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

3

4

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24 **Key words:** Circadian clock, Evening Complex, Thermomorphogenesis

1 **Glossary**

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

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

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

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

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

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

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

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

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

26

1 **Abstract**

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

15

1 **Temperature Fluctuation and Plant Thermomorphogenesis**

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

17

18 *The central role of PIF4*

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

1 activates the expression of rate-limiting BR biosynthetic genes [12]. As a
2 consequence, increases in the BR hormone facilitate increases in growth.

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

29

1 *How are warm temperature cues perceived and transduced to PIF4?*

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

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

19 In addition to phyB, other types of thermosensors are also recently identified.
20 RNA structures are dynamic and sensitive to environmental perturbation in the
21 ambient temperature range. RNA folding can endow transcripts with
22 thermosensing capacity [3]. Indeed, in Arabidopsis, the secondary structure of
23 the 5' UTR of *PIF7* RNA adopts a more relaxed, yet distinct conformation at
24 warmer temperatures, resulting in enhanced protein translation initiation of
25 *PIF7* [28]. *PIF7* belongs to the same family as *PIF4*, and it directly promotes
26 thermomorphogenic growth by inducing the transcription of key genes, such as
27 *YUC8*, alone or in combination with *PIF4* [28,29]. Further, rapid shift of
28 proteins between active and inactive states could serve as an alternative
29 thermosensory mechanism. EARLY FLOWERING 3 (ELF3) was initially

1 identified as a circadian clock component [30]. Genetic association studies
2 revealed that genetic variation in *ELF3* is responsible for the natural variation
3 in warm temperature-induced hypocotyl elongation in arabidopsis [31,32] and
4 *ELF3* negatively regulates the function of *PIF4* [33,34]. Interestingly, the
5 polyglutamine (polyQ) repeats in *ELF3* were proposed to serve as a direct
6 sensing domain for thermal responsiveness [35]. The polyQ tract was reported
7 to resemble a prion-like domain that undergoes **liquid-liquid phase**
8 **separation (LLPS)**, reducing the *ELF3* activity in response to increasing
9 temperature *in vitro*. This is in agreement with the function of *ELF3* in
10 arabidopsis in which the inhibitory effects of *ELF3* on *PIF4* is released under
11 warm temperature conditions [35]. In summary, temperature cues are sensed
12 by several distinct mechanisms in plants and integrated into a regulatory
13 network to promote morphological changes in plants.

14

15 **Can the Circadian Clock Gate Thermoresponsive Growth in Plants?**

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

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

1 ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY)
2 repress the expression of afternoon *PSEUDO-RESPONSE REGULATOR*
3 (*PRR*) genes such as *PRR1/TIMING OF CAB EXPRESSION 1 (TOC1)*, *PRR5*,
4 *PRR7*, and *PRR9*; in turn, TOC1 and other PRR proteins inhibit the expression
5 of *CCA1* and *LHY*, forming a negative feed-back loop [42,43]. CCA1 and LHY
6 also repress the expression of evening genes encoding components in the EC;
7 again, EC represses the expression of afternoon *PRR* genes [38,44,45]. In
8 contrast, midday-expressed MYB-like transcription factors, REVEILLE 4/6/8
9 (*RVE4/RVE6/RVE8*), together with NIGHT LIGHT-INDUCIBLE AND
10 CLOCK-REGULATED 1/2 (*LNK1/LNK2*), are able to activate the expression of
11 several clock genes including *TOC1*, *PRR5*, and EC components, but the
12 expression of *RVE8* is repressed by TOC1 and other PRRs, adding another
13 complex of regulation in the network [46-49]. CCA1/LHY and
14 *RVE4/RVE6/RVE8* bind to similar *cis*-elements. Notably, CCA1/LHY are
15 repressors and *RVE4/RVE6/RVE8* are activators of gene expression. The
16 balance between the expression levels of clock repressors and activators is
17 important to ensure robust rhythmicity under adverse environmental conditions
18 [50].

