Light Controls Cytokinin Signaling via Transcriptional Regulation of Constitutively Active Sensor Histidine Kinase CKI1[1][OPEN]

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In plants, the multistep phosphorelay (MSP) pathway mediates a range of regulatory processes, including those activated by cytokinins. The cross talk between cytokinin response and light has been known for a long time. However, the molecular mechanism underlying the interaction between light and cytokinin signaling remains elusive. In the screen for upstream regulators we identified a *LONG PALE HYPOCOTYL* (*LPH*) gene whose activity is indispensable for spatiotemporally correct expression of *CYTOKININ INDEPENDENT1* (*CKI1*), encoding the constitutively active sensor His kinase that activates MSP signaling. *lph* is a new allele of *HEME OXYGENASE1* (*HY1*) that encodes the key protein in the biosynthesis of phytochromobilin, a cofactor of photoconvertible phytochromes. Our analysis confirmed the light-dependent regulation of the *CKI1* expression pattern. We show that *CKI1* expression is under the control of phytochrome A (phyA), functioning as a dual (both positive and negative) regulator of *CKI1* expression, presumably via the phyA-regulated transcription factors (TF) PHYTOCHROME INTERACTING FACTOR3 and CIRCADIAN CLOCK ASSOCIATED1. Changes in *CKI1* expression observed in *lph/hy1-7* and *phy* mutants correlate with misregulation of MSP signaling, changed cytokinin sensitivity, and developmental aberrations that were previously shown to be associated with cytokinin and/or CKI1 action. Besides that, we demonstrate a novel role of phyA-dependent *CKI1* expression in the hypocotyl elongation and hook development during skotomorphogenesis. Based on these results, we propose that the light-dependent regulation of *CKI1* provides a plausible mechanistic link underlying the well-known interaction between light- and cytokinin-controlled plant development.

Phytohormones from the cytokinin group regulate many fundamental physiological and developmental programs in plants, including embryo and seed development, germination, photomorphogenesis, plant growth and expansion, organ formation, vascular development, leaf senescence, plant immunity, and regulation of circadian rhythms (Werner and Schmülling, 2009; Kieber and Schaller, 2014). Cytokinin responses in Arabidopsis (*Arabidopsis thaliana*) are transduced via a multistep phosphorelay (MSP) pathway [for a recent review, see Hwang et al. (2012)]. In the MSP, the cytokinin signal is received by one of three sensor His kinases (HKs), AHK2, AHK3, and AHK4, which act as cytokinin receptors (Higuchi et al., 2004; Nishimura et al., 2004; Riefler et al., 2006). Activated HKs are supposed to phosphorylate His-containing phosphotransfer proteins (AHP1 through AHP5 in Arabidopsis; Mähönen et al., 2006), which act as positive regulators of cytokinin signaling (Hutchison et al., 2006) and are believed to transfer the P group to the nuclear-located final P acceptors, the B-type *ARABIDOPSISRESPONSE REGULATORS* (*B-ARRs*; Argyros et al., 2008). B-ARRs bind DNA and directly regulate the transcription of cytokinin-responsive genes (Sakai et al., 2001). There are also A-type *ARRs* (*A-ARRs*) that provide negative regulatory feedback in MSP signaling (Hwang and Sheen, 2001). *A-ARRs* are rapidly up-regulated upon cytokinin application even in the absence of translation, suggesting that they have roles as cytokinin primary response genes (Brandstatter and Kieber, 1998; D'Agostino et al., 2000). Thus, level of expression of *A-ARRs* can be regarded as a measure of MSP activity (Pernisová et al., 2009).

Sensor HK CKI1 was originally identified in an activation mutagenesis-based screen. Overexpression of *CKI1* led to cytokinin-independent cell division and shoot formation in tissue culture, and thus it was proposed that CKI1 acts as a cytokinin receptor (Kakimoto, 1996).

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Subsequent studies have shown that although CKI1 is unable to either be activated by cytokinins or bind cytokinins (Yamada et al., 2001), it shares downstream signaling partners with the cytokinin signaling pathway (Hwang and Sheen, 2001; Hejátko et al., 2009; Deng et al., 2010). CKI1-mediated MSP signaling has been reported as regulating female gametophyte development (Pischke et al., 2002; Hejátko et al., 2003; Deng et al., 2010; Yuan et al., 2016), vascular tissue formation (Hejátko et al., 2009), and root elongation (Deng et al., 2010). Thus, although the *CKI1* expression is hardly detectable (Hejátko et al., 2003, 2009), it seems to play an important role in MSP signaling and Arabidopsis development.

Light is one of the most important environmental signals for plants, modulating a broad spectrum of developmental processes. Regulation of plant development is strongly dependent on the spectral quality, quantity, direction, and periodicity of light (Chen et al., 2004; Kami et al., 2010). To date, several classes of photoreceptors have been identified in plants: phytochromes, cryptochromes, phototropins, and UVR8 and ZTL family proteins. Of these, phytochromes are the light receptors responsible for the majority of light perception (Quail et al., 1995; Rockwell et al., 2006; Li et al., 2011). Phytochromes are red/far-red absorbing photoconvertible (Pr inactive/Pfractive form) chromoproteins, and their photoconvertibility is mediated by phytochromobilin (PΦB), a covalently bound, light-absorbing cofactor molecule.

The existence of interactions between light- and cytokinin-mediated developmental regulations has been known for a long time. Light stimulates an increase in endogenous cytokinin levels (Mizuno et al., 1971; Qamaruddin and Tillberg, 1989; Kraepiel et al., 1994; Zubo et al., 2008). Cytokinins mediate photomorphogenic responses in etiolated Arabidopsis seedlings (Chory et al., 1994) and control expression and/or protein abundance of light-associated gene products, including the gene for the light receptor phytochrome A (phyA; Cotton et al., 1990; Bolle et al., 2000; Brenner et al., 2005, 2012). The interaction of one of the A-ARRs, ARR4, with another light sensor, phytochrome B (phyB), stabilizes phyB in its active (Pfr) form, resulting in plant hypersensitivity to red light (Sweere et al., 2001). On the other hand, it has been proposed that light-mediated up-regulation of cytokinin signaling interferes with auxin in the regulation of stem cell activity during shoot apical meristem (SAM) organogenesis (Yoshida et al., 2011) and that light through cytokinin signaling up-regulates *WUSCHEL* in SAM (Pfeiffer et al., 2016). However, the molecular mechanism of light-dependent control over cytokinin signaling and/or response remains elusive.

In a forward genetic screen, we identified *LONG PALE HYPOCOTYL* (*LPH*) as an upstream factor defining the spatiotemporal expression pattern of *CKI1*. The *lph* mutation defines *hy1-7*, a novel allele of *HEMEOXYGENASE1* (*HY1*). HY1 is a key protein in the biosynthesis of a cofactor of the photoconvertible light receptors, and disruption of its activity in *hy1-7* results in defective light perception. We show that *CKI1* is controlled by light via phyA-mediated signaling. Deregulation of *CKI1* expression in the *hy1-7* and *phyA* mutant causes disturbance in MSP activity and developmental defects previously linked with the activities of CKI1 and the MSP pathway. These results indicate a conceptually novel mechanism involving signal-mediated control of a constitutively active sensor that further controls its cognate signaling pathway and provides a mechanistic link between light- and MSP-mediated regulation of plant development.

RESULTS

Forward Genetic Screening Identifies *LPH* as an Upstream Regulator of *CKI1* Expression

Overexpression of *CKI1* mimics cytokinin effects allowing de novo organogenesis (shooting) on hormone-free media (Kakimoto, 1996). Thus, control of *CKI1* expression may be an intrinsic regulatory mechanism with important developmental impact. To identify regulatory components defining the spatiotemporal expression pattern of *CKI1*, we employed a forward genetic screen. Previously, the correlation between *CKI1* promoter activity, as monitored by a GUS reporter in the *ProCKI1:uidA* line and the endogenous *CKI1* expression pattern, has been confirmed by both in situ *CKI1* mRNA localization and CKI1 immunolocalization (Hejátko et al., 2003, 2009). On the basis of those results, we mutagenized the *ProCKI1:uidA* line using ethyl-methane-sulfonate to search for genes involved in the determination of *CKI1* expression. Screening for mutants with altered *CKI1* expression was facilitated using an automated microscopy method (Dobisová and Hejátko, 2014).

Among the candidate mutant lines, we identified a chlorotic mutant, *long pale hypocotyl* (*lph*), revealing extensive alterations in the *CKI1* expression pattern in both the shoot and root (Fig. 1A and Supplemental Fig. S1A). Typically, in short-d-grown seedlings carrying *ProCKI1:uidA* in the wild-type background (further in the text referred to just as "wild type"), we found *CKI1* expression in the SAM, and only very weak activity was identifiable in the vasculature of cotyledons (Fig. 1A and Supplemental Fig. S1A). *CKI1* expression was also detectable in the vascular tissue of the root/shoot junction, the more mature (upper) part of the root, and in the lateral root cap (LRC). In contrast, in the *lph* background, the expression of *CKI1* was not detected in the SAM and root/shoot junction, while it was up-regulated in the vasculature of cotyledons and the root meristem transition zone, and higher *CKI1* activity was also detected in the LRC (Fig. 1A). Further changes were detectable in the vasculature of true leaves and in the generative developmental phase. Increase in the *CKI1* expression could be found in vascular tissue of all *lph* floral organs (Supplemental Fig. S1A).

The total amount of *CKI1* mRNA in light-grown mutant seedlings was comparable to that in the wild type (Supplemental Fig. S1B), indicating that the mutation affects a factor determining the spatiotemporal pattern (specificity) rather than the overall strength of *CKI1*

