The Plant Cell, Vol. 25: 2892–2906, August 2013, www.plantcell.org © 2013 American Society of Plant Biologists. All rights reserved.

Heat Shock–Induced Fluctuations in Clock and Light Signaling Enhance Phytochrome B–Mediated *Arabidopsis* Deetiolation[C][W]

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Moderately warm constant ambient temperatures tend to oppose light signals in the control of plant architecture. By contrast, here we show that brief heat shocks enhance the inhibition of hypocotyl growth induced by light perceived by phytochrome B in deetiolating *Arabidopsis thaliana* seedlings. In darkness, daily heat shocks transiently increased the expression of *PSEUDO-RESPONSE REGULATOR7* (*PRR7*) and *PRR9* and markedly enhanced the amplitude of the rhythms of *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*) expression. In turn, these rhythms gated the hypocotyl response to red light, in part by changing the expression of *PHYTOCHROME INTERACTING FACTOR4* (*PIF4*) and *PIF5*. After light exposure, heat shocks also reduced the nuclear abundance of CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) and increased the abundance of its target ELONGATED HYPOCOTYL5 (HY5). The synergism between light and heat shocks was deficient in the *prr7 prr9*, *lhy cca1*, *pif4 pif5*, *cop1*, and *hy5* mutants. The evening element (binding site of LHY and CCA1) and G-box promoter motifs (binding site of PIFs and HY5) were overrepresented among genes with expression controlled by both heat shock and red light. The heat shocks experienced by buried seedlings approaching the surface of the soil prepare the seedlings for the impending exposure to light by rhythmically lowering *LHY*, *CCA1*, *PIF4*, and *PIF5* expression and by enhancing HY5 stability.

INTRODUCTION

Light and temperature are arguably the most important variables of the environment, providing cues for the adjustment of plant body form and function to the prevailing conditions. There are several examples where key plant developmental decisions depend on the combined action of light and temperature cues (Franklin, 2009).

In the control of plant architecture, high average ambient temperatures tend to oppose light signals. Hypocotyl growth is promoted by high ambient temperatures (Gray et al., 1998) and inhibited by light compared with darkness (Fankhauser and Chory, 1997). Upward leaf growth can be induced by increased ambient temperatures (van Zanten et al., 2009) as well as by shade signals, such as low red to far-red ratios (Vandenbussche et al., 2003; Tao et al., 2008; Keuskamp et al., 2010) or low irradiance (Vandenbussche et al., 2003; Mullen et al., 2006). These responses to light and to average temperatures require the basic helix-loop-helix transcription factor PHYTOCHROME INTERACTING FACTOR4 (PIF4) (Koini et al., 2009; Stavang et al., 2009). PIF4 acts in part by promoting auxin biosynthesis at high temperatures (Franklin et al., 2011) and under low red to far-red ratios (Hornitschek et al., 2012).

In addition to nonstressful ambient temperatures, extreme temperature changes also affect plant growth and development (McClung and Davis, 2010). In dark-grown pea (*Pisum sativum*) seedlings, daily heat shock induces some responses typically triggered by the exposure to light, such as inhibition of stem growth, leaf expansion, opening of the apical hook, and increased expression of genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase and chlorophyll *a/b* binding proteins (Kloppstech et al., 1991), and also enhances the responses of the latter genes and of chloroplast development to light (Otto et al., 1992). In dark-grown barley (*Hordeum vulgare*), daily heat shocks generate circadian rhythms of expression of several light-regulated genes (Beator et al., 1992). While our understanding of the molecular mechanisms that account for the opposite effects of light and average temperature has advanced significantly (Franklin et al., 2011), we are largely ignorant of the signaling players involved in the synergistic actions of light and heat shocks. These effects of heat shock treatments could be the result of either the distortion of normal light signaling by high temperature events (i.e., interference or crosstalk) or the use of temperature fluctuations as a source of information (i.e., signal convergence).

Upon emergence from the soil, the seedling is exposed to light. In *Arabidopsis thaliana*, this signal is perceived mainly by the red/far-red photoreceptors phytochrome A (phyA) and phyB and the blue light photoreceptors cryptochrome 1 (cry1) and cry2. These photoreceptors initiate the deetiolation process, where the growth rate of the hypocotyl is reduced, the cotyledons become

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www.plantcell.org/cgi/doi/10.1105/tpc.113.114306

expanded and unfolded, and the photosynthetic and photoprotective systems are established (Kami et al., 2010). During this dark-to-light transition, the seedling can experience the high temperatures often achieved by the upper layers of the soil. The aim of this work was to investigate the molecular mechanisms of action of transient high-temperature events on light signaling during deetiolation in *Arabidopsis*.

RESULTS

Transient Exposure to High Temperatures Enhances phyB-Mediated Inhibition of Hypocotyl Growth

To investigate whether heat shock and light signals interact to regulate early plant development, 1-d-old seedlings were grown in constant darkness at 22°C (control), daily exposed for three consecutive days to 1.5 h at 37°C in darkness (heat shock) or 6 h of white light (12 μmol m^{-2} s^{-1}) at 22°C, or the combination of both treatments (i.e., 1.5 h at 37°C in darkness immediately followed by 6 h of white light) (Figure 1A). The heat shock was provided in darkness to avoid direct effects caused by the stress of the photosynthetic apparatus (Larkindale and Knight, 2002).

The heat shock treatment had no effect on hypocotyl length in darkness but significantly increased the response to light (Figures 1B and 1C). Thus, the combination of a brief exposure to high temperature and light synergistically inhibited hypocotyl growth. This synergism was observed over a wide range of white light irradiances during the 6-h photoperiod (Figure 1D) and increased with the intensity of the 1.5-h heat shock (Figure 1E). In contrast with the synergistic effect of a transient exposure to high temperature and light, increasing the constant ambient temperature reduced the effect of light on hypocotyl elongation (Gray et al., 1998) (see Supplemental Figure 1 online).

The transcription factor ELONGATED HYPOCOTYL5 (HY5) integrates the action of different photoreceptors (Chen et al., 2004), and the *hy5* mutant failed to show a synergism between heat shock and light (Figure 2A). Compared with the wild type, the *phyA*, *cry1*, and *cry2* photoreceptor mutants showed a minor reduction of the synergism between heat shock and light, whereas in the *phyB* mutant, the synergistic response was absent (Figure 2A).

Since phyB is a red light photoreceptor, we replaced the 6-h white light photoperiod by 6 h red light (12 μmol m^{-2} s^{-1}) in all of the subsequent experiments. In addition to phyA and phyB, we tested mutants of several genes involved in red light signaling, such as *PIF3*, *PIF4*, and *PIF5* (Huq and Quail, 2002; Kim et al., 2003; Bauer et al., 2004; Fujimori et al., 2004; Shin et al., 2009; Leivar and Quail, 2011), *HY5*, *CONSTITUTIVE PHOTOMORPHOGENIC1* (*COP1*) (Lau and Deng, 2012), *EARLY FLOWERING3* (*ELF3*) (Zagotta et al., 1996; Nusinow et al., 2011), *GIGANTEA* (*GI*) (Huq et al., 2000), *LATE ELONGATED HYPOCOTYL* (*LHY*), *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*), *TIMING OF CAB EXPRESSION1* (*TOC1*), *PSEUDO-RESPONSE REGULATOR3* (*PRR3*), *PRR5*, *PRR7*, and *PRR9* (Kaczorowski and Quail, 2003; Más et al., 2003; Ito et al., 2007). In the wild type, hypocotyl length was synergistically inhibited by the combination of heat shocks and red light photoperiods (Figure 2B, top panel).

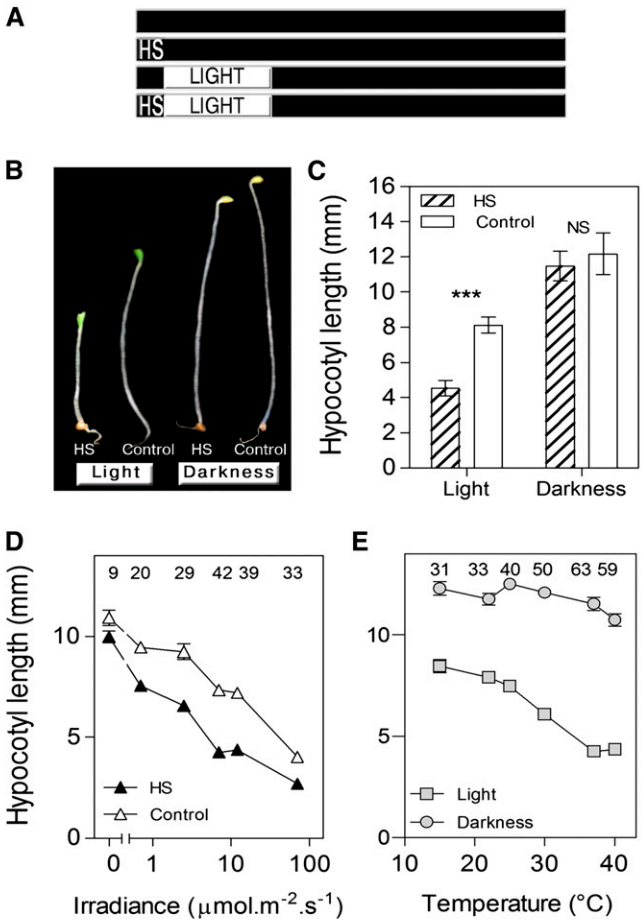

Figure 1. Synergism between Heat Shock and Light on Hypocotyl Growth Inhibition.