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

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

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

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

19

20 **What is the Role of EC in Regulating Plant Thermomorphogenesis?**

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

28 Besides the transcriptional control, *ELF3* also interacts with *PIF4*, which
29 prevents *PIF4* from activating its transcriptional targets. Warm temperature

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

15

16 **Do the Components of EC Function Individually or Collectively in Plant** 17 **Thermomorphogenesis?**

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

27 Structure analysis of LUX^{MYB} complexed with DNA and protein-DNA
28 binding assays revealed that LUX alone binds DNA with high affinity, and the
29 LUX-ELF3 complex is a relatively poor binder of DNA, adding ELF4 to the

1 complex significantly enhances the binding affinity [37]. Although both LUX
2 and ELF3 bind to and regulate circadian targets, strong LUX binding to many
3 of the same binding sites as the EC in an *elf3* mutant suggests that LUX has
4 an *in vivo* ELF3-independent genome-wide DNA-binding ability [57]. Thus, it is
5 possible that LUX represses *PIF4* expression independent on EC in
6 arabidopsis plants.

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

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

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

1

2 **How do the Components of EC Sense and Transduce Ambient** 3 **Temperature Signals?**

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

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

25 Recently, XB3 ORTHOLOG 1 IN ARABIDOPSIS THALIANA (*XBAT31*)
26 and *XBAT35* were shown to interact with both *ELF3* and *BBX18*. It was
27 proposed that *BBX18* recruits *XBAT31* and *XBAT35* to ubiquitinate and
28 degrade *ELF3* at warm ambient temperatures, which releases the inhibitory
29 effect of *ELF3* on *PIF4* activity (**Figure 3**) [68,69]. Because *ELF3* is an

1 important component of EC, the BBX18-XBAT31-XBAT35 regulatory module
2 may also affect the protein stability of the whole EC at warm temperatures.
3 Both *BBX18* and *XBAT31* transcripts are increased by warm temperatures,
4 and the protein level of BBX18 is also increased in response to warm
5 temperatures [66,68]. These results support that warm temperature signals
6 could be directly sensed by ELF3, and/or sensed by other unknown
7 thermosensor and then transduced to ELF3. In either circumstance, signals
8 are relayed to PIF4, the central hub, for downstream gene expression and
9 growth regulations in plant thermomorphogenesis.

10

11 **Concluding Remarks and Future Perspectives**

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

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

1 foci [16,35,68]. Whether the formation of ELF3 condensates contributes to
2 ubiquitination and degradation of ELF3 awaits further investigation.

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

7

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15

16 **References**

- 17 1. Xu, C. et al. (2020) Future of the human climate niche. *Proc. Natl. Acad. Sci. USA* 117
18 (21), 11350-11355.
- 19 2. Legris, M. et al. (2017) Perception and signalling of light and temperature cues in plants.
20 *Plant J.* 90 (4), 683-697.
- 21 3. Vu, L.D. et al. (2019) Feeling the heat: Searching for plant thermosensors. *Trend. Plant*
22 *Sci.* 24 (3), 210-219.
- 23 4. Quint, M. et al. (2016) Molecular and genetic control of plant thermomorphogenesis.
24 *Nat. Plant.* 2 (1), 15190.
- 25 5. Park, Y.J. et al. (2021) External and internal reshaping of plant thermomorphogenesis.
26 *Trend. Plant Sci.* 10.1016/j.tplants.2021.01.002
- 27 6. Casal, J.J. and Balasubramanian, S. (2019) Thermomorphogenesis. *Annu. Rev. Plant*
28 *Biol.* 70, 321-346.
- 29 7. Koini, M.A. et al. (2009) High temperature-mediated adaptations in plant architecture
30 require the bHLH transcription factor PIF4. *Curr. Biol.* 19 (5), 408-413.
- 31 8. Martinez, C. et al. (2018) Convergent regulation of PIFs and the E3 ligase COP1/SPA1
32 mediates thermosensory hypocotyl elongation by plant phytochromes. *Curr. Opin. Plant*
33 *Biol.* 45, 188-203.
- 34 9. Vu, L.D. et al. (2019) Developmental plasticity at high temperature. *Plant Physiol.* 181
35 (2), 399-411.
- 36 10. Franklin, K.A. et al. (2011) PHYTOCHROME-INTERACTING FACTOR 4 (PIF4)

1 regulates auxin biosynthesis at high temperature. Proc. Natl. Acad. Sci. USA 108 (50),
2 20231-20235.