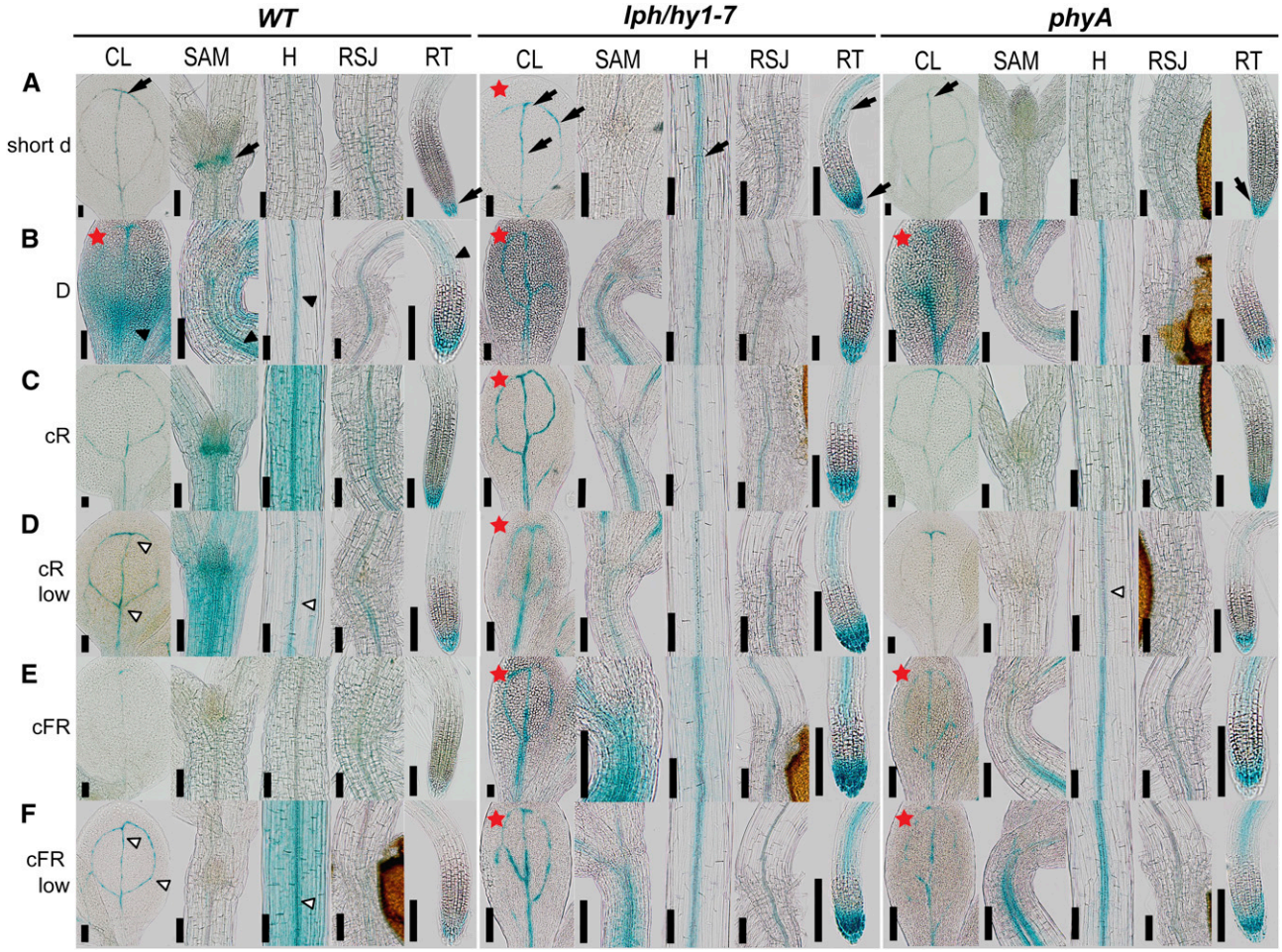

Figure 1. *CKI1* expression under various light conditions (A to F) in wild-type, *lph/hy1-7*, and *phyA* seedlings. *CKI1* expression was analyzed in 6-d-old *ProCKI1:uidA* seedlings grown under short day (short d; 8-h light/16-h darkness), darkness (D), continuous red (cR), and far-red (cFR) light (both 50 μmol m^{-2} s^{-1}) and cR-low and cFR-low (both 2 μmol m^{-2} s^{-1}). In the wild-type, developmental and light-controlled changes in *CKI1* expression could be observed. Onset of etiolation leads to loss of *CKI1* expression in the SAM, while it is up-regulated in the vasculature of hypocotyl and root transition zone. R light stimulates, but FR inhibits *CKI1* activity. *lph/hy1-7* disturbs light-dependent changes in *CKI1* expression pattern and with some minor changes (see the main text), resembles the situation in the wild type under darkness. *phyA* largely phenocopies *CKI1* expression in the *lph/hy1-7*. Note the absence of *CKI1* activity in SAM of *phyA* under short-d and R conditions, suggesting phyA as a positive regulator of *CKI1* in the absence of FR as the dominant light source. phyA mediates the negative effect of FR light on *CKI1* activity indicated by persisting *CKI1* expression in the *phyA* under FR conditions, suggesting the dual role of phyA in the control of *CKI1*. Arrows: Specific *ProCKI1:uidA* signal in individual tissues under the short d conditions. Red asterisk: Conditions leading to etiolation-specific *CKI1* expression pattern. Black arrowhead: *ProCKI1:uidA* signal specific for etiolated seedling phenotype, shown only on wild type as an example. White arrowhead: *CKI1* specific signal related to partially etiolation response under low light intensity. Scale bars: 100 μm. CL, cauline leaf; SAM, shoot apical meristem; H, hypocotyl; RSJ, root, shoot junction; RT, root tip.

expression. Besides the altered *CKI1* expression pattern, the mutant was characterized by chlorosis and elongated hypocotyls. A segregation ratio of 3:1 (green and chlorotic plants) in the mapping population generated by crossing *lph* with L*er-0* suggests that the *lph* mutation is monogenic and recessive in nature.

lph Is a Novel Allele of *HEME OXYGENASE1*

To determine the molecular basis of the *lph* mutation, we employed map-based cloning. *lph* was found to be tightly linked to the SSLP marker nga1126, and, based on the phenotype of *lph*, *HY1* was selected as the most likely candidate gene in this region. We found a point mutation in the genomic DNA of *lph* located close to the donor splicing site in the first intron of *HY1* (Supplemental Fig. S2A). The corresponding region of the *HY1* cDNA sequence was amplified (Supplemental Fig. S2B), and sequencing revealed the presence of an 81 bp deletion in the first exon of *HY1* as a result of the formation of an alternative donor splicing site. This deletion corresponds to a loss of

27 amino acids from the N-terminal portion of HY1 and a G115D mutation (Supplemental Fig. S2, C and D). A genetic test for allelism (based on the phenotype of an F1 population after crossing *lph* with series of *hy* mutants; Supplemental Fig. S3) confirmed that *lph* is allelic with previously identified independent alleles of *hy1* (*hy1-1*, *hy1.6*, and *hy1-100*). Based on these results, *lph* was redesignated *hy1-7*.

To determine whether there is a causal link between *HY1* insufficiency and the control of *CKI1* expression, *ProCKI1*:*uidA* was introgressed into a *hy1-1* background. In the shoots of *hy1-1*/*ProCKI1*:*uidA* plants, we observed changes in *CKI1* expression comparable to those in *hy1-7*/*ProCKI1*:*uidA*. However, in the root of *hy1-1*/*ProCKI1*:*uidA*, we detected a wild-type-like *CKI1* expression pattern (Supplemental Fig. S3B). These findings are consistent with the fact that the *hy1-7* phenotype exhibits the strongest defects in the shoot and the root development of all the tested *hy1* mutants (Supplemental Fig. S3C and later in the text). In contrast, *hy1-1* is a weaker allele, resembling the *hy1-7* phenotype in the shoot, but exhibiting a wild-type-like root (Supplemental Fig. S3, C and D; and data not shown).

Overall, these data prove that there is a causal link between *HY1* function and *CKI1* expression, and show a positive correlation between changes in *CKI1* expression and the strength of the *hy1* phenotype.

Light Regulates Expression of *CKI1*

HY1 has been described as the key member of the heme oxygenase family (Davis et al., 1999; Muramoto et al., 1999). It is of crucial importance in the biosynthetic pathway of PΦB, a cofactor necessary for the formation of photoconvertible phytochromes and thus for proper light sensing (Parks and Quail, 1991; Rockwell et al., 2006). Light signaling deficiency, together with heme accumulation, also affects chlorophyll biosynthesis in *hy1* (Chory et al., 1989; Terry and Kendrick, 1999). As indicated by the name that we originally used to designate the mutant line (*lph*), *hy1-7* exhibits marked photomorphogenic defects, a phenotype that is consistent with the previously described role of HY1 in light perception (Davis et al., 1999; Muramoto et al., 1999). In *hy1-7*, total chlorophyll content is significantly decreased (Supplemental Fig. S4A) and hypocotyls of light-grown *hy1-7* seedlings are longer in comparison to wild type (Supplemental Fig. S4, B and C). Taken together, the molecular nature of HY1, together with the previously reported interaction of light and cytokinin signaling pathways [reviewed by Zdarska et al. (2015)], led us to study the role of light in the control of *CKI1* expression.

We inspected *CKI1* expression under different light conditions. In addition to the previously tested short-d conditions (Fig. 1A), *ProCKI1*:*uidA* seedlings were grown in darkness (Fig. 1B) and in continuous red (cR) and far-red (cFR), both at photon fluxes of 50 μmol m^{-2} s^{-1} (Figs. 1, C and E; for the entire hypocotyl length see Supplemental Fig. S4E). In the etiolated (dark-grown) *ProCKI1*:*uidA* seedlings, the activity of the *CKI1* promoter increased in the vasculature of the cotyledons, hypocotyl, and the transition zone of the root when compared with short-d conditions, while it was strongly down-regulated in the SAM. cR had mostly stimulatory effects on *CKI1* activity: under cR illumination, *CKI1* was up-regulated in the vasculature of the hypocotyl and cotyledons, in the SAM and in the nonvascular tissues of hypocotyl (both epidermis and ground tissues). In contrast, in cFR-grown seedlings, the *CKI1* signal was almost absent from all tissues, suggesting a strong negative effect of cFR on *CKI1* expression (Fig. 1E).

To investigate potential light dose-dependent effect on *CKI1* expression, *ProCKI1*:*uidA* seedlings were grown in the presence of R and FR light of decreased intensity (cR-low and cFR-low, respectively, both at 2 μmol m^{-2} s^{-1}). The overall expression pattern, when compared to that observed in the presence of cR and cFR, did not change, particularly in the SAM and root tip, suggesting wavelength-specific effects on *CKI1* expression in those tissues. However, under the cR-low and cFR-low conditions we observed quantitative changes in the *CKI1* activity in cotyledons and the hypocotyl (compare Fig. 1, D and F, and Supplemental Fig. S4E). In comparison to cR up-regulating *CKI1* in both apical and basal parts of the hypocotyl, cR-low stimulated the *CKI1* expression just under the SAM but not in the more distant (basal) hypocotyl portion. Under cFR-low (similarly to cFR), we observed inhibition of *CKI1* in the SAM and the root tip. However, the *CKI1* activity in cotyledons and the lower part of the hypocotyl was up-regulated when compared to short d (compare Fig. 1, A and F, and Supplemental Fig. S4E).

In contrast to wild type, the *hy1-7* line exhibited dramatically decreased sensitivity of *CKI1* to different light treatments. When compared to short-d conditions, partial light sensitivity of *CKI1* expression in *hy1-7* remained apparent only under cFR, leading to a slightly enhanced signal in the hypocotyl under the SAM (Fig. 1E and Fig. S4F). However, cFR light was unable to inhibit *CKI1* expression in any tissue in the *hy1-7* background. In general, irrespective of light conditions, the *CKI1* expression pattern in *hy1-7* resembled the *CKI1* expression in the etiolated wild-type seedlings. However, some differences were found between the etiolated wild type and *hy1-7*. We have seen up-regulated *CKI1* in the vasculature of cotyledons in *hy1-7* while higher activity of *CKI1* was apparent in the hypocotyl of wild type, and diffuse staining was apparent at the base of wild-type cotyledons (Fig. 1B and Supplemental Fig. S4F). These differences might have an important role in the changed behavior of wild type and *hy1-7* during skotomorphogenesis (see below).

