Article:

Zhang, Lin-Lin, Luo, Anni, Davis, Seth Jon orcid.org/0000-0001-5928-9046 et al. (1 more author) (2021) Timing to grow:roles of clock in thermomorphogenesis. TRENDS IN PLANT SCIENCE. pp. 1248-1257. ISSN 1360-1385

https://doi.org/10.1016/j.tplants.2021.07.020

Timing to Grow in Warmth: The Essential Role of Circadian Clock in Thermomorphogenesis

Lin-Lin Zhang[1], Anni Luo[1], Seth Jon Davis[2,3] and Jian-Xiang Liu [1,*]

[1]State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310027, China.

[2]Department of Biology, University of York, Heslington, York, YO105DD, UK.

[3]Key Laboratory of Plant Stress Biology, School of Life Sciences, Henan University, Kaifeng 475004, China

[*]Correspondence：jianxiangliu@zju.edu.cn (J.X.Liu)

Tel: 0086-571-88208114; Homepage: http://person.zju.edu.cn/embl_507

Article type: Opinion

ORCIDs

Lin-Lin Zhang: 0000-0002-0509-1138

Anni Luo: 0000-0002-7938-2181

Seth Jon Davis: 0000-0001-5928-9046

Jian-Xiang Liu: 0000-0003-0791-1301

Key words: Circadian clock, Evening Complex, Thermomorphogenesis

Glossary

Biomolecular Condensates: membraneless compartments that are non-stoichiometric phase-separated and concentrated with biomolecules full of proteins and nucleic acids.

Evening Complex (EC): a complex composed of three distinct proteins ELF3, ELF4 and LUX, which is an essential component of the circadian clock in plants.

Liquid-liquid phase separation (LLPS): a phenomenon in which a homogeneous solution spontaneously separates into two (or more) distinct solution phases and forms liquid droplets under certain conditions.

Temperature compensation: an effect that the circadian oscillator speed is buffered from changes in ambient temperature.

Temperature entrainment: an effect that the circadian oscillator speed is reset by changes in ambient temperature.

Thermomorphogenesis: morphological changes including hypocotyl/petiole elongation, leaf hyponasty, and accelerated flowering in higher plants, which is induced by ambient (non-stressful) temperature shifts.

Thermoperiodism: a sum of plant responses to cycles of day and night fluctuating temperatures.

Thermosensor: a protein perceiving ambient temperature shifts and undergoing property (structure, activity, *et al.*) changes that trigger temperature-dependent physiological/morphological read-outs.

Vernalization: a phenomenon in which a period of exposure to low ambient (non-stressful) temperature at vegetative stage is required for the transition from vegetative to reproductive development in plants.

Abstract

Plants coordinate their growth and developmental programs with changes in temperature. This process is termed thermomorphogenesis. The underlying molecular mechanisms have begun to emerge in these non-stressful responses to adjustments in prevailing temperature. The circadian clock is an internal timekeeper that ensures growth, development, and fitness across a wide range of environmental conditions and it responds to thermal changes. Here, we highlight how the circadian clock gates thermoresponsive hypocotyl growth in plants, with an emphasis on different action mode of Evening Complex (EC) in thermomorphogenesis. We also discuss the biochemical and molecular mechanisms of EC in transducing temperature signals to the key integrator PIF4. This provides future perspectives on unanswered questions on EC-associated thermomorphogenesis.

Temperature Fluctuation and Plant Thermomorphogenesis

Global warming has dramatic impact on plant ecosystems as well as on crop productivity, and impacts from climate change are projected to increase in the near future [1]. Plants are able to sense ambient temperatures and transduce these signals to trigger subsequent responses for physiological adjustment and developmental adaptation (**Figure 1**) [2,3]. In higher plants, mild-warm temperature induces morphological changes that include hypocotyl/petiole elongation, leaf hyponasty, and accelerated flowering, in a process called collectively **thermomorphogenesis** [4,5]. The term plant thermomorphogenesis has recently been extended to include **vernalization**, the process of promoting of flowering by long-term exposure to low ambient temperature that occurs by over-wintering, and **thermoperiodism** in which cycles of day and night temperature alternations regulate seed germination and hypocotyl growth [6]. In the context of this review paper, we focus on non-detrimental warmth as a temperature regime that induces thermomorphogenesis in plants.

The central role of PIF4

The bHLH family transcription factor Phytochrome interacting factor 4 (PIF4) is a central regulator of thermoresponsive hypocotyl growth in arabidopsis (*Arabidopsis thaliana*) [7-9]. PIF4 recognizes G-box (CACGTG)-containing *cis*-elements in the promoter regions of downstream target genes. These include *Tryptophan aminotransferase of Arabidopsis 1* (*TAA1*), *Cytochrome P450 79B2* (*CYP79B2*), and *YUCCA 8* (*YUC8*), all of which are involved in auxin biosynthesis, and *Small auxin up RNA 19* (*SAUR19*) in the auxin-signaling pathway, responsible for cell expansion and hypocotyl elongation [10,11]. PIF4-mediated thermomorphogenesis also involves other phytohormones, such as brassinosteroid (BR). Warm temperatures facilitate the formation of a PIF4-BRI1 EMS SUPPRESSOR 1 (BES1) complex, which activates the expression of rate-limiting BR biosynthetic genes [12]. As a consequence, increases in the BR hormone facilitate increases in growth.

