The Plant Cell, Vol. 15, 2654–2665, November 2003, www.plantcell.org © 2003 American Society of Plant Biologists

Arabidopsis *PSEUDO-RESPONSE REGULATOR7* Is a Signaling Intermediate in Phytochrome-Regulated Seedling Deetiolation and Phasing of the Circadian Clock

Karen A. Kaczorowski and Peter H. Quail[1]

Department of Plant and Microbial Biology, University of California, Berkeley, California 94720, and United States Department of Agriculture/Agricultural Research Service, Plant Gene Expression Center, Albany, California 94710

To identify new components in the phytochrome (phy) signaling network in Arabidopsis, we used a sensitized genetic screen for deetiolation-defective seedlings. Two allelic mutants were isolated that exhibited reduced sensitivity to both continuous red and far-red light, suggesting involvement in both phyA and phyB signaling. The molecular lesions responsible for the phenotype were shown to be mutations in the Arabidopsis *PSEUDO-RESPONSE REGULATOR7* (*PRR7*) gene. *PRR7* is a member of a small gene family in Arabidopsis previously suggested to be involved in circadian rhythms. A PRR7–β-glucuronidase fusion protein localized to the nucleus, implying a possible function in the regulation of photoresponsive gene expression. Consistent with this suggestion, *prr7* seedlings were partially defective in the regulation of the rapidly light-induced genes *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*), observable as a premature increase in expression level during the second peak of the biphasic induction profile that is elicited upon initial exposure of dark-grown seedlings to light. A similar 3- to 6-h coordinated advance in peak free-running expression of *CCA1*, *LHY*, and *TIMING-OF-CAB1*, which are considered to encode the molecular components of the circadian oscillator in Arabidopsis, was observed in entrained fully green *prr7* seedlings compared with wild-type seedlings. Collectively, these data suggest that PRR7 functions as a signaling intermediate in the phytochrome-regulated gene expression responsible for both seedling deetiolation and phasing of the circadian clock in response to light.

INTRODUCTION

Light has a profound impact on plant growth and development. It influences numerous developmental processes, such as seed germination, seedling deetiolation, neighbor sensing, and phototropism, enabling plants to grow in a way that optimizes their ability to capture light for photosynthesis. Plants possess a number of informational photoreceptors, including phytochromes, cryptochromes, and phototropins, which enable them to monitor specific regions of the light spectrum and to regulate the changes in gene expression that underlie their responses to the light environment (for a recent review, see Quail, 2002). Light also controls photoperiodic responses, such as flowering, through the circadian clock, thereby permitting the synchronization of reproduction with seasonal progression (Mouradov et al., 2002, and references therein). The circadian clock also allows the plant to anticipate and prepare for the regular daily changes in the light environment (Harmer et al., 2000). Both the phytochromes (red/far-red light receptors) and the cryptochromes (blue light receptors) mediate the entrainment of the circadian clock by light (Somers et al., 1998a). Because many light-regulated processes in plants, including elongation growth of stems and hypocotyls, leaf movements, stomatal opening, and transcription of *CHLOROPHYLL A/B BINDING PROTEIN* (*CAB*) genes, also are modulated by the circadian clock (Dowson-Day and Millar, 1999), it is not unlikely that one or more components of the central oscillator are positioned as integral intermediates at an early point in the light signaling pathway.

Genetic screens have generated mutants that have helped to define the photosensory and physiological roles of the various members of the phytochrome family (designated phyA to phyE in Arabidopsis). The evidence indicates that the different family members have differential, albeit partially overlapping, photosensory and/or physiological functions (Quail, 1998). Mutants also have provided insight into how the phytochrome signal is transmitted. The dual responses of light inhibition—cell elongation in seedling hypocotyls and the simultaneous promotion of cell expansion in cotyledons during seedling deetiolation—provide valuable phenotypic markers of light responsiveness. For example, a bona fide light signaling mutant may be distinguished from a mutant affected globally in cell elongation when both of these reciprocal responses are considered. Screens for mutants with reduced sensitivity to light or hypersensitivity to light have identified numerous loci putatively involved in phytochrome signaling (reviewed by Hudson, 2000), and this pathway seems to be far from saturated. Although some components appear to be required for deetiolation in response to either continuous far-red light (FRc) or continuous red light (Rc) exclusively, many appear to affect both pathways.

In addition, many Arabidopsis mutants that show defects in seedling photomorphogenesis during deetiolation also are

[1] To whom correspondence should be addressed. E-mail quail@nature.berkeley.edu; fax 510-559-5678.
Article, publication date, and citation information can be found at www.plantcell.org/cgi/doi/10.1105/tpc.015065.

The Plant Cell

The Plant Cell

affected in clock function in entrained deetiolated seedlings. These include *early flowering3* (*elf3*), which has reduced sensitivity to both Rc and continuous blue light (Reed et al., 2000), *gigantea* (*gi*) and *sensitivity to red light reduced*, which are specifically insensitive to Rc (Huq et al., 2000; Staiger et al., 2003), *zeitlupe*, which is hypersensitive to Rc (Somers et al., 2000), and *timing-of-cab1* (*toc1*), which has reduced sensitivity to both Rc and FRc (Mas et al., 2003). The apparent specificity of these defects for red and/or far-red light indicates that these mutations interfere with phytochrome-dependent seedling photomorphogenesis. Because almost all of these components are regulated by the circadian clock in entrained seedlings, they may be involved in the diurnal control of growth or function as feedback components that regulate light signaling to the clock and other plant light responses. This observation indicates that the circadian system shares many components with the phytochrome signaling pathway.

Considerable progress has been made in recent years in identifying the molecular components of the central oscillator in Arabidopsis (Harmer et al., 2001). There is now considerable evidence that *TOC1*, along with *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*), are integral components of the circadian oscillator that function in a temporally offset feedback loop to generate mutually opposed oscillations (Yanovsky and Kay, 2001). *CCA1* and *LHY* encode myb-related transcription factors that have been independently implicated in the maintenance of circadian rhythms in Arabidopsis. When the rhythmic expression of either of these genes is ablated, many circadian outputs display altered rhythmicity (Schaffer et al., 1998; Wang and Tobin, 1998; Matsushika et al., 2002b). Recent studies with plants reduced in both *LHY* and *CCA1* activity show that these genes have partially redundant functions in the control of circadian rhythms (Alabadi et al., 2002; Mizoguchi et al., 2002). The *TOC1* locus was identified genetically in a screen for mutants that misexpress a transgenic *CAB-luciferase* reporter gene (Millar et al., 1995). The mutant was further shown to have aberrant circadian rhythms in numerous outputs under all conditions; thus, it was believed to encode a component important for central oscillator function (Somers et al., 1998b). More recent studies with the *toc1-2* null mutant have demonstrated that *TOC1* is required for *CCA1* and *LHY* transcriptional oscillations, providing compelling evidence for the feedback loop that suggests that these components likely constitute the circadian oscillator in Arabidopsis (Alabadi et al., 2001).

Transcription of both *CCA1* and *LHY* is induced within 1 h of exposure of dark-grown seedlings to Rc or FRc in a phytochrome-dependent manner (Wang et al., 1997; Tepperman et al., 2001; J. Tepperman and P. Quail, unpublished data). Evidence that PHYTOCHROME-INTERACTING FACTOR3 (PIF3) binds to a fragment of the *CCA1* promoter in vitro and that Rc-induced expression of CCA1 is attenuated in PIF3-deficient seedlings suggests a possible direct mechanism by which *CCA1* transcription is controlled by light via phytochrome action (Martinez-Garcia et al., 2000). This transcriptional control may constitute the link between light signals perceived by phytochromes and the induction of the initial oscillations of the clock components that occurs during seedling deetiolation as well as the subsequent synchronization of these oscillations to daily cycles of light and darkness in fully green seedlings.