In conclusion, both light quality and quantity are important factors controlling the spatiotemporal specificity of *CKI1* expression in Arabidopsis. As may be expected, considering the molecular nature of the mutation, *hy1-7* inhibited light control over *CKI1*, leading to a changed, light-insensitive expression pattern, largely resembling the *CKI1* expression observed in etiolated

wild type irrespective of light growth conditions. The absence of *CKI1* activity in the SAM of the dark-grown wild type and *hy1-7* (under all light conditions) and expression of *CKI1* in the SAM of R-grown seedlings implies the R light (not excluding the other components of the white light spectrum) as a positive regulator of *CKI1* expression in the SAM. On the other side, FR light inhibits *CKI1* expression in the SAM and columella/LRC.

CKI1 Expression Is Under Control of the phyA Signaling

The strong inhibitory effect of FR light on *CKI1* expression suggested an important role for the light sensor phyA that is solely responsible for FR-mediated signaling in Arabidopsis (Nagatani et al., 1993; Reed et al., 1994). To further demonstrate the regulation of *CKI1* by phyA in planta, we introduced the *ProCKI1*:*uidA* construct by crossing into the *phyA* background. Under short d, *phyA* partially phenocopied *hy1-7*, leading to the up-regulation of *CKI1* in cotyledons with absence of *CKI1* expression in the SAM. Further, again similarly to *hy1-7*, *phyA* impaired all the light-mediated changes of *CKI1* activity observed in the wild type, i.e. cR-dependent up-regulation in the hypocotyl and cFR-mediated inhibition of *CKI1* in the root tip (Fig. 1, C and E). The specific role of *phyA* in the cFR-mediated down-regulation of *CKI1* in SAM cannot be determined, as there is no *CKI1* activity in the SAM of *phyA*, irrespective of light conditions (as also seen in the case of *hy1-7*). However, in contrast to *hy1-7*, we did not see up-regulation of *CKI1* in the vasculature of hypocotyl and root transition zone or in the LRC of short-d-grown *phyA* (compare wild type, *hy1-7*, and *phyA* in Fig. 1A). Based on the etiolated seedling phenotype of *hy1-7* on short d and our further observations (discussed later in the text), these differences seem to be attributable to the etiolation-specific expression pattern of *CKI1* (see below).

As the phyA is solely responsible for FR-mediated signaling, the etiolated phenotype is observed in FR-grown *phyA*. To confirm the importance of phyA in the FR-mediated inhibition of *CKI1* expression, irrespective of possible etiolation-specific developmental regulations, we performed another type of experiment. Immediately after the dark adaptation phase, 4-d-old etiolated seedlings were grown for 2 d on cFR (Fig. 2, A and B). In the wild, under these conditions we detected only residual *CKI1* activity (compare Figs. 1B and 2A), while in *phyA*, the *CKI1* expression remained unchanged (compare Figs. 1B and 2B), thus confirming our previous results.

Under cR light, *phyA* shows a *CKI1* expression pattern comparable to that of short-d conditions (compare *phyA* in Fig. 1, A and C, and Supplemental Fig. 4G). This is consistent with the fact that other phytochromes (probably dominantly phyB as the main R light receptor), are still active in *phyA*, and are able to inhibit the etiolation response. However, in contrast to wild type, even under cR light, phyB alone (or in cooperation with other phytochromes) is not sufficient to induce *CKI1*

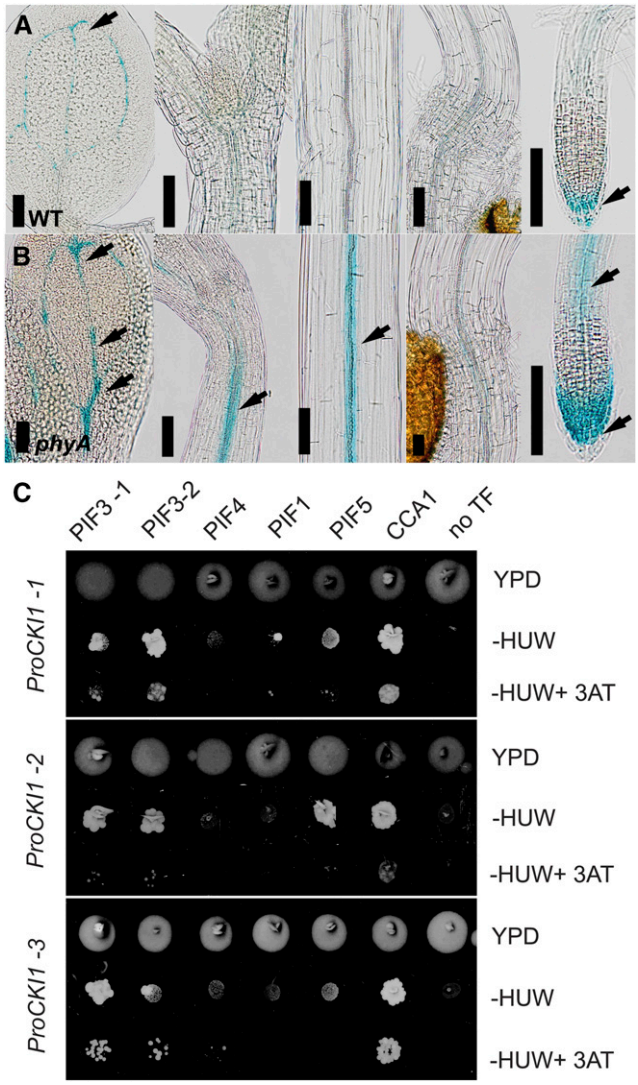

Figure 2. *CKI1* acts downstream of phyA-mediated signaling. A and B, In contrast to wild type, far-red light is unable to inhibit *CKI1* expression in *phyA* (see also Fig. 1). To avoid possible bias due to developmental stage-specific changes, etiolated wild type and *phyA* carrying *ProCKI1*:*uidA* construct were treated with continuous far-red light (50 μmol m^{-2} s^{-1}) immediately after the dark adaptation phase (4 d in darkness, 2 d on far-red). While in *phyA*, the *CKI1* signal is still clearly detectable (arrows), only residual *CKI1* activity can be detected in wild type, mostly in the vascular tissue of the cotyledons and in the LRC (arrows). Scale bars: 100 μm. C, Interactions between light-associated TFs and selected fragments of the *CKI1* promoter (*ProCKI1-1*, *ProCKI1-2*, and *ProCKI1-3*) enriched in phyA-regulated motifs (see Supplemental Table S1 and Supplemental Fig. S5), identified by Y1H. Growth of yeast clones carrying the HIS3 reporter under the control of *ProCKI1-1*, *ProCKI1-2*, or *ProCKI1-3* and selected TFs from the REGIA-REGULATORS collection was recorded after incubation for 6 d on either vector-selective media, interaction-selective media lacking His, uracil, and Trp or interaction-selective media supplemented with 10 mM 3AT, a competitive inhibitor of the HIS3 gene product. Only those yeast clones growing under the latter (more stringent) interaction-selective conditions were considered to be carrying interactors. PIF3 (both of the two independent clones available in the collection) specifically recognizes *ProCKI1-1* and *ProCKI1-2*, while CCA1 interacts with all the fragments tested. HUW, His, uracil, and Trp; YPD, vector-selective media.

expression in the SAM and/or in the hypocotyl in the absence of phyA (compare wild type and *phyA* in Fig. 1C and Supplemental Fig. S4, E and G). This, together with the down-regulation of *CKI1* expression in the SAM of *phyA* under short-d conditions (Fig. 1A), implies the role of phyA as a positive regulator of *CKI1* activity in the SAM in the absence of FR as the only light source.

To summarize, we show that white and R light up-regulates *CKI1* promoter activity in a phyA-dependent way in the SAM and/or hypocotyl, respectively. Also, we show that *CKI1* activity in LRC (and probably also in SAM) is under the phyA-mediated negative regulation by FR light.

phyA-controlled TFs Bind *CKI1* Promoter

The aforementioned data clearly show that *CKI1* is under control of phyA. Consistent with this, we found several phyA-responsive elements (Hudson and Quail, 2003) in the *CKI1* promoter sequence. The presence of circadian rhythm-responsible elements, solid elements, and a large number of G-box and GATA motifs, is in a good accordance to our experimental data providing additional evidence that *CKI1* is likely to be a light-regulated gene (Supplemental Table S1). To further substantiate the role of light-controlled TFs in the regulation of *CKI1*, we employed a Y1H assay to study the interaction of *CKI1* promoter fragments enriched in these elements (Supplemental Fig. S5) with a selected subset of phy-regulated TFs (Obayashi et al., 2014). We found that a basic helix-loop-helix TF PHYTOCHROME INTERACTING FACTOR3 (PIF3) specifically recognizes two out of the three assayed *CKI1* promoter regions, and the MYB-related TF CIRCADIAN CLOCK ASSOCIATED1 (CCA1) binds all three of the fragments (Fig. 2C). Thus, interaction of phytochrome-controlled TFs with *CKI1* promoter indicates that *CKI1* could be a direct target of phyA-mediated signaling.

CKI1 Expression Reveals Rhythmical Changes Integrating Both Light-Independent and Light-Regulated Control

It was previously shown that nuclear localization of phyA, and the activity of the *phyA* promoter, are under circadian control (Tóth et al., 2001; Kircher et al., 2002). Tóth et al. (2001) also showed that circadian oscillation within the phytochrome family is tissue specific, with the diurnal-controlled activity of phyA happening predominantly in the SAM. Our data showing binding of CCA1 to the *CKI1* promoter implied possible circadian regulation of *CKI1*.

To investigate possible diurnal rhythms in *CKI1* activity, we analyzed *CKI1* expression in 6-d-old wild-type seedlings grown under long-d in various time intervals within a 48-h period. The time intervals for sample collection were set according to the published *CCA1* oscillation pattern, where at the end of the light and night phase *CCA1* reaches its expression minima and maxima, respectively (Gutiérrez et al., 2008; Flis et al., 2015; Supplemental Fig. S6). Certain variability in *CKI1* expression pattern was observed even among seedlings collected in the frame of the individual time points. However, approximately 15 out of 20 seedlings were well synchronized, showing diurnal periodicity in the *CKI1* expression (Supplemental Fig. S6). Under long-d conditions, *CKI1* peaked in approximately 12-h intervals (with expression maxima at 12, 24, 36 and 48 h; Supplemental Fig. S6), thus covering both minima and maxima of *CCA1*. *CKI1* predominantly fluctuated in the SAM and hypocotyl, implying possible tissue specificity of diurnal *CKI1* regulation. During the light period, *CKI1* activity was gradually increasing, reaching the maxima at the end of the light phase, followed by partial decrease of *CKI1* expression early after dusk. Another maximum located just at the beginning of the following light phase and after partial decrease, the intensity of *CKI1* expression was growing, again peaking just at the end of the light phase (Supplemental Fig. S6).