Collectively many intersecting signaling pathways use PIF4 as a hub in warming-induced growth (**Figure 1**).In the light, *PIF4* expression is directly suppressed by the bZIP transcription factor LONG HYPOCOTYL 5 (HY5) [13]. Reciprocal competition for mutual regulatory DNA-binding sites between PIF4 and HY5 also exists [14]. Furthermore, Cryptochrome 1 (CRY1) directly interacts with PIF4 in a blue light-dependent manner to repress the transcriptional activation activity of PIF4 [15]. Thus, part of the repression of PIF4 responses to warming is by association to neighboring *cis*-element in competing DNA-binding. Elevated temperature triggers nuclear import of the ubiquitin E3-ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1). This is associated with reduced protein stability of the COP1 target HY5, which in turn releases the repression to promote *PIF4* expression. Thereby COP1 degradation of HY5 leads to PIF4-mediated hypocotyl growth [13,16]. Warm temperatures activate a mitogen activated protein kinase TARGET OF TEMPERATURE3 (TOT3) to adjust BR signaling [17]. BRASSINAZOLE-RESISTANT 1 (BZR1), a key transcription factor in BR signaling, binds to the promoters of *PIF4* and other temperature responsive genes. This promotes their expression under elevated ambient temperature [18,19]. Finally, warm temperatures induce *PIF4* expression also through other mechanisms, such as histone deacetylation and eviction of H2A.Z histone variant [20,21]. In addition, BLADE-ON-PETIOLE proteins BOP1 and BOP2 act in E3 ubiquitin ligase complexes to control PIF4 abundance under both normal and elevated ambient temperature [22]. By contrast, DE-ETIOLATED 1 (DET1), a key regulator of light signaling, regulates thermoresponsive hypocotyl elongation through stabilizing PIF4 protein [23]. Taken together, PIF4 is regulated at both transcriptional and posttranslational levels.

How are warm temperature cues perceived and transduced to PIF4?

According to recently proposed stringent criteria [3], several **thermosensors** were recently identified (**Figure 1**). They can receive temperature cues and have property changes at the molecular level, conveying this information on prevailing temperature to downstream components such as PIF4 to regulate downstream gene expression, and temperature-dependent physiological and morphological read-outs [24].

Phytochromes are photo-interconvertible photoreceptors that quickly switch from an inactivated Pr form to an activated Pfr form upon absorbing red- and far-red light, respectively. There is also a slow reversion to Pr form from Pfr form in a process that has been termed "dark reversion." Recent studies have established phytochrome B (phyB) as an important thermosensor assessing the levels of evening warmth, as just after dusk, a warm evening accelerates the conversion of phyB from active Pfr to inactive Pr to lift the inhibitory effects of phyB on PIF4 [25,26]. phyB also coveys the temperature signal to HEMERA (HMR) that interacts with PIF4 and increases PIF4 protein accumulation at warm temperature under long-day conditions under red light [27]..

In addition to phyB, other types of thermosensors are also recently identified. RNA structures are dynamic and sensitive to environmental perturbation in the ambient temperature range. RNA folding can endow transcripts with thermosensing capacity [3]. Indeed, in arabidopsis, the secondary structure of the 5' UTR of *PIF7* RNA adopts a more relaxed, yet distinct conformation at warmer temperatures, resulting in enhanced protein translation initiation of PIF7 [28]. PIF7 belongs to the same family as PIF4, and it directly promotes thermomorphogenic growth by inducing the transcription of key genes, such as *YUC8*, alone or in combination with PIF4 [28,29]. Further, rapid shift of proteins between active and inactive states could serve as an alternative thermosensory mechanism. EARLY FLOWERING 3 (ELF3) was initially identified as a circadian clock component [30]. Genetic association studies revealed that genetic variation in *ELF3* is responsible for the natural variation in warm temperature-induced hypocotyl elongation in arabidopsis [31,32] and ELF3 negatively regulates the function of PIF4 [33,34]. Interestingly, the polyglutamine (polyQ) repeats in ELF3 were proposed to serve as a direct sensing domain for thermal responsiveness [35]. The polyQ tract was reported to resemble a prion-like domain that undergoes **liquid-liquid phase separation (LLPS),** reducing the ELF3 activity in response to increasing temperature *in vitro*. This is in agreement with the function of ELF3 in arabidopsis in which the inhibitory effects of ELF3 on PIF4 is released under warm temperature conditions [35]. In summary, temperature cues are sensed by several distinct mechanisms in plants and integrated into a regulatory network to promote morphological changes in plants.

Can the Circadian Clock Gate Thermoresponsive Growth in Plants?

ELF3, together with EARLY FLOWERING 4 (ELF4) and LUX ARRHYTHMO (LUX, also known as PHYTOLOCK1), assembles into an **Evening Complex (EC)** that is an essential regulator of the circadian clock [36]. Among the three components of EC, LUX is a SHAQKYF-type MYB transcription factor that directly binds to target DNA sites with high affinity [37]. In contrast, LUX does not directly interact with ELF4; ELF3 functions as a scaffold to bridge LUX and ELF4 together [36], and ELF4 promotes the nuclear localization of ELF3 [38]. Loss-of-function of any of the EC genes results in misexpression of clock genes and impaired hypocotyl growth [39]. The circadian clock allows plants to anticipate predictable environment and coordinate their growth and development with the environment [40].

This circadian oscillator is a complex network consisting of repressors and activators that form multiple interconnected feedback loops (**Figure 2**) [41]. In brief, morning expressed transcription factors CIRCADIAN CLOCK

ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) repress the expression of afternoon *PSEUDO-RESPONSE REGULATOR* (*PRR*) genes such as *PRR1*/*TIMING OF CAB EXPRESSION 1* (*TOC1*), *PRR5*, *PRR7*, and *PRR9;* in turn, TOC1 and other PRR proteins inhibit the expression of *CCA1* and *LHY*, forming a negative feed-back loop [42,43]. CCA1 and LHY also repress the expression of evening genes encoding components in the EC; again, EC represses the expression of afternoon *PRR* genes [38,44,45]. In contrast, midday-expressed MYB-like transcription factors, REVEILLE 4/6/8 (RVE4/RVE6/RVE8), together with NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED 1/2 (LNK1/LNK2), are able to activate the expression of several clock genes including *TOC1*, *PRR5*, and EC components, but the expression of *RVE8* is repressed by TOC1 and other PRRs, adding another complex of regulation in the network [46-49]. CCA1/LHY and RVE4/RVE6/RVE8 bind to similar *cis*-elements. Notably, CCA1/LHY are repressors and RVE4/RVE6/RVE8 are activators of gene expression. The balance between the expression levels of clock repressors and activators is important to ensure robust rhythmicity under adverse environmental conditions [50].