Recent evidence has shown that TOC1 is required for normal phytochrome-dependent seedling deetiolation in Rc and FRc and for the phytochrome-dependent induction of *CCA1* by pulsed red light (Mas et al., 2003). *TOC1* is a member of a small gene family in Arabidopsis (Imamura et al., 1998). These genes were independently named Arabidopsis *PSEUDO-RESPONSE REGULATORS* (*APRR*s, now synonymous with *PRR*s) because the proteins lack the conserved, phospho-accepting Asp of the bacterial response regulators (Makino et al., 2000). Close examination of the expression pattern of this gene family revealed that transcripts for each gene oscillate with a circadian expression pattern under free-running conditions, suggesting that these genes are regulated by the circadian clock (Makino et al., 2000; Matsushika et al., 2000). Plants that constitutively express *PRR5* or *PRR9* exhibit a hypersensitive seedling deetiolation phenotype in Rc and flower early, indicating that the aberrant expression of these genes interferes with normal phytochrome responses (Matsushika et al., 2002a; Sato et al., 2002). The precise molecular function of the PRRs is unknown, but TOC1, also known as PRR1, was shown to interact with PIF3 and a related basic helix-loop-helix transcription factor protein in vitro (Makino et al., 2002). This finding raises the possibility that TOC1 also may play a role in phytochrome-dependent transcriptional regulation.

To identify potential novel components of phytochrome signaling, we used a sensitized screen that exploits the synergistic effects of the *phyA* and *phyB* mutations on seedling deetiolation in Rc. *phyA* null mutants are almost completely insensitive to FRc but have a normal response to higher fluence rates of Rc (Parks and Quail, 1993). *phyB* null mutants have a more pleiotropic phenotype. Seedlings exhibit reduced sensitivity to Rc as well as reduced chlorophyll accumulation and early flowering, implicating phyB in both early light responses during deetiolation and the repression of flowering (Reed et al., 1993). *phyA phyB* double mutants have an even more severe defect in Rc perception at the seedling deetiolation stage, which indicates that phyA plays a role in Rc perception that is secondary to that of phyB (Reed et al., 1994; Smith et al., 1997). By screening for Rc-insensitive mutants in the *phyA-101* null mutant (Quail et al., 1994) background, we hoped to enhance the phenotype of mutants in the phyB signaling pathway, with the goal of identifying components specific to the phyB response. This screen has allowed the identification of a new mutant defective in both the phytochrome-mediated response to Rc and FRc and the regulation of the circadian clock.

RESULTS

Isolation of a Light-Insensitive Mutant

Many seedlings were selected in a screen for reduced sensitivity to Rc, but two independently isolated M3 lines, which were suspected to be novel mutants, are described here. Allelism tests performed with M4 plants from these lines showed that the long-hypocotyl phenotype was not complemented in the F1 generation, which demonstrated that these two lines consti-

tuted one complementation group (data not shown). Further tests showed that these mutant alleles complemented the *phyA-101 phyB-1* double mutant and thus did not represent new alleles of *phyB* (data not shown). The long-hypocotyl phenotype of each line was mapped in the F2 generation from a cross of the mutant in the *phyA-101* (RLD ecotype) background to *phyA-211* (Columbia ecotype) (Quail et al., 1994) using PCR-based markers. The mutant locus was mapped to the top of chromosome V, distal to the marker CTR1.2, in a region not known to contain any other loci involved in photomorphogenesis. Recombinant F2 individuals on either side of the mutant locus defined a region spanned by four BACs (Figure 1A).

Candidate gene sequencing in this region revealed lesions in Arabidopsis *PRR7* (At5g02810) in both mutant lines (Figure 1B). This gene is a member of a small family that includes *TOC1*. This family has been studied with respect to gene expression and has been referred to as the *TOC1/APRR1* family. Here, in keeping with community guidelines for mutant gene nomenclature, our mutants were given the three-letter abbreviation *prr7*. Both alleles contain G-to-A transversions that would result in early termination of the predicted protein in a highly conserved domain homologous with the receiver domain (Figures 1B and 1C).

PRR7 Is a Nuclear Protein

A PROSITE (Hofmann et al., 1999) scan revealed a potential bipartite nuclear localization signal near the C terminus of PRR7 (amino acids 680 to 696). A PRR7–β-glucuronidase (GUS) fusion protein was expressed transiently in leek epidermal cells after bombardment with a plasmid carrying a strong 35S promoter upstream of a fusion of the *PRR7* coding region with the

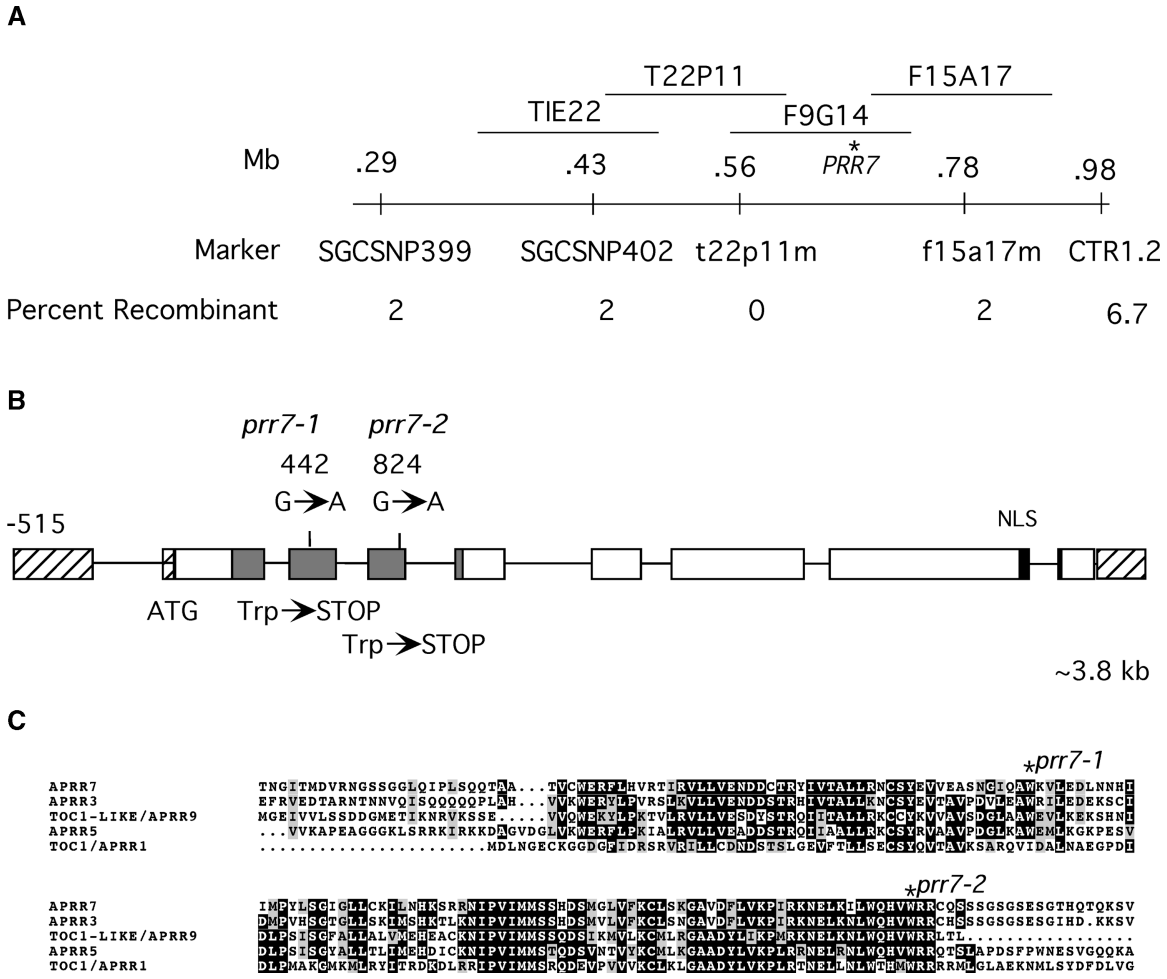

Figure 1. Identification of the *prr7* Locus.

(A) Map positions of the *PRR7* locus and closely linked markers on chromosome 5.

(B) Structure of the *PRR7* gene, showing the positions of the *prr7-1* and *prr7-2* mutations and the putative nuclear localization signal (NLS; black rectangle). The gray shaded area represents the conserved receiver domain shown in **(C)**.

(C) Predicted protein sequence alignment of the conserved receiver domain of the TOC1/APRR1 family showing the positions of the stop codons introduced by *prr7-1* and *prr7-2*.

