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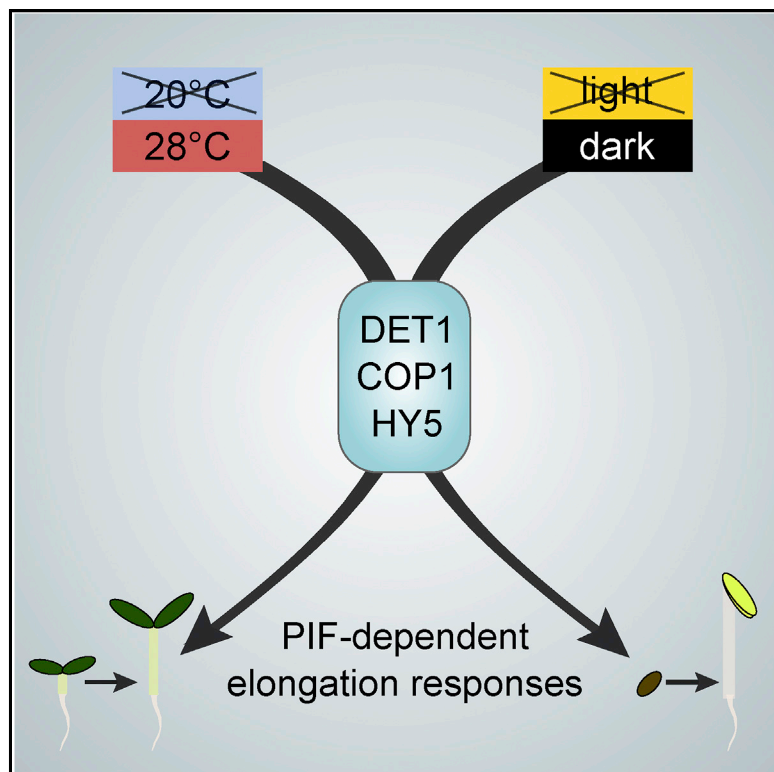
The DET1-COP1-HY5 Pathway Constitutes a Multipurpose Signaling Module Regulating Plant Photomorphogenesis and Thermomorphogenesis

Graphical Abstract



Highlights

- DET1-COP1-HY5 mutants show altered temperature-induced elongation growth
- *PIF4* expression and downstream regulation is controlled by DET1-COP1-HY5
- Light and temperature signaling share essential elements in signal transduction

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In Brief

Delker et al. show that the DET1-COP1-HY5 signaling cascade regulates PIF4 to induce elongation in response to elevated ambient temperature. The multipurpose modular function of signaling elements indicates a co-evolution of light and ambient temperature signal transduction.

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The DET1-COP1-HY5 Pathway Constitutes a Multipurpose Signaling Module Regulating Plant Photomorphogenesis and Thermomorphogenesis

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SUMMARY

Developmental plasticity enables plants to respond to elevated ambient temperatures by adapting their shoot architecture. On the cellular level, the basic-helix-loop-helix (bHLH) transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4) coordinates this response by activating hormonal modules that in turn regulate growth. In addition to an unknown temperature-sensing mechanism, it is currently not understood how temperature regulates PIF4 activity. Using a forward genetic approach in *Arabidopsis thaliana*, we present extensive genetic evidence demonstrating that the DE-ETIOLATED 1 (DET1)-CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1)-ELONGATED HYPOCOTYL 5 (HY5)-dependent photomorphogenesis pathway transcriptionally regulates PIF4 to coordinate seedling growth in response to elevated temperature. Our findings demonstrate that two of the most prevalent environmental cues, light and temperature, share a much larger set of signaling components than previously assumed. Similar to the toolbox concept in animal embryonic patterning, multipurpose signaling modules might have evolved in plants to translate various environmental stimuli into adaptational growth processes.

INTRODUCTION

Within the context of globally increasing ambient temperatures, it is imperative to improve our understanding of the basic processes plants employ to react and adapt to environmental perturbations. Consistent with the term photomorphogenesis, thermomorphogenesis describes the effect of ambient temperature on plant morphogenesis (Erwin et al., 1989). Hypocotyl elongation (Gray et al., 1998) and increased leaf epinasty (van Zanten et al., 2009) are among the earliest thermomorphogenic changes in response to elevated temperatures. Physiologically, these coordinated responses likely enhance evaporative leaf cooling (Crawford et al., 2012; Bridge et al., 2013) and thus enable plants to adapt to warmth.

