PLANT SCIENCE

Phytochromes function as thermosensors in *Arabidopsis*

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Plants are responsive to temperature, and some species can distinguish differences of 1°C. In *Arabidopsis*, warmer temperature accelerates flowering and increases elongation growth (thermomorphogenesis). However, the mechanisms of temperature perception are largely unknown. We describe a major thermosensory role for the phytochromes (red light receptors) during the night. Phytochrome null plants display a constitutive warm-temperature response, and consistent with this, we show in this background that the warm-temperature transcriptome becomes derepressed at low temperatures. We found that phytochrome B (phyB) directly associates with the promoters of key target genes in a temperature-dependent manner. The rate of phyB inactivation is proportional to temperature in the dark, enabling phytochromes to function as thermal timers that integrate temperature information over the course of the night.

Plant development is responsive to temperature, and the phenology and distribution of crops and wild plants have already altered in response to climate change (*1*, *2*). In *Arabidopsis thaliana*, warm temperature–mediated elongation growth and flowering are dependent on the basic helix-loop-helix transcription factors PHYTOCHROME INTERACTING FACTOR4 (PIF4) and PIF5 (*3*–*6*). Growth at 27°C reduces the activity of the evening complex (EC), resulting in greater *PIF4* transcription. The EC is a transcriptional repressor made up of the proteins EARLY FLOWERING3 (ELF3), ELF4, and LUX ARRHYTHMO (LUX) (*7*–*9*). To test whether the EC is also required for hypocotyl elongation responses below 22°C, we examined the behavior of *elf3-1* and *lux-4* at 12° and 17°C. Hypocotyl elongation in *elf3-1* and *lux-4* is largely suppressed at lower temperatures (Fig. 1, A and B), which is consistent with cold temperatures being able to suppress *PIF4* overexpression phenotypes (*10*). Because *PHYTOCHROME B* (*PHYB*) was identified as a quantitative trait locus for thermal responsiveness and PIF4 activity is regulated by phytochromes (*8*, *11*), we investigated whether these red light receptors control hypocotyl elongation in the range 12° to 22°C. phyABCDE, lacking phytochrome activity (*12*) shows constitutively long hypocotyls at 12° and 17°C. Thus, phytochromes are essential for responding to temperature (Fig. 1, C and D, and fig. S1).

We used transcriptome analysis to determine whether disrupted thermomorphogenesis in *phyABCDE* is specific for temperature signaling or is a consequence of misregulated growth pathways. To capture diurnal variation in thermoresponsiveness, we sampled seedlings over 24 hours at 22° and 27°C. Clustering analysis revealed 20 groups of transcripts (Fig. 2A and fig. S3; described in supplement). Thermomorphogenesis occurs predominantly at night and is driven by *PIF4*. Consistent with this, we observed that *PIF4* is present in cluster 20, which is more highly expressed at 27°C during darkness. Clusters 15 and 16 represent the other major groups of nighttime thermally responsive genes, and these are strongly induced in *phyABCDE*. These clusters are enriched for genes involved in hormone signaling and elongation growth (*YUCCA8*, *YUCCA9*, *BRASSINOSTEROID INSENSITIVE2*, *LIKE AUXIN RESISTANT1*, *AUXIN RESPONSE FACTOR7* and *9*, *BIG*, and *TRANSPORT INHIBITOR1*) (data S2 and S3). These results indicate that the nighttime warm-temperature transcriptome is specifically affected when phytochrome activity is altered.

We used principal components analysis to investigate the overall responses of the transcriptome to temperature and phytochromes. For principal component PC1, which explains 40.8% of the expression variance, *phyABCDE* at 22°C occupies a similar position to the wild type at 27°C (Fig. 2B). At nighttime, there is a positive correlation between gene expression changes in response to temperature and in response to *phyABCDE*; this relationship is lost during the day (Fig. 2C). Phytochromes are interconvertible photoreceptors (*13*–*15*): phyB switches between an inactive Pr state and an active Pfr state upon absorbing red and far-red light, respectively. In a thermal relaxation reaction, Pfr in the dark spontaneously reverts to the Pr state (*16*–*18*). We hypothesized