The diurnal oscillations of *CKI1* could be caused either by alternation in light conditions or by activity of the internal circadian clock. To investigate the possibility of *CKI1* regulation by circadian clock, several experiments were performed. As expected, the periodicity of *CKI1* expression was partially impaired in seedlings grown for 6 d under continuous light. However, a certain level of periodicity still could be recognized, arguing in a favor of possible circadian regulation of *CKI1* (Supplemental Fig. S6). Besides that, under continuous light conditions, we observed higher *CKI1* activity in the SAM when compared to long-d (Supplemental Fig. S6), thus confirming our previous results indicating the positive role of light and the light dose, in the control of *CKI1* in the SAM (Fig. 1; and Supplemental Table S2). In another experiment, the diurnal phase of 6-d-old long-d-grown seedlings was disturbed by placing the plants to the constant conditions (continuous light or dark) of the opposite phase (i.e. light phase was replaced by extended darkness; and vice versa, dark phase was replaced by continuous light). Under both the prolonged darkness and light phases, the trend in the *CKI1* expression observed under short d was partially retained (Supplemental Fig. S6), further implying possibility of circadian regulation. However, both phase shifts also revealed light-controlled changes in *CKI1* expression. Incubation in prolonged darkness apparently enhanced *CKI1* expression in the hypocotyl close to the SAM and petioles, indicating a possibility of developmental-specific *CKI1* expression pattern associated with the onset of etiolation. In the case of the prolonged light phase, after the initial decrease (circadian regulation), *CKI1* expression in the SAM gradually increased, thus reflecting the light-mediated positive regulation observed in the continuous light grown seedlings.

In conclusion, *CKI1* expression exhibits rhythmical changes, with an atypical 12-h (semidiurnal) cycle. However, besides the circadian regulation, changes in the light conditions seem to contribute to observed diurnal cycling of *CKI1* expression.

CKI1 Shows Etiolation-Specific Expression Pattern

The observed up-regulation of *CKI1* in the hypocotyl correlating with the onset of etiolation response under extended dark phase conditions (Supplemental Fig. S6) suggested possible developmental control over *CKI1* expression, associated with dark adaptation. Accordingly, specific *CKI1* expression pattern could be identified in all inspected genotypes, wild type, *hy1-7*, and *phyA*, when grown under conditions leading to the etiolation response (marked by an asterisk in Fig. 1). The etiolation-specific pattern could be characterized by up-regulated *CKI1* in the vasculature of cotyledons, hypocotyl, and the root just above the transition zone and in columella/LRC (black arrowheads, Fig. 1). These developmental-specific effects seem to be responsible for most of the differences between *hy1-7* and *phyA* (higher *CKI1* expression in the vasculature of cotyledons, hypocotyl, and transition zone of the root in *hy1-7*) under short-d conditions, when *hy1-7* (in contrast to *phyA*) shows an etiolation-like phenotype (Fig. 1 A and Supplemental Fig. S4, F and G). Up-regulated *CKI1* activity in the hypocotyl vasculature could also be detected in the wild type and *phyA* under cR-low and wild type under cFR-low light, consistent with the increased hypocotyl length observed under these conditions, suggesting the onset of etiolation (Supplemental Fig. S4, D, E, and G). On the other side, the absence of *CKI1* expression in the SAM of etiolated seedlings seems to be an effect of absent phyA signaling rather than an etiolation-specific developmental regulation, as the same effect (absence of *CKI1* activity) could be observed in both short-d- and R-grown and thus deetiolated *phyA* (see above; and Fig. 1C and Supplemental Fig. S4G).

To sum up, *CKI1* expression is under direct light (developmental-independent) control, particularly in the SAM and in columella/LRC. However, at the same time, *CKI1* activity observed within the cotyledons, hypocotyl, and vasculature of the root transition zone seems to correspond to light-controlled developmental responses, particularly those associated with dark adaptation (onset of etiolation) and/or low light intensity, such as hypocotyl elongation and root shortening.

CKI1 Expression Controls Skotomorphogenesis

Changes in *CKI1* expression associated with the initiation of etiolation (Supplemental Fig. S6), as well as the specific *CKI1* expression pattern in the fully etiolated seedlings (Fig. 1), suggested a possible role of CKI1 during dark-adapted plant growth (skotomorphogenesis). To investigate this, we assayed the etiolated seedlings phenotype in *hy1-7*, *phyA*, and *35S:CKI1* plants. Hypocotyl elongation is one of the first responses to the lower-light intensity/darkness. Both hypocotyl growth dynamics, as well as final hypocotyl length in dark-grown seedlings, differed in opposite ways (i.e. being faster in *hy1-7* and *phyA*, and slower in *35S:CKI1*, when compared with respective wild-type controls; Supplemental Fig. S7, A, C, and D).

Besides elongated hypocotyls, dark-grown seedlings typically form an apical hook that protects the fragile shoot meristem during soil penetration (Raz and Ecker, 1999). Hook formation, and its opening, was previously demonstrated to be under hormonal and light control (Vriezen et al., 2004; Zádniková et al., 2010; Smet et al., 2014). Considering that, we investigated the potential impact of *hy1-7* and *phyA* on the *CKI1* expression during hook development and real-time kinetics of individual stages of the process, i.e. hook formation, maintenance, and opening (Raz and Ecker, 1999). Interestingly, the *CKI1* expression pattern dynamically changed during the apical hook development (Supplemental Fig. S7B). In the wild-type seedlings at 30 h after germination (HAG), corresponding to the end of the hook formation stage, the *CKI1* activity was rather weak and delimited to the hypocotyl vasculature. However, at 42 HAG, corresponding to the start of the hook opening, *CKI1* was strongly up-regulated in the apical hypocotyl portion, including the bended (hook) part (Supplemental Fig. S7B). In *hy1-7* and *phyA*, the *CKI1* reporter activity at 30 HAG was comparable to the wild type, except of slightly higher *CKI1* activity in the hypocotyl vasculature of *hy1-7* (Supplemental Fig. S7B). However, at the later interval (42 HAG), both *hy1-7* and *phyA* showed much weaker *CKI1* expression in the apical portion of the hypocotyl proximally to SAM (Supplemental Fig. S7B). Accordingly, we observed dramatically prolonged maintenance phase in both *hy1-7* and *phyA* (hook opens slower) when compared to that of wild type (Supplemental Fig. S7, E and F). Opposite differences in apical hook development were observed in the *35S:CKI1* line, where we found the hook opening phase shortened (the hook opens faster) in comparison to the wild type.

To sum up, the dynamic expression of *CKI1* during etiolated seedling growth is deregulated in both *hy1-7* and *phyA*, which corresponds with changes in hook development and hypocotyl elongation. Importantly, the developmental defects observed in dark-grown *phyA* mutant imply a light-independent role of phyA during skotomorphogenesis.

Light Signaling Controls Cytokinin Response in the Root

CKI1 acts through MSP and activates downstream responses, among them the transcription of type *A-ARRs* (Hwang and Sheen, 2001; Hejátko et al., 2009; Deng et al., 2010), the cytokinin primary response genes (D'Agostino et al., 2000). Altered *CKI1* expression as a result of attenuated light perception in the *hy1-7* mutant might, therefore, directly impact cytokinin responses, including *A-ARRs* transcription. Of eight *A-ARRs* tested, we found that *ARR3* was strongly down-regulated in *hy1-7* (Fig. 3A), suggesting a possible defect in cytokinin

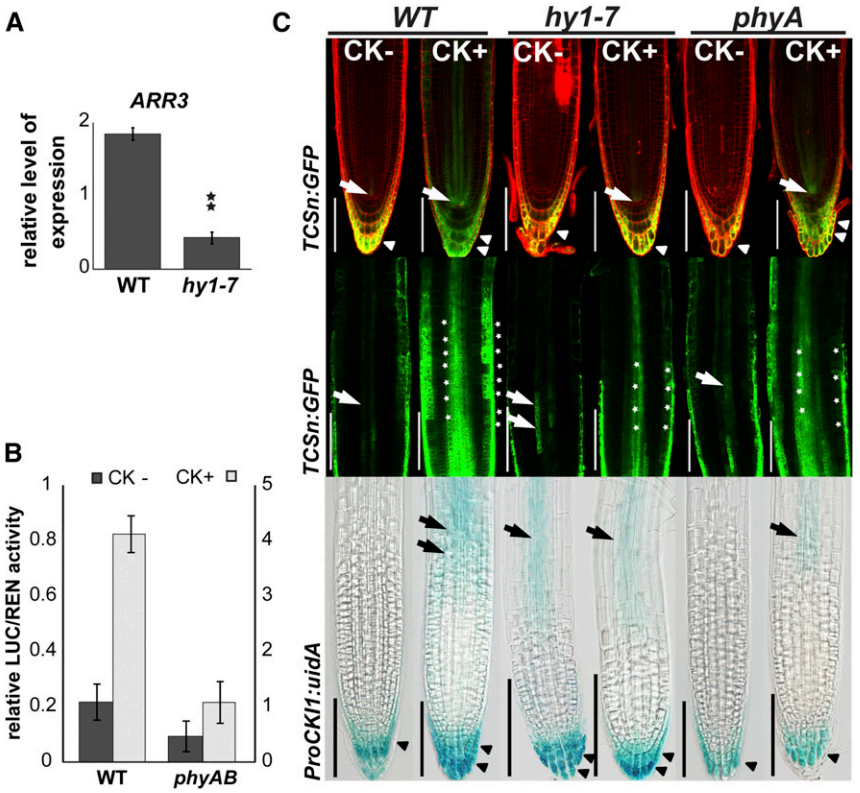

Figure 3. *hy1-7* exhibits disturbed MSP activity and attenuated cytokinin sensitivity. A, Relative quantification by qRT-PCR of *ARR3* expression in 6-d-old wild-type and *hy1-7* seedlings grown under short-d conditions. Data were normalized to *UBIQUITIN 10*. Statistically significant difference at $\alpha = 0.01$ is shown (**; $n = 3$). B, Relative expression of the cytokinin-responsive MSP reporter (*TCS*:*LUC*) in protoplasts from suspension cultures of wild-type and the *phyA phyB* mutant line; error bars show SD. Impaired MSP signaling in *phyA phyB* is apparent in both control (values on *y* axis on the left) and cytokinin-induced (0.1 μM BA) protoplasts (values on *y* axis on the right). C, Disturbed MSP signaling and inducibility of *CKI1* expression in root tips of 6-d-old seedlings by cytokinin (0.1 μM BA). Upper row: Expression of cytokinin-responsive MSP reporter *TCSn*:*GFP* in the root apical meristem. In the absence of exogenous cytokinins, MSP activity in wild type is detected in the stele close to the quiescent center (arrow) and in the LRC and columella (arrowhead). In *hy1-7* and *phyA*, the MSP activity in the stele close to the QC center is missing. In *hy1-7* and partially also in *phyA*, the *TCSn*:*GFP* signal is decreased in LRC and almost missing in the cells of columella. In wild type, exogenous cytokinin treatment strongly up-regulates the *TCSn*:*GFP* signal in the stele, LRC (double arrowhead) and columella. In contrast, only modest cytokinin-mediated up-regulation is apparent in LRC (double arrowhead) and columella of *phyA*, and only very weak increase of MSP activity is detectable in case of cytokinin-treated *hy1-7* (single arrow/arrowhead). PI contrastaining was used (red signal). Middle row: MSP activity as measured by *TCSn*:*GFP* expression in the root including proximal meristem and transition zone of wild type, *hy1-7*, and *phyA*. In comparison to wild type (single arrow), both mutants show increased activity of MSP signaling in the root vasculature including the transition zone (double arrow) in the absence of exogenous cytokinins. However, both *phyA* and particularly *hy1-7* show only weak increase in MSP activity upon exogenous cytokinin treatment in the vascular tissue and epidermis (*). Bottom row: Disturbed cytokinin inducibility of *CKI1* as shown by *ProCKI1*:*uidA* activity. Cytokinin up-regulates expression of *CKI1* in the root transition zone (arrow) and columella/LRC (double arrowhead) of wild type, but not in the cytokinin-insensitive *hy1-7* line; cytokinin only partially activated *CKI1* in the transition zone and columella/LRC of *phyA*. Note the higher level of expression in the vasculature of the transition zone and in the columella/LRC in *hy1-7* in the absence of exogenous cytokinin. In *phyA*, the *CKI1* expression is slightly decreased in the LRC (arrowhead) in the absence of cytokinins. Scale bars: 100 μm.