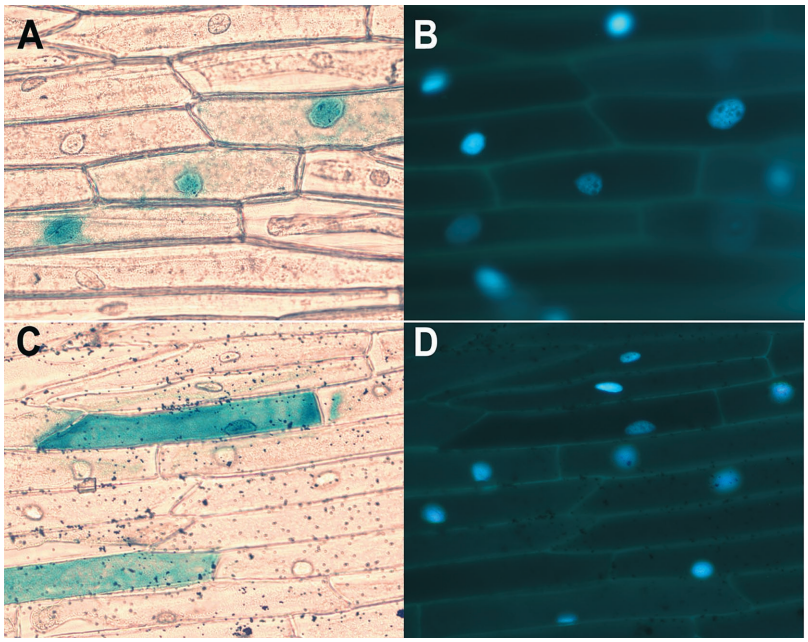

Figure 2. PRR7 Is Localized to the Nucleus.

(A) Bright-field image showing that PRR7-GUS is localized to the nucleus in leek epidermal peels.
(B) 4′,6-Diamidino-2-phenylindole (DAPI) staining of the cells shown in **(A)**.
(C) Bright-field image showing that the GUS control is distributed throughout the cell.
(D) DAPI staining of the cells shown in **(C)**.

GUS marker gene. The GUS activity in dark-maintained leek epidermal peels transformed with the PRR7-GUS fusion protein was observed primarily in the nucleus (Figures 2A and 2B). In parallel experiments, GUS alone was detected throughout the cytoplasm (Figures 2C and 2D). These data provide evidence that PRR7 likely is a nuclear protein.

prr7 Seedlings Have Reduced Sensitivity to Rc and FRc, and the Rc Phenotype Is Enhanced by *phyA-101*

Fluence rate response curves in Rc and FRc were determined to quantitatively characterize the effect of the *prr7* mutation on seedling sensitivity to these two diagnostic wavelengths. *prr7* seedlings were 1 to 2 mm taller than wild-type seedlings at all fluence rates >0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ Rc (Figures 3A and 3B), an effect that indicates reduced responsiveness to light of this wavelength. The reduced sensitivity of *prr7* to Rc also was evident in the expansion of the cotyledons. Cotyledon expansion was reduced in *prr7* with respect to the wild type (Figure 3C). Sensitivity to FRc also was reduced in *prr7* with respect to the wild type (Figures 3A and 3D). Comparison of *phyA prr7* double mutants with monogenic mutants showed that the *prr7* mutant phenotype in Rc was more dramatic in the *phyA-101* background, especially in the range from 0.1 to 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Figures 3B and 3D). However, in FRc (Figure 3D), *phyA prr7* double mutant seedlings were indistinguishable from *phyA* seedlings, which suggests that *phyA* and *prr7* do not have an additive effect under these light conditions. This is not surprising considering that phyA is the sole photoreceptor for far-red light and that *phyA* seedlings are essentially blind to FRc. *prr7* seedlings did not have a long-hypocotyl phenotype when grown under continuous white light with a fluence rate of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (data not shown).

The Hyposensitive Phenotype of *prr7* Mutants Is Not the Result of Reduced Levels of phyB

To test the possibility that PRR7 is involved in the control of *PHYB* expression, the levels of phyB protein in wild-type and *prr7* seedlings were analyzed. Figure 4 shows that phyB levels were normal in the mutants both in the dark and under Rc. This finding suggests that the *prr7* phenotype is not a consequence of altered phyB levels and that PRR7 likely functions downstream of the phyB photoreceptor in Rc signal transduction.

Light-Induced Gene Expression in *prr7*

To test the involvement of PRR7 in the regulation of early-responding, light-induced genes (Tepperman et al., 2001), we compared the transcriptional induction of *CCA1* and *LHY* in response to Rc in the mutant and the wild type. In the wild type, *CCA1* and *LHY* both were induced rapidly and transiently in response to light, exhibiting biphasic patterns with an acute peak of expression at 1 h after transfer to light, followed by a trough from 6 to 12 h and a second peak at ~18 h (Figures 5A and 5B), as observed previously (Wang et al., 1997; Schaffer et al.,

The Plant Cell

1998; Wang and Tobin, 1998; Tepperman et al., 2001; J. Tepperman and P. Quail, unpublished data). No oscillation in *CCA1* transcript level was detectable in dark-grown wild-type seedlings during the 24-h period preceding the transfer to light (data not shown), establishing that these oscillations occurred only in response to the light signal under these conditions. The *prr7* mutants also showed an initial peak and partial immediate downregulation of *CCA1* expression upon first exposure to light, but *CCA1* transcript levels began to increase again prematurely at 6 h and peaked at 18 h, coincident with the peak in the wild type at this level of resolution (Figure 5A). Thus, the depth of the trough of *CCA1* expression was attenuated in *prr7*.

Figure 5B shows a similar but less severe defect in the expression of *LHY* in the *prr7* mutants. These results suggest that PRR7 may be required for the negative regulation of *CCA1* and *LHY* during the early trough in expression of these genes in Rc.

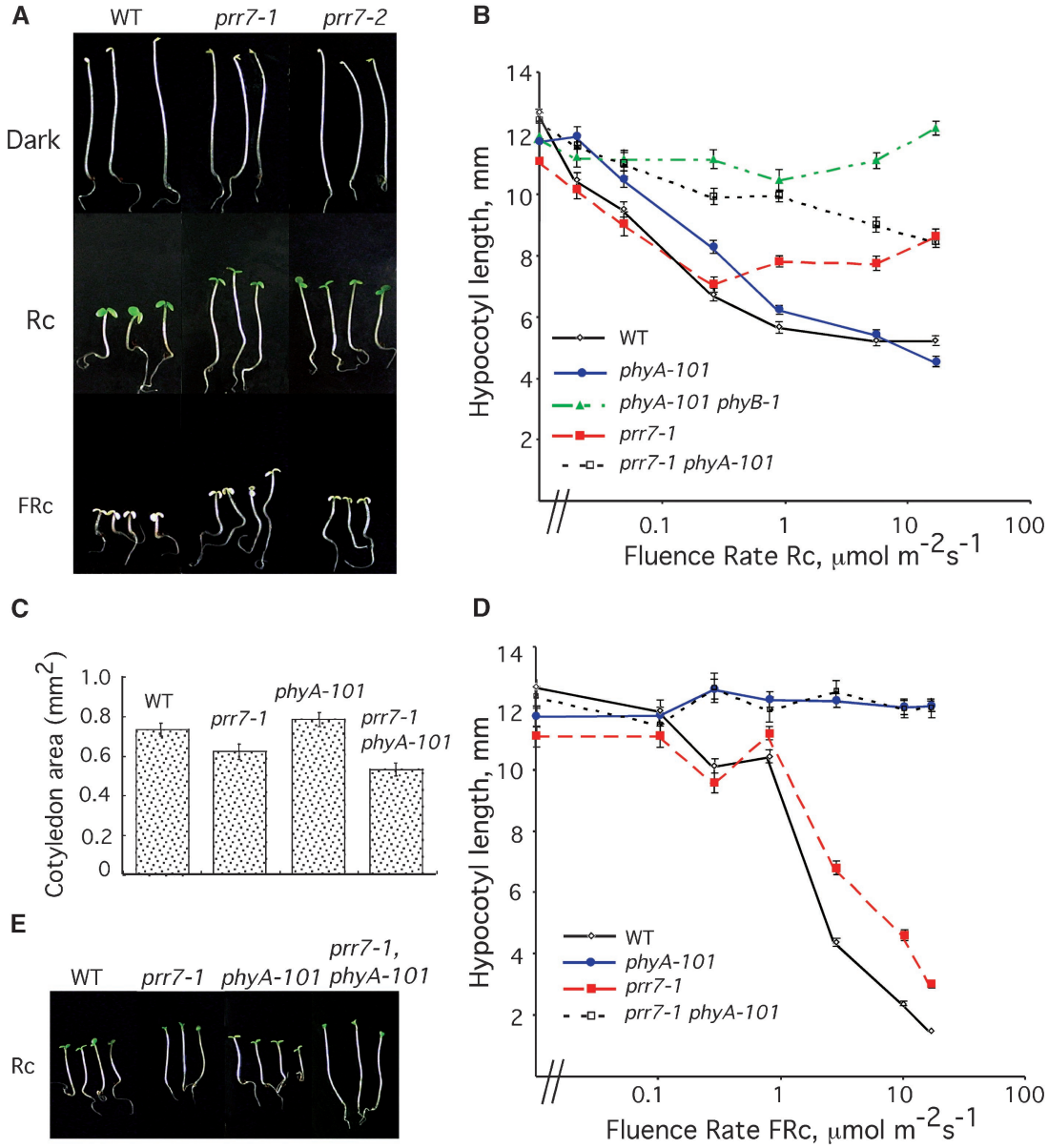

Figure 3. *prr7* Mutants Have Reduced Sensitivity to both Rc and FRc.