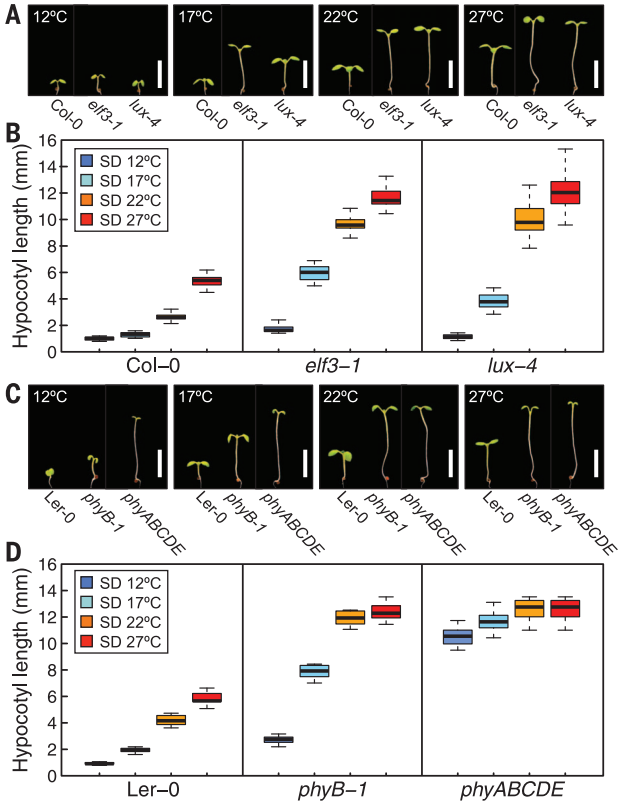

Fig. 1. Phytochromes are essential for correct thermomorphogenesis at lower temperatures. (**A**) Seedlings for Col-0, *elf3-1*, and *lux-4* (left to right in each panel) were grown at the indicated temperatures for 8 days under short photoperiods. Scale bars, 5 mm. (**B**) Hypocotyl length box plots for the indicated genotypes grown at different temperatures as in (A). (**C**) Seedlings for Ler, *phyB-1*, and *phyABCDE* (left to right in each panel) were grown at the indicated temperatures for 8 days under short photoperiods. Scale bars, 5 mm. (**D**) Hypocotyl length box plots for the indicated genotypes grown at different temperatures as in (C). In (B) and (D), each box is bounded by the lower and upper quartiles, the central bar represents the median, and the whiskers indicate minimum and maximum values.

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that the reversion of Pfr to the inactive Pr state may contribute to the subsequent increase in expression of the warm-temperature transcriptome during the night. We therefore examined plants containing a constitutively active version of phyB. The $Tyr^{276} \rightarrow$ His point mutation in phyB (YHB) prevents the dark reversion reaction (*19*, *20*), locking phyB in the active Pfr state. This results in constitutive repression of the warm-temperature transcriptome throughout the night (Fig. 2A and fig. S3). *YHB* plants also phenocopy cool-grown plants, showing little thermoresponsive elongation growth even at 27°C (fig. S2). Across the time course, we found that 79% of the temperature transcriptome is misregulated in *phyABCDE*, *YHB*, or both backgrounds (Fig. 2D).

PhyA is able to bind promoters (*21*), and PIF3 can recruit phyB to G-boxes, motifs bound by PIFs, in vitro (*22*), so we tested whether phyB controls temperature-responsive genes directly. Chromatin immunoprecipitation of epitope-tagged phyB (phyB-Myc) coupled with sequencing (ChIP-seq) revealed phyB to bind >100 sites (~95 promoters), with more targets bound at 17°C than at 27°C (Fig. 3A). Phytochrome signaling is reduced at warmer temperatures, and even for the 33 sites bound by phyB at both temperatures, less binding occurred at 27°C (Fig. 3B). Most phyB target genes are expressed at night in response to temperature (fig. S4). It has been suggested