signaling due to disturbed light signaling. To further corroborate the role of phytochromes in the control of cytokinin signaling, we assayed cytokinin-mediated activation of MSP signaling using a *TCS*:*LUC* reporter (Müller and Sheen, 2008) in transiently transformed protoplasts prepared from a suspension culture of a double loss-of-function mutant defective in *phyA* and *phyB*, previously shown to be affected in the majority of phytochrome-mediated light responses (Reed et al., 1994). Our results show that in both mock- and cytokinin-treated samples, MSP activity was strongly reduced in *phyA phyB* protoplasts compared to that of wild type (Fig. 3B), suggesting decreased cytokinin responsiveness in the light signaling-defective mutant.

To study the possible spatial-specific changes of cytokinin signaling in the light signaling-defective lines, we introduced the cytokinin signaling reporter *TCSn*:*GFP* (Zürcher et al., 2013) by crossing into the

hy1-7 and *phyA* mutants. In the mock-treated wild type, *TCSn*:*GFP* was active in columella/LRC and only weak signal was detectable in the (pro)vascular tissue close to the root tip. In comparison to that, we observed up-regulated cytokinin signaling (MSP output) in cell clusters of the root vasculature in both *hy1-7* and *phyA* mutants in the absence of exogenous cytokinins (Fig. 3C). Exogenous cytokinin treatment led to the strong up-regulation of *TCSn*:*GFP* activity in the root tip of wild type, including the vasculature and epidermis of the transition zone and columella/LRC, as also described in Zürcher et al. (2013). In contrast, cytokinin induced almost no increase of *TCSn*:*GFP* in *hy1-7* (weak up-regulation was apparent only in the root vasculature of *hy1-7*), and only partial activation was detectable in the vascular tissue and columella/LRC of *phyA* (Fig. 3C). Thus, in line with the protoplast assays, these data imply aberrant MSP signaling and disturbed ability of *phyA* and particularly *hy1-7* to respond to cytokinins.

Interestingly, we found that not only light, but also cytokinins, control expression of *CKI1*. In the wild type, the presence of exogenous cytokinin led to *CKI1* up-regulation in the vasculature of the root, particularly in the transition zone and in the columella/LRC (Fig. 3C). As mentioned previously, even in the absence of exogenous cytokinin, *CKI1* was up-regulated in the vasculature of the transition zone in *hy1-7*, while wild-type-like expression (absent or very weak *CKI1* activity) was observed in the transition zone of *phyA* (Fig. 3C). Consistent with the cytokinin insensitivity of *TCSn*:*GFP* reporter in *hy1-7*, *CKI1* expression was not further up-regulated by cytokinin in the transition zone and columella/LRC of *hy1-7* roots. Similar response, i.e. only partial up-regulation of *CKI1*, was observed in cytokinin-treated *phyA* (Fig. 3C).

Overall, we demonstrate that disrupting phytochrome-mediated light perception results in aberrant MSP signaling and disturbed ability of MSP pathway to respond to cytokinin. We also show that changes in *CKI1* expression in light signaling-defective *hy1-7* and *phyA* partially overlap with the changes in MSP output observed in those mutants.

Light-Regulated *CKI1* Controls Root Growth

To further investigate the possible developmental consequences of disturbed cytokinin signaling in the root of light signaling mutants, wild-type, *hy1-7*, *phyA*, *phyB*, and *phyA phyB* seedlings were grown for two weeks on media supplemented with cytokinins. In the wild type, cytokinin treatment induced root hair formation and thickening of the differentiated (root hair-forming) portion of the root. In contrast, *hy1-7* exhibited almost complete resistance to cytokinin in root hair formation and in primary root thickening (Fig. 4A and Supplemental Fig. S8A). Cytokinin-induced root hair formation was also disturbed in *phyA* and *phyB* single mutant lines. Interestingly, additive effects were apparent in *phyA phyB* double mutant, thus showing a role for both light receptors in cytokinin-regulated development (Fig. 4A). This is also in line with the previously documented role of phytochromes in root hair formation (De Simone et al., 2000).

Similar results, confirming the lowered cytokinin sensitivity of *hy1-7*, *phyA*, and *phyA phyB*, were observed in cytokinin-mediated root shortening, another well-established cytokinin sensitivity assay (Supplemental Fig. S8B). Root growth is largely dependent on the RAM activity. In particular, the equilibrium between cell division and cell differentiation in the root transition zone defines the size of the RAM and thus the extent of root growth (Dello Ioio et al., 2007). As mentioned in the previous section, in *hy1-7* grown under control conditions (i.e. in the absence of exogenous cytokinins), the activity of the *CKI1* promoter is enhanced in the vascular tissue of the root transition zone, which associates with the increase of MSP signaling in the root vasculature (Fig. 3C). We observed that this correlates with reduced root growth and smaller RAM, which is a phenotype reminiscent of that in plants overexpressing *CKI1* (Fig. 4, B to D). Slightly up-regulated MSP signaling (weaker effect when compared with *hy1-7*) in the root vasculature of *phyA* in the absence of exogenous cytokinins (Fig. 3C), also correlates with the partial reduction of root length and RAM size (Fig. 4, B and C). In *phyA phyB* this effect becomes even stronger, indicating the role of phyB in the control of RAM size too (Fig. 4, B and C).

Altogether, disturbed cytokinin signaling and the reduced RAM size observed in *hy1-7*, *phyA*, and *35S*:*CKI1* lines, together with ectopic activation of MSP signaling in *hy1-7* and partially also in *phyA*, suggests that the up-regulated *CKI1* in both light-signaling mutants negatively affects RAM activity. This consequently inhibits root growth via up-regulation of MSP signaling in the root tip.

Altered *CKI1* Expression Pattern Associates with Defects in Vascular Tissue Formation

The expression of *CKI1* in vascular bundles (VBs) and the cortex of the inflorescence stem and its significance in VB development via control of procambial activity have been described previously (Hejátko et al., 2009). We therefore examined the impact of an altered *CKI1* expression pattern in *hy1-7* on VB development. In wild type, *CKI1* transcription was mainly located in the phloem adjacent to the procambium. By contrast, the signal of *CKI1* in *hy1-7* was located mostly in the xylem, and weaker and less focused *CKI1* activity was detectable in the phloem (Fig. 4E). These changes in *CKI1* expression were confirmed by in situ CKI1 immunolocalization (Fig. 4F). Consistent with the observed changes in the *CKI1* expression profile, we found VBs in *hy1-7* to be severely affected in both number and architecture. The diameter of the *hy1-7* inflorescence stem was drastically reduced, having a maximum of only six VBs in comparison to the more usual eight VBs in the wild type (Fig. 4G). In the individual VBs of *hy1-7*, the cell number of all three tissue types (phloem, procambium,