(A) Wild-type RLD (WT) and *prr7* mutants grown at 25°C in darkness, Rc (17 $\mu mol \cdot m^{-2} \cdot s^{-1}$), or FRc (10.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

(B) Rc fluence rate response curves for *prr7*.

(C) Cotyledon expansion in Rc is inhibited in *prr7*. Seedlings were grown in Rc (0.79 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

(D) FRc fluence rate response curve for *prr7*.

(E) The *prr7* mutant phenotype is enhanced by *phyA-101*. Seedlings were grown in Rc (0.89 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

The Plant Cell

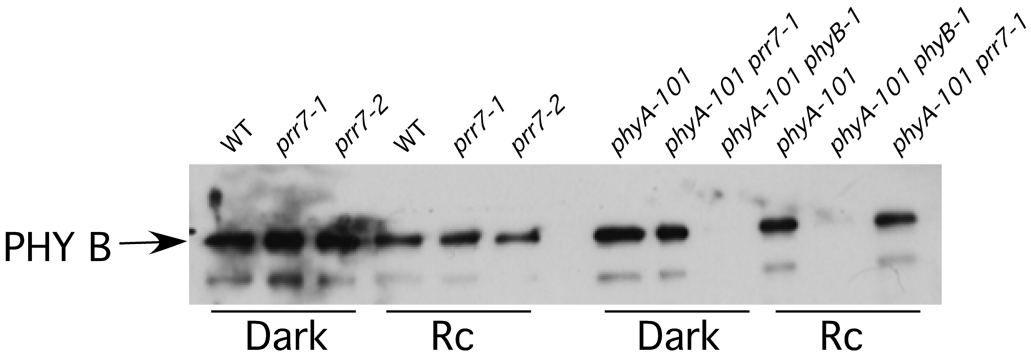

Figure 4. phyB Levels Are Not Affected in *prr7*.

Immunoblot of total protein extracted from 3-day-old seedlings grown either in darkness or Rc and probed with monoclonal antibody to phyB. WT, wild-type ecotype RLD.

A similar pattern of *CCA1* expression was observed in FRc (data not shown). We observed a small difference in responsiveness between the *prr7-1* and *prr7-2* alleles both at 1 h after transfer to Rc and again at 18 to 24 h after transfer to Rc, but these differences are not likely to be significant in this experiment.

Circadian Clock–Regulated Gene Expression in *prr7*

As a result of the proposed role of PRR7 in circadian clock function (Makino et al., 2001) and the initial misexpression of the clock genes *CCA1* and *LHY* at early stages after transfer to red light observed here, we examined the circadian clock–regulated expression of these genes in *prr7-1*. Figure 6A shows that *CCA1* mRNA oscillated with circadian rhythmicity in free-running continuous light conditions in fully green, wild-type seedlings that had been entrained to 12-h-light/12-h-dark cycles. In these seedlings, *CCA1* expression began to increase in anticipation of subjective dawn to peak at zeitgeber time 0 (ZT0) to ZT3. *prr7-1* also showed robust oscillations in *CCA1* under these conditions (Figure 6A). However, the peaks of *CCA1* expression in the mutant occurred before subjective dawn at ZT18 to ZT21 and in each cycle occurred 3 to 6 h before the wild-type peaks. *CCA1* expression in the wild type increased most rapidly from ZT15 to ZT18, but in *prr7-1*, the trough was attenuated and *CCA1* levels had already started to increase at ZT12. The mean period of expression for *CCA1* was 20.7 $\pm$ 2.3 h in the wild type and 20.7 $\pm$ 2.8 h in *prr7-1*.

A similar phenomenon was observed for *LHY*, which also oscillated robustly in *prr7-1* and the wild type, with peak expression occurring at ZT0 in the wild type and at ZT21 in the mutant. The mean free-running period of *LHY* expression was calculated to be 21 $\pm$ 2.6 h in the wild type and 22 $\pm$ 2.1 h in *prr7-1*. The average periods of expression of *CCA1* and *LHY* did not differ significantly between the mutant and the wild type at the resolution level of this experiment. Amplitude values for both *CCA1* and *LHY* were comparable between *prr7* and the wild type, but with a tendency for expression to be higher in the troughs of the oscillations in *prr7*. The same is true for the *TOC1* transcript (Figure 6C), for which peak expression in the wild type occurred at ZT9 to ZT12 but occurred earlier in *prr7* at ZT6 to ZT9. Thus, the peak of expression for all three of the genes examined occurred earlier in *prr7-1*, without a detectable aberration in period length. These results indicate that the absence of PRR7 causes a significant, coordinated shift in the phasing of the oscillatory expression of the central components of the circadian clock.

DISCUSSION

In this study, a sensitized genetic screen was used to identify new loci that are required for the Rc-induced deetiolation response. Two mutant alleles of *PRR7* were identified. Both contain a recessive mutation that results in early termination of the predicted protein in the conserved receiver domain, and neither is likely to make a functional protein. A reduction in the levels or activity of the phyB photoreceptor would have been sufficient to account for the insensitivity to Rc that we observed in *prr7*. However, no reduction in the levels of phyB were detected in the mutant by immunoblot analysis. Therefore, these data provide evidence that PRR7 functions downstream of phyB in the Rc-induced deetiolation response. The reduced response of *prr7* to Rc in both the cotyledons and the hypocotyl was enhanced by the *phyA-101* mutation. *prr7* also had a defect in its responsiveness to FRc, apparent as reduced inhibition of hypocotyl elongation. We interpret these data to indicate that PRR7 functions in a positive manner downstream of the convergence of the phyA and phyB signaling pathways, which regulate seedling photomorphogenesis in Rc and FRc.

Evidence has emerged in recent years to indicate that phytochromes can enter the nucleus to directly regulate gene expression in response to light (Kircher et al., 1999; Huq et al., 2003). A number of proteins implicated in phytochrome signaling are known to be localized to the nucleus, including CCA1, PIF3, PIF4, *FAR-RED-IMPAIRED RESPONSE1*, *SUPPRESSOR OF PHYA1*, GI, ELF3, and TOC1 (Wang et al., 1997; Ni et al., 1998; Hoecker et al., 1999; Hudson et al., 1999; Huq et al., 2000; Makino et al., 2000; Strayer et al., 2000; Huq and Quail,

The Plant Cell

2002). The nuclear localization of PRR7 suggests a possible regulatory function in phytochrome-controlled gene expression.