(A) Daily protocol: Seedlings were grown in darkness at 22°C (black bar) interrupted by 1.5 h at 37°C in darkness (heat shock [HS]) and/or 6 h of white light (12 μmol m^{-2} s^{-1}) at 22°C (LIGHT, white bar).

(B) Representative seedlings after 3 d treatment.

(C) Hypocotyl length after 3 d of treatment. Data are means and SE of nine boxes of seedlings. Interaction between light and temperature conditions: $P < 0.05$. ***$P < 0.001$; NS, not significant.

(D) The synergism between light and heat shock is observed over a wide range of irradiances. The seedlings were treated as in **(A)** but with different irradiance levels. Data are means and SE of nine boxes of seedlings. Interaction between light and temperature conditions: $P < 0.0001$. The percentage of inhibition of hypocotyl growth by heat shock compared with continuous 22°C is indicated for each irradiance.

(E) The synergism increases with the magnitude of the heat shock. The seedlings were treated as in **(A)** but with different temperatures during the 1.5-h heat shock (i.e., the first two points involve no temperature rise above the control at constant 22°C). Data are means and SE of nine boxes of seedlings. Interaction between light and temperature conditions: $P < 0.0001$. The percentage of inhibition of hypocotyl growth by light compared with darkness is indicated for each temperature.

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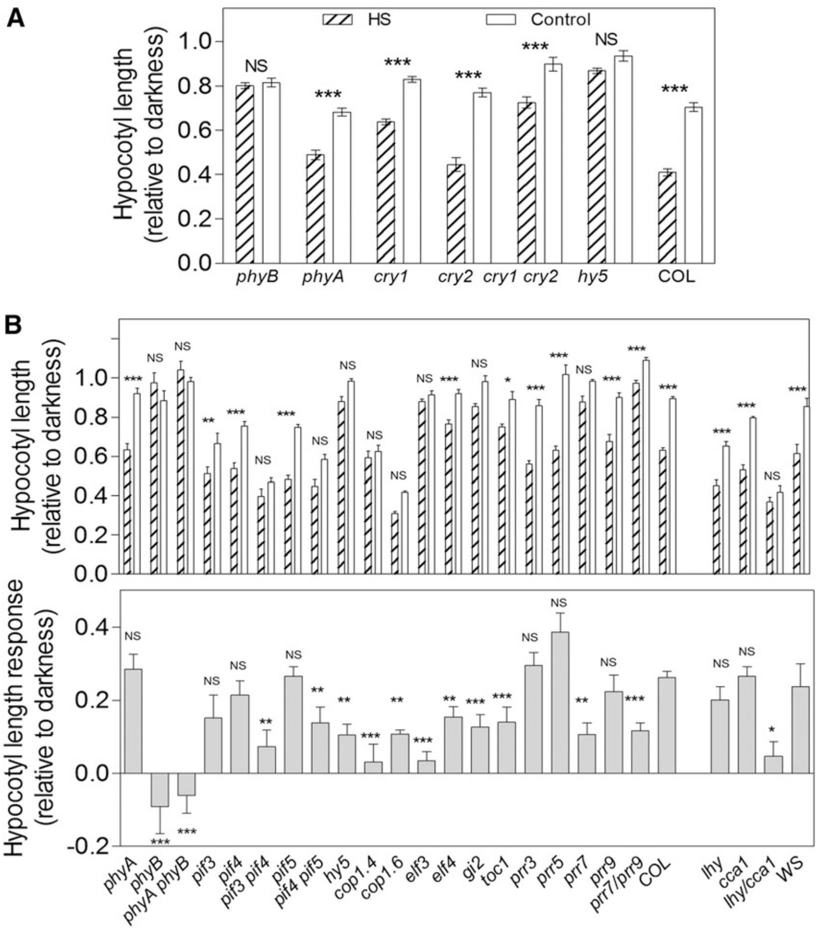

Figure 2. The Synergism between Heat Shocks and Light Signals Requires Intact phyB Signaling.

(A) Seedlings were grown in darkness at 22°C interrupted by 1.5 h at 37°C in darkness (HS) and/or 6 h of white light (12 μmol m^{-2} s^{-1}) at 22°C (as in Figure 1A).

(B) Red light (12 μmol m^{-2} s^{-1}) was used instead of white light. In the bottom box, the difference between the control and heat shock is presented for each genotype and compared with its wild type. COL, Columbia; WS, Wassilewskija.

Data are means and SE of three to nine boxes of seedlings. Hypocotyl length of heat shock–treated and control seedlings exposed to 6 h light is presented relative to their respective heat shock and control lengths in darkness to increase accuracy. Effect of heat shocks compared with control temperature (**[A]** and **[B]**, top panel) or the magnitude of the effect of the heat shock in each genotype compared with its wild type (**[B]**, bottom panel): ***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$; NS, not significant.

To quantitatively analyze the synergism, we calculated the difference between the heat shock and control conditions under red light photoperiods for each genotype (Figure 2B, bottom panel). The synergism was absent in the *phyB* and *phyA phyB* mutants and reduced in *pif3 pif4*, *pif4 pif5*, *cop1*, *hy5*, *elf3*, *elf4*, *gi*, *toc1*, *prr7*, *prr7 prr9*, and *lhy cca1*. Single *pif3*, *pif4*, *pif5*, *prr9*, *lhy*, or *cca1* mutants showed no significant reduction in synergism, indicating some degree of redundancy.

Heat Shocks Generate a Rhythm of Hypocotyl Growth Sensitivity to Red Light

To investigate the molecular mechanisms underlying the synergism between heat shocks and red light, we described the kinetics of the physiological output in detail and then searched for expression patterns matching those kinetics among the genes required for the synergism (Figure 2B).

The combination of heat shocks and red light exposures generated a strong rhythm of hypocotyl elongation rate in subsequent darkness (prolonged night), not observed in seedlings exposed only to light, only to heat shocks or in the controls (see Supplemental Figure 2 online). The period of the rhythm was close to 30 h, suggesting its circadian nature.

The Pfr (active) form of phytochrome can be present for several hours in darkness after exposure to light (Downs et al., 1957; Casal, 1996). Therefore, the growth rhythm admits two explanations: (1) The combination of light and heat shocks is required to entrain a rhythm of hypocotyl growth; (2) heat shocks are required to entrain a rhythm of sensitivity to red light. To test the latter idea, the seedlings were either kept in darkness at constant temperature or entrained under heat shock conditions for 2 d, transferred to constant 22°C (darkness) for periods of different duration, and only then given 6 h of red light before measurements of hypocotyl growth rate (Figure 3A). This protocol separates in time the light and temperature inputs. The heat shocks generated rhythmic changes in the sensitivity of hypocotyl growth rate to red light (Figure 3B).

Alternating temperatures are able to entrain circadian rhythms (Salomé et al., 2008; Troein et al., 2011). In particular, warm-cold cycles of 12 h, 24°C/12 h, 18°C gate the response of *ProCAB*:*LUC* to red light (Troein et al., 2011). To investigate whether the effect of our heat shock protocol can be equated to the effect of alternating temperatures, we repeated the experiment described in Figure 3A and recorded the activity of *ProCAB*:*LUC*. Red light and the heat shock promoted *ProCAB*:*LUC* activity synergistically (time 0, Figure 3C). However, no heat shock–mediated gating of the *ProCAB*:*LUC* response to red light was observed (Figure 3C).

Heat Shocks Reduce phyB Nuclear Body Formation under Red Light

If heat shocks operate via changes at the receptor level, phyB activity should be enhanced by heat shocks at the time when sensitivity to red light is enhanced. The expression of *PHYB* was unaffected by heat shocks (control, 504 ± 37; heat shock, 469 ± 23; $P > 0.1$, data from microarray experiments described below). The nuclear fluorescence of PHYB-GFP (for green fluorescent protein) increased in response to red light (Van Buskirk et al., 2012) and was unaffected by heat shocks (Figure 4B). Weak phyB photobodies (Van Buskirk et al., 2012) were already present in darkness and increased during the first 4 h of exposure to red light (Figure 4C). Bright photobodies were absent during the first 2 h of red light treatment and accumulated subsequently (Figures 4A and 4C). Heat shocks reduced both types of photobodies. Since heat shocks did not increase nuclear accumulation of phyB or the formation of phyB photobodies when they increase sensitivity to red light, the latter effect should involve other signaling components.