Oscillating light and temperature are two essential entraining signals for setting the central circadian clock. One key clock output is as an important regulator of plant thermomorphogenesis (**Figure 2**). Recent studies showed that warm temperature-induced hypocotyl growth in the *cca1 lhy* double mutant was much-reduced under warm temperature conditions, suggesting that CCA1 and LHY promote thermoresponsive growth [51]. CCA1 interacts with SHORT HYPOCOTYL UNDER BLUE 1 (SHB1). With mid-day approaches, light intensity and ambient temperature tend to increase, CCA1 recruits SHB1 and binds to the promoter of *PIF4* to sustain *PIF4* expression and trigger thermomorphogenic responses under red-light conditions [51].

GIGANTEA (GI) is another important component in the circadian oscillator.

In a reciprocal manner, GI activates the expression of *CCA1* and *LHY* while CCA1 and LHY represses *GI* expression [41]. GI also acts as a molecular chaperone to stabilize the GA-signaling mediator REPRESSOR OF *ga1-3* (RGA), which acts as a suppressor of PIF4 under long-day conditions at warm temperature. However, GI abundance becomes relatively lower under short-day conditions, which decreases the inhibitory effect of RGA on PIF4 and promotes thermomorphogenic growth under short-day conditions at warm temperatures [52].

The clock protein TOC1 directly interacts with PIF4 and inhibits its ability to activate target gene expression, loss of function of *TOC1* and its close homologue *PRR5* enhances thermosensitivity in the evening, demonstrating that TOC1 reduces thermoresponses in the evening [53]. The LOV-domain blue light photoreceptor ZEITLUPE (ZTL) is an F-box protein in the SCF complex that controls the protein stability of TOC1 [54]. Mutation at *ZTL* reduces plant sensitivity to warm temperature in terms of hypocotyl growth. Perhaps this is as there are high levels of TOC1 in the *ztl* mutant plants [53]. Taken together, these studies confirmed that the circadian clock gates thermoresponsive growth in plants.

What is the Role of EC in Regulating Plant Thermomorphogenesis?

The EC night-time repressor in the circadian clock is involved in numerous clock-output pathways, including thermomorphogenesis [44]. Under short-day conditions, EC is recruited to the promoter regions of *PIF4*/*PIF5* and suppresses their gene expression, and as the level of EC decreases as dawn approaches, the inhibition of *PIF4*/*PIF5* expression is released and the downstream genes involved in auxin biosynthesis and signaling are increased and this is associated to increases in hypocotyl growth [33].

Besides the transcriptional control, ELF3 also interacts with PIF4, which prevents PIF4 from activating its transcriptional targets. Warm temperature alleviates this ELF3 inhibition, which promotes PIF4 activity [34]. Loss-of-function mutations of *ELF4* or *LUX* also confer arrhythmic circadian clock with output phenotypes including altered hypocotyl growth and flowering time similar to that of *elf3* mutants [55]. Recently, micro-grafting assays and mathematical analyses showed that ELF4 moves from shoots to roots to regulate circadian rhythms, and high ambient temperature decreased such movement, leading to a faster clock in roots [56]. *In vitro*, the full EC has been hypothesized to act as a direct thermosensor, with stronger DNA binding at a cooler temperature and weaker binding at a warmer temperature. The addition of ELF4 is reported to restore EC binding even at high temperature [37]. However, whether EC directly senses warm temperatures in plants is not known. Nevertheless, previous studies demonstrate that EC functions in regulating hypocotyl growth by repressing PIF4 activity, and warm temperature suppresses EC activity to promote plant thermomorphogenesis (**Figure 2**).

Do the Components of EC Function Individually or Collectively in Plant Thermomorphogenesis?

The expression peak of all transcripts encoding EC components overlaps under short-day conditions and mutation of any of the three genes results in arrhythmic phenotypes. Therefore, ELF3, ELF4, and LUX are originally considered to function together in a protein complex [36]. Indeed, genome-wide Chromatin Immunoprecipitation-Sequencing (ChIP-Seq) results demonstrated that ELF3, ELF4, and LUX have many overlapping binding sites, and the ability of EC to bind to genome-wide targets depends on ambient temperature [57]. Therefore, EC components could function collectively to repress PIF4 activity and warm temperature counteracts with such inhibition.

Structure analysis of LUX^{MYB} complexed with DNA and protein-DNA binding assays revealed that LUX alone binds DNA with high affinity, and the LUX-ELF3 complex is a relatively poor binder of DNA, adding ELF4 to the complex significantly enhances the binding affinity [37]. Although both LUX and ELF3 bind to and regulate circadian targets, strong LUX binding to many of the same binding sites as the EC in an *elf3* mutant suggests that LUX has an *in vivo* ELF3-independent genome-wide DNA-binding ability [57]. Thus, it is possible that LUX represses *PIF4* expression independent on EC in arabidopsis plants.

An EC-independent function of ELF3 has also been proposed. ELF3 interacts with PIF4 and directly prevents PIF4 from activating downstream targets. Overexpression of *ELF3* does not affect the expression of EC-targeted circadian genes, but reduces the expression of PIF4-targeted genes [34]. In agreement with the EC-independent role of ELF3, reduced but significant binding of ELF3 to EC targets were also observed in the *lux* mutant background [57], although NOX/BROTHER OF LUX ARRHYTHMO (NOX/BAO), a paralog of LUX [58], could possibly incorporate into the EC to complement the function of LUX in the *lux* mutant plants.

GI interacts with PIFs and modulates their transcriptional activities [59]. A previous report showed that ELF4 interacts with GI and sequesters GI to nuclear bodies to regulate photoperiodic flowering under long-day conditions, in which the function of ELF4 seems to be EC-independent [60]. Whether ELF4 could function independently on EC to regulate PIF4 activity during plant thermomorphogenesis awaits further investigation.

Mathematic modeling results support that EC activity is rate-limited by LUX and NOX, by ELF3-ELF4 complex formation, and by free ELF3 levels [61]. Further, the expression peak of the EC shifts from evening to afternoon under long-day conditions [62], and the expression peaks of LUX and ELF4 may not necessarily always overlap with that of ELF3 [63]. Therefore, despite that the three core components of EC act collectively to regulate thermoresponsive hypocotyl growth, all of ELF3, ELF4, and LUX could potentially function individually to control plant thermomorphogenesis (**Figure 3**).