Consistent with this possibility, phytochrome-dependent gene induction was affected in *prr7*. Here, we show that PRR7 was required for the negative regulation of *CCA1* and *LHY* in seedlings in Rc during a defined temporal window in the early phases of deetiolation (Figure 5). A biphasic waveform pattern of expression for *CCA1* and *LHY* was detectable in wild-type etiolated seedlings upon transfer to Rc. After the initial rapid induction of *CCA1* and *LHY*, which peaked at ~1 to 3 h after transfer from darkness to Rc, transcript levels declined sharply, remained low for 6 h, and then increased again at 12 h. By contrast, in both *prr7-1* and *prr7-2*, *CCA1* and *LHY* transcript levels increased prematurely between 6 and 12 h. The *prr7* mutants had fourfold to sixfold higher levels of *CCA1* and *LHY* transcript than the wild type during the 6- to 12-h period after transfer to Rc. This early and precise misexpression caused by the loss of PRR7 indicates that it functions as a negative modulator of the inductive phytochrome signaling pathway, suggesting that PRR7 plays a role in controlling the initial establishment of *CCA1* and *LHY* oscillations.

prr7 also displayed a clear defect in the sustained circadian expression pattern of *LHY* and *CCA1* (Figures 6A and 6B). In entrained, fully green *prr7* seedlings under free-running conditions of continuous white light, the peak expression of both genes was shifted by 3 to 6 h earlier than in the wild type. The rising phase of the *CCA1* and *LHY* transcriptional oscillations that occurred earlier in *prr7* resembled the premature increase in transcript level observed after the initial transfer of dark-grown seedlings to Rc (Figure 5). Oscillation of *TOC1* mRNA

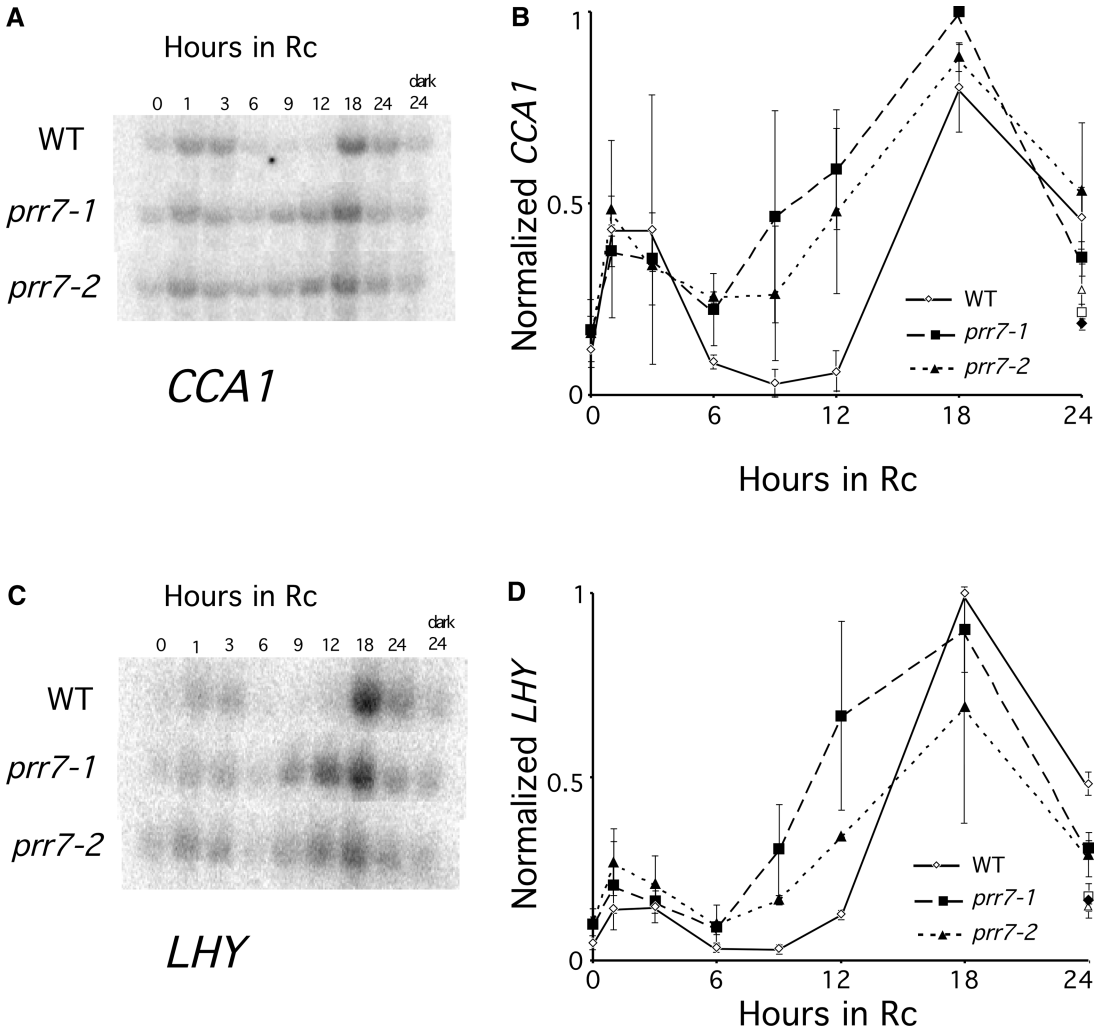

Figure 5. Rc-Induced Expression of *CCA1* and *LHY* Is Altered in *prr7*.

Induction of *CCA1* **(A)** and *LHY* **(B)** in wild-type RLD (WT), *prr7-1*, and *prr7-2*. Seedlings were grown for 96 h in darkness and transferred to Rc. Tissue was collected at the times indicated. Average values for two biological replicates are plotted, and error bars represent ranges. One representative RNA gel blot is shown for each transcript.

The Plant Cell

The Plant Cell

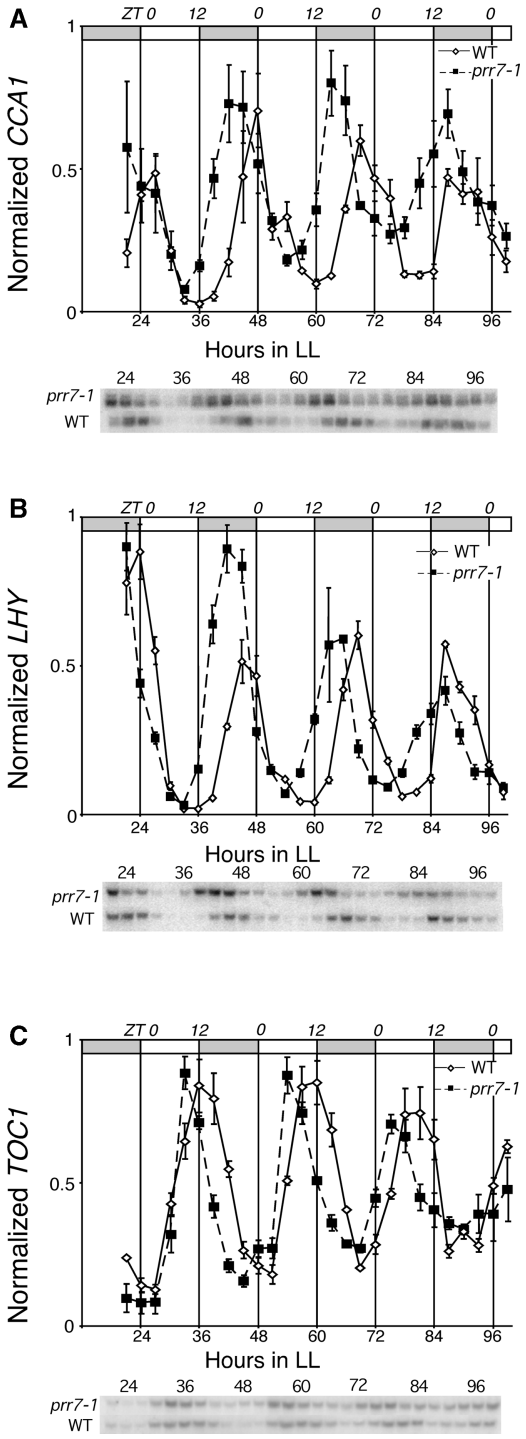

Figure 6. Circadian Oscillations of Clock Transcripts Are Altered in *prr7*.

Expression in *prr7-1* mutants and wild-type ecotype RLD (WT) under free-running continuous light conditions (LL) for *CCA1* mRNA **(A)**, *LHY* mRNA **(B)**, and *TOC1* mRNA **(C)**. Shaded areas in the bars at top represent subjective night, and the upper axis indicates zeitgeber time. Expression level measurements were performed by RNA gel blot quantitation, and the values were corrected for gel loading relative to 18s rRNA hybridization. These values then were scaled such that the highest expression value for each experiment was 1, and the resulting values were averaged. The mean values from three independent biological replicates are plotted. Error bars represent standard errors of the mean. A representative gel blot image of one replicate is shown.

under free-running conditions was shifted similarly in *prr7* (Figure 6C), demonstrating that mutation in *PRR7* affected the clock genes expressed in both the morning (*CCA1* and *LHY*) and evening (*TOC1*) phases of the diurnal cycle and that the interlocked, opposed oscillations of *CCA1/LHY* and *TOC1* were maintained in register in this mutant. No differences in the mean free-running period length between the mutant and the wild type were detectable at the resolution of the assays used here.