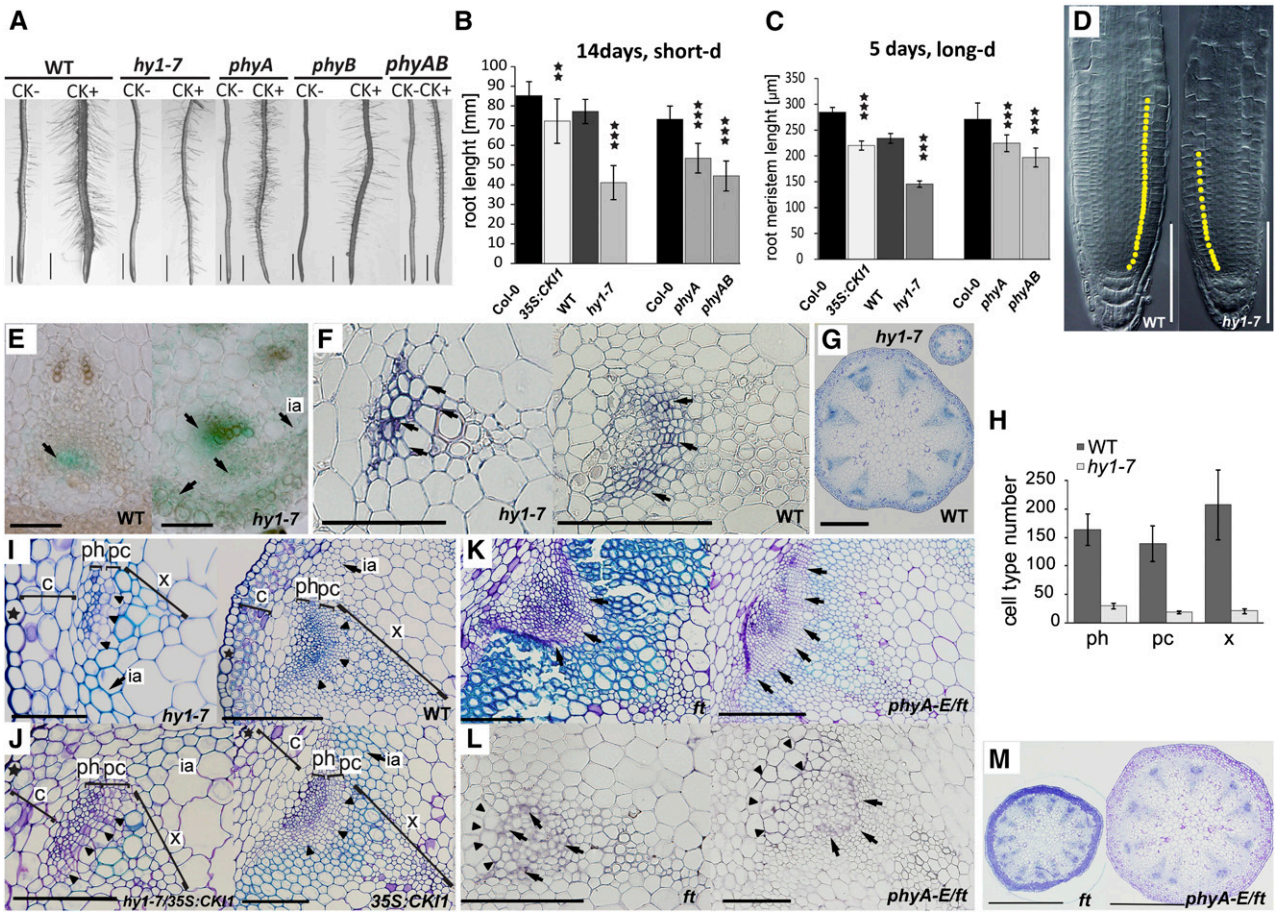

Figure 4. Changes in the spatiotemporal specificity of *CKI1* expression are associated with changes in Arabidopsis meristematic activity. A, *hy1-7* is insensitive to cytokinin with respect to root hair formation and root thickening (see also Supplemental Fig. S8). Additive loss of sensitivity to cytokinins is apparent in *phyA*, *phyB*, and *phyA phyB* mutants. Representative images show 14-d-old seedlings grown under short-d conditions in the presence (+) or absence (−) of cytokinin (0.1 μM BA). B to D, *CKI1* is a negative regulator of root growth. Analysis of the root (B; $n = 15$) and the root apical meristem length (C; $n = 20$) in seedlings grown under white light. In comparison to wild type, *hy1-7*, like *35S:CKI1*, has shorter roots (B) and reduced root apical meristem size (D); yellow dots in (D) indicate meristem cells. Note that the wild-type (*ProCKI1:GUS*) line is used as a control for *hy1-7*, while the Col-0 ecotype is used as a control for *35S:CKI1*. Root length shortening, as well as root apical meristem reduction, is observed in the phytochrome mutants *phyA* and *phyA phyB*. Note additive effect of both phy mutations. E, *ProCKI1:uidA* expression profile in the vascular tissue of the inflorescence stem; arrows indicate *CKI1* activity. In the wild type, *CKI1* expression is predominantly localized in phloem adjacent to procambium, while in *hy1-7*, the *CKI1* expression maximum is found mostly in the xylem. Weak activity in the phloem is also detectable. In *hy1-7*, up-regulation of *CKI1* is apparent in the cortex, and ectopic *CKI1* activity is detectable in the interfascicular arcs. F, Immunolocalization of CKI1 protein (arrows) in the vascular tissue of the inflorescence stem. In wild type, CKI1 is predominantly localized to the procambial cells, whereas the *hy1-7* signal is shifted to the xylem cells. G, *hy1-7* inflorescence stem show decrease in the number and size of VBs. Toluidine blue staining was used for VBs structure characterization. H, Clear reduction in numbers of all VB cell types is observed in *hy1-7* ($n = 3$). I, Precocious lignification (light-blue stained cell walls, arrow) and reduction in the procambial cell layer (arrowheads) are observed in the basal portion of the inflorescence stem in *hy1-7* when compared to wild type. Toluidine blue staining was used for VBs structure characterization. J, Partial rescue of *hy1-7* phenotype by the *35S:CKI1*, showing stronger, but dominantly wild-type-like (procambial) CKI1 localization (Hejátko et al., 2009). For the complementation of independent *hy1-1* line, see Supplemental Fig. S9. In comparison to the parent *hy1-7* line, *hy1-7/35S:CKI1* reveals partial restoration of the procambial cell layer associated with increase of VB size and decreased lignification, particularly in the interfascicular arcs. Toluidine blue staining was used for VB structure characterization. K, Cross sections of the inflorescence stem of *ft* and *phyA-E/ft* mutant. In comparison to *ft*, *phyA-E/ft* reveals decrease in lignification (light-blue stained cell walls) and increased number of procambial cells (arrows). Toluidine blue staining was used for VB structure characterization. L, Immunolocalization of CKI1 protein (arrows) in the vascular tissue of *ft* and *phyA-E/ft* mutant line. Ectopic, mostly phloem localization of CKI1 in *ft* mutant is partially restored in the presence of *phyA-E*. However, ectopic CKI1 localization in the VB sheath cells (arrowheads) is still apparent in *phyA-E/ft*. M, Up-regulation of VBs number in *ft* is partially restored in the *phyA-E/ft* background. Toluidine-blue staining was used for VB structure characterization. Scale bars: 1 mm (A), 500 μm (G, M), 100 μm (D, E, F, I, J, K, L), and 50 μm (only for *hy1-7* in E, F, and J). In all graphs, mean and SD are presented. Statistical significance determined by a *t* test at α-levels of 0.05, 0.01, and 0.001 (*, **, and ***, respectively) is shown. Star symbol (★), epidermal cell layer; c, cortex; ph, phloem; pc, procambium; x, xylem; ia, interfascicular arcs., long-d, long day; short d, short day.

and xylem) was substantially reduced (Fig. 4H). We also identified changes in the lignification of interfascicular arcs of both *hy1-7* and *35S:CKI1* (Fig. 4, I and J).

Up-regulation of *CKI1* in *35S:CKI1* was demonstrated to accelerate procambial activity (demonstrated by the thicker procambial layer; Fig. 4J) and thus the radial growth in the Arabidopsis inflorescence stem. Interestingly, stronger but mostly wild-type-like procambial localization of CKI1 has been demonstrated in *35S:CKI1*, implying possible posttranscriptional regulations involved in the localization of CKI1 to the procambium (Hejátko et al., 2009). To confirm that changes in VB development observed in the *hy1* background could be attributed to changes in the *CKI1* expression pattern, we attempted to rescue the *hy1-7* and independent *hy1-1* allele phenotypes by crossing with the *35S:CKI1* line. In both *hy1-7/35S:CKI1* and *hy1-1/35S:CKI1*, the VBs enlarged, the amount of the individual cell types per VB increased, and ectopic lignification decreased, thus presenting partial phenotypical rescue (Fig. 4J and Supplemental Fig. S9).

The importance of phyA in the number of developmental aspects was previously shown (Franklin and Quail, 2010). Its specificity within the developmental processes is highly dependent on the role and action of other light receptors (Cerdán and Chory, 2003; Franklin and Quail, 2010; Strasser et al., 2010). Thus, phyA-specific *CKI1* regulation could differ in the early (seedling) and late (generative) plant development. Accordingly, when compared to wild type, we observed an increase in the VB number and diameter of the inflorescence stem in *phyA*, and the structure of VBs in *phyA* was also comparable to that of wild type (Supplemental Fig. S10). Thus, the role of phyA in the control over VB development seems to be minor and/or backed-up by other factors, most probably other phytochromes. To decipher the possible influence of other phytochromes on VB development, the quintuple mutant *phyA-E* in the *flowering locus T* (*ft*) mutant background was studied (Fig. 4, K to M). The *phyA-E/ft* was used because of the ability of *ft* to override the seedling developmental arrest observed in white light-grown *phyA-E/FT* (Strasser et al., 2010). Noteworthy, *FT* itself was found to be under phyB control (Cerdán and Chory, 2003). Firstly, we inspected the phenotype of *ft* mutant as a control to the *phyA-E/ft* line. Surprisingly, we observed increased number of VBs and highly up-regulated lignification in the *ft* inflorescence stems when compared to wild type. These developmental defects can be associated with ectopic, mostly phloem-specific and rather random localization of CKI1 (Fig. 4, K, L, and F). Interestingly, the *phyA-E/ft* mutants exhibit partial restoration of the VBs structure as compared to *ft* background. When compared with *ft*, the *phyA-E/ft* phenotype has a typical decreased number of VBs with thicker procambial layers, increased diameter of inflorescence stems, and fewer lignified xylem and interfascicular arc cells (Figs. 4, K to M). Accordingly, in the *phyA-E/ft* we found CKI1 to be arranged mostly to the phloem-adjacent procambial cells, thus partially overlapping with wild-type localization. However, persisting mislocalization of CKI1 into phloem and VB sheath was still apparent (Fig. 4L), confirming the role of phytochromes in the *CKI1* control and VBs development.

In conclusion, altered *CKI1* expression and CKI1 localization in *hy1-7* largely correlates with phenotypic aberrations previously linked with *CKI1* activity in the regulation of vascular development. Changes in the CKI1 localization and VB development could also be observed in multiple *phyA-E/ft* and *ft* mutant, providing further evidence that light signaling and light signaling-controlled processes impact CKI1-dependent plant development.

DISCUSSION

Light Controls *CKI1* Expression

Here we identified HY1 as an upstream factor controlling the expression pattern of *CKI1*. HY1 in chloroplasts catalyzes cleavage of heme to form biliverdinIXa, which is further metabolized to the final product PΦB. PΦB is an essential cofactor covalently bound to phytochrome apoproteins, thus allowing formation of photoconvertible phytochromes (Weller et al., 1996; Emborg et al., 2006). In the Arabidopsis genome, four members of the heme oxygenase family (HY1, HO2, HO3, and HO4) have been identified. Further analysis showed that *hy1* mutants contain no detectable holophytochrome and are almost insensitive to FR and R light. These findings suggest a dominant role for HY1 within the HO family in PΦB synthesis (Parks and Quail, 1991; Davis et al., 2001). In addition to attenuated light signaling, other developmental aberrations were reported in the heme oxygenase-deficient plants. Defects in chlorophyll biosynthesis and chloroplast development due to nonfunctional phytochrome signaling in *hy1* mutants were described (Chory et al., 1989). Furthermore, chlorophyll biosynthesis in *hy1* plants is also inhibited via a negative feedback loop mediated by heme accumulation (Terry and Kendrick, 1999). Based on our comparison of the *lph/hy1-7* phenotype with the phenotypes of other *hy1* mutants identified to date, it appears that *hy1-7* represents one of the strongest alleles (Supplemental Fig. S3; Davis et al., 1999; Muramoto et al., 1999). Thus, besides aberrant light perception, light-independent defects could also be attributable to the *hy1-7* phenotype, and only part of these defects being associated with changes in *CKI1* expression. However, we have shown in two independent *hy1* alleles (*hy1-1* and *hy1-7*) that the phenotype strength correlates with the magnitude of changes in *CKI1* activity. Furthermore, our data showing phenocopy of *CKI1* expression in the independent *phyA* mutant provides evidence that compromised phyA signaling is the key factor contributing to the changes of *CKI1* expression observed in *hy1-7* seedlings.

Besides the role of phyA, the importance of phyB and/or other light receptors in the modulation of *CKI1* expression cannot be completely excluded. In particular,

the dual role of phyA in the control of *CKI1* expression in the SAM (positive in the presence of white or R light while negative upon FR irradiation) might be explained by the existence of another light-controlled factor acting in synergy with phyA in a developmental-specific context. Interestingly, the light conditions under which *CKI1* is active in the SAM can be explained by the presence of the two main Arabidopsis light receptors phyA and phyB in the nucleus (compare Fig. 1 and Supplemental Table S2). The necessity of coordinated action between phyA and another R/FR light receptor in the control of *CKI1* activity is supported by the impaired ability of R light to up-regulate *CKI1* in the SAM and hypocotyl of *phyA* (Fig. 1, C and D). Furthermore, it has been shown that not only phytochrome localization (cytoplasmic versus nuclear) but also the phytochrome content is essential for mediating light responses (Boylan and Quail, 1991; Whitelam et al., 1993). Under continuous light treatments most phyA is degraded, leading to the dramatic changes in the ratio among individual phytochromes (Sharrock and Clack, 2002). Under such conditions, we detected stronger *CKI1* signal in SAM (compare Fig. 1, A and C, and Supplemental Fig. S6), implying that phyA abundance (possibly phyA to other phy ratio) could contribute to the control over *CKI1* activity. That, however, remains to be identified.

