

ZEITLUPE positively regulates hypocotyl elongation at warm temperature under light in *Arabidopsis thaliana*

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Abbreviations: FKF1, FLAVIN-BINDING KELCH REPEAT F-BOX 1; LKP2, LOV KELCH PROTEIN 2; PIF, PHYTOCHROME-INTERACTING FACTOR; phyB, phytochrome B; ZTL, ZEITLUPE.

Hypocotyl cell elongation has been studied as a model to understand how cellular expansion contributes to plant organ growth. Hypocotyl elongation is affected by multiple environmental factors, including light quantity and light quality. Red light inhibits hypocotyl growth via the phytochrome signaling pathways. Proteins of the FLAVIN-BINDING KELCH REPEAT F-BOX 1 / LOV KELCH PROTEIN 2 / ZEITLUPE family are positive regulators of hypocotyl elongation under red light in *Arabidopsis*. These proteins were suggested to reduce phytochrome-mediated inhibition of hypocotyl elongation. Here, we show that ZEITLUPE also functions as a positive regulator in warmth-induced hypocotyl elongation under light in *Arabidopsis*.

Plants use light not only as an energy source, but also as an environmental signal for growth and development. Among numerous genes and proteins involved in the perception and transduction of environmental signals, those for photoreceptors are well investigated.^{1,2}

The FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1) / LOV KELCH PROTEIN 2 (LKP2) / ZEITLUPE (ZTL) family is a group of blue-light photoreceptors in *Arabidopsis*.^{3–6} These proteins determine the period of circadian oscillation, regulate photoperiodic flowering, and are involved in light-controlled hypocotyl elongation.^{3–6} *fkf1* mutants have short hypocotyls under continuous blue or red light.⁴ LKP2-overproducing plants have elongated hypocotyls under continuous blue, red, or white light.⁵ *ztl* mutants are indistinguishable from wild type under blue light but have short hypocotyls under continuous red light;⁶ ZTL-overproducing plants have elongated hypocotyls under blue, red, or white light.⁴ These results suggest that, even though they are blue-light photoreceptors, proteins of this family promote hypocotyl growth under red or white light by inhibiting the phytochrome B (phyB)-mediated signal transduction pathway, as phyB is the main receptor mediating red light-induced inhibition of hypocotyl elongation.^{1,2}

Hypocotyl growth is affected by both light and temperature. The hypocotyls of *Arabidopsis* plants grown at 28°C are longer than those grown at 22°C.⁷ The basic helix-loop-helix transcriptional regulator PHYTOCHROME-INTERACTING

FACTOR (PIF) 4 functions as a positive regulator in this temperature-mediated hypocotyl elongation, as indicated by the fact that the *pir4* mutant does not show enhanced hypocotyl elongation at 28°C under continuous light.⁷ PIF4 is destabilized by photoactivated phyB, indicating that the stability of PIF4 is modulated by light.⁸ These facts suggest that signals from light and temperature are integrated at PIF4 for hypocotyl growth regulation. Therefore, factors that inhibit phyB signaling, such as FKF1/LKP2/ZTL family proteins, may promote temperature-mediated hypocotyl elongation.

To assess this possibility, we grew *ztl-3*, *ztl-105*, *fkf1-t lkp2-1 ztl-105*,⁹ and Columbia seedlings under continuous white light (80 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or in the dark on 1/2-basal-salt Murashige and Skoog agar (0.8% w/v) at 22 or 28°C, and measured the hypocotyl length as described previously (Fig. 1A–D).¹⁰ At 22°C under continuous white light, hypocotyls of the mutants were slightly but significantly shorter than those of the wild type (by 25% for *ztl-3*, 19% for *ztl-105*, and 29% for *fkf1-t lkp2-1 ztl-105*; Fig. 1A). At 28°C under the same light conditions (Fig. 1C), hypocotyls of the wild type and mutants were longer than at 22°C (Fig. 1A): $\times 3.1$ for wild type but only $\times 2.2$ for both *ztl3* and *ztl-105* and $\times 2.0$ for *fkf1-t lkp2-1 ztl-105*. There were no statistically significant differences between the hypocotyl lengths of dark-grown wild-type and mutant plants either at 22°C (Fig. 1B) or at 28°C (Fig. 1D), indicating that the differences in hypocotyl length between wild type and mutants were light dependent.