Heat Shock Treatments Generate Circadian Rhythms of *CCA1* and *LHY* in Darkness

We reasoned that entrainment under heat shock compared with constant temperature conditions in darkness might induce oscillations in the expression of genes affecting phyB-mediated signaling. We therefore measured by real-time RT PCR the mRNA levels of clock genes (Pokhilko et al., 2012) that are required for the synergism according to the results presented in Figure 2B. The seedlings were either kept in darkness at constant 22°C or entrained at 22°C interrupted by 1.5-h heat shocks (i.e., the two conditions used for the physiological outputs in Figure 3) and then transferred to constant 22°C (darkness) (Figure 5; see Supplemental Figure 3 online). To maximize the resolution of the data, for gene expression we used seedlings entrained in darkness for 3 d because older seedlings yield more fresh weight, whereas for hypocotyl growth the seedlings were entrained in darkness for 2 d because growth rate decreases with age. However, the pattern of hypocotyl growth was similar in seedlings entrained for either 2 d (Figure 3) or 3 d (see Supplemental Figure 4 online), indicating that the comparison between expression and growth data is meaningful.

In control seedlings, *LHY* and *CCA1* showed weak oscillations in expression only during the first 24 h of the recorded period. In darkness, some genes can show poor rhythms when analyzed by real-time PCR and more robust rhythms when analyzed by reporters (Kikis et al., 2005; Salomé et al., 2008), suggesting that the latter reveal the patterns of expression in a more restricted and synchronized group of cells than whole seedling RNA samples. The heat shocks induced a rhythm of *LHY* and *CCA1* expression with increased amplitude, which persisted during the second 24-h period (Figure 5A). The expression of *TOC1* showed apparent rhythmicity, which was confirmed by recording luciferase activity under the control of the *TOC1* promoter, but the oscillations were already present in darkness and the effect of heat shocks was mainly to advance the phase (see Supplemental Figure 3 online). The expression of *GI* showed a weak rhythm apparently phase-shifted by heat shocks. The phase advance observed for the dusk-expressed genes *TOC1*, *CCR2*, and *GI* is consistent with the peak observed at 18 h for *LHY* and *CCA1* (Pokhilko et al., 2012). Heat shocks induced an acute promotion of *PRR7* and *PRR9* expression, which was followed by rhythmic expression during the first 24 h (see Supplemental Figure 3 online). The expression of *ELF3* and *ZEITLUPE* was not obviously affected by the heat shocks during the first 24 h under free-running conditions (despite a weak rhythmic pattern in the case of *ELF3*) (see Supplemental Figure 3 online).

Since *LHY* and *CCA1* are direct targets of PRR7 and PRR9 (Nakamichi et al., 2010), we investigated the levels of *LHY* and *CCA1* in *prr7 prr9*. The *prr7 prr9* mutant showed at most weak oscillations of *LHY* or *CCA1* mRNA (Figure 5A). In Figure 5A, expression values are normalized to the median to focus on the patterns of fluctuations but, as expected (Nakamichi et al., 2010), values not normalized to the median confirm enhanced *LHY* and *CCA1* expression in *prr7 prr9* (see Supplemental Table 1 online).

We also investigated the reciprocal control (i.e., whether the transient promotion of *PRR7* and *PRR9* expression by heat shocks required *LHY* and *CCA1*). The *lhy cca1* mutant showed reduced background *PRR7* and *PRR9* expression consistent with previous reports (Farré et al., 2005; Nakamichi et al., 2010) but enhanced responses to heat shock (Figure 5B). This indicates that the promotion of *PRR7* and *PRR9* expression by heat shocks does not require *LHY* or *CCA1*.

Temperature Gating of the Hypocotyl Growth Response to Red Light Requires *CCA1*, *LHY*, *PRR9*, and *PRR7*

The oscillations in *LHY* and *CCA1* mRNA were in antiphase with sensitivity to red light (Figure 6A). Since LHY and CCA1 promote growth under red light (Ito et al., 2007), these results provide correlative evidence for the hypothesis that temperature shifts gate the sensitivity to red light by inducing oscillations in *LHY* and *CCA1* expression. This idea is consistent with the observation that the double *lhy cca1* mutant shows no significant synergism between red light and temperature (Figure 2B). The corollary of this hypothesis is that in hypocotyl growth gating

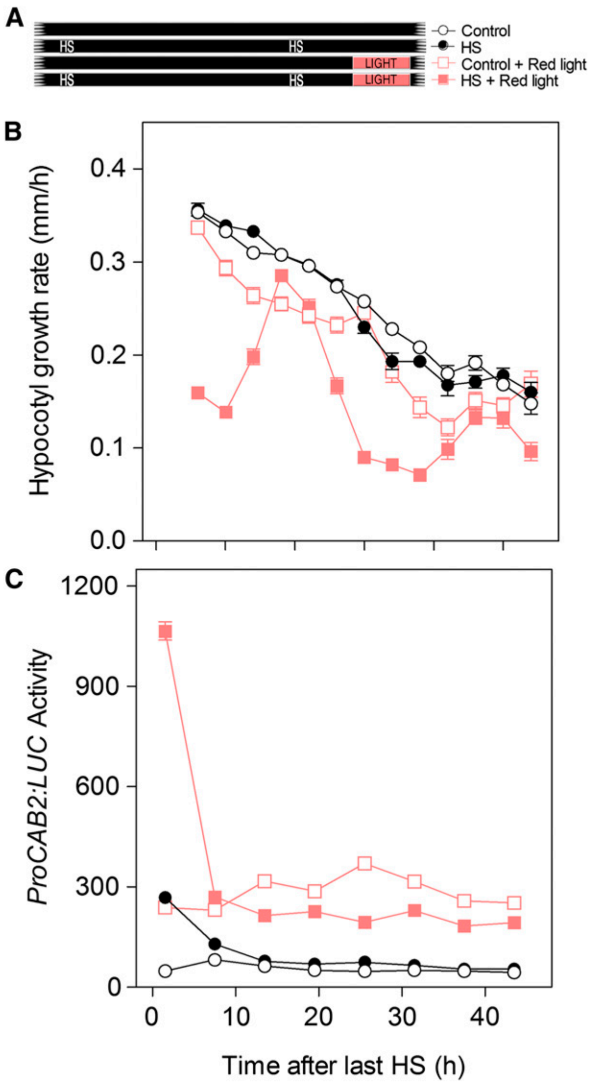

Figure 3. Heat Shocks Gate Hypocotyl Growth but Not *ProCAB:LUC* Expression upon Exposure to Red Light.

(A) Protocol: Seedlings were either kept at constant 22°C (black bar) or entrained at 1.5 h at 37°C (HS) followed by 22.5 h at 22°C for 2 d in darkness. Immediately after the second heat shock (time 0), the seedlings were transferred to constant darkness at 22°C for periods of different duration (abscissas) and then were either exposed or not to red light.

(B) and **(C)** The first time point corresponds to seedlings that received red light immediately after the end of the last heat shock. Controls grown simultaneously are also plotted against the time after the heat shock was received by the treated seedlings. Data are means and SE (whenever larger than the symbols) from 20 to 45 **(B)** or 40 **(C)** seedlings.

(B) Hypocotyl growth rate measured during the 18 h in darkness following the exposure to 6 h red light (i.e., when maximum differences in growth rate are observed according to Supplemental Figure 2 online), plotted against time between the last heat shock and the end of red light exposure (i.e., the beginning of the 18-h growth period in darkness). The interaction between light and heat shock ($P < 0.0001$) indicates a stronger effect of light compared with darkness in heat shock–treated than in control plants, at 6 and 30 h but not at 18 and 42 h.

(C) *ProCAB:LUC*-induced luminescence recorded after 1.5 h of exposure to red light (i.e., during the acute response to red light) (Anderson et al., 1997), plotted against the time between the last heat shock and the end of red light treatment. Interaction between light and temperature conditions: $P < 0.0001$. No rhythmic pattern is observed.

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experiments, as the wild type, the *lhy cca1* mutant should show elevated hypocotyl growth rates in darkness independently of the heat shock entrainment. However, in contrast with the wild type, the *lhy cca1* mutant should show constitutive reduced growth rates in response to red light (strong inhibition response). The latter response should not require the heat shock entrainment and should not be rhythmic because, according to the proposed hypothesis, in the wild type the temperature entrainment is required to rhythmically reduce *LHY* and *CCA1* expression and favor the response to red light, while in the *lhy cca1* mutant the levels are constantly null. The observations fulfilled all of these expectations (Figure 6B).

Since PRR7 and PRR9 have been implicated in temperature entrainment of the clock (Salomé and McClung, 2005; Yamashino et al., 2008), are required for the full heat shock–induced rhythms of *LHY* and *CCA1* expression (Figure 5A), are positive regulators of the response to red light (Ito et al., 2007), and are required for the synergism between light and temperature (Figure 2B), we investigated their role in the gating response. As the wild type, the *prr7 prr9* mutant showed a rate of hypocotyl growth that was elevated in darkness independently of previous heat shock or constant temperature conditions (Figure 6B). However, in contrast with the wild type, the *prr7 prr9* mutant also showed an elevated growth rate even after exposure to heat shock (i.e., the *prr7 prr9* mutant showed no gating of hypocotyl growth sensitivity to red light) (Figure 6B). The single *prr7* mutant also showed defects (Figure 2B; see Supplemental Figure 5 online).

