.

The previous observations prompted us to examine the light responses of the WES1-overexpressing wes1-D and the knockout wes1 mutants. Dark-grown hypocotyls of the mutants were indistinguishable from control hypocotyls. However, light-grown hypocotyls of wes1-D were significantly shorter than control hypocotyls (Fig. 1A). The light
dependence of the \textit{wes1-D} hypocotyls is similar to that of \textit{dfll-D} (Nakazawa et al. 2001) but different from that of \textit{ydkl-D}. The \textit{ydkl-D} hypocotyls are shorter in both the light and darkness (Takase et al. 2004). These observations suggest that the function of \textit{WES1} is distinct from those of other GH3 enzymes. Furthermore, the light-dependent short hypocotyl phenotype of \textit{wes1-D} was more evident under high light fluence than under low light fluence (Fig. 1B). In contrast, the \textit{wes1} hypocotyls were slightly but reproducibly longer than control hypocotyls in the light, and the differences were more evident under low light fluence. These observations suggest that \textit{WES1} plays a role in light-regulated hypocotyl growth.

We next examined the effects of different light wavelengths on hypocotyl growth of \textit{wes1-D} and \textit{wes1}. The \textit{wes1-D} hypocotyl growth was most significantly reduced when grown under red (R) light (Fig. 2A). It was also reduced under blue (B) light to a moderate level. In contrast, the effects of light wavelengths on hypocotyl growth were the least in seedlings grown under FR light. Meanwhile, the \textit{wes1} hypocotyls were slightly but reproducibly longer under R and B light in repeated measurements. The slight difference might be due to functional redundancy among the GH3 proteins (also see Fig. 3). These results suggest that \textit{WES1} function in hypocotyl growth is regulated primarily by phytochrome B. Consistent with this notion, \textit{WES1} expression was induced by EOD-FR light as well as by dark treatment (Fig. 2B), providing support for \textit{WES1} expression being regulated by the light-stable phytochrome B.

To investigate further the phytochrome B regulation of \textit{WES1}, plants containing mutations in various photoreceptor genes were grown in white light, and \textit{WES1} expression was examined. \textit{WES1} transcript levels were very low in control plants and in the phytochrome A mutant (\textit{phyA}). However, it was significantly up-regulated in the phytochrome B mutant (\textit{phyB}) as well as in the \textit{phyAphyB} double mutant (Fig. 2C). It was also up-regulated slightly in the cryptochrome mutants (\textit{cry1} and \textit{cry2}). These results provide support for \textit{WES1} expression being repressed primarily by phytochrome B. Consistent with this notion, the \textit{wes1} hypocotyls showed a reduced EOD-FR response. Whereas they were longer than control hypocotyls in control photoperiods (12 h light and 12 h dark), they were
shorter in the same light conditions but with EOD-FR treatments (Fig. 2D).

To confirm the results, control plants and wes1-D and wes1 mutants were grown under increasing light fluences. The differences in hypocotyl lengths between wes1-D and control plants gradually increased as light fluences increased when grown under R light (Fig. 3). A similar pattern was also observed under B light, but to a lesser degree. In contrast, the differences were much less under FR light. The wes1 hypocotyl length was not significantly different from that of control plants under B and R light. However, the difference was reproducibly observed in at least four independent measurements and was more evident under higher fluences of R light. Together, these results verify that WES1 is regulated principally by phytochrome B.
Light is critical for stress responses (Karpinski et al. 2003, Genoud et al. 2004). Functional phytochromes are essential for the hypersensitive reaction (HR) and PR induction. Cold-induced CBF expression also depends on phytochrome-regulated light signals (Kim et al. 2002). In particular, we previously observed that expression of PRs and CBFs was up-regulated in the wes1-D mutant (Park et al. 2007b). It was therefore hypothesized that stress-responsive genes might be regulated by light wavelengths, like WES1.

To examine this hypothesis, expression of PR genes was investigated in plants grown under different light wavelengths. Individual PR genes exhibited distinct responses to different light wavelengths. Transcript levels of PR1, PR2 and PR5 were higher in light-grown plants, whereas those of PR3 and PR4 were higher in dark-grown plants (Fig. 4A). Interestingly, whereas PR1 expression was markedly repressed by R light, like WES1, PR2 expression was induced by R light.

To examine whether WES1 plays a role in the light responses of PR genes, their transcript levels were measured in wes1-D and wild type grown under different light wavelengths. Notably, those of PR1, PR2 and PR5 were greatly reduced in wes1, whereas they were up-regulated in wes1-D (Fig. 4B). Together, these observations indicate that individual PR genes are differentially regulated by light wavelengths and that WES1 is linked to light regulation of PR genes. In addition, our data suggest that WES1 may be a cross-talk point at which phytochrome-mediated light signals would be incorporated into stress signaling pathways.