On the cellular level, elevated temperature stimuli are transduced into altered gene expression by affecting chromatin status (Kumar and Wigge, 2010) and, specifically, by controlling expression of the basic-helix-loop-helix transcription factor PHYTOCHROME INTERACTING FACTOR 4 (PIF4) (Koini et al., 2009). The downstream mechanisms through which PIF4 regulates ambient temperature signaling are beginning to emerge. Although other hormones play important roles, PIF4-mediated activation of auxin responses is central to increased elongation growth (Franklin et al., 2011; Sun et al., 2012; Proveniers and van Zanten, 2013; de Wit et al., 2014). In contrast, upstream elements of the pathway are poorly understood, in particular, temperature sensing and the mechanism(s) by which temperature influences PIF4 activity.

Thus, the identification of signaling components upstream of PIF4 will be a major advance in the dissection of ambient temperature signaling (Wigge, 2013). Here, we uncover a large signaling module that controls PIF4-dependent thermomorphogenesis.

RESULTS AND DISCUSSION

The *okapi1* Mutant Encodes an Allele of the Photomorphogenesis Regulator DE-ETIOLATED 1

Temperature-induced hypocotyl elongation (TIHE) is widely used as a phenotypic readout for responsiveness to warmth (Gray et al., 1998; Stavang et al., 2009; Oh et al., 2012). Mutant alleles of *PIF4* and of several auxin genes that execute temperature information transmitted via PIF4 display TIHE defects (Stepanova et al., 2008; Gray et al., 1998; Figure S1A). We therefore reasoned that mutant alleles of as of yet unknown genes acting upstream of *PIF4* would also be deficient in translating temperature stimuli into elongation growth and designed a mutagenesis screen based on the TIHE phenotype. Ethylmethanesulfonate-mutagenized seeds were germinated for 4 days at 20°C and the seedlings then shifted to 28°C and cultivated for another 4 days. In comparison to constant growth at 20°C, wild-type seedlings significantly elongate their hypocotyls after transfer to high temperature at permissive light

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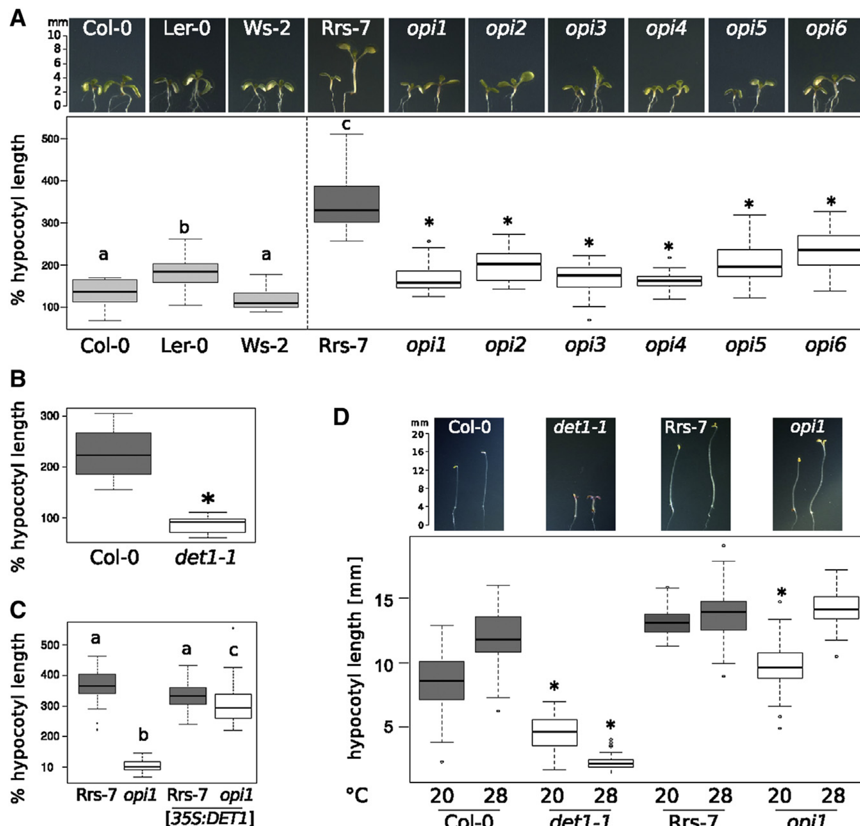

Figure 1. Isolation of the *okapi1* Mutant

(A) Temperature-induced hypocotyl elongation (TIHE) under high-light conditions (250 μmol m^{-2} s^{-1}) in commonly used *A. thaliana* accessions, Rrs-7 and *opi* mutants.