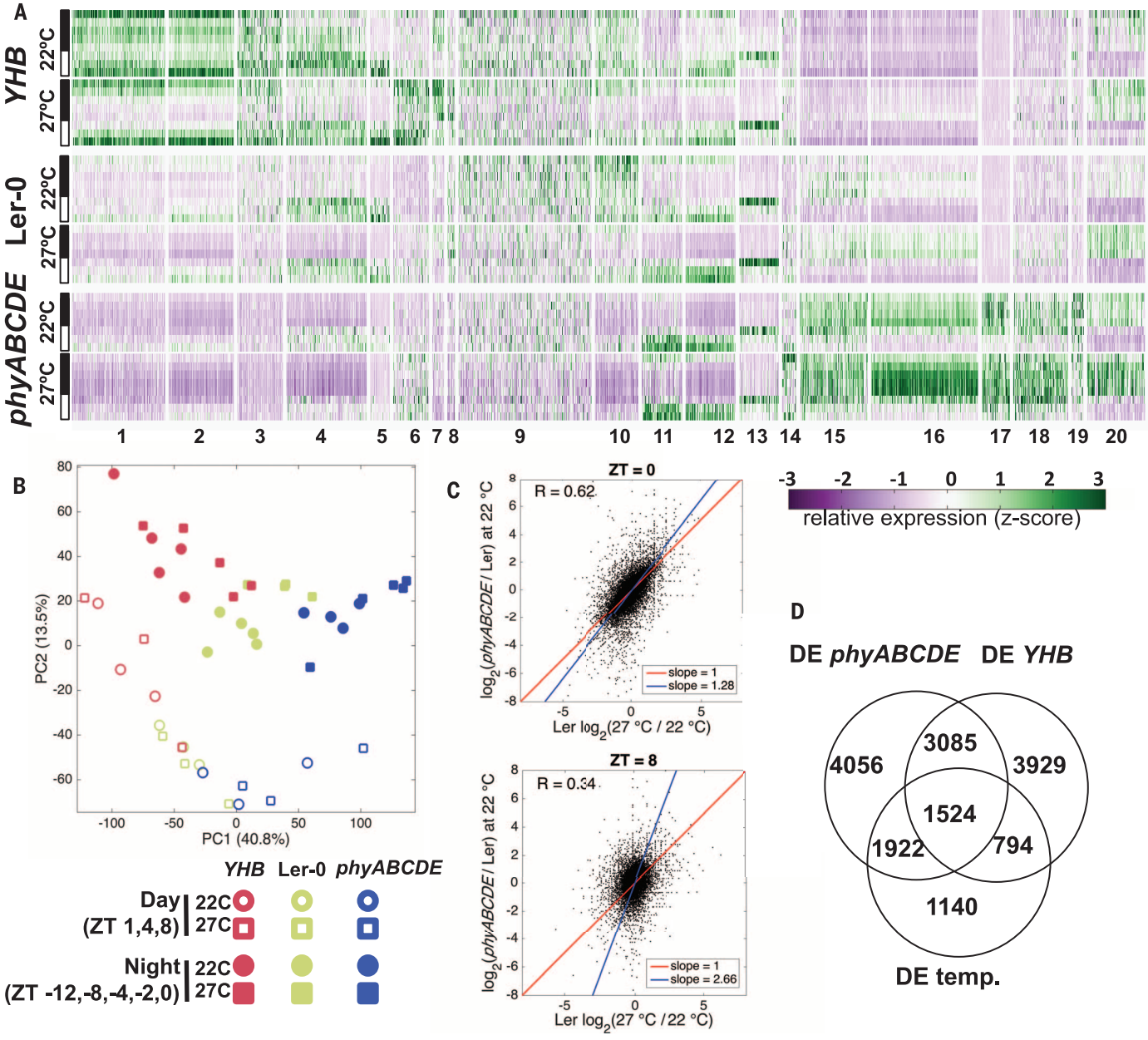

Fig. 2. Phytochromes control the temperature transcriptome at night. (**A**) Clustering of all RNA sequencing (RNA-seq) time course samples for *phyABCDE*, Ler-0, and *YHB* at two different temperatures, 22°C and 27°C. Samples were collected every 4 hours from ZT (zeitgeber time) = 0 with additional time points at ZT = 1 and ZT = 22. Black bars (far left) indicate night, white bars day. Clustering was performed on the expression-filtered data set using a Gaussian mixture model. The number of clusters was assumed to be a random variable and was automatically learned using an empirical Bayes approach (variational Bayesian inference). (**B**) Principal components analysis on the expression-filtered data set using genes as features. (**C**) Transcripts up-regulated in the *phyABCDE* background correlated positively with genes induced by temperature at dawn (ZT = 0, top) but not at dusk (ZT = 8, bottom). (**D**) Sets of differentially expressed (DE) genes from phytochrome and temperature transcriptomes overlap.