How do the Components of EC Sense and Transduce Ambient Temperature Signals?

Which specific tissues are critical for plant thermomorphogenesis? Organ-specific transcriptomic analysis showed that thermomorphogenesis involves both autonomous and organ-interdependent temperature sensing and signaling [64]. A recent study demonstrated that the epidermis-specific expression, but not vasculature-specific expression of *PIF4*, induces constitutive long hypocotyls through the phyB-PIF4-auxin pathway in Arabidopsis. This led to the suggestion that epidermal surface is essential for thermoresponsive hypocotyl growth [65].

The expression of *ELF3* is induced by warm temperatures, notably at midnight times, while the expression of *ELF4* appears to be insensitive to changes in temperature [44,66]. However, EC activity is reduced at high temperatures [44,57]. How is ELF3 regulated at the protein level? Under normal growth temperature conditions, COP1 mediates ubiquitination and proteasomal degradation of ELF3, and COP1 also destabilizes GI. Together this is proposed to modulate light input signal to the circadian clock [67]. Whether warming intersects in the relations of GI and ELF3 from COP1 awaits investigations. In response to warming temperatures, the protein level of ELF3 is decreased around dusk and increased around midnight, in which COP1 plays a minor role [66]. In contrast, B-box 18 (BBX18) and BBX23 interact with ELF3 and act as scaffolding proteins to promote ELF3 degradation under warm temperature conditions [66,68].

Recently, XB3 ORTHOLOG 1 IN ARABIDOPSIS THALIANA (XBAT31) and XBAT35 were shown to interact with both ELF3 and BBX18. It was proposed that BBX18 recruits XBAT31 and XBAT35 to ubiquitinate and degrade ELF3 at warm ambient temperatures, which releases the inhibitory effect of ELF3 on PIF4 activity (**Figure 3**) [68,69]. Because ELF3 is an important component of EC, the BBX18-XBAT31-XBAT35 regulatory module may also affect the protein stability of the whole EC at warm temperatures. Both *BBX18* and *XBAT31* transcripts are increased by warm temperatures, and the protein level of BBX18 is also increased in response to warm temperatures [66,68]. These results support that warm temperature signals could be directly sensed by ELF3, and/or sensed by other unknown thermosensor and then transduced to ELF3. In either circumstance, signals are relayed to PIF4, the central hub, for downstream gene expression and growth regulations in plant thermomorphogenesis.

Concluding Remarks and Future Perspectives

Ambient temperatures act as an input, resetting cues to establish the plant circadian clock in a process termed **temperature entrainment.** In turn plants have the ability of **temperature compensation**, in which the oscillator keeps a constant oscillation period of about 24 hr within a wide range of ambient temperatures [47,70]. How the circadian oscillation is buffered by temperature changes is still less understood (see Outstanding Questions).

Elevated ambient temperature induces LLPS of ELF3 *in vitro* [35]. In many cases, phase separation leads to the formation of membrane-less compartments (**biomolecular condensates**) [71]. LLPS is an evolutionarily ancient mechanism for the organization of intracellular material: dicing bodies are plant-specific dynamic condensates involved in miRNA processing while processing bodies are involved in mRNA decay [71-73]. Recently, salicylic acid-induced NPR1 condensates (SINCs) were found to be formed in the cytoplasm when plants were treated with high levels of SA, a phytohormone involved in plant immunity, and multiple protein components in SINCs are subjected to NPR1-mediated ubiquitination and degradation [74]. ELF3, XBAT31, and COP1 have all been found to accumulate in discrete subnuclear foci [16,35,68]. Whether the formation of ELF3 condensates contributes to ubiquitination and degradation of ELF3 awaits further investigation.

In conclusion, circadian clock especially the EC has important roles in gating plant thermomorphogenesis, and accumulative evidence supports that EC components work not only collectively but also individually to regulate thermoresponsive growth in plants.

Acknowledgements

We apologize to those researchers whose work has not been cited in the manuscript owing to space limitations. This project was financially supported by grants from the National Natural Science Foundation of China (31625004), the Zhejiang Provincial Talent Program (2019R52005), the Natural Science Foundation of Zhejiang, China (LD21C020001), the 111 Project (B14027), and the BBSRC (BB/N018540/1).

References

1. Xu, C. et al. (2020) Future of the human climate niche. Proc. Natl. Acad. Sci. USA 117 (21), 11350-11355.
2. Legris, M. et al. (2017) Perception and signalling of light and temperature cues in plants. Plant J. 90 (4), 683-697.
3. Vu, L.D. et al. (2019) Feeling the heat: Searching for plant thermosensors. Trend. Plant Sci. 24 (3), 210-219.
4. Quint, M. et al. (2016) Molecular and genetic control of plant thermomorphogenesis. Nat. Plant. 2 (1), 15190.
5. Park, Y.J. et al. (2021) External and internal reshaping of plant thermomorphogenesis. Trend. Plant Sci. 10.1016/j.tplants.2021.01.002
6. Casal, J.J. and Balasubramanian, S. (2019) Thermomorphogenesis. Annu. Rev. Plant Biol. 70, 321-346.
7 Koini, M.A. et al. (2009) High temperature-medated adaptations in plant architecture require the bHLH transcription factor PIF4. Curr. Biol. 19 (5), 408-413.
8. Martinez, C. et al. (2018) Convergent regulation of PIFs and the E3 ligase COP1/SPA1 mediates thermosensory hypocotyl elongation by plant phytochromes. Curr. Opin. Plant Biol. 45, 188-203.
9. Vu, L.D. et al. (2019) Developmental plasticity at high temperature. Plant Physiol. 181 (2), 399-411.
10. Franklin, K.A. et al. (2011) PHYTOCHROME-INTERACTING FACTOR 4 (PIF4)

regulates auxin biosynthesis at high temperature. Proc. Natl. Acad. Sci. USA 108 (50), 20231-20235.

11. Sun, J. et al. (2012) PIF4-mediated activation of *YUCCA8* expression integrates temperature into the auxin pathway in regulating Arabidopsis hypocotyl growth. PLoS Genet. 8 (3), e1002594.