(B) The *det1-1* mutant (Col-0 background) phenocopies the *opi1* phenotype (90 μmol m^{-2} s^{-1}).

(C) Transgenic complementation of *opi1* with the genomic *DET1* fragment of Rrs-7 (250 μmol m^{-2} s^{-1}).

(D) Hypocotyl length of 5-day-old etiolated seedlings. Asterisks denote statistical differences ($p < 0.05$) as assessed by one-way ANOVA and Tukey HSD.

(A–C) Statistical differences were assessed by two-way ANOVA ($p < 0.05$) of the absolute data presented in Figure S1. Different letters denote significant differences among all samples; asterisks highlight significant differences to the wild-type response. Box plots show relative (28°C/20°C in %; A–C) or absolute (D) hypocotyl length. For additional *opi1* phenotypes and *opi1* mapping, see Figures S2 and S3, respectively. (A and D) Photographs show representative seedlings grown at 20°C (left) and 28°C (right).

conditions. We specifically screened for mutants with short hypocotyls, indicative of a defect in translating the 28°C stimulus into elongation growth.

We applied high white light intensities (250 μmol m^{-2} s^{-1}) and low seedling densities to favor identification of temperature-specific response regulators and to prevent hypocotyl elongation caused by either low light or shade avoidance responses. Whereas reference accessions like Col-0, Ler-0, and Ws-2 were temperature responsive under light intensities of 90 μmol m^{-2} s^{-1} (Figure S1B), TIHE was drastically decreased in these accessions at 250 μmol m^{-2} s^{-1} (Figures 1A and S1C). Other accessions, like Rrs-7, responded strongly even under high light intensities (Figures 1A and S1C). Hence, the mutagenesis screen was conducted in the extremely temperature-sensitive natural accession Rrs-7. Due to the resemblance of the TIHE phenotype to the long neck of a giraffe, we designated the "short-necked" mutants as *okapi* (*opi*), named after the short-necked species from the Giraffidae family. The *opi* mutants were placed into six complementation groups (Figures 1A and S1D), of which *opi1* is the subject of this study.

Although not responsive to warmth, hypocotyl elongation in *opi1* could be induced by applying either gibberellic acid, brassinosteroids, or the synthetic auxin picloram (Figure S2A), indicating that the elongation defect in *opi1* is specific for temperature stimuli upstream of phytohormone action. In addition to the TIHE phenotype, *opi1* mutants displayed several temperature-dependent developmental phenotypes, including decreased petiole elongation and temperature-dependent delayed transition to flowering (Figures S2B–S2D).

Mapping by sequencing (Schneeberger et al., 2009; James et al., 2013; Schneeberger, 2014) identified a non-synonymous mutation in *AT4G10180* of *opi1*, causing a glycine to glutamic acid change (Figure S3). *AT4G10180* encodes DE-ETIOLATED 1 (DET1). A mutant allele (*det1-1*) in the Col-0 background (Chory et al., 1989) displayed *opi1*-like TIHE defects when grown under 90 μmol m^{-2} s^{-1} white light (Figures 1B and S1E). Complementation of the *opi1* mutant with the Rrs-7 wild-type *DET1* allele confirmed *DET1* as the gene responsible for the temperature-related defects in *opi1* (Figures 1C and S1F). As DET1 contains no known domains other than a bipartite nuclear localization signal and structure-function associations are poorly understood, it remains speculative as to how the *opi1* mutation might affect protein function.

Photomorphogenesis Mutants Display Defects in Temperature-Induced Hypocotyl Elongation

The best-described function of DET1 is its central role in the repression of photomorphogenesis (Lau and Deng, 2012). In darkness, *det1* mutants do not etiolate but display light-grown phenotypes including short hypocotyls (Figure 1D; Pepper et al., 1994). In contrast, *opi1* mutants are able to elongate in the dark (Figure 1D). Whereas the elongation at 20°C dark was slightly weaker than wild-type, *opi1* hypocotyls were similar to wild-type at 28°C dark. Thus, *opi1* is a unique *DET1* allele affected primarily in thermomorphogenesis rather than photomorphogenesis. In addition, wild-type-like behavior of *opi1* at 28°C in the dark renders a temperature-sensitive nature of the mutation unlikely.