The evidence that the expression of these genes is perturbed in the mutant after the initial transfer of seedlings to Rc (in the absence of an entrained circadian clock) suggests that the function of PRR7 may be to attenuate phytochrome signaling to the central oscillator during a specific temporal window. The loss of PRR7 appears to result in the early derepression of *CCA1* and *LHY* expression in what would normally be the trough of the waveform. Therefore, we propose that the perturbation observed here in *prr7* during the light-induced initiation of the oscillations in expression of clock-component genes, which occurred upon first exposure of dark-grown seedlings to light, is reiterated daily at dawn in the dark-to-light transition under diurnal cycles and is propagated under free-running conditions in entrained seedlings to generate a phase advance of the circadian oscillations in the clock-component transcripts. Consistent with this conclusion, phyB has been implicated in the control of circadian phase in white light (Salome et al., 2002).

A simplified model of the proposed dual function of PRR7 in the control of seedling deetiolation and phasing of the circadian clock is presented in Figure 7. The observation that PRR7 appears to act positively in phytochrome-regulated deetiolation but negatively in controlling the clock suggests early branching in the phytochrome signaling pathway upstream of these two target processes. However, because *CCA1*, *LHY*, and *TOC1* also have been shown to function in regulating deetiolation (Schaffer et al., 1998; Wang and Tobin, 1998; Mas et al., 2003), these two pathway branches presumably converge somewhere downstream (Figure 7). The initial light-induced transcriptional activation of *CCA1* during deetiolation is postulated to be a direct target of the phytochrome photoreceptor via interaction with PIF3, with few if any other signaling intermediates intervening (Martinez-Garcia et al., 2000). PRR7 functions during the first 24 h of deetiolation to antagonize this initial light-induced expression in a temporally specific manner, creating a trough in the profile and determining the timing of the rise of the second peak of expression (Figure 5). It is proposed that under established circadian conditions, PRR7 continues to function in this manner, thereby controlling the phasing of the oscillator by determining the timing of release from repression of *CCA1/LHY* expression at each cycle.

The mechanism of repression is unknown. However, in principle, it appears likely that PRR7 antagonizes the promotive ac-

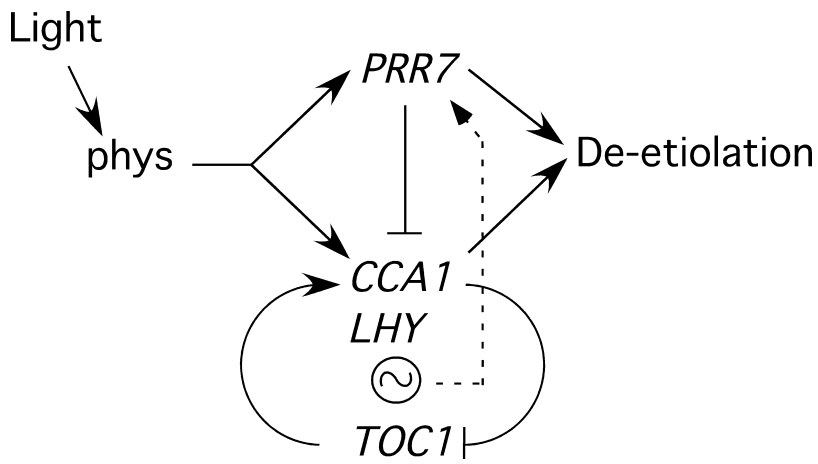

Figure 7. Model of the Proposed PRR7 Function in Phytochrome-Regulated Seedling Deetiolation and Phasing of the Circadian Oscillator.

Light perceived by phytochromes induces both seedling deetiolation and oscillations in expression of the central clock–component genes *CCA1*, *LHY*, and *TOC1*. PRR7 acts positively in the pathway that controls seedling photomorphogenesis but negatively in the circadian cycle by transiently repressing or delaying *CCA1* and *LHY* expression during a temporally defined window and by antagonizing or repressing the promotive activity of *TOC1*. *PRR7* expression also is controlled by the circadian clock.

tion of TOC1 on *CCA1/LHY* expression in some way (Figure 7). It is uncertain whether this antagonism by PRR7 is direct or indirect. The expression profiles of *PRR7* and *TOC1* appear to overlap sufficiently for *PRR7* to act directly (Matsushika et al., 2000). On the other hand, because *PRR7* is a member of the "PRR quintet" of genes that are expressed sequentially in the order *PRR9*, *PRR7*, *PRR5*, *PRR3*, *PRR1/TOC1*, it is possible that the effect is exerted indirectly through *PRR5* and/or *PRR3* in a cascade-like manner. Because the expression of this quintet, including *PRR7*, also is controlled by the clock in a feedback manner (Figure 7), it has been proposed that these five components may constitute a second interlocking loop involved in regulating circadian oscillations under diurnal conditions (Makino et al., 2001; Eriksson and Millar, 2003). Regardless, it seems reasonable to propose that this antagonism of TOC1 action is imposed at the post-transcriptional level, modulating only the onset of the period over which TOC1 can exert its promotive activity on *CCA1/LHY* transcription, without altering the onset of *TOC1* expression. This configuration permits the phasing of the circadian oscillations to be varied without changing the peak-to-peak period. Possible mechanisms of such post-transcriptional regulation might include inhibitory protein–protein interactions, providing transient sequestration or inactivation of TOC1, and direct transcriptional repression, by competitive displacement of TOC1 from *CCA1/LHY* promoter target sites.

The model presented here exemplifies the concept that certain key early components of the phytochrome signaling network have been recruited to function also as integral components of the circadian oscillator, thereby imposing oscillatory behavior on a broad spectrum of downstream genes in the light-regulated transcriptional cascade (Harmer et al., 2000; Eriksson and Millar, 2003). Such a configuration provides an elegant mechanism by which the extensive and complex network of light-regulated processes on which the plant depends can be attuned to the diurnal light cycle. The strategy used here of directly monitoring the behavior of the molecular components of the clock during the initial induction of oscillations upon first transfer from darkness to light provides a powerful means of dissecting the primary sequence of events involved in inducing and establishing the necessary feedback loops that drive the oscillator under steady state conditions. We anticipate that the use of this strategy, in conjunction with mutants compromised in various aspects of light responsiveness, will provide additional insights into the hierarchy and interactions among the molecular components involved in the function and regulation of the circadian clock.

METHODS

Mutagenesis and Screening

A total of 40,000 *Arabidopsis thaliana phyA-101* (ecotype RLD) seeds were treated with 0.3% ethyl methanesulfonate for 12 h, washed, and planted on soil. After stratification for 4 days at 4°C, flats were transferred to the greenhouse under continuous white light. M2 seeds were harvested from families of 1,000 M1 plants and desiccated for 14 days in Drierite (W.A. Hammond Drierite Company, Xenia, OH). M2 seeds were sown on growth media (Valvekens et al., 1988) without sucrose, stratified for 5 days at 4°C, given a synchronizing 3-h white light treatment and 21-h dark treatment, and then grown for 72 h in continuous red light (Rc; 2 to 4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on vertical plates at 21°C (a total of 96 h from germination). Light sources and fluence measurements have been described elsewhere (Huq et al., 2000). Seedlings with long hypocotyls were selected and allowed to self. Rescreening of the resulting M3 seeds identified a number of lines with a heritable long-hypocotyl phenotype. M3 seeds were plated on biliverdin-supplemented medium to eliminate chromophore biosynthetic mutants (Parks and Quail, 1991). M3 plants were crossed to the *phyA-101 phyB-1* introgressed line (described by

The Plant Cell

Smith et al., 1997) to identify new alleles of the phyB photoreceptor mutant. Candidate M3 lines were backcrossed to RLD and crossed to *phyA-211* in the Columbia ecotype (Reed et al., 1994) for mapping.

Mapping and Sequencing

The mutant locus was mapped from the F2 population of the mutant by *phyA-211* cross using simple sequence length polymorphism, cleaved amplified polymorphic sequence, and single nucleotide polymorphism markers polymorphic between RLD and Columbia. Plant DNA was prepared according to Edwards et al. (1991).

Nuclear Localization Experiments

The *PRR7* open reading frame was amplified by PCR from cDNA using primers containing restriction sites for ClaI and XbaI and inserted into the modified pRTL2-GUS/NiaDBam vector described by Hoecker et al. (1999). Constructs were sequenced for accuracy. Leek (*Allium porrum*) epidermal peels were bombarded with this construct (35S:GUS-APRR7) and pRTL2-GUS and incubated in darkness for 24 h. Bombardment and GUS staining were performed as described by Ni et al. (1998).