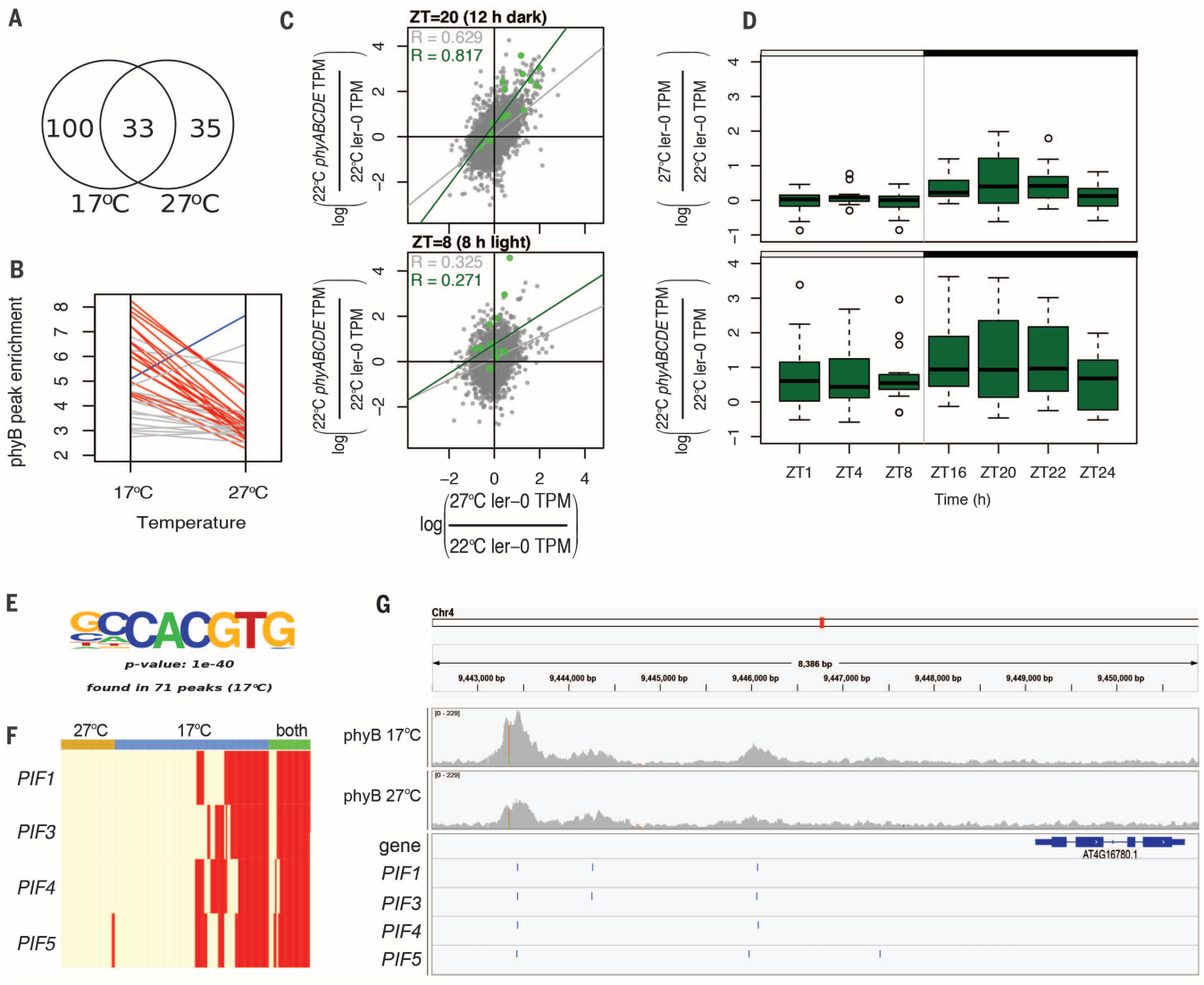

Fig. 3. phyB binds to promoters of temperature-responsive genes. (**A**) Comparison of phyB-Myc ChIP-seq peaks identified at 17° and 27°C using model-based analysis of ChIP-seq (MACS2). In six instances, a single broad 17°C peak was identified as two smaller peaks at 27°C, each of which is considered separately in the subsequent analysis. (**B**) Among the 33 peaks that were found at both 17° and 27°C, we compared the phyB-Myc peak enrichment [calculated as the logarithm of change relative to input (log-fold change) by MACS2] at 17° and 27°C. Values that decreased by >50% are colored red, those that increased by >50% are blue, and the remainder are gray. (**C**) Log-fold change in expression in *phyABCDE* compared to log-fold change in expression caused by elevated temperature (27°C versus 22°C), at two time points (ZT = 8 and 20) in the RNA-seq time course from Fig. 2. The green points depict the transcripts of the 15 genes that are adjacent to the 33 peaks that are found in both 17°C and 27°C. Pearson's *R* is calculated for all genes (gray) and the phyB target genes (green). TPM denotes transcripts per million. (**D**) Distributions of log-fold change in expression caused by *phyABCDE* and temperature elevation for 15 phyB target genes. Open circles represent outliers (greater than the upper quartile value by more than 1.5 times the interquartile domain, and vice versa for points of low value). The horizontal lines represent the largest and smallest points that fall within this range. (**E**) From the 17°C peaks (127, considering the six broad peaks as a single peak), a G-box was the strongest de novo motif identified by Homer2 software for motif analysis, using shuffled sequence to form the background distribution. (**F**) Each column represents a different phyB peak, sorted by whether it was found at 27°C, 17°C, or