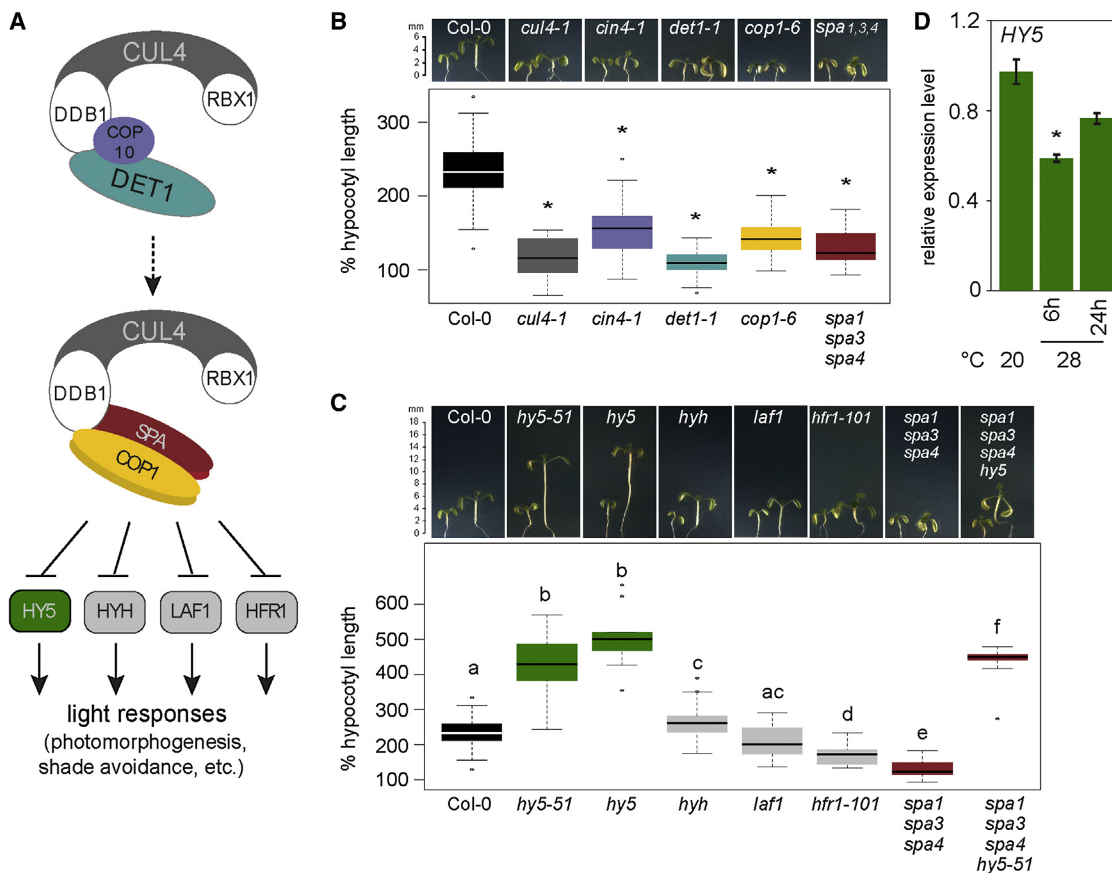

Figure 2. Mutants Impaired in Regulators of Photomorphogenesis Are Affected in Ambient Temperature Responses

(A) Simplified model of known components involved in DET1-dependent light signal transduction.

(B) TIHE is significantly reduced in CDD or COP1/SPA complex mutants.

(C) TIHE analysis of known COP1/SPA targets reveals a hypersensitive response in *hy5* loss-of-function mutants and epistasy of *HY5* over the COP1/SPA-complex-encoding genes.

(D) *HY5* expression (qRT-PCR) in 5-day-old seedlings is significantly reduced 6 hr after transfer to 28°C ($p = 0.004$) and slightly reduced after 24 hr of 28°C ($p = 0.056$). Bar plots show means ± SEM ($n = 4$). Statistical differences were assessed by one-way ANOVA and Tukey HSD test.

(B and C) Photographs show representative seedlings grown at 20°C (left) and 28°C (right). Box plots show relative hypocotyl length (28°C/20°C in %; $n > 20$ per genotype and temperature). Statistical differences in temperature responses were assessed by two-way ANOVA ($p < 0.05$) of the absolute data presented in Figure S1. (B–D) Light intensities in all experiments were 90 μmol m^{-2} s^{-1}. Asterisks or different letters denote significant differences ($p < 0.05$) to the wild-type or among samples, respectively.