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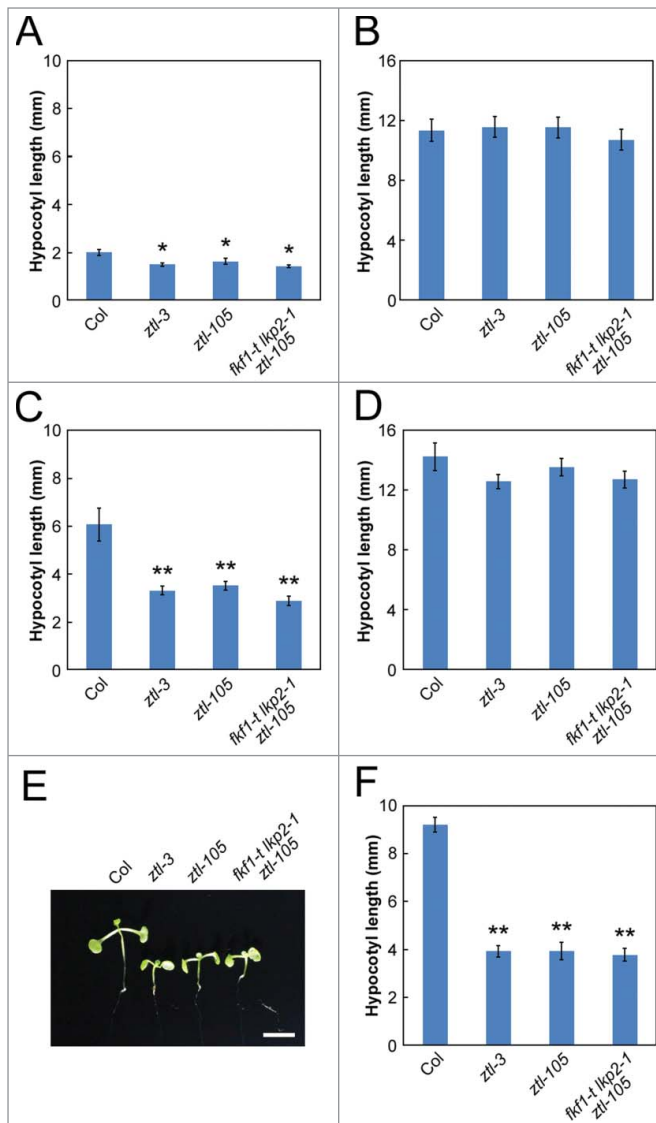


Figure 1. Hypocotyl length of *Arabidopsis thaliana* plants grown under different temperature and light conditions. Seedlings of control wild-type Columbia (Col) plants and 3 mutants (*ztl-3*, *ztl-105*, and *fkf1-t lkp2-1 ztl-105*) were grown on 1/2-basal-salt Murashige and Skoog agar (0.8% w/v) medium. (A, B) Hypocotyl length of seedlings grown for 8 days at 22°C under continuous white light ($80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (A) or in the dark (B). (C, D) Hypocotyl length of seedlings grown for 3 days at 22°C and then for 5 days at 28°C under continuous white light ($80 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (C) or in the dark (D). (E, F) A photograph and hypocotyl length of seedlings grown for 3 days at 22°C and then for 5 days at 28°C under continuous red light ($10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Error bars represent standard error of the mean ($n = 7\text{--}27$); * $P < 0.05$ and ** $P < 0.001$ (Student's *t*-test) in comparison with Col. Scale bar = 5 mm.