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

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

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

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

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

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

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

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

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

24 20. Kim, Y.J. et al. (2016) POWERDRESS and HDA9 interact and promote histone H3
25 deacetylation at specific genomic sites in Arabidopsis. Proc. Natl. Acad. Sci. USA 113 (51),
26 14858-14863.

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

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

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

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

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

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

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

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

- 1 29. Fiorucci, A.S. et al. (2020) PHYTOCHROME INTERACTING FACTOR 7 is important
2 for early responses to elevated temperature in *Arabidopsis* seedlings. *New Phytol.* 226 (1),
3 50-58.
- 4 30. Thines, B. and Harmon, F.G. (2010) Ambient temperature response establishes ELF3
5 as a required component of the core *Arabidopsis* circadian clock. *Proc. Natl. Acad. Sci.*
6 *USA* 107 (7), 3257-3262.
- 7 31. Box, M.S. et al. (2015) ELF3 controls thermoresponsive growth in *Arabidopsis*. *Curr.*
8 *Biol.* 25 (2), 194-199.
- 9 32. Anwer, M.U. et al. (2014) Natural variation reveals that intracellular distribution of
10 ELF3 protein is associated with function in the circadian clock. *eLife* 3, e02206.
- 11 33. Nomoto, Y. et al. (2012) Circadian clock- and PIF4-controlled plant growth: A
12 coincidence mechanism directly integrates a hormone signaling network into the
13 photoperiodic control of plant architectures in *Arabidopsis thaliana*. *Plant Cell Physiol.* 53,
14 1950-1964.
- 15 34. Nieto, C. et al. (2015) ELF3-PIF4 interaction regulates plant growth independently of
16 the Evening Complex. *Curr. Biol.* 25, 187-193.
- 17 35. Jung, J.H. et al. (2020) A prion-like domain in ELF3 functions as a thermosensor in
18 *Arabidopsis*. *Nature* 585 (7824), 256-260.
- 19 36. Nusinow, D.A. et al. (2011) The ELF4-ELF3-LUX complex links the circadian clock to
20 diurnal control of hypocotyl growth. *Nature* 475 (7356), 398-402.
- 21 37. Silva, C.S. et al. (2020) Molecular mechanisms of Evening Complex activity in
22 *Arabidopsis*. *Proc. Natl. Acad. Sci. USA.* 117, 6901-6909.
- 23 38. Herrero, E. et al. (2012) EARLY FLOWERING4 recruitment of EARLY FLOWERING3
24 in the nucleus sustains the *Arabidopsis* circadian clock. *Plant Cell* 24, 428-443.
- 25 39. Huang, H. and Nusinow, D.A. (2016) Into the evening: Complex interactions in the
26 *Arabidopsis* circadian clock. *Trend. Genet.* 32, 674-686.
- 27 40. Oakenfull, R.J. and Davis, S.J. (2017) Shining a light on the *Arabidopsis* circadian
28 clock. *Plant Cell Environ.* 40 (11), 2571-2585.
- 29 41. Creux, N. and Harmer, S. (2019) Circadian rhythms in plants. *Cold Spring Harb.*
30 *Perspect. Biol.* 11 (9), a034611.
- 31 42. Alabadi, D. et al. (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within
32 the *Arabidopsis* circadian clock. *Science* 293 (5531), 880-883.
- 33 43. Kamioka, M. et al. (2016) Direct repression of evening genes by CIRCADIAN
34 CLOCK-ASSOCIATED1 in the *Arabidopsis* circadian clock. *Plant Cell* 28 (3), 696-711.
- 35 44. Mizuno, T. et al. (2014) Ambient temperature signal feeds into the circadian clock
36 transcriptional circuitry through the EC night-time repressor in *Arabidopsis thaliana*. *Plant*
37 *Cell Physiol.* 55 (5), 958-976.
- 38 45. Li, G. et al. (2011) Coordinated transcriptional regulation underlying the circadian
39 clock in *Arabidopsis*. *Nature Cell Biol.* 13 (5), 616-622.
- 40 46. Rawat, R. et al. (2011) REVEILLE8 and PSEUDO-RESPONSE REGULATOR5 form a
41 negative feedback loop within the *Arabidopsis* circadian clock. *PLoS Genet.* 7 (3),
42 e1001350.
- 43 47. Hsu, P.Y. and Harmer, S.L. (2014) Wheels within wheels: The plant circadian system.
44 *Trend. Plant Sci.* 19 (4), 240-249.