The *det1-4* allele in the Col-0 background (Pepper et al., 1994) is affected in the same amino acid residue as *opi1* (gly > arg). Interestingly, in comparison to *det1-1*, the etiolation defect of *det1-4* seedlings was less severe. This suggests a specific role of this domain of DET1 in temperature responses or, alternatively, the presence of accession-specific modifiers in the Rrs-7 background.

On the cellular level, DET1 assembles with CONSTITUTIVE PHOTOMORPHOGENIC 10 (COP10) and DAMAGED DNA BINDING PROTEIN 1 (DDB1) into the CULLIN 4 (CUL4)-based E3 ubiquitin ligase CUL4-DDB1$^{COP10\text{-}DET1}$ (CDD) (Schroeder et al., 2002; Yanagawa et al., 2004). The CDD complex is thought to enhance the activity of CUL4-DDB1-COP1-SUPPRESSOR OF PHYTOCHROME A-105 (SPA) E3 ubiquitin ligases (COP1-SPA) (Lau and Deng, 2012; Nixdorf and Hoecker, 2010; Figure 2A). COP1-SPA targets negative regulators of elongation growth such as the bZIP transcription factor ELONGATED HYPOCOTYL 5 (HY5) for proteasomal degradation, resulting in suppression of photomorphogenesis and therefore long hypocotyls in darkness (Koornneef et al., 1980; Oyama et al., 1997).

Based on the similar phenotypic output (hypocotyl elongation) in response to both stimuli, light and temperature, we asked whether DET1 possibly recruits the same signaling components to regulate TIHE in light-grown seedlings. If so, loss-of-function alleles of both CDD and COP1-SPA complex subunits should exhibit reduced TIHE, and, in contrast, *hy5* mutants should hyperelongate. Because mutant alleles of these genes were not available in the Rrs-7 background, mutant analyses were performed in the Col-0 background at TIHE-permissive light intensities of 90 μmol m^{-2} s^{-1}. In full support of the described scenario, *cul4-1* (Bernhardt et al., 2006), *COP10*$^{cin4\text{-}1}$ (Vogel et al., 1998), *cop1-6* (McNellis et al., 1994), and *spa1,3,4*

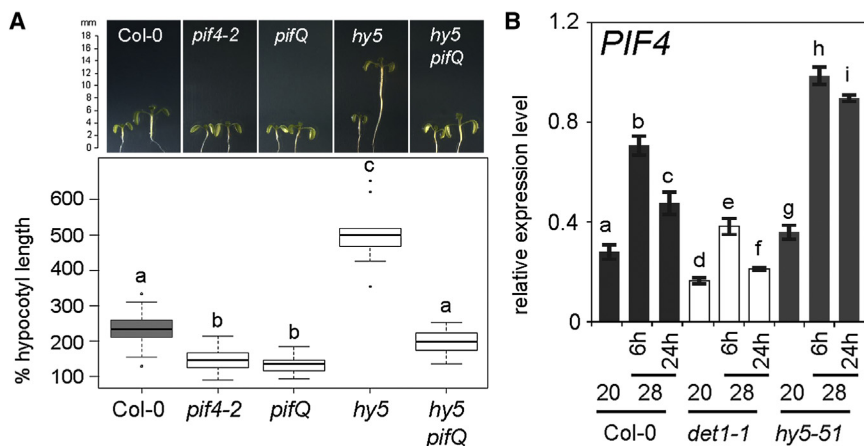

Figure 3. HY5 Is a Negative Regulator of PIF4

(A) The hypersensitive TIHE response of *hy5* was genetically suppressed by mutations in *PIF*s (90 μmol m^{-2} s^{-1}). Photographs show representative seedlings grown at 20°C (left) and 28°C (right). Box plots show relative hypocotyl length (28°C/20°C in %; n > 15). Different letters denote statistical differences in temperature responses as assessed by two-way ANOVA ($p < 0.05$) of the absolute data presented in Figure S1.

(B) A null mutation of *DET1* or *HY5* results in decreased or increased expression of *PIF4*, respectively. Relative expression levels were determined in 5-day-old seedlings by qRT-PCR. Bar plots show means ± SEM (n = 4). Statistical differences between samples are highlighted by different letters and were assessed by one-way ANOVA and Tukey HSD test.