According to this model, phyA translocates to the nucleus in a response to the light of several wave lengths (blue, R, FR; Hisada et al., 2000; Kim et al., 2000; Kircher et al., 2002) and (directly or indirectly) mediates phosphorylation of PIF3, a basic helix-loop-helix TF. In the response to FR light, phyA enters the nucleus and forms nuclear bodies, which, in their early form, colocalize with PIF3 (Bauer et al., 2004). PIF3 phosphorylation leads to its degradation and up-regulation of PIF3-inhibited gene expression (Al-Sady et al., 2006; Ni et al., 2013). PIF3 was found to be degraded under both R and FR conditions (Bauer et al., 2004), though with higher efficiency in the presence of R light (Al-Sady et al., 2006). Thus, PIF3 seems to integrate both R and FR signaling. Interestingly, PIF3 might act as both a positive and a negative transcriptional regulator (Sentandreu et al., 2011; Leivar and Monte, 2014). This is in line with our findings, suggesting a dual role of phyA in positive and negative control of *CKI1* activity in the SAM, and with control of *CKI1* expression in a response to both R (in the hypocotyl) and FR light (in the SAM and LRC). The spatial distribution of the phyA-mediated *CKI1* expression also fits well with the *phyA* expression pattern, showing the strongest signal in the hypocotyl including SAM and in the LRC (Hall et al., 2001). Also, our data suggesting the role of PIF3 in the control over *CKI1* are supported by an independent study reporting on PIF3 binding to the *CKI1* promoter via ChIP-seq (Zhang et al., 2013). However, *CKI1* expression at the whole-seedling level does not seem to be regulated by PIF3 (Tepperman et al., 2006; Zhang et al., 2013). That corresponds with our data showing an altered *CKI1* expression pattern but only slightly and statistically insignificant changed total levels of the *CKI1* transcript in *hy1-7* (Supplemental Fig. S1B).

In parallel to PIF3, we detected binding of the *CKI1* promoter to another light-associated TF, CCA1. CCA1 is a MYB-related TF involved in the regulation of circadian clock-regulated genes in Arabidopsis (Wang et al., 1997; Green and Tobin, 1999). Similarly to PIF3, CCA1 also acts downstream of phytochrome signaling (Tepperman et al., 2006) and may function as a transcription repressor or activator (Alabadí et al., 2001; Nagel and Kay, 2012). Interestingly, PIF3 has been supposed to up-regulate *CCA1* via direct binding to its promoter (Martínez-García et al., 2000). Nevertheless, in *pif3*, *CCA1* expression was largely unaffected, thus calling the importance of PIF3 in the regulation of *CCA1* into question (Stephenson et al., 2009). In support of our Y1H results, the previously published CCA1 ChIP-Seq data (bioviz.org/igb/index.html; Nicol et al., 2009; Nagel et al., 2015) suggest that two of the three regions of the *CKI1* promoter tested in our Y1H assay overlap with the CCA1-recognized genomic regions (Supplemental Fig. S11). The circadian cycling of *CKI1* in SAM and adjacent apical hypocotyl portion suggest a possible role of CCA1 in this process (Supplemental Fig. S6). However, the observed semidiurnal (12 h) periodicity cannot be explained by the *CCA1* expression itself, implying a more complex regulatory mechanism. These might include other factors such as both the morning- and evening-phased regulatory circuits (Pruneda-Paz and Kay, 2010) and/or light-controlled *CKI1* expression, as apparent in both dark- and light-phase extension experiments. Alternatively, the semidiurnal periodicity might be explained as a lunar tides response, as described in the case of semidiurnal cycling of Arabidopsis root growth (Barlow et al., 2013).

Collectively, our data show that light quality and quantity are translated into changes in the spatiotemporal specificity of *CKI1* expression via an as-yet unknown mechanism that includes the action of phyA and possibly other phytochromes, light receptors, or modulators of the light response. The diurnal oscillations in *CKI1* activity seem to integrate both light-controlled and circadian (and/or other) regulations that might include further, yet elusive factors.

Light-Regulated Expression of *CKI1* Provides a Molecular Link among Light, Cytokinin Signaling, and Meristematic Activity

Our data clearly show that the light-mediated regulation of *CKI1* has important developmental consequences. We demonstrate that alterations in the spatiotemporal pattern of *CKI1* expression in light perception-defective *hy1-7* and *phyA*, as well as impaired light signaling in both single *phyA phyB* and double *phyA phyB* mutant, correspond with reduced cytokinin responsiveness of the MSP pathway and

disturbed root cytokinin response. This implies a molecular link among phy-mediated control of the *CKI1* expression, MSP pathway activity, and cytokinin-regulated development.

The phenotypic changes that we see due to disturbed expression of *CKI1* in *hy1-7* and *phyA* mutants are consistent with the previously identified role of cytokinin in the regulation of plant meristematic activity. A positive role for cytokinins and MSP-mediated cytokinin signaling in the regulation of procambial cell proliferation has been reported previously (Matsumoto-Kitano et al., 2008; Nieminen et al., 2008; Hejátko et al., 2009; Immanen et al., 2016). Manipulating *CKI1* activity has been shown to affect procambial cell number, suggesting the role of CKI1 in the maintenance of procambial cell identity and/or regulation of procambial cell proliferation (Hejátko et al., 2009). The reduction in the size of the procambial layer in the VBs of *hy1-7* is most probably a consequence of down-regulating *CKI1* expression in the tissue adjacent to the procambium and a shift of the *CKI1* expression maxima dominantly to the xylem. This seems to be confirmed by the ability of the *35S:CKI1* line, previously shown to reveal dominantly wild-type-like (i.e. procambial) CKI1 localization (Hejátko et al., 2009), to partially rescue the vascular phenotype of *hy1-7* and *hy1-1*. Interestingly, the partial rescue of CKI1 localization in the *phyA-E/ft* background when compared to *ft* suggests that control over *CKI1* expression integrates more complex regulatory interactions including individual phytochromes and/or phytochrome-controlled genes controlling inflorescence stem development, like *FT*.

In the RAM, cytokinin-activated MSP has been shown to inhibit root growth via induction of cell differentiation. Importantly, the vascular tissue of the root transition zone has been identified as the site of cytokinin action in this process (Dello Ioio et al., 2007). Thus, up-regulation of *CKI1* in the vascular tissue of the transition zone in *hy1-7* appears to mimic the cytokinin effects by activating MSP in this region, leading to a reduction in both the RAM size and root growth. This is further substantiated by the atypical activation of the MSP activity reporter *TCSn:GFP* in the root vasculature including the transition zone of *hy1-7*, which corresponds well with the position of ectopic *CKI1* transcription observed in those tissues in the absence of exogenous cytokinins. The loss of cytokinin responsiveness of *hy1-7* fits well to the previously published results demonstrating that *CKI1* overexpression leads to the activation of MSP response that is, however, further insensitive to the exogenous cytokinin application (Hejátko et al., 2009). Finally, the negative role of *CKI1* in RAM size and shortening of the main root is supported by the phenotype of the *CKI1* overexpressing line, resembling the effects of exogenous cytokinin application (Figs. 3 and 4; Dello Ioio et al., 2007).

Consistent with our results showing the key effect of phyA on the expression of *CKI1*, we also observed RAM shortening in the *phyA* mutant line. In contrast to *hy1-7*, however, in *phyA* we were unable to see up-regulation of *CKI1* in the transition zone of the root. Nonetheless, the additive effect of *phyB* to the RAM and root shortening in the *phyA phyB* background when compared to *phyA* suggests possible contribution of phyB to *CKI1* expression, as discussed above. Thus, in this scenario, the up-regulation of *CKI1* in the transition zone of the root below the detection limit (the *CKI1* activity is very weak in general) would lead to the up-regulation of MSP activity in the root vasculature of *phyA*, as detected using the *TCSn:GFP* reporter and consequently RAM shortening.

LRC is a site of cytokinin biosynthesis (Aloni et al., 2004, 2005) and the functional importance of LRC in the control of RAM size has been demonstrated (Tsugeki and Fedoroff, 1999). Thus, the changes in *CKI1* expression and defects in its cytokinin inducibility observed in the LRC of *hy1-7* and *phyA* might also contribute to the changes in the RAM size of the light signaling mutants. Thus, although other mechanisms leading to RAM shortening in the cases of *phyA* and *phyA phyB* cannot be excluded, the ectopic expression of *CKI1* overlapping with activation of MSP signaling in roots of both *hy1-7* and *phyA* indicates that changes in the *CKI1* expression in the light signaling mutants are at least partially responsible for the observed effects on RAM size.

Altogether, our findings show that transcriptional regulation of *CKI1* by light impacts MSP activity, and affects MSP-mediated responses including cytokinin signaling. This fits well with the previously described role of CKI1 in the regulation of MSP activity (Hwang and Sheen, 2001; Hejátko et al., 2009; Deng et al., 2010), and implies that transcriptional regulation of *CKI1* is an important mechanism mediating developmental regulations in Arabidopsis. As expected for this type of regulation, the spatiotemporal specificity of transcriptional control over *CKI1*, rather than changes in the total amount of the *CKI1* transcript, is an important issue.

CKI1 and phyA Play a Role in Skotomorphogenesis

Our results show not only direct phytochrome-mediated control over *CKI1* expression, but also developmental-specific expression of *CKI1* associated with the light-controlled onset of etiolation. This implies a role for CKI1 during the dark-adapted developmental phase of plant growth (skotomorphogenesis) and/or the shade avoidance response. Surprisingly, we identified that in the absence of light, both *hy1-7* and *phyA* show changes in hypocotyl growth and hook development when compared to wild type. This could show a potential role for (most probably) cytoplasmically located phyA and its importance within skotomorphogenesis. Cytoplasmic phytochrome signaling, and its developmental importance, has been unequivocally reported (Rösler et al., 2010; Hughes, 2013). The phyA response independent of light signaling has also been

demonstrated (Correll and Kiss, 2005). Based on the differences in the rates of root growth between *phyA phyB* and wild type in the darkness, the authors suggested a role for the inactive (Pr) form of phytochromes in the root elongation. Nevertheless, these changes were not observed in other *phyA* mutants like *phyA-201* (*Ler* ecotype), *phyA-101*, and *phyA-105* (both *RLD* ecotype; Reed et al., 1994; Hoecker et al., 1998; Chen et al., 2015). Thus, we cannot exclude that the changes observed during skotomorphogenesis of *phyA-211* could be allele/ecotype specific. In our study, we show similar changes in *CKI1* expression and phenotype response (strong extension of the hook opening phase) in the dark-grown *phyA* (lacking the phyA protein) and *hy1-7* (having a reduced amount of the photoconvertible phyA holoprotein). This suggests that the ability of phyA to be activated by light, rather than the presence of its inactive Pr form (as is the case in *hy1-7*), is necessary for proper *CKI1* expression, hypocotyl elongation, and hook development in the dark. However, the light-independent role of photoconvertible phytochromes in the dark-adapted plants is still rather hypothetical. The effect of light used to activate seed germination (pregermination light) reported to affect hypocotyl elongation in dark-grown Arabidopsis seedlings (Alconada Magliano and Casal, 2004) could provide an alternative explanation.