both; a red bar indicates that this peak overlaps with the center of the reported PIF binding site (*23*). All but three of the peaks found at both 17° and 27°C are bound by multiple PIFs; almost none of the peaks found only at 27°C are bound by PIFs. (**G**) Integrated Genomics Viewer browser view of the *ATHB2* promoter, showing overlap between phyB-Myc peaks at 17° and 27°C and the PIF1, 3, 4, and 5 peaks previously described (*23*).

that phyB may modulate transcription (*22*), and our results indicate that it acts as a transcriptional repressor; most targets were up-regulated under conditions that reduce phytochrome activity during the night, whereas during daytime this relationship was lost (Fig. 3, C and D, and figs. S6 and S9). We did not observe a change in *PHYB* expression or phyB protein levels in response to temperature, consistent with the effect of temperature on phyB being direct (fig. S11).

Most of the phyB-bound peaks contained G-boxes (Fig. 3E and fig. S7). We found overlap between phyB peaks and reported binding sites for PIF1, 3, 4, and 5 (*23*–*25*) (Fig. 3, E and F). phyB-preferring sites that are bound by all four PIFs were enriched at both 17° and 27°C. [Only 7% of the identified peaks are shared among all four PIFs in (*23*).] Of the loci that were bound by phyB at both 17° and 27°C, only three were not bound by PIFs. Relative to other sites, phyB at PIF binding sites showed stronger enrichment (fig. S5). These loci likely represent sites for facilitated PIF binding and coenrichment for phyB. The *ATHB2* promoter illustrates how phyB binding correlates to the binding sites of PIFs (Fig. 3G and fig. S8). To further resolve the phyB peaks, we used cross-linking ChIP (X-ChIP) (*26*). Verified multiple PIF-bound sites corresponded to phyB-bound sites (fig. S10). Although light induces degradation of PIFs, readily detectable levels of