Next, we grew *ztl-3*, *ztl-105*, *fkf1-t lkp2-1 ztl-105*, and Columbia seedlings on the same medium under continuous red

References

- Chen M, Chory J, Fankhauser C. Light signal transduction in higher plants. *Annu Rev Genet* 2004; 38:87-117; PMID:15568973; <http://dx.doi.org/10.1146/annurev.genet.38.072902.092259>

- Nagatani A. Phytochrome: structural basis for its functions. *Curr Opin Plant Biol* 2010; 13:565-70; PMID:20801708; <http://dx.doi.org/10.1016/j.pbi.2010.07.002>
- Kiyosue T, Wada M. LKP1 (LOV kelch protein 1): a factor involved in the regulation of flowering time in

Arabidopsis. *Plant J* 2000; 23:807-15; PMID:10998191; <http://dx.doi.org/10.1046/j.1365-313x.2000.00850.x>

- Nelson DC, Lasswell J, Rogg LE, Cohen MA, Bartel B. FKF1, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. *Cell* 2000; 101:331-

light ($10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at 28°C (Fig. 1E, F). Hypocotyls of the mutants were significantly shorter than those of the wild-type seedlings (by 57% for *ztl-3* and *ztl-105* and by 59% for *fkf1-t lkp2-1 ztl-105*; Fig. 1F). The differences in hypocotyl length between *ztl* and *fkf1 lkp2 ztl* mutants were small at 28°C under either continuous white or red light. These data suggest that, among FKF1/LKP2/ZTL family members, ZTL is the major contributor to hypocotyl elongation at 28°C under white or red light in wild-type plants.

How does ZTL reduce light-induced hypocotyl growth inhibition and promote warm temperature-mediated hypocotyl elongation? Upon absorbing red light, light-activated phyB enters the nucleus, where it interacts with several regulatory proteins, including PIFs, and modulates their activity or stability, thereby inducing light responses by altering the expression of various genes.¹¹ Dark-grown *pif* mutants show constitutive photomorphogenic phenotypes, including short hypocotyls, as though they grew in red light; PIFs therefore function negatively in this phytochrome-mediated red light signaling.^{12,13} Under red light, PIF4 accumulates in seedlings in response to warm temperatures.¹⁴ In response to warm temperature under white light, PIF4 binds to promoter regions of 2 genes for auxin biosynthesis enzymes, *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1* and *CYP79B2*, promoting gene expression, which leads to elevated indole-3-acetic acid levels in seedlings and thus to hypocotyl elongation.¹⁵ Light-activated phyB mediates PIF4 degradation by the proteasome.⁸ ZTL and PHYB apoprotein can interact.¹⁶ Interaction between ZTL and phyB might inhibit that between phyB and PIF4 and thus increase the level of free PIF4. Inhibition of the phyB-PIF4 interaction by ZTL could explain the role of ZTL in the promotion of warm temperature-induced hypocotyl elongation under red light if ZTL level, activity, or both are increased in response to elevated temperatures. In this respect, it may be relevant that heat shock protein HSP90 has been reported to be essential for ZTL accumulation and function.¹⁷