Impaired *PIF4* and *PIF5* Expression in *lhy cca1*

PIF4 and *PIF5* are required for the synergism between light and temperature shifts (Figure 2B), show rhythmic expression after light entrainment (Nozue et al., 2007), and show high expression in CCA1 overexpressors (Nozue et al., 2007; Niwa et al., 2009; Lu et al., 2012) and low expression in *lhy cca1* mutants (Niwa et al., 2009). PIF4 and PIF5 are negative regulators of the response to red light (Huq and Quail, 2002; Fujimori et al., 2004), and the antiphase oscillation of their expression levels (see Supplemental Figure 3 online) with the sensitivity to red light (Figure 6A) would be consistent with their contribution to the rhythm of growth response.

Nine hours after the heat shock treatment, the wild type Wassilewskija showed reduced *PIF4* and *PIF5* expression compared with constant temperature controls (all in darkness). The *lhy cca1* mutant showed reduced levels of *PIF4* and *PIF5* expression and a weaker response to the heat shock (Figure 7A). In the *pif4 pif5* double mutant, the ability of hypocotyl growth to respond to red light was constitutive, at most weakly enhanced by the entrainment with heat shocks (Figure 7B). This observation is consistent with the idea that daily heat shocks cause rhythms of *LHY* and *CCA1*, which in turn cause oscillations of *PIF4* and *PIF5*, which modulate the ability to respond to red light.

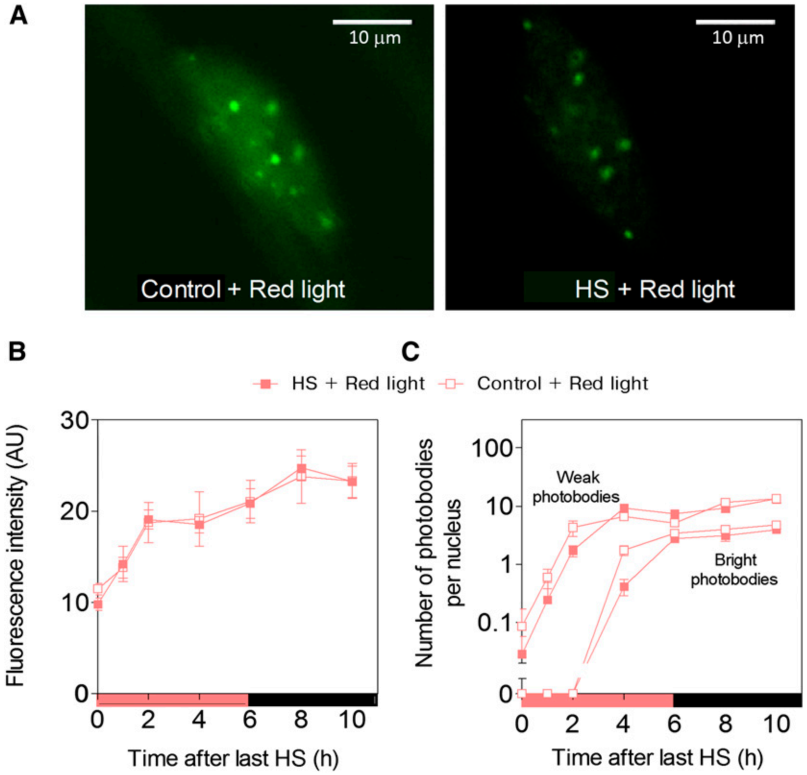

Figure 4. Heat Shocks Reduce the Accumulation of Nuclear Photobodies of phyB.

Seedlings were either kept at constant 22°C or entrained at 1.5 h 37°C (HS) followed by 22.5 h at 22°C for 3 d in darkness. Immediately after the second heat shock (time 0), the seedlings were transferred to 6 h of red light followed by darkness (22°C). The protocol corresponds to the time of maximum sensitivity to red light in hypocotyl gating experiments (Figures 3A and 3B).

(A) Representative nuclei after 4 h of red light.

(B) Nuclear fluorescence of PHYB-GFP. AU, arbitrary units.

(C) Number of weak and bright nuclear photobodies containing phyB-GFP plotted against time after the beginning of red light exposure. Interaction between temperature conditions and time (0 to 4 h): $P < 0.0001$ (bright photobodies) and $P < 0.01$ (weak photobodies). Each data point is the mean and SE of at least seven seedlings (five nuclei were averaged per seedling).

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Heat Shocks Enhance the Stability of HY5

The synergism between heat shocks and light required HY5 (Figure 2), but heat shocks did not induce rhythms of *HY5* expression. *HY5* expression tended to be low when the responses to red light are high and vice versa (see Supplemental Figure 3 online). The HY5 transcription factor is regulated at different levels, including via targeted degradation in the 26S proteasome by the E3 ligase COP1 (Osterlund et al., 2000). We investigated the abundance of nuclear HY5 using a transgenic line expressing a physiologically active HY5-YFP (for yellow fluorescent protein) fusion protein (Oravecz et al., 2006). As expected, fluorescence was low in darkness and increased upon exposure to red light (Figure 8A). In the seedlings entrained with heat shocks, fluorescence was higher than in constant temperature controls at the end of the 6-h photoperiod and in subsequent darkness (when the levels decreased) (Figures 8A and 8B). Therefore, heat shocks increase the apparent stability of HY5.

To investigate whether heat shocks induce a circadian rhythm of HY5 abundance response to red light, we measured HY5-YFP fluorescence 8 h after the beginning of the red light treatment (i.e., 2 h after the end of the red light treatment) in seedlings that received red light, 0, 12, or 24 h after the end of the last daily heat shock. The 0-h time point in Figure 8C is equivalent to the 8-h time point in Figure 8A (but data come from an independent experiment). Heat shocks increased HY5-YFP fluorescence in red light–treated seedlings, but the effect was not circadian, as it decreased with time after the last heat shock without an obvious recovery at 24 h (Figure 8C).

Heat Shocks Reduce Nuclear Abundance of COP1

Since COP1 regulates HY5 stability (Osterlund et al., 2000), we investigated the abundance of nuclear COP1 at the time when heat shocks increase HY5 abundance using a biologically active YFP-COP1 fusion protein (Oravecz et al., 2006). Heat shocks reduced nuclear COP1 fluorescence and the formation of nuclear speckles containing COP1 (Figure 9A). The effect decreased with time after the heat shock without showing a recovery at 24 h (Figure 9B).

Convergence of Light and Temperature to Control the Seedling Transcriptome

To provide a more comprehensive description of the interactions between heat shocks and light signaling, we investigated whether heat shock treatments affect transcriptome responses

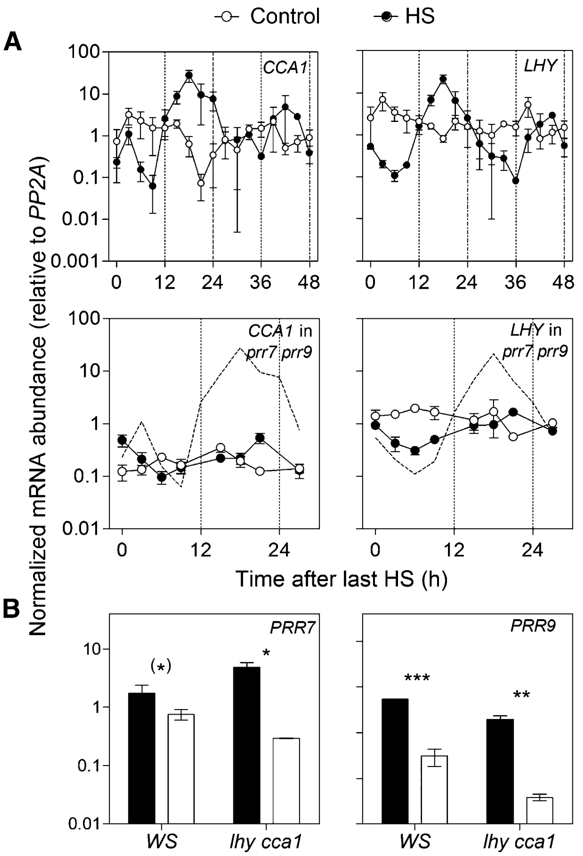

Figure 5. Heat Shocks Generate Rhythms of *LHY* and *CCA1* mRNA Abundance in Darkness.

Seedlings were either kept at constant 22°C or entrained at 1.5 h at 37°C (HS) followed by 22.5 h at 22°C in darkness and transferred to free-running conditions of constant darkness at 22°C immediately after the last heat shock (time 0). Protocol is as in Figure 3A (conditions without red light). Each data point is mean and SE of three biological replicates.

(A) *CCA1* and *LHY* mRNA levels were recorded for 48 h in wild-type Columbia seedlings and for 24 h in the *prr7 prr9* mutant (dotted lines show Columbia data). Three-way ANOVA shows significant interaction ($P < 0.005$ for *CCA1*; $P < 0.0001$ for *LHY*) among temperature conditions, time, and genotype, indicating that heat shocks enhance the rhythmic oscillations in the wild type more than in the *prr7 prr9* mutant.

(B) The transient promotion of *PRR7* and *PRR9* expression by heat shocks does not require CCA1 and LHY (samples harvested 0 h after the last heat shock). *** $P < 0.001$, **$P < 0.01$, *$P < 0.05$, and $^{(*)}P < 0.1$. WS, Wassilewskija.