Light affects diverse aspects of plant growth and developmental processes through complex signaling networks (Gazzarrini and McCourt 2003, Katagiri 2004). Interactions of light with auxin, SA and ABA signals have been extensively documented (Swarup et al. 2002). SA-induced PR induction and HR to pathogenic infections depend on phytochrome-mediated light signals (Genoud et al. 2002, Karpinski et al. 2003). Roles of auxin signaling mediators have been demonstrated in diverse photomorphogenic processes, such as phototropism, stem and hypocotyl growth, and shade avoidance response (Hagen and Guilfoyle 2002).

In this work, we demonstrated that WES1 is regulated by phytochrome B-regulated light signals in hypocotyl growth.
growth. It is induced by EOD-FR, which is perceived primarily by phytochrome B. It is also induced in the phyB mutant. However, phyB mutations cause long, slender hypocotyls. It is therefore likely that WES1 induction in the phyB mutant would be due to a higher auxin response and that WES1 would be a component of a negative feedback, as has been proposed (Park et al. 2007b). In response to shaded light, primarily mediated by phytochrome B, elongation of hypocotyls/stems and petioles is accelerated, and leaf expansion is reduced (Smith and Whitelam 1997). The previous observations and those of our present study suggest that the WES1 gene may play a role in plant responses, particularly hypocotyl growth, to shaded light.

Notably, at least a subset of PR genes is also regulated by R light in a similar pattern to WES1, suggesting that WES1 is a genetic component linking environmental light signals to biotic stress responses. The light regulation of WES1 may be modulated by altering the endogenous auxin pool. Alternatively, light and auxin signals are integrated into stress signal pathways through the WES1-mediated negative feedback loop to balance plant growth and stress responses.

Materials and Methods

All Arabidopsis thaliana lines used were in the Columbia background (Col-0) except for cryptochrome mutants, which were in the Lansberg erecta (Ler) background. Arabidopsis plants were grown in a controlled culture room at 22°C under long day conditions (16 h light and 8 h dark) with white light illumination (120 μmol m⁻² s⁻¹) provided by fluorescent FLR40D/A tubes (Osram, Seoul, Korea). The wsl-D and wsl (SALK 151766.46.55X, Arabidopsis Biological Resource Center, Ohio State University) mutants have been described previously (Park et al. 2007b). Absence of WES1 expression in wsl was confirmed by reverse transcription–PCR (RT–PCR).

For treatments with different light wavelengths, seedlings were grown in darkness or under R, FR or B light (12, 8 and 15 μmol m⁻² s⁻¹), respectively, in a VS-940L-DUAL incubator (Vision, Seoul, Korea) equipped with R, FR or B light-emitting diodes. Hypocotyl lengths of 30 seedlings grown for 5 d were measured and averaged for each treatment. Statistical significance of the measurements was determined using a Student t-test.

Transcript levels were measured by semi-quantitative RT–PCR. Total RNA samples were extracted from appropriate plant materials using the RNeasy Plant Total RNA Isolation Kit (Qiagen, Valencia, CA, USA). The first-strand cDNA was synthesized from 1 μg of total RNA in a 20 μl reaction volume using the Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The RT–PCR runs consisted of 15–35 cycles, depending on the linear range of PCR amplification for individual genes. Each PCR cycle included incubations at 94°C for 0.5 min, at 55°C for 30 s and at 72°C for 3 min. One additional cycle at 72°C for 7 min was included after the last cycle to allow completion of incomplete polymerizations. The PCR primer sequences have been described previously (Park et al. 2007b), except for CAB1. Those for the CAB1 gene were 5'-GAGCTACCTTACCGGAGAGT for the forward primer and 5'-CTCCTTCACCTTCACTCAG for the reverse primer.

RT-PCR-based Southern blot hybridization was performed by analyzing PCR products on a 1% agarose gel and transferring to a Hybond-N nylon membrane (Amersham-Pharmacia, Piscataway, NJ, USA). The membranes were hybridized with gene-specific probes labeled with [³²P]dCTP using the Megaprime DNA labeling system (Amersham-Pharmacia).

Acknowledgments

This work was supported by the Biogreen 21 (20050301034456) and National Research Laboratory (ROA-2005-000-10002-0) Programs and by grants from the Plant Signaling Network Research Center and Korea Research Foundation (2005-070-C00129).

References


(Received May 4, 2007; Accepted June 28, 2007)