(Fittinghoff et al., 2006) displayed significantly shorter hypocotyls, whereas *hy5* behaved in the opposite hypersensitive manner (Figures 2B, 2C, S1G, and S1H).

In addition to *hy5*, mutant alleles of other known COP1 targets involved in different light-signaling pathways were tested for their temperature response. In contrast to *hy5*, loss-of-function alleles of *LONG AFTER FAR-RED LIGHT 1*, *HY5-HOMOLOG*, and *LONG HYPOCOTYL IN FAR-RED 1* (Fankhauser and Chory, 2000; Duek and Fankhauser, 2003; Jang et al., 2005) showed no or much less pronounced alterations in the TIHE response (Figures 2C and S1H), demonstrating the predominant role of *HY5* in temperature signaling. In addition, temperature responsiveness of *spa1,3,4 hy5* quadruple mutants (Rolauffs et al., 2012) was similar to *hy5* single mutants (Figures 2C and S1H), indicating that *HY5* is epistatic over the *SPAs* and acts downstream of COP1-SPA.

Adopting the photomorphogenesis model, this genetic evidence suggests that protein levels of the suppressor of elongation growth HY5 are not only low in darkness but also in the light at elevated temperatures. Indeed, HY5 protein levels have recently been shown to increase in the cold (Catalá et al., 2011) and decrease at elevated temperatures (Toledo-Ortiz et al., 2014). Furthermore, HY5 seems to function in light-dependent repression of cell expansion at lower temperatures (Johansson et al., 2014). Remarkably, increased HY5 protein levels at low temperature occur also in the dark and coincide with exclusion of COP1 from the nucleus (Catalá et al., 2011). In addition to protein stability, temperature-responsive changes in *HY5* transcript levels may also contribute. Together, this would relieve HY5's suppressive function and result in hypocotyl elongation. In line with this model, we find that light-grown seedlings exhibit lower *HY5* transcript levels at elevated temperatures (Figure 2D).

HY5 Negatively Regulates *PIF4* Transcription

We next examined a possible connection of the CDD/COP1-SPA/HY5-signaling module with the ambient temperature signaling hub PIF4. As *hy5* and *pif4* or *pifQ* (*pif1*,*3*,*4*,*5*; Leivar et al., 2008) mutants display contrasting TIHE phenotypes (Figure 3A), *hy5 pifQ* quintuple mutants could potentially reveal genetic interactions between these two branches. Our analysis of *hy5 pifQ* (Jia et al., 2014) mutants revealed that the loss of PIFs neutralized the hypersensitive TIHE response exhibited by the *hy5* mutant (Figures 3A and S1I). Taking into account that *PIF4* is the dominant *PIF* family member in temperature signaling (Koini et al., 2009), this genetic interaction indicates that *PIF4* is largely epistatic over *HY5*. Consequently, the CDD/COP1-SPA pathway likely regulates *PIF4* activity via HY5.

Interestingly, Lee et al. (2007) reported that HY5 binds to the *PIF4* promoter. Based on this study, we sought evidence for potential transcriptional regulation of *PIF4* by HY5, and we performed quantitative RT-PCR (qRT-PCR) analyses of light-grown seedlings that were shifted from 20°C to 28°C for 6 or 24 hr. In comparison to wild-type, temperature-induced upregulation of *PIF4* transcripts was significantly increased in *hy5* mutants (Figure 3B), suggesting that HY5 negatively regulates *PIF4* gene expression in response to elevated ambient temperatures. Furthermore, reduced *PIF4* expression in *det1-1* (Figure 3B) supports the general hierarchy of the CDD/COP1-SPA/HY5-signaling module. As HY5 and PIFs both recognize G-box *cis*-regulatory elements, they might compete for the same binding sites and form a dynamic activation-suppression transcriptional module, as recently shown for the expression of photosynthetic genes (Toledo-Ortiz et al., 2014).