12. Martinez, C. et al. (2018) PIF4-induced BR synthesis is critical to diurnal and thermomorphogenic growth. EMBO J. 37 (23), e99552.

13. Delker, C. et al. (2014) The DET1-COP1-HY5 pathway constitutes a multipurpose signaling module regulating plant photomorphogenesis and thermomorphogenesis. Cell Rep. 9, 1983-1989.

14. Toledo-Ortiz, G. et al. (2014) The HY5-PIF regulatory module coordinates light and temperature control of photosynthetic gene transcription. PLoS Genet. 10 (6), e1004416.

15. Ma, D. et al. (2016) Cryptochrome 1 interacts with PIF4 to regulate high temperature-mediated hypocotyl elongation in response to blue light. Proc. Natl. Acad. Sci. USA 113 (1), 224-229.

16. Park, Y.J. et al. (2017) COP1 conveys warm temperature information to hypocotyl thermomorphogenesis. New Phytol. 215 (1), 269-280.

17. Vu, L.D. et al. (2021) The membrane-localized protein kinase MAP4K4/TOT3 regulates thermomorphogenesis. Nat. Commun. 12. 10.1038/s41467-021-23112-0

18. Oh, E. et al. (2012) Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat. Cell Biol. 14 (8), 802-809.

19. Ibanez, C. et al. (2018) Brassinosteroids dominate hormonal regulation of plant thermomorphogenesis via BZR1. Curr. Biol. 28 (2), 303-310.

20. Kim, Y.J. et al. (2016) POWERDRESS and HDA9 interact and promote histone H3 deacetylation at specific genomic sites in Arabidopsis. Proc. Natl. Acad. Sci. USA 113 (51), 14858-14863.

21. van der Woude, L.C. et al. (2019) HISTONE DEACETYLASE 9 stimulates auxin-dependent thermomorphogenesis in Arabidopsis thaliana by mediating H2A.Z depletion. Proc. Natl. Acad. Sci. USA 116 (50), 25343-25354.

22. Zhang, B. et al. (2017) BLADE-ON-PETIOLE proteins act in an E3 ubiquitin ligase complex to regulate PHYTOCHROME INTERACTING FACTOR 4 abundance. eLife 6, e26759.

23. Gangappa, S.N. and Kumar, S.V. (2017) DET1 and HY5 control PIF4-mediated thermosensory elongation growth through distinct mechanisms. Cell Rep. 18 (2), 344-351.

24. Lin, J.Y. et al. (2020) Emerging plant thermosensors: From RNA to protein. Trend. Plant Sci. 25 (12), 1187-1189.

25. Jung, J.H. et al. (2016) Phytochromes function as thermosensors in Arabidopsis. Science 354 (6314), 886-889.

26. Legris, M. et al. (2016) Phytochrome B integrates light and temperature signals in Arabidopsis. Science 354 (6314), 897-900.

27. Qiu, Y.J. et al. (2019) Daytime temperature is sensed by phytochrome B in Arabidopsis through a transcriptional activator HEMERA. Nat. Commun.10, 140.

28. Chung, B.Y.W. et al. (2020) An RNA thermoswitch regulates daytime growth in Arabidopsis. Nat. Plant. 6 (5), 522-532.

29. Fiorucci, A.S. et al. (2020) PHYTOCHROME INTERACTING FACTOR 7 is important for early responses to elevated temperature in Arabidopsis seedlings. New Phytol. 226 (1), 50-58.

30. Thines, B. and Harmon, F.G. (2010) Ambient temperature response establishes ELF3 as a required component of the core Arabidopsis circadian clock. Proc. Natl. Acad. Sci. USA 107 (7), 3257-3262.

31. Box, M.S. et al. (2015) ELF3 controls thermoresponsive growth in Arabidopsis. Curr. Biol. 25 (2), 194-199.

32. Anwer, M.U. et al. (2014) Natural variation reveals that intracellular distribution of ELF3 protein is associated with function in the circadian clock. eLlife 3, e02206.

33. Nomoto, Y. et al. (2012) Circadian clock- and PIF4-controlled plant growth: A coincidence mechanism directly integrates a hormone signaling network into the photoperiodic control of plant architectures *in Arabidopsis thaliana*. Plant Cell Physiol. 53, 1950-1964.

34. Nieto, C. et al. (2015) ELF3-PIF4 interaction regulates plant growth independently of the Evening Complex. Curr. Biol. 25, 187-193.

35. Jung, J.H. et al. (2020) A prion-like domain in ELF3 functions as a thermosensor in Arabidopsis. Nature 585 (7824), 256-260.

36. Nusinow, D.A. et al. (2011) The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature 475 (7356), 398-402.

37. Silva, C.S. et al. (2020) Molecular mechanisms of Evening Complex activity in Arabidopsis. Proc. Natl. Acad. Sci. USA. 117, 6901-6909.

38. Herrero, E. et al. (2012) EARLY FLOWERING4 recruitment of EARLY FLOWERING3 in the nucleus sustains the Arabidopsis circadian clock. Plant Cell 24, 428-443.

39. Huang, H. and Nusinow, D.A. (2016) Into the evening: Complex interactions in the Arabidopsis circadian clock. Trend. Genet. 32, 674-686.

40. Oakenfull, R.J. and Davis, S.J. (2017) Shining a light on the Arabidopsis circadian clock. Plant Cell Environ. 40 (11), 2571-2585.

41. Creux, N. and Harmer, S. (2019) Circadian rhythms in plants. Cold Spring Harb. Perspect. Biol. 11 (9), a034611.

42. Alabadi, D. et al. (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. Science 293 (5531), 880-883.

43. Kamioka, M. et al. (2016) Direct repression of evening genes by CIRCADIAN CLOCK-ASSOCIATED1 in the Arabidopsis circadian clock. Plant Cell 28 (3), 696-711.

44. Mizuno, T. et al. (2014) Ambient temperature signal feeds into the circadian clock transcriptional circuitry through the EC night-time repressor in *Arabidopsis thaliana.* Plant Cell Physiol. 55 (5), 958-976.

45. Li, G. et al. (2011) Coordinated transcriptional regulation underlying the circadian clock in Arabidopsis. Nature Cell Biol. 13 (5), 616-622.