Further investigation is necessary to assess this possibility and understand how ZTL regulates hypocotyl growth.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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- 40; PMID:10847687; [http://dx.doi.org/10.1016/S0092-8674\(00\)80842-9](http://dx.doi.org/10.1016/S0092-8674(00)80842-9)
5. Schultz TF, Kiyosue T, Yanovsky M, Wada M, Kay SA. A role for LKP2 in the circadian clock of *Arabidopsis*. *Plant Cell* 2001; 13:2659-70; PMID:11752379; <http://dx.doi.org/10.1105/tpc.010332>
 6. Somers DE, Schultz TF, Milnamow M, Kay SA. ZEITLUPE encodes a novel clock-associated PAS protein from *Arabidopsis*. *Cell* 2000; 101:319-29; PMID:10847686; [http://dx.doi.org/10.1016/S0092-8674\(00\)80841-7](http://dx.doi.org/10.1016/S0092-8674(00)80841-7)
 7. Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC, Franklin KA. High temperature-mediated adaptations in plant architecture require the bHLH transcription factor PIF4. *Curr Biol* 2009; 19:408-13; PMID:19249207; <http://dx.doi.org/10.1016/j.cub.2009.01.046>
 8. Lorrain S, Allen T, Duek PD, Whitelam GC, Fankhauser C. Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. *Plant J* 2008; 53:312-23; PMID:18047474; <http://dx.doi.org/10.1111/j.1365-313X.2007.03341.x>
 9. Takase T, Nishiyama Y, Tanihigashi H, Ogura Y, Miyazaki Y, Yamada Y, Kiyosue T. LOV KELCH PROTEIN2 and ZEITLUPE repress *Arabidopsis* photoperiodic flowering under non-inductive conditions, dependent on FLAVIN-BINDING KELCH REPEAT F-BOX1. *Plant J* 2011; 67:608-21; PMID:21518052; <http://dx.doi.org/10.1111/j.1365-313X.2011.04618.x>
 10. Miyazaki Y, Yoshizumi T, Takase T, Matsui M, Kiyosue T. Overexpression of LOV KELCH PROTEIN 2 enhances cell elongation and increases cell number and ploidy in the hypocotyl of *Arabidopsis thaliana*. *Plant Biotech* 2011; 28:267-72; <http://dx.doi.org/10.5511/plantbiotechnology.11.0105a>
 11. Jeong J, Choi G. Phytochrome-interacting factors have both shared and distinct biological roles. *Mol Cells* 2013; 35:371-80; PMID:23708772; <http://dx.doi.org/10.1007/s10059-013-0135-5>
 12. Leivar P, Monte E, Oka Y, Liu T, Carle C, Castillon A, Huq E, Quail PH. Multiple phytochrome-interacting bHLH transcription factors repress premature seedling photomorphogenesis in darkness. *Curr Biol* 2008; 18:1815-23; PMID:19062289; <http://dx.doi.org/10.1016/j.cub.2008.10.058>
 13. Leivar P, Monte E, Al-Sady B, Carle C, Storer A, Alonso JM, Ecker JR, Quail PH. The *Arabidopsis* phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. *Plant Cell* 2008; 20:337-52; PMID:18252845; <http://dx.doi.org/10.1105/tpc.107.052142>
 14. Foreman J, Johansson H, Hornitschek P, Josse EM, Fankhauser C, Halliday KJ. Light receptor action is critical for maintaining plant biomass at warm ambient temperatures. *Plant J* 2011; 65:441-52; PMID:21265897; <http://dx.doi.org/10.1111/j.1365-313X.2010.04434.x>
 15. Franklin KA, Lee SH, Patel D, Kumar SV, Spartz AK, Gu C, Ye S, Yu P, Breen G, Cohen JD, et al. PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) regulates auxin biosynthesis at high temperature. *Proc Natl Acad Sci USA* 2011; 108:20231-5; PMID:22123947; <http://dx.doi.org/10.1073/pnas.1110682108>
 16. Jarillo JA, Capel J, Tang RH, Yang HQ, Alonso JM, Ecker JR, Cashmore AR. An *Arabidopsis* circadian clock component interacts with both CRY1 and phyB. *Nature* 2001; 410:487-90; PMID:11260718; <http://dx.doi.org/10.1038/35068589>
 17. Kim TS, Kim WY, Fujiwara S, Kim J, Cha JY, Park JH, Lee SY, Somers DE. HSP90 functions in the circadian clock through stabilization of the client F-box protein ZEITLUPE. *Proc Natl Acad Sci USA* 2011; 108:16843-8. PMID:21949396; <http://dx.doi.org/10.1073/pnas.1110406108>