The Photomorphogenesis Pathway Regulates PIF4-Dependent Transcriptional Auxin Responses

The described PIF4-mediated temperature response involves the direct transcriptional activation of auxin response genes (Franklin et al., 2011), which ultimately cause elongation growth. To complete the analysis of this known chain of events, we investigated whether the CDD/COP1-SPA/HY5 regulation of *PIF4* is reflected at this level. The *SMALL AUXIN UP-REGULATED RNA* (*SAUR*) *19-24* and *INDOLE-3-ACETIC ACID* (*IAA*) *29* genes have been previously shown to be activated by PIF4 in a temperature-dependent manner (Franklin et al., 2011). Overexpression or gain-of-function mutants in these genes can significantly alter hypocotyl elongation in response to diverse stimuli (Spartz et al., 2012). We find that temperature-induced expression as well as absolute expression levels of all six genes were significantly decreased in both *pifQ* and *det1-1* mutants but significantly increased in the *hy5* mutant

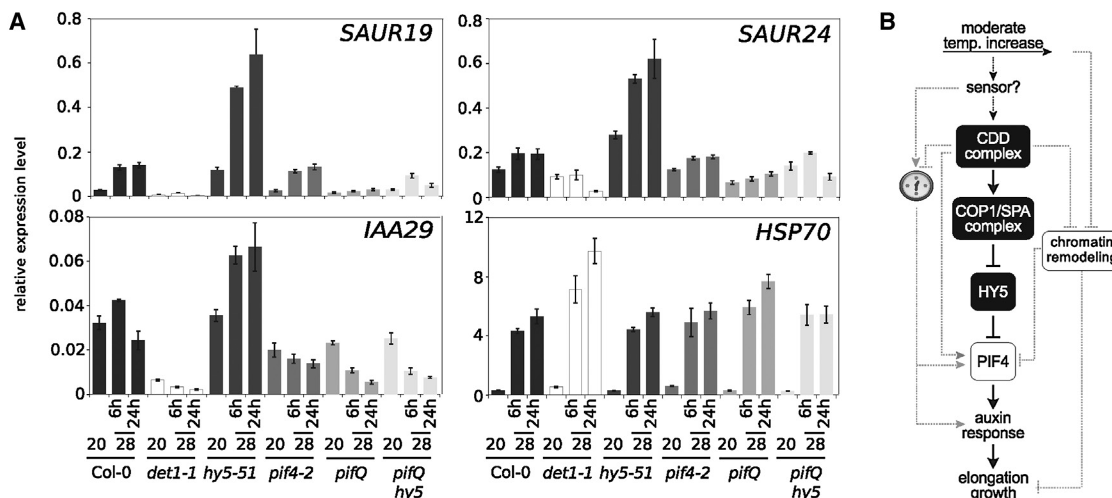

Figure 4. Expression Analyses Confirm a Model of CDD/COP1/HY5-Mediated Regulation of PIF4 in Response to Elevated Temperature

(A) Expression (qRT-PCR) of auxin-responsive, PIF4-regulated genes (*SAUR19-24* and *IAA29*) are significantly reduced and increased in *det1-1* and *hy5-51*, respectively (see Figure S4 for the complete *SAUR* gene set). The epistasis of the *pif* mutations over the *hy5* loss of function is reflected by the expression levels of *IAA29* and *SAUR19-24*. Expression of temperature-sensitive *HSP70* is similar to the wild-type for most of the analyzed mutants with exception of *det1-1*. Bar plots show means ± SEM (n = 4). Statistical analysis between samples was assessed by one-way ANOVA and Tukey HSD test and is summarized in Figure S4B.

(B) Updated and simplified model of the temperature-signaling network. The signaling elements upstream of PIF4 identified in this study are highlighted by black boxes. Hypothetical connections and effects of DET1 described for other biological processes might putatively contribute to the temperature response and are depicted by gray dashed lines.

(Figures 4A and S4). Furthermore, *pifQ*-like gene expression patterns of the *SAUR* and *IAA* genes in the *hy5 pifQ* background (Figures 4A, S4A, and S4B) confirmed the above-described epistatic interactions between *PIF4* and *HY5* (Figure 3A). Together, these results indicate that the aberrant TIHE phenotypes of the photomorphogenesis mutants are caused by their inability to activate PIF4-dependent transcriptional auxin responses in elevated temperatures.

An increase in ambient temperature is known to cause global transcriptome changes, which are to a large extent mediated via the eviction of H2A.Z histone variants that prevent transcription factor binding to DNA at lower temperatures (Kumar and Wigge, 2010). Albeit a connection of the H2A.Z- and the PIF-mediated pathway remains hypothetical, we wished to assess whether the H2A.Z-dependent temperature response of the analyzed mutants is still functional. We thus quantified transcript levels of *HEAT SHOCK PROTEIN 70* (*HSP70/AT3G12580*), which serves as a marker gene for the H2A.Z-mediated temperature pathway.