46. Rawat, R. et al. (2011) REVEILLE8 and PSEUDO-REPONSE REGULATOR5 form a negative feedback loop within the Arabidopsis circadian clock. PLoS Genet. 7 (3), e1001350.

47. Hsu, P.Y. and Harmer, S.L. (2014) Wheels within wheels: The plant circadian system. Trend. Plant Sci. 19 (4), 240-249.

48. Xie, Q.G. et al. (2014) LNK1 and LNK2 are transcriptional coactivators in the Arabidopsis circadian oscillator. Plant Cell 26 (7), 2843-2857.

49. Hsu, P.Y. et al. (2013) Accurate timekeeping is controlled by a cycling activator in Arabidopsis. eLife 2, e00473.

50. Shalit-Kaneh, A. et al. (2018) Multiple feedback loops of the Arabidopsis circadian clock provide rhythmic robustness across environmental conditions. Proc. Natl. Acad. Sci. USA 115 (27), 7147-7152.

51. Sun, Q.B. et al. (2019) SHB1 and CCA1 interaction desensitizes light responses and enhances thermomorphogenesis. Nat. Commun. 10, 3110.

52. Park, Y.J. et al. (2020) GIGANTEA shapes the photoperiodic rhythms of thermomorphogenic growth in Arabidopsis. Mol. Plant 13 (3), 459-470.

53. Zhu, J.Y. et al. (2016) TOC1-PIF4 interaction mediates the circadian gating of thermoresponsive growth in Arabidopsis. Nat. Commun. 7, 13692.

54. Mas, P. et al. (2003) Targeted degradation of TOC1 by ZTL modulates circadian function in Arabidopsis thaliana. Nature 426 (6966), 567-570.

55. Hazen, S.P. et al. (2005) LUX ARRHYTHMO encodes a Myb domain protein essential for circadian rhythms. Proc. Natl. Acad. Sci. USA 102 (29), 10387-10392.

56. Chen, W.W. et al. (2020) A mobile ELF4 delivers circadian temperature information from shoots to roots. Nat. Plant. 6 (4), 416-426.

57. Ezer, D. et al. (2017) The evening complex coordinates environmental and endogenous signals in Arabidopsis. Nat. Plant. 3 (7), 17087.

58. Dai, S. et al. (2011) BROTHER OF LUX ARRHYTHMO is a component of the Arabidopsis circadian clock. Plant Cell 23 (3), 961-972.

59. Nohales, M.A. et al. (2019) Multi-level modulation of light signaling by GIGANTEA regulates both the output and pace of the circadian clock. Dev. Cell 49 (6), 840-851.

60. Kim, Y. et al. (2013) ELF4 regulates GIGANTEA chromatin access through subnuclear sequestration. Cell Rep. 3 (3), 671-677.

61. Fogelmark, K. and Troein, C. (2014) Rethinking transcriptional activation in the Arabidopsis circadian clock. PLoS Comput. Biol.10 (7). e1003705.

62. Webb, A.A.R. et al. (2019) Continuous dynamic adjustment of the plant circadian oscillator. Nat. Commun. 10, 550.

63. Mockler, T.C. et al. (2007) The Diurnal project: Diurnal and circadian expression profiling, model-based pattern matching, and promoter analysis. Cold Spring Harb. Symp. Quant. Biol. 72, 353-363.

64. Bellstaedt, J. et al. (2019) A mobile auxin signal connects temperature sensing in cotyledons with growth responses in hypocotyls. Plant Physiol. 180, 757-766.

65. Kim, S. et al. (2020) The epidermis coordinates thermoresponsive growth through the phyB-PIF4-auxin pathway. Nat. Commun. 11 (1), 1053.

66. Ding, L. et al. (2018) Two B-box domain proteins, BBX18 and BBX23, interact with ELF3 and regulate thermomorphogenesis in Arabidopsis. Cell Rep. 25 (7), 1718-1728.

67. Yu, J.W. et al. (2008) COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. Mol. Cell 32 (5), 617-630.

68. Zhang, L.L. et al. (2021) XBAT31 regulates thermoresponsive hypocotyl growth through mediating degradation of the thermosensor ELF3 in Arabidopsis. Sci Adv. 7,

eabf4427.

69. Zhang, L.L. et al. (2021) The E3 ligase XBAT35 mediates thermoresponsive hypocotyl growth by targeting ELF3 for degradation in Arabidopsis. J Integr Plant Biol. 63(6):1097-1103.

70. Salome, P.A. et al. (2010) The role of the Arabidopsis morning loop components CCA1, LHY, PRR7, and PRR9 in temperature compensation. Plant Cell 22 (11), 3650-3661.

71. Emenecker, R.J. et al. (2020) Emerging roles for phase separation in plants. Dev. Cell 55 (1), 69-83.

72. Luo, Y. et al. (2018) P-bodies: Composition, properties, and functions. Biochemistry 57 (17), 2424-2431.

73. Liu, Q. et al. (2012) Dicing bodies. Plant Physiol. 158 (1), 61-66.

74. Zavaliev, R. et al. (2020) Formation of NPR1 condensates promotes cell survival during the plant immune response. Cell 182 (5), 1093-1108.