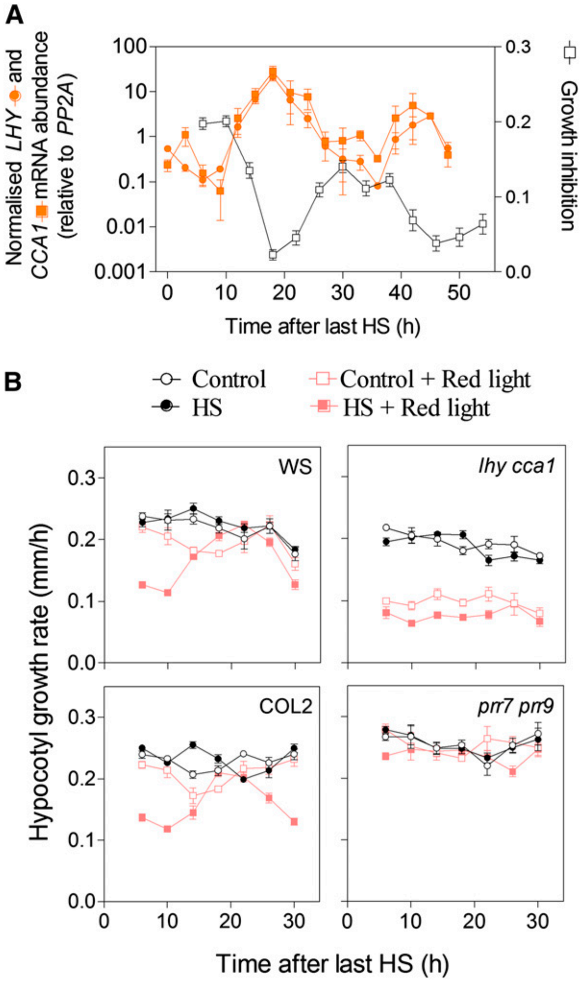

Figure 6. The Generation of Circadian Rhythm of Sensitivity of Hypocotyl Growth to Red Light Requires *LHY* and *CCA1*.

(A) Anticorrelation between the levels of *LHY* or *CCA1* mRNA and the hypocotyl sensitivity to red light. The levels of *LHY* mRNA from seedlings entrained under heat shock conditions (data from Figure 5A), and hypocotyl growth inhibition caused by red light in seedlings entrained under the same conditions is plotted against time. Growth inhibition was calculated as the difference in hypocotyl growth rate between seedlings with or without exposure to red light after entrainment under heat shock conditions (data from Figure 3). The anticorrelation between expression and growth inhibition is significant for *LHY* ($R^2 = 0.67$, $P < 0.0005$) and *CCA1* ($R^2 = 0.67$, $P < 0.0005$).

(B) The *lhy cca1* and *prr7 prr9* mutants lack the heat shock–induced gating of hypocotyl growth to red light. Seedlings were either kept at constant 22°C or entrained at 1.5 h at 37°C (HS) followed by 22.5 h at 22°C for 2 d in darkness. Immediately after the second heat shock (time 0), the seedlings were transferred to constant darkness at 22°C for periods of different duration and then were either exposed to red light or left as nonirradiated controls (protocol as in Figure 3). Data are means and SE (whenever larger than the symbols) of 20 to 36 seedlings. Significant interaction ($P < 0.0001$ for *lhy cca1*; $P < 0.01$ for *prr7 prr9*) among temperature conditions, light conditions, and genotype indicates that heat shocks enhance the sensitivity to light in the wild type more than in the *lhy1 cca1* or the *prr7 prr9* mutants. COL, Columbia; WS, Wassilewskija.

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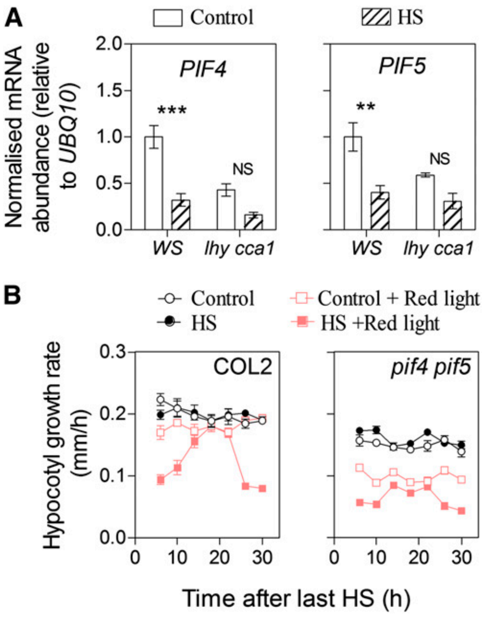

Figure 7. The Generation of Circadian Rhythm of Full Sensitivity of Hypocotyl Growth to Red Light Requires *PIF4* and *PIF5*.

(A) Expression of *PIF4* and *PIF5* in wild-type Wassilewskija (WS) and *lhy cca1*. Seedlings were either kept at constant 22°C or entrained at 1.5 h at 37°C followed by 22.5 h at 22°C for 3 d in darkness and harvested 9 h after the last heat shock. Data are means and SE of three biological replicates. ***$P < 0.001$ and **$P < 0.01$; NS, not significant.

(B) The *pif4 pif5* mutant shows weak heat shock–induced gating of hypocotyl growth to red light. Seedlings were incubated for 2 d in darkness under 22°C with or without 1.5 h at 37°C (HS). Immediately after the second heat shock (time 0), the seedlings were transferred to constant darkness at 22°C for periods of different duration and then were either exposed to red light or left as nonirradiated controls (protocol as in Figure 3). Data are means and SE (whenever larger than the symbols) of 15 to 22 seedlings. Significant interaction ($P < 0.005$) among temperature conditions, light conditions, and genotype indicates that heat shocks enhance the sensitivity to light in the wild type more than in the *pif4 pif5* mutant. COL, Columbia.

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to a subsequent exposure to light (as these treatments do with hypocotyl growth responses). The seedlings were grown in darkness either under constant temperature or heat shock conditions and then exposed to 6 h of red light or darkness before harvest for RNA extraction to investigate gene expression profiles. Processed samples were hybridized to Affymetrix microarrays. We selected 3677 genes that showed significant effects of treatments ($P < 0.05$, $q < 0.05$) above a fold change cutoff (see Supplemental Data Set 1 online). Despite major differences in protocols and genetic backgrounds, we observed a highly significant correlation between the effects of our red light or heat shock treatments and publicly available data (Busch et al., 2005; Tepperman et al., 2006) (see Supplemental Figure 6 online), and this argues in favor of the robustness of these effects. The number of genes affected by both red light and temperature (378) was significantly higher than expected by chance, indicating a significant convergence of both signals to control gene expression (Figure 10A). However, the nature of the convergence is specific for different sets of genes as within those genes affected by both treatments (378) some are promoted or inhibited by both treatments (206) and others are promoted by one treatment and inhibited by the other (172) (Figure 10A, χ^2 test with Yates correction: $P > 0.7$).

CCA1 and *LHY* expression decreased in response to heat shock and red light (Figure 10B). In adult plants, the expression of *LHY* decreases 20 and 60 min after the beginning of the heat stress (Kant et al., 2008). Reduced *LHY* and *CCA1* expression 6 h after the heat shock is confirmed by our real-time PCR data (Figure 5A). The expression of *CCA1* and *LHY* decreases with

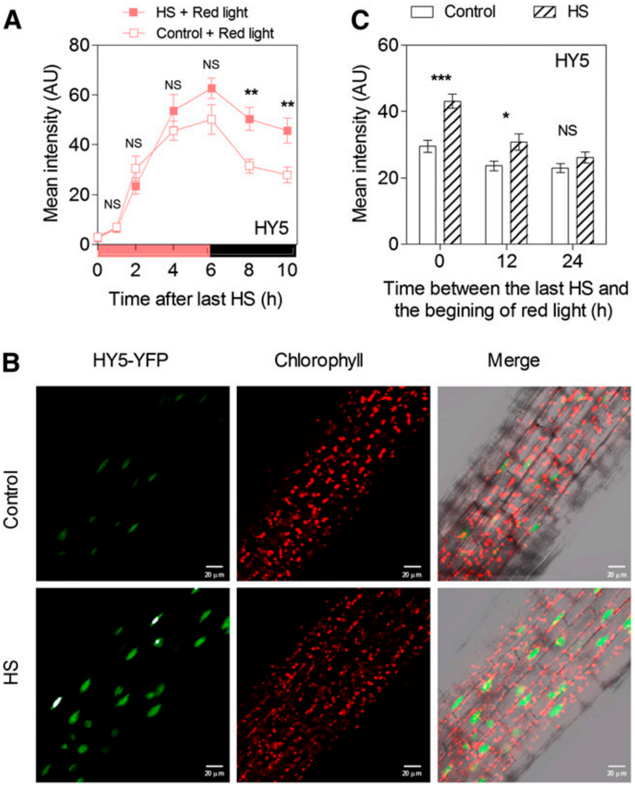

Figure 8. Heat Shocks Increase HY5 Abundance upon Exposure to Red Light.

(A) Kinetics of accumulation of HY5-YFP in the nucleus. Seedlings were either kept at constant 22°C or entrained at 1.5 h at 37°C (HS) followed by 22.5 h at 22°C in darkness and transferred to 6 h of red light at 22°C immediately after the last heat shock (time 0). AU, arbitrary units.