CONCLUSIONS

Identification of *hy1-7* in a screen for upstream regulators of the *CKI1* expression revealed an important molecular link between light and MSP signaling. Based on our findings, we propose a model in which the light-regulated expression of *CKI1* controls MSP activity and thus sensitivity to cytokinins. In the model, light quality and quantity controls spatiotemporal specificity of *CKI1* expression via an as-yet unknown mechanism that includes both positive and negative regulation of *CKI1* via phyA-mediated signaling. The constitutive (cytokinin-independent) activity of CKI1 subsequently contributes to MSP signaling leading to changes in cytokinin sensitivity (Fig. 5A). This provides a direct molecular link between light signaling and several important aspects of cytokinin-mediated developmental regulation (including the cytokinin-regulated *CKI1* expression), particularly the control of meristematic activity. Both light-mediated (developmental) and light-induced (nondevelopmental) effects participate in the control over *CKI1* expression (Fig. 5B).

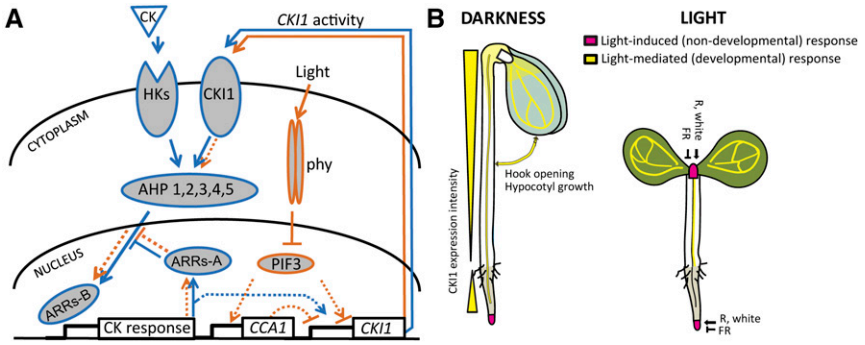

Figure 5. Light-dependent *CKI1* expression controls cytokinin signaling. A, Model of interaction between light and cytokinin signaling via light-controlled *CKI1*. Light initiates autophosphorylation and nuclear transport of phytochromes that interact with PIF3 and mediate its light-dependent degradation. PIF3, which is able to act as both positive and negative regulator, binds to the promoter of transcriptional repressor CCA1 and controls its expression. Both PIF3 and CCA1 bind directly to the *CKI1* promoter. Via an as-yet unknown mechanism that includes the action of phyA, and possibly other light receptors, light quality is translated into changes in the spatiotemporal specificity of *CKI1* expression that include both up- and down-regulation of *CKI1* activity. Besides that, *CKI1* is also under cytokinin control. The resulting changes in the expression of constitutively active CKI1 modulate MSP activity at the level of *ARRs-A* expression and regulate cytokinin sensitivity. The exact position at which cytokinin signaling is negatively regulated via *ARRs-A* is still not clear. For the sake of simplicity, it is shown here as inhibiting the final step in the MSP signaling. The nuclear transport of AHPs and phytochromes is not part of the model as well. (Color code: blue, CK signaling pathway and response; orange, phytochrome signaling pathway and light-mediated response.) B, *CKI1* expression is both under direct (nondevelopmental) and indirect (developmental) light control. Light-induced (direct, nondevelopmental) control over *CKI1* is apparent in the SAM and LRC, where R light, potentially together with other components of the white light, positively regulates *CKI1* via phyA and possibly other light receptors. In parallel, phyA mediates negative regulation of *CKI1* by FR light in those tissues. Light-mediated (indirect, developmental) regulation of *CKI1* associates with etiolation, leading to up-regulation of *CKI1* in the vasculature of cotyledons, hypocotyl, and root transition zone. The spatiotemporal specificity of *CKI1* expression in etiolated seedlings controls apical hook opening, hypocotyl elongation, and root growth.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis (*Arabidopsis thaliana*) seeds of lines *ProCKI1*:*uidA* (Hejátko et al., 2003, 2009), the *phyA-211* mutant (Reed et al., 1994; N6223), the *phyA-211 phyB-9* double mutant (Strasser et al., 2010), *35S*:*CKI1*(CKI1 2-2; Hejátko et al., 2009), Col-0 (the background ecotype of all the above lines; N1092), *hy1-1* (NW67, background ecotype L*er*; Koornneef et al., 1980), *hy1-100* [also known as *hy6* (cs236); background Col-0, LOS5; Chory et al., 1989] and *hy1.62* (background Col-0, PoCA108; Vinti et al., 2000), and *ft-1* (Lee et al., 2000) were used. *ProCKI1*:*uidA* was introduced to the *phyA-211* and *hy1-1* by crossing. The original *phyA-211 cab3*:*uidA* construct was outcrossed and does not interfere with the results. TCSn:GFP was introduced to *phyA-211* and *hy1-7* mutant lines by crossing, and two stable lines were used for phenotype analysis. Seeds were sterilized using 70% (v/v) ethanol and plated on full Murashige & Skoog salt media, 1% (w/v) plant agar, 1% (w/v) Suc (Duchefa Biochemie), supplemented when required with 0.1 μMBA (6-benzylaminopurine, dissolved in DMSO) or with 0.01% (v/v) DMSO (control). Plants were cultivated in growth chambers with controlled light, temperature (21°C/19°C, light/dark, respectively), and relative air humidity (50%). For histological analysis, and for GUS staining of leaves and inflorescences, we used flowering plants in phase II (when the first silique appears; Altamura et al., 2001). Light conditions were as follows: short d, photoperiod of 8/16 h (light/dark); long-d, photoperiod 16/8 h (light/dark); continuous light: photoperiod 24/0 (light/dark); (continuous) darkness (D): 24 h of induction by light (to initiate germination) followed by continuous cultivation under dark conditions. White light at an intensity of 150 μmol m^{-2} s^{-1} was used. For cultivation under light of defined quality, plants were cultivated in growth chambers (CLF floraLEDs; CLF Plant Climatics) equipped with diodes emitting 670 nm (red) and 740 nm (far-red), photoperiod 24/0 (light/dark), and light intensity 50 or 2 μmol m^{-2} s^{-1} for each wavelength.

Analysis of the *lph* Mutation

For chromosome linkage analysis (using at least two SSLP markers per chromosome), and for fine mapping with the nga1126 marker, we used 22 and 50 *lph* mutant plants, respectively, from the mapping population (*lph* plants from the F2 progeny of the *lph* cross with L*er*-0 background). A genomic fragment of *HY1* was isolated using primers HO1-u (5′-CAT TCA CCC TCT CAT CGT TAT CTT-3′) and HO1-l (5′-TGT ATT TGA GCT ATA AAA CGG CAG-3′). Sequencing of DNA was done with primers HO1-u, HO1-l, and HO1-ui (5′-CCT TCT TAT CTT CTT GTT ATG-3′). For analysis of cDNA length, and for cDNA sequencing, we used primers HO1-1exon (5′-GG TGA GAA AGA GAC TAA ATC-3′) and HO1-2exon (5′-TAG ATG TTG TAG AAG TGA CA-3′). In silico translation and protein comparison were performed with SDSC Biology WorkBench tools (http://workbench.sdsc.edu/).

Phenotypic Analysis

6- and 14-d-old seedlings were photographed and the root and hypocotyl parameters were evaluated using ImageJ (http://rsbweb.nih.gov/ij/). Measurement of the root meristem zone was performed on 5-d-old seedlings grown under long-d conditions. Cleared root tips were analyzed with a differential interference contrast microscope (model no. BX61; Olympus). Confocal microscopy was carried out on an inverted Observer.Z1 equipped with a LSM780 confocal unit and ×40 water immersion objective (Carl Zeiss). An appropriate set of filters was used for GFP imaging (excitation 488 nm, emission 507 nm). Roots of inspected lines were stained with propidium iodide (PI; 10 g/mL in water for 5 min) and observed using appropriate filter sets (PI excitation at 488 to 543 nm and emission at 610 nm). All measurements were done on at least two biological replicates and two technical replicates, each consisting of at least 15 samples. The mean and SD values or SES are shown on representative graphs. The hook opening assay and dynamics of hypocotyl growth were performed under an infrared light source (900 nm) as described by Zádniková et al. (2010). For the circadian experiment, plants were cultivated in growth chambers with controlled light (white light, 150 μmol m^{-2} s^{-}), temperature (21°C/19°C, light/dark, respectively), and relative air humidity (50%) under long-d conditions: 16/8 h (light/dark) and moved to continuous light: 24/0 (light/dark) or darkness (D): 0/24 (light/dark) conditions. The end of the dark phase when the plants reached the age of 6 d was set as a time point 0 h. Possible circadian regulation was tested in long-d-grown seedlings by replacing the light phase with darkness (long-d > D) at the time point 0 or 24 h or vice versa replacing the dark phase by continuous light (long-d > continuous light) at the time point 16 or 40 h (see also Supplemental Fig. S6).

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: *CKI1* (AT2G47430), *ARR3* (AT1G59940), *HY1* (AT2G26670), *PHYA* (AT1G09570), *PHYB* (AT2G18790), *PIF3* (AT1G09530), *CCA1* (AT2G46830), *PIF1* (AT2G20180), *PIF4* (AT2G43010), *PIF5* (AT3G59060).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. *CKI1* expression during wild-type and *lph* development-specificity in the vegetative and generative development.

Supplemental Figure S2. Molecular analysis of the *lph*/*hy1-7* allele.

Supplemental Figure S3. Phenotypes of known mutations in the *HY1* gene.

Supplemental Figure S4. Light-dependent phenotype of the *hy1-7* mutant line.

Supplemental Figure S5. *CKI1* promoter fragments, used in the Y1H binding assay.

Supplemental Figure S6. Circadian regulation of *CKI1* expression.

Supplemental Figure S7. Early seedling development-correlation to *CKI1* activity.

Supplemental Figure S8. Sensitivity of roots to the CK treatment.

Supplemental Figure S9. Partial phenotype rescue of *hy1-1* by *35S*:*CKI*.

Supplemental Figure S10. *phyA* phenotype in VB specific development.

Supplemental Figure S11. Normalized tag counts by location in the genome for CCA1 target CKI1.

Supplemental Table S1. Analysis of *CKI1* promoter for the presence of phyA-responsive elements.

Supplemental Table S2. Schematic nuclear localization of phyA and phyB the main light receptors in relation to CKI1 expression in SAM.

Supplemental Materials and Methods. Supplemental methods.

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