Figures

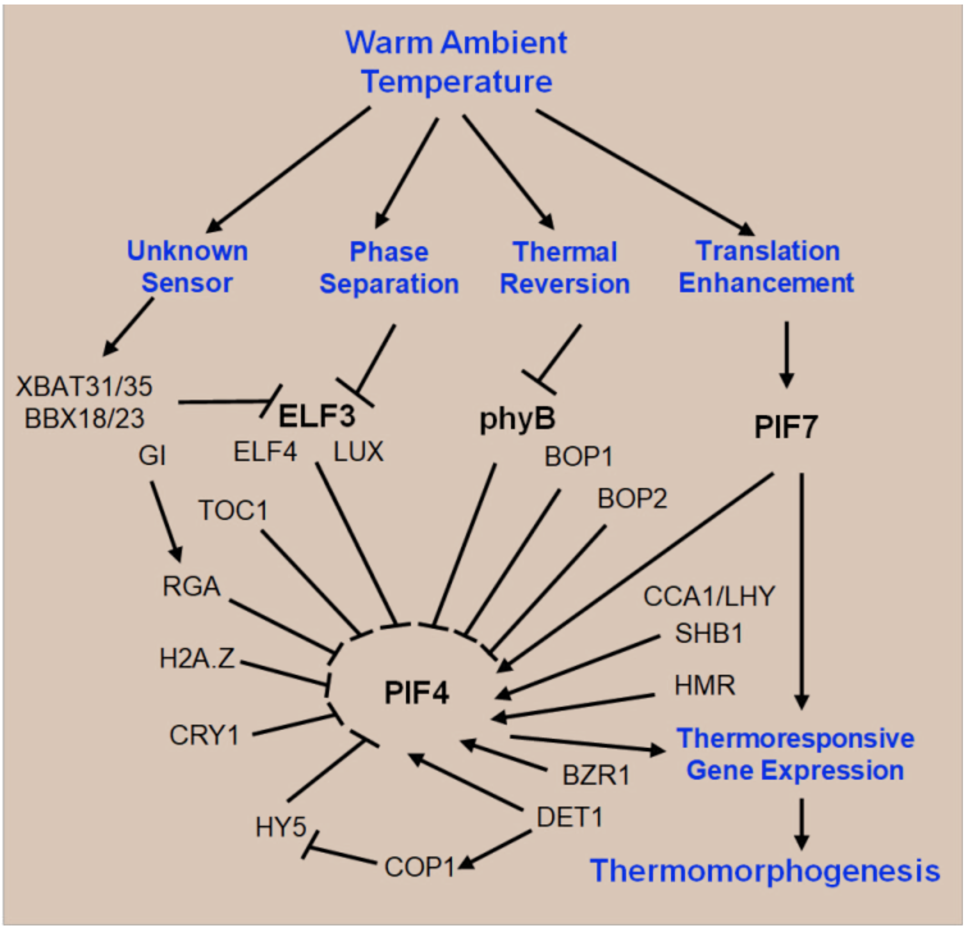

Figure 1. An overview of the regulatory networks of plant thermomorphogenesis.

Warm temperature conditions promote thermal reversion of phyB and liquid-liquid phase separation of ELF3 to inactivate these two thermosensors, respectively. The protein stability of ELF3 is also controlled by XBAT31/35 in association with BBX18/23. Warm temperature conditions enhance the translation efficiency of PIF7, which activates thermoresponsive gene expression alone or together with PIF4 to promote thermomorphogenesis in plants. Both ELF3 and phyB are negative regulators of PIF4, a central transcription regulator of plant thermomorphogensis. Other positive or negative regulators of PIF4 are also depicted. Positive and negative regulatory actions are indicated by arrows and lines with bars, respectively. Abbreviations: BBX18/23, B-box 18/23; BOP1/2, BLADE-ON-PETIOLE 1/2; BZR1,

BRASSINAZOLE-RESISTANT 1; CCA1, CIRCADIAN CLOCK ASSOCIATED 1; COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; CRY1, Cryptochrome 1; DET1, DE-ETIOLATED 1; ELF3/4, EARLY FLOWERING 3/4; GI, GIGANTEA; HMR, HEMERA; HY5, LONG HYPOCOTYL 5; H2A.Z, histone H2A protein variant Z; LHY, LATE ELONGATED HYPOCOTYL; LUX, LUX ARRHYTHMO; PHYB, phytochrome B; PIF4/7, Phytochrome interacting factor 4/7; RGA, REPRESSOR OF *ga1-3*; SHB1, SHORT HYPOCOTYL UNDER BLUE 1; TOC1, TIMING OF CAB EXPRESSION 1, XBAT31/35, XB3 ORTHOLOG 1/5 IN ARABIDOPSIS THALIANA.

Key Figure

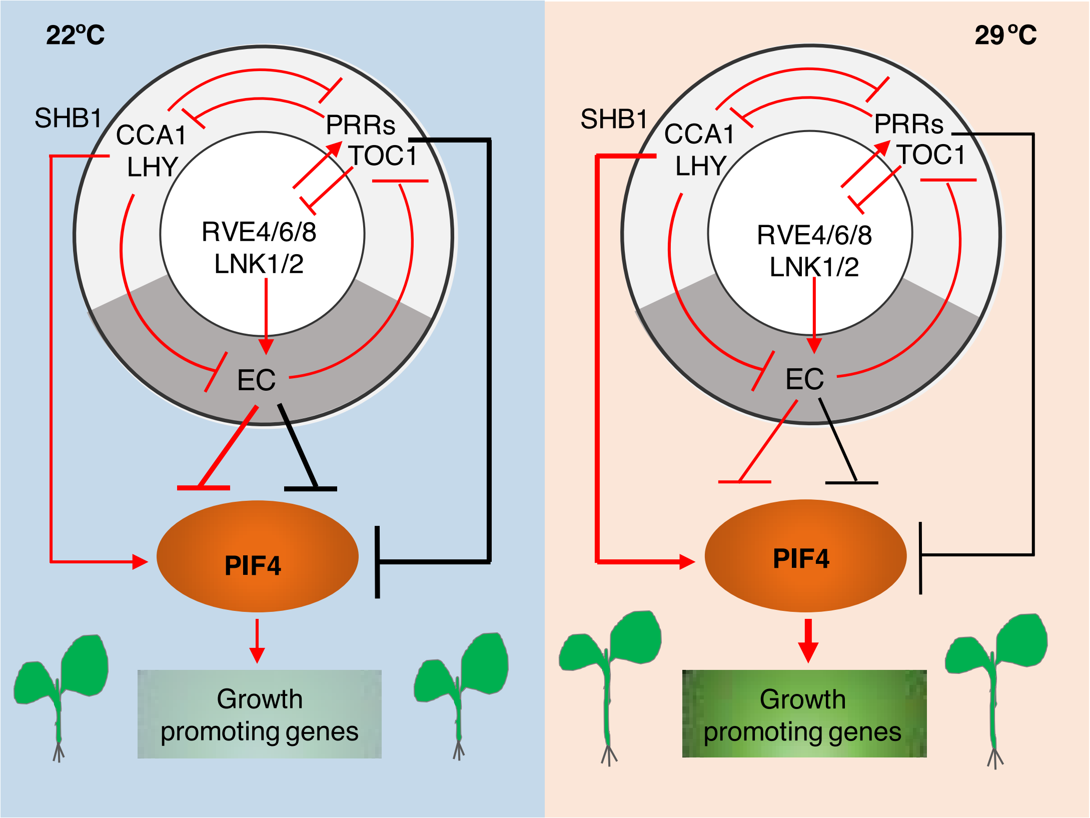

Figure 2. Summary of the function of circadian clock regulators in plant thermomorphogenesis.