(B) Representative hypocotyls harvested at time 8 h.

(C) The effect of the heat shocks gradually disappears. Seedlings were treated as in **(A)**, but a period of darkness (0, 12, or 24 h) was interposed between the last heat shock and the beginning of the 6-h red light treatment, and observations were made 2 h after the end of the red light treatment (i.e., time 0 corresponds to 8 h in **[A]**, but data come from independent experiments).

***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$; NS, not significant. Each data point is the mean and SE of 10 to 12 **(A)** or 60 **(C)** seedlings.

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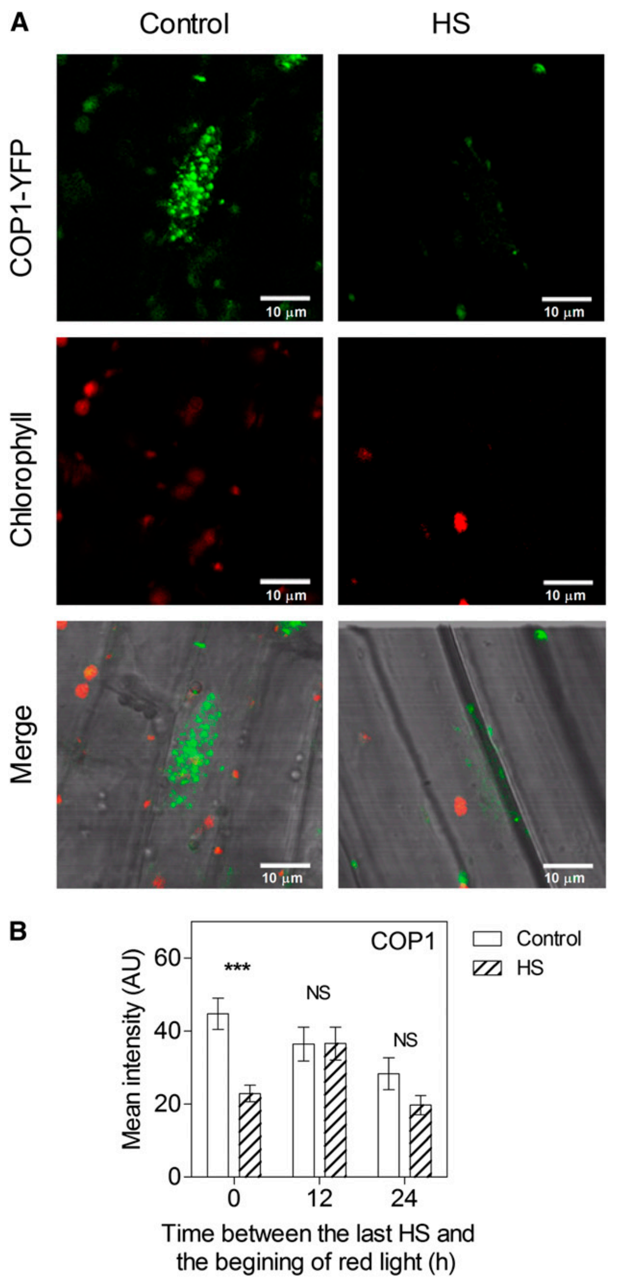

Figure 9. Heat Shocks Reduce Nuclear Abundance of COP1.

(A) Accumulation of YFP-COP1 in the nucleus. Seedlings were incubated either at constant 22°C or 1.5 h at 37°C (HS) followed by 22.5 h at 22°C in darkness and transferred to 6 h of red light and 2 h darkness at 22°C immediately after the last heat shock (the photographs correspond to 8 h in Figure 8A).

(B) The effect of the heat shocks gradually disappears. Seedlings were treated as in **(A)**, but a period of darkness (0, 12, or 24 h) was interposed between the last heat shock and the beginning of 6-h red light treatment. $^{***}P < 0.001$; NS, not significant. Each data point is the mean and SE of 60 seedlings. AU, arbitrary units.

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exposure to 6 h of red light (Kikis et al., 2005). The evening element motif, a binding site of LHY/CCA1, and the G-box motif (5′-CACGTG-3′), bound by PIF5 and other PIF proteins (Zhang et al., 2013) and HY5 (Lee et al., 2007), are overrepresented among the genes with expression controlled in the same direction by red light and heat shocks (Figure 10A). To test the functional significance of this finding, we compared the promotion of *ABSCISIC ACID RESPONSIVE ELEMENT BINDING FACTOR1* (*ABF1*), At2g46600, and *C-REPEAT/DRE BINDING FACTOR2* (*CBF2*) expression (three genes bearing the evening element in their promoters) by heat shocks in darkness in wild-type and *lhy cca1* seedlings and observed that the first two of these genes show impaired responses in the mutant (Figure 10C). The normal reduction of *CBF2* expression in *lhy cca1* was unexpected because both the amplitude of the circadian oscillations and cold induction of *CBF2* are impaired in this double mutant and *CBF2* is a direct target of CCA1 (Dong et al., 2011). *PIF4* and *PIF5* show reduced expression 9 h after the heat shock but not at 6 h (Figure 7); therefore, it is not surprising that they showed no response in microarray experiments. However, their targets could be affected by the heat shocks given in previous days. The genes promoted by light and heat shock also include the transcription factor gene *ABF1* (Figure 10B), whose product binds the ABA-responsive element (ABRE) motif, and ABRE or ABRE-like promoter motifs were enriched in several gene groups (see Supplemental Table 2 online).

DISCUSSION

Exposure to heat shocks enhanced the hypocotyl responsiveness to light, without affecting growth in the absence of light (Figure 1). The synergism between high temperature and light signals was observed only when the exposure to high temperatures was transient (heat shock), as increasing the constant growth temperature reduced rather than increased the effect of light (Gray et al., 1998) (see Supplemental Figure 1 online). Transcriptome analysis revealed a significant convergence between light and heat shock signaling. However, many genes responded only to light, without obvious interference by the heat shock (Figure 10B), indicating that the effects of heat shocks were selective.

The convergence involves the action of heat shocks mainly on phyB signaling, as heat shocks failed to enhance the inhibition of hypocotyl growth in the *phyB* mutant and had reduced effects in several mutants of genes involved in phyB signaling, such as *pif3 pif4*, *pif4 pif5*, *elf3*, *gi*, *toc1*, and *lhy cca1* (Zagotta et al., 1996; Huq et al., 2000; Huq and Quail, 2002; Kaczorowski and Quail, 2003; Kim et al., 2003; Más et al., 2003; Bauer et al., 2004; Fujimori et al., 2004; Ito et al., 2007; Shin et al., 2009; Leivar and Quail, 2011; Nusinow et al., 2011) (Figure 2). Heat shocks affected at least three aspects of phyB signaling: (1) the formation of nuclear photobodies containing phyB, (2) the nuclear abundance of COP1 and HY5, and (3) the kinetics of expression of the *PRR7/PRR9-LHY/CCA1-PIF4/PIF5* pathway.

The enhanced hypocotyl growth response to active phyB (Figure 2) is not obviously accounted for by the delayed formation of phyB nuclear photobodies in heat shock–treated seedlings

(Figure 4) because phyB photobodies appear positively related to phyB-mediated responses (Van Buskirk et al., 2012).

Heat shocks enhanced the nuclear abundance of HY5 (Figure 8), a transcription factor required for full photomorphogenesis (Lau and Deng, 2012) and for the synergism between heat shocks and light (Figure 2). This indicates that heat shocks increase the response to red light in part by increasing HY5 abundance (Figure 11). The effect of heat shocks was more obvious during the decay of nuclear HY5 after exposure to red light (Figure 8A), when the effects of heat shocks on hypocotyl growth were also more pronounced (see Supplemental Figure 2A online). These kinetics suggest that heat shocks increase HY5 stability and, in agreement with this interpretation, heat shocks reduced the nuclear abundance of COP1 (Figure 9), which targets HY5 for degradation in the proteasome (Osterlund et al., 2000). This effect decreased with time after the heat shock and did not evidence a role of the circadian clock (Figures 8 and 9). The increased abundance of HY5 could account for the enhanced *ProCAB:LUC* activity observed in seedlings exposed to light and heat shocks (time 0 in Figure 3C) (Andronis et al., 2008). It is noteworthy that low temperatures also stabilize HY5 through nuclear depletion of COP1 during cold acclimation (Catalá et al., 2011). Other signaling events, such as the elevation of cytosolic free calcium levels, occur both during cold acclimation and in response to heat shocks (McClung and Davis, 2010).