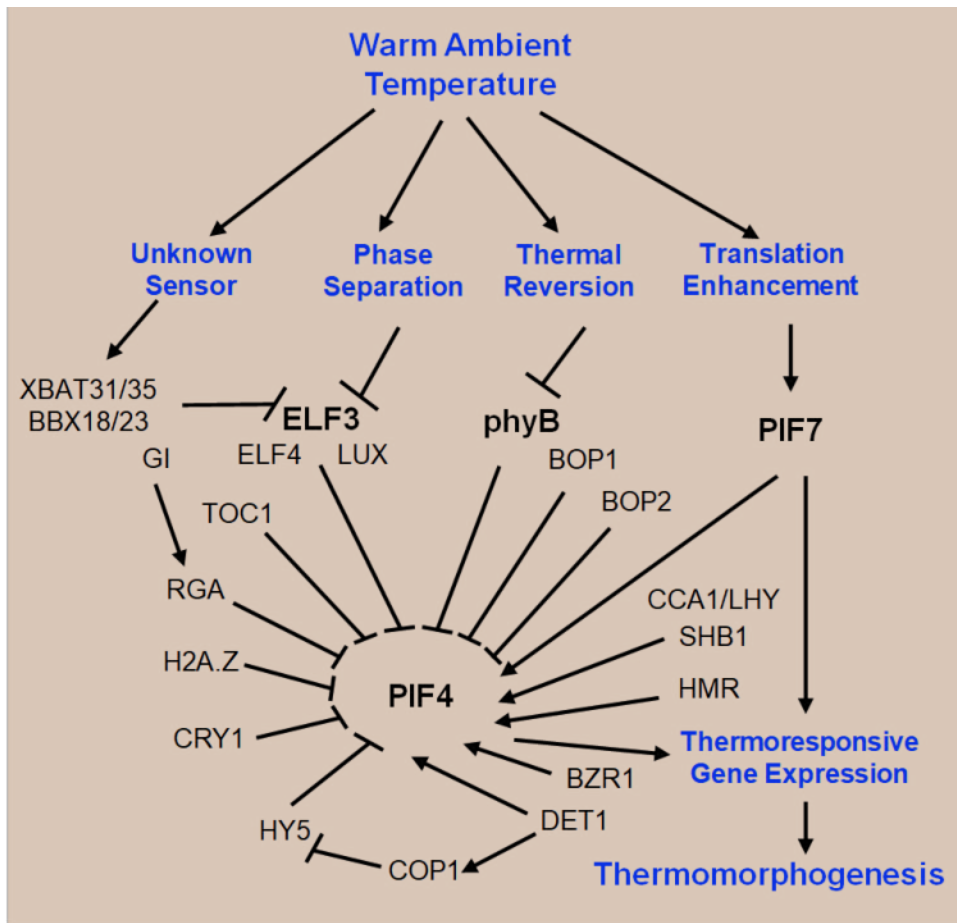
- 1 48. Xie, Q.G. et al. (2014) LNK1 and LNK2 are transcriptional coactivators in the
2 Arabidopsis circadian oscillator. *Plant Cell* 26 (7), 2843-2857.
- 3 49. Hsu, P.Y. et al. (2013) Accurate timekeeping is controlled by a cycling activator in
4 Arabidopsis. *eLife* 2, e00473.
- 5 50. Shalit-Kaneh, A. et al. (2018) Multiple feedback loops of the Arabidopsis circadian
6 clock provide rhythmic robustness across environmental conditions. *Proc. Natl. Acad. Sci.*
7 *USA* 115 (27), 7147-7152.
- 8 51. Sun, Q.B. et al. (2019) SHB1 and CCA1 interaction desensitizes light responses and
9 enhances thermomorphogenesis. *Nat. Commun.* 10, 3110.
- 10 52. Park, Y.J. et al. (2020) GIGANTEA shapes the photoperiodic rhythms of
11 thermomorphogenic growth in Arabidopsis. *Mol. Plant* 13 (3), 459-470.
- 12 53. Zhu, J.Y. et al. (2016) TOC1-PIF4 interaction mediates the circadian gating of
13 thermoresponsive growth in Arabidopsis. *Nat. Commun.* 7, 13692.
- 14 54. Mas, P. et al. (2003) Targeted degradation of TOC1 by ZTL modulates circadian
15 function in Arabidopsis thaliana. *Nature* 426 (6966), 567-570.
- 16 55. Hazen, S.P. et al. (2005) LUX ARRHYTHMO encodes a Myb domain protein essential
17 for circadian rhythms. *Proc. Natl. Acad. Sci. USA* 102 (29), 10387-10392.
- 18 56. Chen, W.W. et al. (2020) A mobile ELF4 delivers circadian temperature information
19 from shoots to roots. *Nat. Plant.* 6 (4), 416-426.
- 20 57. Ezer, D. et al. (2017) The evening complex coordinates environmental and
21 endogenous signals in Arabidopsis. *Nat. Plant.* 3 (7), 17087.
- 22 58. Dai, S. et al. (2011) BROTHER OF LUX ARRHYTHMO is a component of the