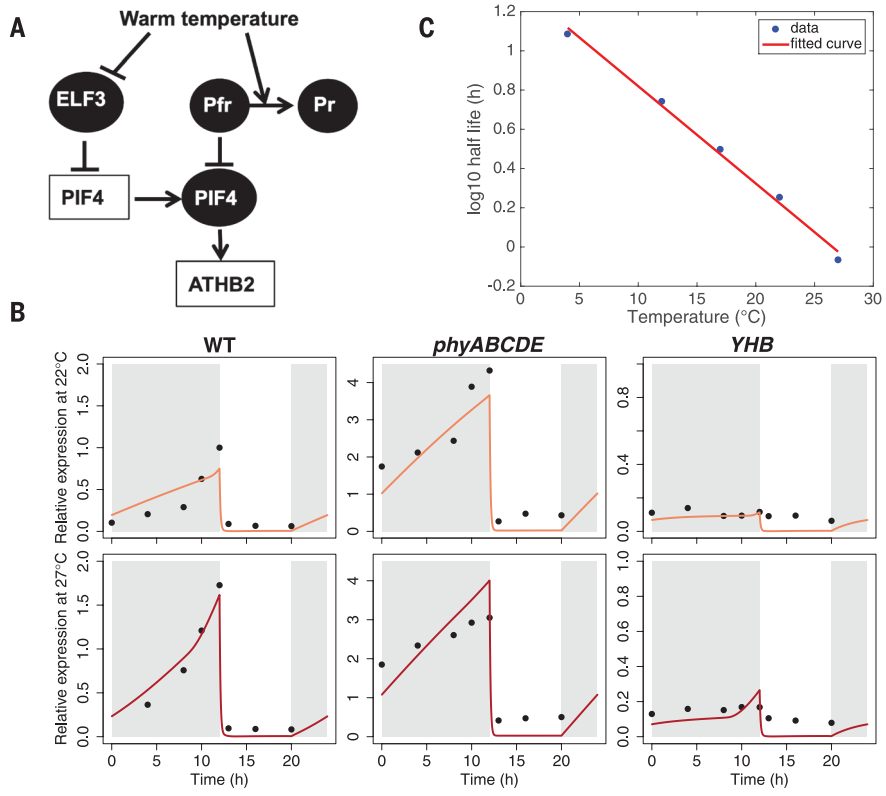

Fig. 4. A thermoresponsive gene expression network. (**A**) Regulatory network integrating temperature information through ELF3 activity and Pfr dark reversion. Temperature information is integrated through activity of ELF3 (which acts as a repressor of PIF4) and deactivation of phyB (active Pfr state switching to inactive Pr state). Ovals and rectangles denote proteins and squares, respectively. (**B**) Modeled gene expression (orange and red lines) agrees with measured values (black dots). (**C**) The phyB Pfr half-life varies exponentially with temperature (assayed in etiolated seedlings). At lower temperatures, active Pfr persists at the end of the night, whereas at higher temperatures most Pfr is depleted within a few hours.

natively expressed PIF4 were seen during the daytime (*27*). Thus, phyB at promoters may recruit or modulate the action of other regulators, providing a mechanism to increase the dynamic range of the transcriptional regulation of target genes. Precedent exists for a transcriptional activator being converted into a repressor by a ligand; for example, FD (ATBZIP14) is an activator when bound to FLOWERING LOCUS T and a repressor when bound to TERMINAL FLOWER1 (*28*).

Changes in the activity of a transcriptional repressor, R, can explain the thermoresponsive expression of *PIF4* and *LUX*, and the thermal responsiveness of R can be accounted for by *ELF3* (*8*). However, transcripts of PIF4 target genes such as *ATHB2* cluster separately from *PIF4* and show greater responsiveness to phytochrome signaling (Fig. 2A). This led us to conclude that phytochromes provide additional temperature-dependent regulation of PIF4. Thus, active phytochromes (Pfr) repress the activity of PIF4 and its ability to activate *ATHB2* (Fig. 4A). In the dark, the amount of Pfr is determined by the dark reversion rate, *b*. We used *ATHB2* expression data from L*er*, *phyABCDE*, and *YHB* to calibrate this model (fig. S12). Using parameters from the previous model (*8*), we allowed the model to determine which values of *b* are consistent with the thermoresponsive gene expression profiles (table S1). The model selects values of *b* that vary as a function of temperature: The half-life of Pfr is 2.09 hours at 22°C and 1.53 hours at 27°C. If *b* is not allowed to vary with temperature, the model does not fit the data as well (fig. S13). Our model recapitulates *ATHB2* expression in response to temperature, which suggests that dual control of *PIF4* at transcriptional and posttranslational levels explains regulation of gene expression to control expansion growth (Fig. 4B).

Our model infers a temperature-responsive half-life for Pfr. Dark reversion of phytochromes from various species is a thermal relaxation reaction (*14*). We observed that the in vivo dark reversion rate of phyB:phyB$_{Pfr}$, in terms of half-life ($t_{1/2}$), showed an exponential relationship to temperature over one order of magnitude between 4° and 27°C [$t_{1/2} = 10^{(-0.0496T + 1.315)}$, where T is temperature (°C); $R^2 = 0.99$] (Fig. 4C and fig. S14). The half-lives at 22° and 27°C ($t_{1/2}$ = 1.79 hours and 0.86 hours, respectively) are comparable to those seen in the model.

Phytochromes are central regulators of plant responses to the environment; hence, their acquisition of a thermally responsive behavior suggests an evolutionary route to integrate temperature information into development. Because single amino acid changes as well as posttranslational modifications can alter the dark reversion rate, sequence diversification among phytochromes may afford diversity in thermal responsiveness for different climates (*18*, *20*, *29*–*31*). The Pfr dark reversion rate represents a sensitive mechanism for integrating small differences in temperature during the night in order to make developmental decisions.

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SUPPLEMENTARY MATERIALS

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Model Description
Gene Expression Cluster Descriptions
Materials and Methods
Figs. S1 to S14
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Combining heat and light responses

Plants integrate a variety of environmental signals to regulate growth patterns. Legris *et al.* and Jung *et al.* analyzed how the quality of light is interpreted through ambient temperature to regulate transcription and growth (see the Perspective by Halliday and Davis). The phytochromes responsible for reading the ratio of red to far-red light were also responsive to the small shifts in temperature that occur when dusk falls or when shade from neighboring plants cools the soil.

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