The combination of heat shock and phyB signaling generated a rhythm of hypocotyl growth not observed in seedlings treated only with either red light or heat shocks (see Supplemental Figure 2 online). The temporal separation of the heat shocks and light treatments demonstrated that, in darkness, daily heat shocks generate a rhythm of sensitivity that gates the response to red light perceived by phyB (Figure 3). We searched for genes showing a rhythm of expression induced by the heat shocks in darkness, with a phase that could account for the changes in hypocotyl sensitivity to light (i.e., in phase for genes promoting the hypocotyl response to red light and antiphase for genes reducing the response to red light). *LHY* and *CCA1* (Figure 5A) and *PIF4* and *PIF5* (see Supplemental Figure 3 online) fulfilled this criterion. The response of *PIF4* and *PIF5* expression to heat shocks was deficient in *lhy cca1* (Figure 7A). The expression of *PRR7* and *PRR9* showed transient oscillations in response to the heat shock (see Supplemental Figure 3 online). PRR7 and PRR9 repress the expression of *LHY* and *CCA1* by binding to their promoters (Nakamichi et al., 2010), are involved in the temperature input to

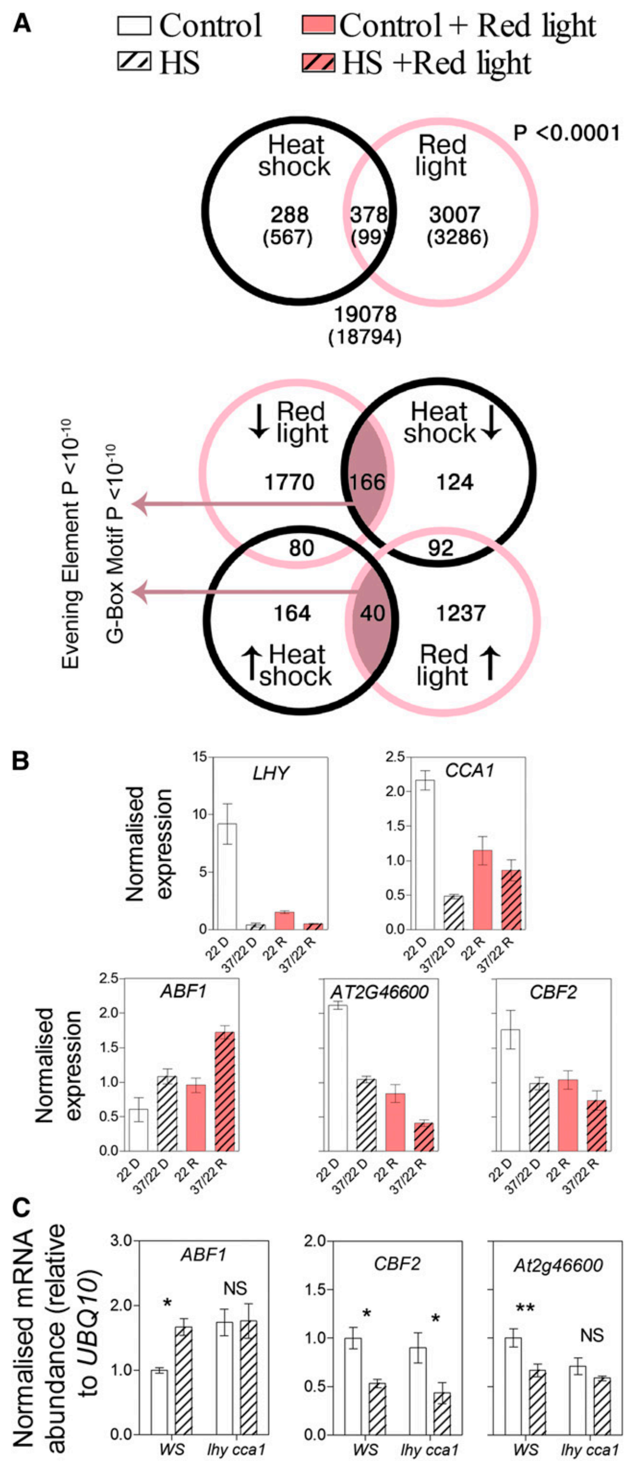

Figure 10. Transcriptome Responses to Red Light and Heat Shocks.

Seedlings were grown in darkness at 22°C interrupted by 1.5 h at 37°C in darkness (HS) and/or 6 h of red light (12 μmol m^{-2} s^{-1}) at 22°C (protocol as in Figure 1A but with red light).

(A) Number of genes showing significant effects of red light (red circle) and/or heat shocks (black circle) (predicted values are shown in parentheses, and the significance of the χ^2 test with Yates correction is indicated) and number of genes promoted (upwards arrows) or inhibited (downwards arrows) by red light and/or heat shocks. The seedlings were harvested 6 h after the last heat shock, and their processed RNA was hybridized to ATH1 Affymetrix microarrays.

(B) Expression of selected genes from the experiment presented in **(A)**, showing effects of red light and heat shocks in Columbia seedlings. Statistics based on the analysis of microarray data. D, dark; R, red light.

(C) Expression of *ABF1*, At2g46600, and *CBF3* in wild-type Wassilewskija (WS) and *lhy cca1* seedlings (samples were harvested 9 h after the last heat shock). **P < 0.01 and *P < 0.05; NS, not significant. Each data point is the mean and SE of three biological replicates.

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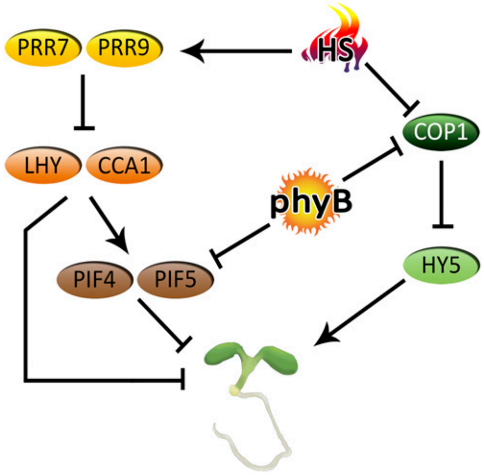

Figure 11. Model of the Synergism between Heat Shocks and Light Signaling.

In the presence of daily heat shocks (HS), two signaling pathways converge to enhance the sensitivity of hypocotyl growth to light perceived by phyB. First, heat shock decreases accumulation of COP1 and hence increases nuclear accumulation of HY5, which is a positive regulator of photomorphogenesis. Second, daily heat shocks induce transient oscillations in the expression of *PRR7* and *PRR9*, which leads to oscillations in the expression of *CCA1* and *LHY* and, in turn, to oscillations in *PIF4* and *PIF5* expression, which are negative regulators of photomorphogenesis.
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the clock (Salomé and McClung, 2005; Yamashino et al., 2008), and are required for the heat shock–induced oscillations of *LHY* and *CCA1* mRNA abundance (Figure 5A). Conversely, the transient promotion of *PRR7* and *PRR9* expression did not require LHY or CCA1 (Figure 5B). The *lhy cca1*, *pif4 pif5*, and *prr7 prr9* mutants showed weak oscillations in hypocotyl sensitivity to red light, which was constitutively high in *lhy cca1* and *pif4 pif5* and constitutively low in *prr7 prr9* (Figures 2B, 6B, and 7B). Based on these observations, we propose a model (Figure 11) where transient oscillations in *PRR7* and *PRR9* expression in response to the heat shock initiate a relatively robust rhythm in the expression of *LHY* and *CCA1*, which in turn induces oscillations in the expression of *PIF4* and *PIF5*. When the expression of *PIF4* and *PIF5* is high, phyB Pfr established by red light is less effective and when the expression of *PIF4* and *PIF5* is low, red light is more effective (Figure 7). An inverse relationship between *PIF4* or *PIF5* expression and response to red light has been demonstrated in experiments with overexpressors or mutants (Huq and Quail, 2002; Fujimori et al., 2004), suggesting (among other possibilities) that Pfr could more effectively inactivate a smaller pool of PIFs. PIFs reduce phyB stability in long-term experiments (Leivar and Quail, 2011), but the oscillations in *PIF4* and *PIF5* expression were not associated with changes in nuclear phyB in the context of these experiments (Figure 4B).

High constant temperatures, alternating temperatures (involving several hours of warm temperatures), and heat shocks (brief exposures to high temperature) have partially different impacts on the clock. Temperature compensation mechanisms buffer the speed of the oscillator against differences in constant temperature (McClung and Davis, 2010). At constant temperature, the amplitude and maximum peaks of *LHY* and *CCA1* mRNA rhythms decrease at 27°C, compared with 12°C, but these alterations are balanced by changes in the expression of *GI* and other genes; thus, the clock is temperature compensated (Gould et al., 2006). Daily alternating temperatures reset or entrain the circadian oscillator and set the phase of expression of *CCA1*, *LHY*, *TOC1*, *PRR7*, and *PRR9* (Somers et al., 1998; Salomé and McClung, 2005; Salomé et al., 2008; McClung and Davis, 2010; Wenden et al., 2011). Apparently, there are two oscillators in *Arabidopsis*; one is synchronized preferentially by light-dark cycles and the other responds preferentially to temperature cycles (Michael et al., 2003). Heat shocks can reset the clock (Tamaru et al., 2011), as clearly observed for the circadian rhythm of expression of *TOC1* and the output gene *CCR2* (see Supplemental Figure 3 online). However, the entrainment caused by heat shocks was rudimentary when compared with the well-known effects of alternating temperatures (Salomé et al., 2008; Wenden et al., 2011) as the rhythms were often not robustly sustained (see Supplemental Figure 3 online) and heat shocks failed to gate the response of *CAB2* to red light (Figure 3B). In addition, heat shocks enhanced the amplitude of oscillation of expression of some genes (*LHY*, *CCA1*, *PRR9*, *PIF4*, and *PIF5*; Figures 5 and 7; see Supplemental Figure 3 online). During deetiolation, light but not alternating temperatures increases the amplitude of circadian rhythms (Salomé et al., 2008), indicating another difference between heat shocks and classical alternating temperatures.