23 Arabidopsis circadian clock. *Plant Cell* 23 (3), 961-972.
- 24 59. Nohales, M.A. et al. (2019) Multi-level modulation of light signaling by GIGANTEA
25 regulates both the output and pace of the circadian clock. *Dev. Cell* 49 (6), 840-851.
- 26 60. Kim, Y. et al. (2013) ELF4 regulates GIGANTEA chromatin access through
27 subnuclear sequestration. *Cell Rep.* 3 (3), 671-677.
- 28 61. Fogelmark, K. and Troein, C. (2014) Rethinking transcriptional activation in the
29 Arabidopsis circadian clock. *PLoS Comput. Biol.* 10 (7). e1003705.
- 30 62. Webb, A.A.R. et al. (2019) Continuous dynamic adjustment of the plant circadian
31 oscillator. *Nat. Commun.* 10, 550.
- 32 63. Mockler, T.C. et al. (2007) The Diurnal project: Diurnal and circadian expression
33 profiling, model-based pattern matching, and promoter analysis. *Cold Spring Harb. Symp.*
34 *Quant. Biol.* 72, 353-363.
- 35 64. Bellstaedt, J. et al. (2019) A mobile auxin signal connects temperature sensing in
36 cotyledons with growth responses in hypocotyls. *Plant Physiol.* 180, 757-766.
- 37 65. Kim, S. et al. (2020) The epidermis coordinates thermoresponsive growth through the
38 phyB-PIF4-auxin pathway. *Nat. Commun.* 11 (1), 1053.
- 39 66. Ding, L. et al. (2018) Two B-box domain proteins, BBX18 and BBX23, interact with
40 ELF3 and regulate thermomorphogenesis in Arabidopsis. *Cell Rep.* 25 (7), 1718-1728.
- 41 67. Yu, J.W. et al. (2008) COP1 and ELF3 control circadian function and photoperiodic
42 flowering by regulating GI stability. *Mol. Cell* 32 (5), 617-630.
- 43 68. Zhang, L.L. et al. (2021) XBAT31 regulates thermoresponsive hypocotyl growth
44 through mediating degradation of the thermosensor ELF3 in Arabidopsis. *Sci Adv.* 7,