Seedling Growth Conditions and Measurements

The F2 progeny of the first backcross to RLD were analyzed with derived-cleaved amplified polymorphic sequence markers (Neff et al., 1998) to select lines homozygous for *prr7-1* and *prr7-2*. These plants also were tested for the *phyA-101* mutation to obtain *prr7-1 phyA-101* sibling lines for fluence response measurements. Seeds were sown, stratified, and synchronized as described above. After 72 h in Rc or continuous far-red light (96 h after germination) on horizontal plates containing growth media (Valvekens et al., 1988) without sucrose, seedlings were photographed with a digital camera and hypocotyl length was measured using NIH Image software (Bethesda, MD). For cotyledon area measurements, seedlings were sown as described above except that after 96 h of Rc (120 h after germination) at a fluence rate of 0.79 $\mu mol \cdot m^{-2} \cdot s^{-1}$, cotyledons were flattened to the agar surface and photographed with a digital camera and the area of individual cotyledons was measured using NIH Image software.

RNA Isolation

For red-light transcriptional induction experiments, seedlings were sown and stratified as described above but after synchronization were grown in darkness for 93 h before transfer to Rc (7.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (time 0). Samples for RNA were taken at specified intervals. For circadian entrainment experiments, seeds were plated and stratified and immediately transferred to a 12-h-light/12-h-dark photoperiod for 6 days at a constant temperature of 21°C. On day 7, seedlings were transferred to continuous white light at a fluence rate of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and seedlings were collected at 3-h intervals. Seedling RNA was isolated and analyzed using the Qiagen Plant RNeasy kit (Valencia, CA) according to the manufacturer's instructions. Two to 10 μg of total RNA was separated on 1.2% formaldehyde gels and blotted to Ambion Brightstar membranes (Austin, TX). Hybridization and washes were performed at 65°C according to Church and Gilbert (1984). Probes were labeled by random priming using the Redi-Prime II kit (Amersham Pharmacia). Probes for *CCA1* and *LHY* were described by Martinez-Garcia et al. (2000). The probe for *TOC1* was amplified by PCR from genomic DNA using primers described by Makino et al. (2002).

Signal was quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and ImageQuant for Mac version 1.2 and corrected for loading using the 18s rRNA hybridization signal. These values were normalized so that the highest value for each transcript was equal to 1. For circadian experiments, the mean of three biological replicates was plotted, and error bars represent standard errors of the mean. For red light transcriptional induction experiments, the average normalized value (corrected for even loading with 18s rRNA signal, with the highest value set to 1) of two biological replicates was plotted, and the ends of the error bars represent the actual normalized values of each replicate. Period estimates for *CCA1* and *LHY* oscillations were the average time between peaks of expression from three biological replicates over three cycles.

Protein Isolation and Immunoblot Analysis

Seedling protein isolation and immunoblotting and detection procedures were performed as described by Martinez-Garcia et al. (1999). Total protein was isolated from 3-day-old seedlings grown in darkness or in Rc and separated by SDS-PAGE (8%). Ten micrograms of total protein was loaded for dark-grown seedlings, and 30 μg was loaded for Rc-grown seedlings. To detect PHYB, membranes were probed with the monoclonal antibodies B1 and B7 (1:500 dilution each) described by Hirschfield et al. (1998).

Upon request, materials integral to the findings presented in this publication will be made available in a timely manner to all investigators on similar terms for noncommercial research purposes. To obtain materials, please contact Peter H. Quail, quail@nature.berkeley.edu.

ACKNOWLEDGMENTS

We thank members of our laboratory for helpful discussion, M. Hudson and D. Somers for critical reading of the manuscript, A. Smith for technical assistance, and D. Hantz for excellent plant care. This work was supported by National Institutes of Health Grant GM47475, Department of Energy Grant DE-FG03-87ER13742, and U.S. Department of Agriculture–Agricultural Research Service Current Research Information Service number 5335-21000-017-00D.

Received June 27, 2003; accepted September 3, 2003.

REFERENCES

Alabadi, D., Oyama, T., Yanovsky, M.J., Harmon, F.G., Mas, P., and Kay, S.A. (2001). Reciprocal regulation between *TOC1* and *LHY/CCA1* within the *Arabidopsis* circadian clock. Science **293,** 880–883.

Alabadi, D., Yanovsky, M.J., Mas, P., Harmer, S.L., and Kay, S.A. (2002). Critical role for *CCA1* and *LHY* in maintaining circadian rhythmicity in *Arabidopsis*. Curr. Biol. **12,** 757–761.

Church, G.M., and Gilbert, W. (1984). Genomic sequencing. Proc. Natl. Acad. Sci. USA **81,** 1991–1995.

Dowson-Day, M.J., and Millar, A.J. (1999). Circadian dysfunction causes aberrant hypocotyl elongation patterns in Arabidopsis. Plant J. **17,** 63–71.

Edwards, K., Johnstone, C., and Thompson, C. (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. **19,** 1349.

Eriksson, M.E., and Millar, A.J. (2003). The circadian clock: A plant's best friend in a spinning world. Plant Physiol. **132,** 732–738.

Harmer, S.L., Hogenesch, J.B., Straume, M., Chang, H.-S., Han, B., Zhu, T., Wang, X., Kreps, J.A., and Kay, S.A. (2000). Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. Science **290,** 2110–2113.

Harmer, S.L., Panda, S., and Kay, S.A. (2001). Molecular bases of circadian rhythms. Annu. Rev. Cell Dev. Biol. **17,** 215–253.

Hirschfield, M., Tepperman, J.M., Clack, T., Quail, P.H., and Sharrock, R.A. (1998). Coordination of phytochrome levels in phyB mutants of *Arabidopsis* as revealed by apoprotein-specific monoclonal antibodies. Genetics **149,** 523–535.

Hoecker, U., Tepperman, J.M., and Quail, P.H. (1999). SPA1, a WD-repeat protein specific to phytochrome A signal transduction. Science **284,** 496–499.

Hofmann, K., Bucher, P., Falquest, L., and Bairoch, A. (1999). The PROSITE database, its status in 1999. Nucleic Acids Res. **27,** 215–219.

Hudson, M., Ringli, C., Boylan, M.T., and Quail, P.H. (1999). The FAR1 locus encodes a novel nuclear protein specific to phytochrome A signaling. Genes Dev. **13,** 2017–2027.

Hudson, M.E. (2000). The genetics of phytochrome signalling in Arabidopsis. Semin. Cell Dev. Biol. **11,** 475–483.

Huq, E., Al-Sady, B., and Quail, P.H. (2003). Nuclear translocation of the photoreceptor phytochrome B is necessary for its biological function in seedling photomorphogenesis. Plant J. **35,** 660–665.

Huq, E., and Quail, P.H. (2002). PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in *Arabidopsis*. EMBO J. **21,** 2441–2450.

Huq, E., Tepperman, J.M., and Quail, P.H. (2000). GIGANTEA is a nuclear protein involved in phytochrome signaling in Arabidopsis. Proc. Natl. Acad. Sci. USA **97,** 9789–9794.

Imamura, A., Hanaki, N., Umeda, H., Nakamura, A., Suzuki, T., Ueguchi, C., and Mizuno, T. (1998). Response regulators implicated in His-to-Asp phosphotransfer signaling in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **95,** 2691–2696.

Kircher, S., Kozma-Bognar, L., Kim, L., Adam, E., Harter, K., Schafer, E., and Nagy, F. (1999). Light quality–dependent nuclear import of the plant photoreceptors phytochrome A and B. Plant Cell **11,** 1445–1456.

Makino, S., Kiba, T., Imamura, A., Hanaki, N., Nakamura, A., Suzuki, T., Taniguchi, M., Ueguchi, C., Sugiyama, T., and Mizuno, T. (2000). Genes encoding pseudo-response regulators: Insight into His-to-Asp phosphorelay and circadian rhythm in *Arabidopsis thaliana*. Plant Cell Physiol. **41,** 791–803.

Makino, S., Matsushika, A., Kojima, M., Oda, Y., and Mizuno, T. (2001). Light response of the circadian waves of the APRR1/TOC1 quintet: When does the quintet start singing rhythmically in *Arabidopsis*? Plant Cell Physiol. **42,** 334–339.