The convergence between heat shocks and red light signaling could affect a wide spectrum of processes as revealed by the transcriptome analysis (Figure 10). These responses could still depend on the heat shock–enhanced amplitude of *LHY* and *CCA1* expression and abundance of HY5, as the evening element, bound by LHY and CCA1, and the G-box motif, bound by PIFs and HY5, were overrepresented among the genes regulated by light and heat shocks in the same direction (Figure 10A). The overrepresentation of the evening element suggests that LHY and CCA1 could also operate directly (i.e., not only via PIF4 and PIF5) (Figure 11). Heat shocks and red light increased the expression of the bZIP-type transcription factor AREB1/ABF1 (Yoshida et al., 2010), which binds the ABRE promoter motif. The heat shock response required *LHY/CCA1* (Figure 10C).

Whereas light penetrates the soil only superficially, the increasing maximum temperatures experienced by the shoot approaching the soil surface in darkness would inform the seedling about its impending emergence. In turn, the transient high-temperature signals enhance the sensitivity to light via the *PRR7/PRR9-LHY/CCA1PIF4/PIF5* and COP1-HY5 pathways, preparing the seedling for deetiolation upon light exposure. In favor of this functional interpretation, heat shocks are more efficient than red light in enhancing the sensitivity to subsequent red light (see Supplemental Figure 7 online), suggesting that the synergism could have evolved as a mechanism to adjust to the sequence of signals that the seedlings are likely to experience in nature.

METHODS

Plant Materials

The mutant alleles and transgenic lines of *Arabidopsis thaliana* used in this study are described in Supplemental Methods 1 online. For growth experiments, 15 seeds were sown in clear plastic boxes (40 × 33 mm^2 × 15 mm height). For mRNA experiments (microarrays and real-time PCR), ~200 seeds were sown in Petri dishes, and to monitor luciferase activity, one seed per well was sown in 96-well Berthlod clear bottom plates containing 5 mL, 25 mL, or 200 μL of 0.8% agar plus 1× Murashige and Skoog salts. Seeds were sown during the afternoon to provide an afternoon cue (Salomé et al., 2008) and kept in darkness at 4°C for 3 d (stratification). Stratified seeds were exposed to red light for 2 h during the morning followed by 22 h in darkness at 22°C before the beginning of different light and/or temperature treatments.

Light and Temperature Treatments

White light (6 h at 12 μmol m^{-2} s^{-1}, unless indicated otherwise) was provided by fluorescent tubes (Philips TLD 15W/54). Red light (6 h at 12 μmol m^{-2} s^{-1}) was provided by fluorescent tubes in combination with red, yellow, and orange acetate filters (LEE Filters 106, 101, and 105). Plants were grown at 22°C and transferred for 90 min to a chamber at 37°C (unless indicated otherwise) wrapped in black plastic foil to prevent any exposure to light during the transfers and temperature treatment. Temperature was checked every day before transfer.

Hypocotyl Growth

To obtain end-point data, hypocotyl length was measured to the nearest 0.5 mm with a ruler after 3 d of treatment in the 10 tallest seedlings of each box. These values were averaged and used as a replicate. To measure hypocotyl growth rate, the position of the boxes containing seedlings was shifted to place the agar plane normal to the horizontal plane after the red light pulse used to induce germination. Photographs of the seedlings were taken under a weak green light every 3 h using a Canon Power Shot A520 camera. Seedling images of successive time points were aligned using Photoshop 7.0 to record hypocotyl length increments.

Real-Time PCR

For real-time PCR, total RNA was treated with DNaseI (Promega), and 2 μg was used for cDNA synthesis using SuperScript III reverse transcriptase (Invitrogen) with custom-made oligo(dT) (15 nucleotides T). Two microliters of eightfold diluted cDNA and 5 μL of FastStart Universal SYBR Green Master (Rox; Roche) were used for the PCR reaction. Fluorescence was detected using an ABI 7500 real-time PCR system (Applied Biosystems). Primers and PCR conditions were as in Supplemental Table 3 online. Each biological replicate is the mean of two subsamples (technical replicates). For each technical replicate, gene expression was normalized against *PROTEIN PHOSPHATASE 2A* or *UBIQUITIN10*.

Confocal Microscopy

Confocal fluorescence images were taken from the epidermis and the first subepidermal layers of the upper third portion of the hypocotyl using a LSM5 Pascal laser scanning microscope (Zeiss) with a water-immersion objective lens (C-Apochromat ×40/1.2; Zeiss). For GFP and YFP fluorescence detection, probes were excited with an argon laser (λ= 488 nm), and fluorescence was detected using a BP 505-530 filter. Chlorophyll detection was performed using a He-Ne laser (λ= 543 nm) for excitation and a LP 560 filter for detection. A transmitted light channel was also configured. Fluorescent nuclei were defined as regions of interest, and fluorescence intensity was measured using NIH ImageJ (Abràmoff et al., 2004). In order to classify the photobodies containing phyB, images were converted to a rainbow palette using the Zeiss LSM Image Browser software, which assigns false colors according to the intensity of fluorescence signals. Documentation of representative cells was performed during the first 15 min of microscopy analysis.

Luciferase Activity

Twenty-four hours before starting to record luciferase activity, 50 μL of 0.5 mM D-luciferin was added to each well. Luciferase activity was detected with a Centro LB 960 (Berthold) luminometer. To highlight the pattern of expression, activities for each seedling at each time point are expressed relative to median activity for that seedling.

Statistics

Data were analyzed by one-way, two-way, or three-way analysis of variance (ANOVA) followed by Bonferroni post tests. In microarray experiments, two-way ANOVA was followed by the calculation of q values (Storey and Tibshirani, 2003) and fold change criteria described in Supplemental Methods 2 online.

Microarray Experiments

Samples were harvested in liquid nitrogen, and total RNA was extracted using the RNeasy plant mini kit (Qiagen). Three biological replicates were obtained for each condition. cDNA and cRNA synthesis and hybridization to ATH1 Affymetrix *Arabidopsis* Gene Chips were performed according to Affymetrix instructions. To identify enrichment of promoter sequences and Gene Ontology terms, we used the ATHENA analysis tool (O'Connor et al., 2005).

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or the GenBank/EMBL databases under the following accession numbers: AT2G18790 (PHYB), AT3G59060 (PIF5), AT2G25930 (ELF3), AT5G61380 (TOC1) and the accession numbers listed in Supplemental Data Set 1 online.

Supplemental Data

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

Supplemental Figure 1. High Constant Temperatures Reduce the Hypocotyl Growth Response to Light.

Supplemental Figure 2. Circadian Rhythm of Hypocotyl Growth in Plants Exposed to Heat Shocks and Red Light Photoperiods.

Supplemental Figure 3. Expression of the Indicated Genes in Response to Entrainment with Daily Heat Shocks.

Supplemental Figure 4. Heat Shocks Gate Hypocotyl Growth to Red Light.

Supplemental Figure 5. Rhythmic Expression of *LHY* and *CCA1* and Sensitivity to Red Light in *prr7*.

Supplemental Figure 6. Comparison of the Microarray Data Reported Here with Publicly Available Data.

Supplemental Figure 7. Heat Shocks and Red Light as Entrainment Cues for Sensitivity of Hypocotyl Growth to Red Light.

Supplemental Table 1. Expression of *CCA1* and *LHY* in Col and *prr7 prr9* Seedlings.

Supplemental Table 2. Enriched Motif and Gene Ontology Terms in the Genes That Responded to Light and/or Heat Shock.

Supplemental Table 3. Primers for Real-Time PCR.

Supplemental Data Set 1. Genes with Expression Significantly Affected by Red Light and/or Heat Shocks.

Supplemental Methods 1. Mutant and Transgenic Lines.

Supplemental Methods 2. Statistics of Microarray Data.

ACKNOWLEDGMENTS

We thank the University of Buenos Aires (Grants G044 and 01/W437) and Agencia Nacional de Promoción Científica y Tecnológica (Grant PICT-2012-1396 to J.J.C.) for financial support. We thank Christian Fankhauser (University of Lausanne) for his kind provision of *pif3-1 pif4* and Roman Ulm (University of Geneva) for his kind provision of *cop1–4 Pro35S*:*YFP-COP1* and *hy5-1 ProHY5*:*HY5-YFP*.

AUTHOR CONTRIBUTIONS

E.K., R.S., M.L., M.J.Y., and J.J.C. designed the research. E.K., R.S., and M.L. performed research. E.K., R.S., M.L., M.J.Y., and J.J.C. analyzed data. J.J.C. wrote the article.

Received May 30, 2013; revised July 1, 2013; accepted July 16, 2013; published August 9, 2013.

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Heat Shock–Induced Fluctuations in Clock and Light Signaling Enhance Phytochrome B–Mediated *Arabidopsis* Deetiolation

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Plant Cell 2013;25;2892-2906; originally published online August 9, 2013;
DOI 10.1105/tpc.113.114306