1 eabf4427.
2 69. Zhang, L.L. et al. (2021) The E3 ligase XBAT35 mediates thermoresponsive hypocotyl
3 growth by targeting ELF3 for degradation in Arabidopsis. *J Integr Plant Biol.*
4 63(6):1097-1103.
5 70. Salome, P.A. et al. (2010) The role of the Arabidopsis morning loop components
6 CCA1, LHY, PRR7, and PRR9 in temperature compensation. *Plant Cell* 22 (11),
7 3650-3661.
8 71. Emenecker, R.J. et al. (2020) Emerging roles for phase separation in plants. *Dev. Cell*
9 55 (1), 69-83.
10 72. Luo, Y. et al. (2018) P-bodies: Composition, properties, and functions. *Biochemistry*
11 57 (17), 2424-2431.
12 73. Liu, Q. et al. (2012) Dicing bodies. *Plant Physiol.* 158 (1), 61-66.
13 74. Zavaliev, R. et al. (2020) Formation of NPR1 condensates promotes cell survival
14 during the plant immune response. *Cell* 182 (5), 1093-1108.
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1 **Figures**

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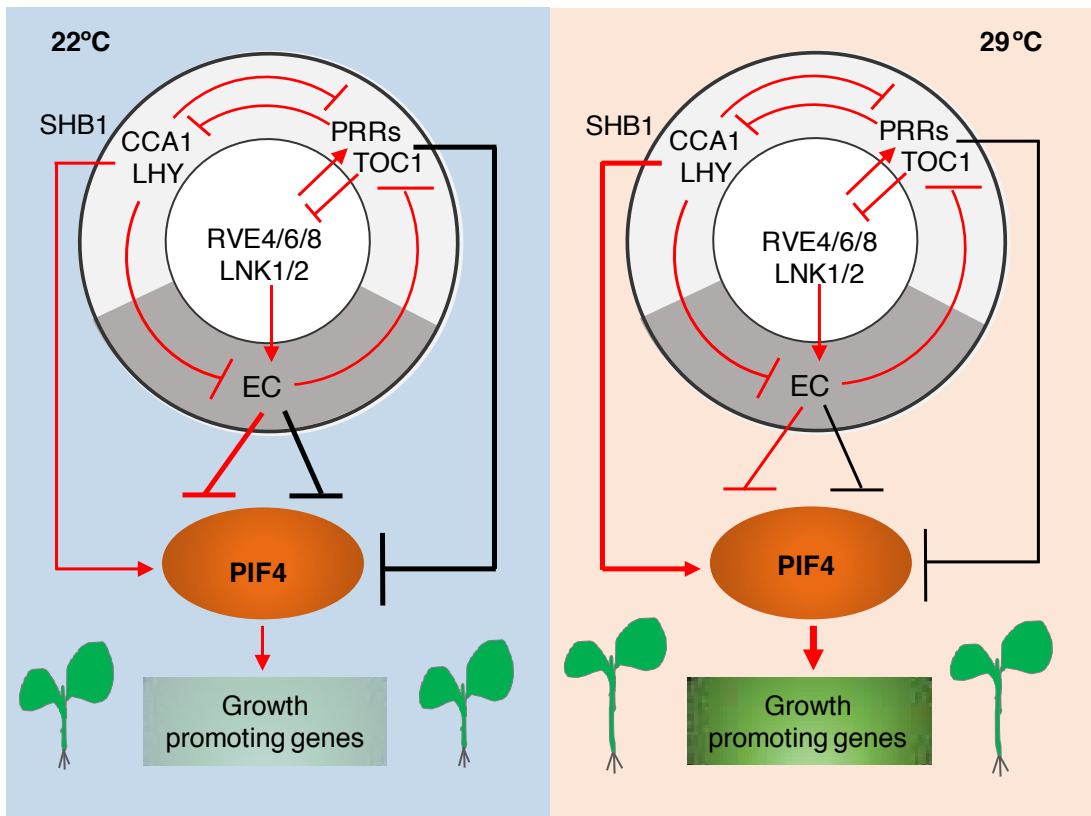
5 **Figure 1. An overview of the regulatory networks of plant**
 6 **thermomorphogenesis.**