Makino, S., Matsushika, A., Kojima, M., Yamashino, T., and Mizuno, T. (2002). The APRR1/TOC1 quintet implicated in circadian rhythms of *Arabidopsis thaliana*. I. Characterization with APRR1-overexpressing plants. Plant Cell Physiol. **43,** 58–69.

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

Martinez-Garcia, J.F., Monte, E., and Quail, P.H. (1999). A simple, rapid and quantitative method for preparing Arabidopsis protein extracts for immunoblot analysis. Plant J. **20,** 251–257.

Mas, P., Alabadi, D., Yanovsky, M., Oyama, T., and Kay, S.A. (2003). Dual role of TOC1 in the control of circadian and photomorphogenic responses in Arabidopsis. Plant Cell **15,** 223–236.

Matsushika, A., Imamura, A., Yamashino, T., and Mizuno, T. (2002a). Aberrant expression of the light-inducible and circadian-regulated APRR9 gene belonging to the circadian-associated APRR1/TOC1 quintet results in the phenotype of early flowering in *Arabidopsis thaliana*. Plant Cell Physiol. **43,** 833–843.

Matsushika, A., Makino, S., Kojima, M., and Mizuno, T. (2000). Circadian waves of expression of the APRR1/TOC1 family of pseudo-response regulators in *Arabidopsis thaliana*: Insight into the plant circadian clock. Plant Cell Physiol. **41,** 1002–1012.

Matsushika, A., Makino, S., Kojima, M., Yamashino, T., and Mizuno, T. (2002b). The APRR1/TOC1 quintet implicated in circadian rhythms of *Arabidopsis thaliana*. II. Characterization with CCA1-overexpressing plants. Plant Cell Physiol. **43,** 118–122.

Millar, A.J., Carre, I.A., Strayer, C.A., Chua, N.-H., and Kay, S.A. (1995). Circadian clock mutants in *Arabidopsis* identified by luciferase imaging. Science **267,** 1161–1163.

Mizoguchi, T., Wheatley, K., Hanzawa, Y., Wright, L., Mizoguchi, M., Song, H.-R., Carre, I.A., and Coupland, G. (2002). *LHY* and *CCA1* are partially redundant genes required to maintain circadian rhythms in *Arabidopsis*. Dev. Cell **2,** 629–641.

Mouradov, A., Cremer, F., and Coupland, G. (2002). Control of flowering time: Interacting pathways as a basis for diversity. Plant Cell **14** (suppl.), S111–S130.

Neff, M.M., Neff, J.D., Chory, J., and Pepper, A.E. (1998). dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: Experimental applications in *Arabidopsis thaliana* genetics. Plant J. **14,** 387–392.

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

Parks, B.M., and Quail, P.H. (1991). Phytochrome-deficient *hy1* and *hy2* long hypocotyl mutants of *Arabidopsis* are defective in phytochrome chromophore biosynthesis. Plant Cell **3,** 1177–1186.

Parks, B.M., and Quail, P.H. (1993). hy8, a new class of Arabidopsis long hypocotyl mutants deficient in functional phytochrome A. Plant Cell **5,** 39–48.

Quail, P.H. (1998). The phytochrome family: Dissection of functional roles and signalling pathways among family members. Philos. Trans. R. Soc. Lond. B Biol. Sci. **353,** 1399–1403.

Quail, P.H. (2002). Phytochrome photosensory signaling networks. Nat. Rev. Mol. Cell. Biol. **3,** 85–93.

Quail, P.H., Briggs, W.R., Chory, J., Hangarter, R.P., Harberd, N.P., Kendrick, R.E., Koornneef, M., Parks, B., and Sharrock, R.A. (1994). Spotlight on phytochrome nomenclature. Plant Cell **6,** 468–471.

Reed, J.W., Nagatani, A., Elich, T.D., Fagan, M., and Chory, J. (1994). Phytochrome A and phytochrome B have overlapping but distinct functions in *Arabidopsis* development. Plant Physiol. **104,** 1139–1149.

Reed, J.W., Nagpal, P., Bastow, R.M., Solomon, K.S., Dowson-Day, M.J., Elumalai, R.P., and Millar, A.J. (2000). Independent action of ELF3 and phyB to control hypocotyl elongation and flowering time. Plant Physiol. **122,** 1149–1160.

Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M., and Chory, J. (1993). Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell **5,** 147–157.

Salome, P.A., Michael, T.P., Kearns, E.V., Fett-Neto, A.G., Sharrock, R.A., and McClung, C.R. (2002). The *out of phase 1* mutant defines a role for PHYB in circadian phase control in *Arabidopsis*. Plant Physiol. **129,** 1674–1685.

Sato, E., Nakamichi, N., Yamashino, T., and Mizuno, T. (2002). Aberrant expression of the *Arabidopsis* circadian-regulated *APRR5* gene belonging to the TOC1/APRR1 quintet results in early flowering and hypersensitiveness to light in early photomorphogenesis. Plant Cell Physiol. **43,** 1374–1385.

Schaffer, R., Ramsay, N., Samach, A., Corden, S., Putterill, J., Carre, I.A., and Coupland, G. (1998). The *late elongated hypocotyl* mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. Cell **93,** 1219–1229.

Smith, H., Xu, Y., and Quail, P.H. (1997). Antagonistic but complementary actions of phytochromes A and B allow optimum seedling de-etiolation. Plant Physiol. **114,** 637–641.

Somers, D.E., Devlin, P.F., and Kay, S.A. (1998a). Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. Science **282,** 1488–1494.

The Plant Cell

Somers, D.E., Schultz, T.F., Milnamow, M., and Kay, S.A. (2000). *ZEITLUPE* encodes a novel clock-associated PAS protein from *Arabidopsis*. Cell **101,** 319–329.

Somers, D.E., Webb, A.A.R., Pearson, M., and Kay, S.A. (1998b). The short-period mutant, *toc1-1*, alters circadian clock regulation of multiple outputs throughout development in *Arabidopsis thaliana*. Development **125,** 485–494.

Staiger, D., Allenbach, L., Salathia, N., Fiechter, V., Davis, S.J., Millar, A.J., Chory, J., and Fankhauser, C. (2003). The *Arabidopsis SRR1* gene mediates phyB signaling and is required for normal circadian clock function. Genes Dev. **17,** 256–268.

Strayer, C., Oyama, T., Schultz, T.F., Raman, R., Somers, D.E., Mas, P., Panda, S., Kreps, J.A., and Kay, S.A. (2000). Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. Science **289,** 768–771.

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

Valvekens, D., Van Montagu, M., and Van Lijsebettens, M. (1988). *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. Proc. Natl. Acad. Sci. USA **85,** 5536–5540.

Wang, Z.-Y., Kenigsbuch, D., Sun, L., Harel, E., Ong, M.S., and Tobin, E.M. (1997). A Myb-related transcription factor is involved in the phytochrome regulation of an Arabidopsis Lhcb gene. Plant Cell **9,** 491–507.

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

Yanovsky, M.J., and Kay, S.A. (2001). Signaling networks in the plant circadian system. Curr. Opin. Plant Biol. **4,** 429–435.

The Plant Cell

Arabidopsis PSEUDO-RESPONSE REGULATOR7 Is a Signaling Intermediate in Phytochrome-Regulated Seedling Deetiolation and Phasing of the Circadian Clock

Karen A. Kaczorowski and Peter H. Quail

PLANT CELL 2003;15;2654-2665; originally published online Oct 16, 2003;
DOI: 10.1105/tpc.015065

This information is current as of February 17, 2011

References	This article cites 54 articles, 38 of which you can access for free at: http://www.plantcell.org/cgi/content/full/15/11/2654#BIBL
Permissions	https://www.copyright.com/ccc/openurl.do?sid=pd_hw1532298X&issn=1532298X&WT.mc_id=pd_hw1532298X
eTOCs	Sign up for eTOCs for *THE PLANT CELL* at: http://www.plantcell.org/subscriptions/etoc.shtml
CiteTrack Alerts	Sign up for CiteTrack Alerts for *Plant Cell* at: http://www.plantcell.org/cgi/alerts/ctmain
Subscription Information	Subscription information for *The Plant Cell* and *Plant Physiology* is available at: http://www.aspb.org/publications/subscriptions.cfm

© American Society of Plant Biologists

ADVANCING THE SCIENCE OF PLANT BIOLOGY