Feed-back loops of the circadian clock under both normal (22°C) and elevated (29°C) ambient temperature conditions are shown in simplified diagrams. The

Evening Complex (EC) is composed of ELF3, ELF4, and LUX or NOX. EC suppresses the expression of *PIF4*, while EC and TOC1 inhibit the protein activity of PIF4, all of which are counteracted by warm temperatures. CCA1/LHY also promote the expression of *PIF4* together with SHB1 under warm temperature conditions. Positive and negative regulatory actions are indicated by arrows and lines with bars, respectively. Red colour represents transcriptional control while black colour represents post-translational control.The thickness of the lines depicts the degree of regulation. Abbreviations: CCA1, CIRCADIAN CLOCK ASSOCIATED 1; LHY, LATE ELONGATED HYPOCOTYL; LNK1/2, NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED 1/2; PIF4, Phytochrome interacting factor 4; PRR, PSEUDO-RESPONSE REGULATOR; RVE4/6/8, REVEILLE 4/6/8; SHB1, SHORT HYPOCOTYL UNDER BLUE 1; TOC1, TIMING OF CAB EXPRESSION 1.

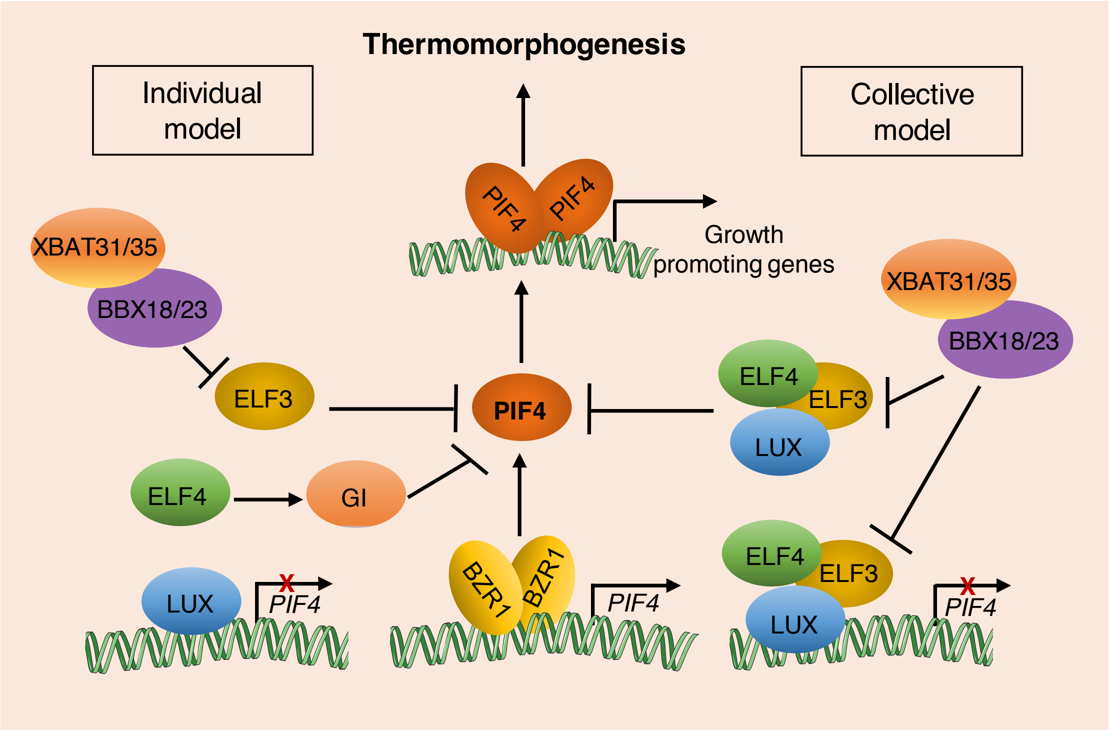

Figure 3. Molecular regulation of PIF4 activity by EC components during thermomorphogenesis. The *PIF4* expression is up-regulated by warm temperature via BZR1 and the PIF4 protein is required to induce downstream gene expression for promoting hypocotyl growth at elevated warm temperautres. In the collective model, ELF3, ELF4, and LUX form the Evening Complex (EC), which inhibits the expression of *PIF4* in the evening, as well as the protein activity of PIF4 in the daytime under normal growth temperatures. In the individual model, LUX-ELF4 inhibits the expression of *PIF4* in the evening, while ELF3 prevents PIF4 from activating downstream genes in an EC-independent manner in the daytime under normal growth-temperature conditions. ELF4 sequesters GI to nuclear bodies in which GI is a negative regulator of *PIF4* under normal growth temperature conditions. In either model, the E3 ligases, XBAT31 and XBAT35, interact with and ubiquitinate ELF3 to regulate ELF3 stability in association with BBX18 and BBX23 under warm temperature conditions. Interestingly, both BBX18 and XBAT31 are responsive to warm ambient temperature in arabidopsis. This figure was created with the aid of BioRender (https://biorender.com/). Positive and negative regulatory actions are indicated by arrows and lines with bars, respectively. Abbreviations: BBX18/23, B-box 18/23; BZR1, BRASSINAZOLE-RESISTANT 1; ELF3/4, EARLY FLOWERING 3/4; GI, GIGANTEA; LUX, LUX ARRHYTHMO; PIF4,

Phytochrome interacting factor 4; XBAT31/35, XB3 ORTHOLOG 1/5 IN ARABIDOPSIS THALIANA.