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

1 BRASSINAZOLE-RESISTANT 1; CCA1, CIRCADIAN CLOCK ASSOCIATED
 2 1; COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; CRY1, Cryptochrome
 3 1; DET1, DE-ETIOLATED 1; ELF3/4, EARLY FLOWERING 3/4; GI,
 4 GIGANTEA; HMR, HEMERA; HY5, LONG HYPOCOTYL 5; H2A.Z, histone
 5 H2A protein variant Z; LHY, LATE ELONGATED HYPOCOTYL; LUX, LUX
 6 ARRHYTHMO; PHYB, phytochrome B; PIF4/7, Phytochrome interacting factor
 7 4/7; RGA, REPRESSOR OF *ga1-3*; SHB1, SHORT HYPOCOTYL UNDER
 8 BLUE 1; TOC1, TIMING OF CAB EXPRESSION 1, XBAT31/35, XB3
 9 ORTHOLOG 1/5 IN ARABIDOPSIS THALIANA.

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Key Figure



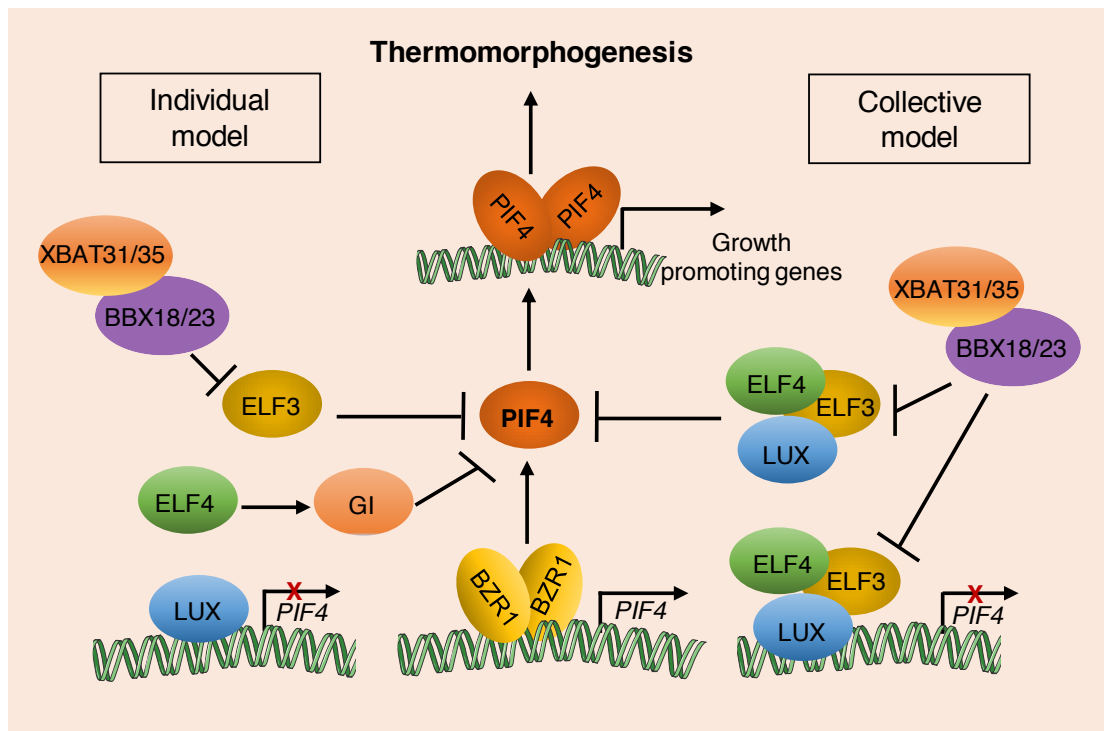
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Figure 2. Summary of the function of circadian clock regulators in plant thermomorphogenesis.

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

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

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

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