Apart from increased expression in *det1-1*, *HSP70* showed wild-type-like induction in all other mutants tested (Figures 4A, S4A, and S4B), indicating that H2A.Z-dependent transcriptional responses were likely not affected. This suggests a largely PIF4-dependent role of the photomorphogenesis pathway in inducing elongation growth in response to elevated temperatures. With few exceptions, other general heat-responsive genes (*HSP17/AT5G12020*, *HSP90/AT5G52640*, and *HSP101/AT1G74310*) showed a similar expression pattern (Figure S4C). The distinct misexpression of *HSP* genes in *det1-1* indicates putative additional functions of DET1 in temperature signaling that are independent of PIF4. As DET1 has previously been reported to bind nonacetylated histone tails (Benvenuto et al., 2002), this might involve chromatin remodeling.

CONCLUSIONS

This study uncovers a large signaling module upstream of the described PIF4-dependent ambient temperature signaling pathway (Figure 4B). A combination of genetic and physiological data indicates that PIF4 activity is regulated by the CDD/COP1-SPA/HY5 module possibly by direct transcriptional regulation of *PIF4* by HY5. A recent report on a direct interaction of DET1 and PIFs in photomorphogenesis signaling (Dong et al., 2014) theoretically represents a shortcut that might bypass COP1/SPA and HY5 if also active in thermomorphogenic responses. Furthermore, known functions of DET1 in chromatin remodeling and the circadian clock, two processes that affect temperature responses, render it a potential master regulator in ambient temperature signaling upstream of PIF4 (Figure 4B). Future studies need to address how this pathway is connected to the thermosensory H2A.Z-containing nucleosomes (Kumar and Wigge, 2010) in the potential regulation of PIF4 transcriptional complexes. Together, our study shows that temperature and light signaling share a much larger set of signaling components than previously assumed. Given that light intensities and ambient temperatures are generally tightly linked in both terrestrial and aquatic environments of land plant precursors, it is likely that the signaling modules described here have co-evolved early on to respond to both stimuli.

EXPERIMENTAL PROCEDURES

Plant Material and Growth Conditions

Accession numbers and providers of wild-type and mutant strains of *Arabidopsis* used in this study are summarized in the Supplemental Experimental Procedures. Plants were grown on vertical plates containing *Arabidopsis thaliana* solution (ATS) medium (Lincoln et al., 1990) or standard soil in Percival Scientific AR66L3 growth cabinets under the growth conditions specified for the individual experiments.

TIHE Assays

Seeds were surface sterilized, stratified in sterile water at 4°C for 3 or 4 days, and placed in horizontal rows on vertically oriented ATS medium-containing plates. Seedlings were cultivated for 4 days at 20°C before being separated into two groups. The control and warmth-induced groups were cultivated for additional 4 days at 20°C and 28°C, respectively. At day 8, digital images of plates were taken. White light intensities for both temperatures were either 90 or 250 μmol m^{-2} s^{-1}, as stated for the individual experiments under a long day diurnal light cycle (16 hr light/8 hr dark). Assays were performed in at least three independent experiments, of which one representative set is shown.

RNA Extraction, cDNA Synthesis, and qRT-PCR

Expression analyses were performed essentially as described previously (Franklin et al., 2011). RNA was extracted from four independent pools of 5-day-old seedlings grown either at 20°C (control) or grown at 20°C with a shift to 28°C for the last 6 or 24 hr of cultivation, respectively. RNA extraction was performed with the RNeasy Plant Mini Kit (QIAGEN) including the on-column DNase digestion step according to the manufacturer's protocols. Integrity of the RNA was verified by agarose gel electrophoresis. One microgram of total RNA was reverse-transcribed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). qRT-PCR analyses were performed using the ABsolute Blue QPCR SYBR Green low ROX Mix and gene-specific oligonucleotides listed in Supplemental Experimental Procedures. qRT-PCRs were performed of four biological replicates per time point using *At1g13320* as a reference gene (Czechowski et al., 2005). Relative expression levels for each analyzed gene were calculated as $2^{(\text{Ct reference gene} - \text{Ct gene of interest})}$.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.11.043.

AUTHOR CONTRIBUTIONS

C.D. and M.Q. designed the experiments and wrote the manuscript. C.D., L.S., P.J., C.I., H.Z., T.P., K.D., S.M., J.Z., S.J.D., G.V.J., K.S., and M.Q. performed experiments and analyzed data.

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