PLANT SIGNALING & BEHAVIOR
2017, VOL. 12, NO. 12, e1407019 (3 pages)
https://doi.org/10.1080/15592324.2017.1407019

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION



# Protein quality control is essential for the circadian clock in plants

Kyung-Eun Gil[a] and Chung-Mo Park[a,b]

[a]Department of Chemistry, Seoul National University, Seoul, Korea; [b]Plant Genomics and Breeding Institute, Seoul National University, Seoul, Korea

**ABSTRACT**

Extreme environmental conditions, such as heat and cold, often disturb cellular proteostasis, resulting in protein denaturation and oxidative damage that threaten cell viability. Therefore, living organisms have evolved versatile protein quality control mechanisms that clear damaged proteins from cellular compartments. It has been shown that a repertoire of molecular chaperones, including heat shock proteins (HSPs), works together with ubiquitin-proteasome systems in this biochemical process in animals and yeast. However, the protein quality control systems have not been well-characterized in plants. We have recently reported that the E3 ubiquitin ligase ZEITLUPE (ZTL), a central component of the plant circadian clock, constitutes a protein quality control system in conjunction with HSP90, which is responsible for clearing denatured protein aggregates at high temperatures. The ZTL-HSP90 protein complexes are colocalized in insoluble fractions in heat-exposed plants. Notably, lack of ZTL reduces protein polyubiquitination and disrupts the robustness of circadian rhythms under heat stress conditions, providing a novel role of ZTL: it mediates a heat-responsive protein quality control to sustain the clock function. We summarize the potential roles of ZTL in thermal responses and stability of the circadian clock in plants.

**ARTICLE HISTORY**
Received 3 November 2017
Accepted 14 November 2017

**KEYWORDS**
ZTL; HSP90; protein quality control; thermotolerance; circadian clock

Eukaryotes have evolved a variety of protein quality control pathways that are capable of eliminating misfolded and denatured proteins to maintain proteostasis.[1] Cellular proteins undergo inevitable misfolding during their synthetic steps.[2] The tertiary conformations of cellular proteins are also destroyed upon exposure to sudden heat or other stressful conditions.[3] The non-natural, denature proteins form insoluble aggregates that cause severe defects in cellular functions.

Molecular chaperones are required for the ubiquitination-mediated degradation of protein aggregates. Heat shock proteins (HSPs), such as HSP70 and HSP90, are highly conserved both in animals and plants and exert their roles in various cellular responses under stressful conditions.[1-3] For example, it has been reported that HSP90 facilitates the rescue of denatured proteins or removes the protein aggregates to ensure cell fitness at high temperatures in animals and yeast.[4,5] The high conservation of molecular chaperones in plants suggests that HSP90 also plays a role in plant thermal responses.

Recently, we have demonstrated that the F-box protein ZTL, a well-known client of HSP90 in *Arabidopsis*, mediates protein turnover at high temperatures.[6] The *ZTL*-defective *ztl-105* mutant exhibits a thermo-susceptible phenotype at high temperatures in darkness. In addition, the level of heat-induced protein polyubiquitination was reduced but that of insoluble protein aggregates were elevated in the mutant, indicating that ZTL, and perhaps HSP90 as well, function in removing insoluble protein aggregates through the ubiquitin-proteasome pathways. It is known that ZTL interacts with HSP90.[7] We found that the formation of ZTL and HSP90 complex was induced, and the protein complex was detected mostly in insoluble fractions rather than in soluble fractions at high temperatures. Consistent with the functional linkage of HSP90 with ZTL, the level of protein polyubiquitination was significantly reduced in HSP90 RNAi plants. Interestingly, the functional relevance of the ZTL-HSP90 module was also proven in maintaining circadian rhythms at high temperatures. Under heat stress conditions, circadian rhythms were rapidly decomposed in *ztl-105* mutant and HSP90 RNAi plants, while Col-0 plants retained robust circadian rhythms and periods at high temperatures.

In our previous report,[6] we have observed that thermotolerance was reduced in the *ztl-105* mutant in darkness. In contrast, it has been found that ZTL protein stability was not changed under identical conditions. We examined the protein stability of ZTL under high temperature conditions in the light. The ZTL protein level was significantly elevated at high temperatures in the light (Fig. 1A). However, the transcript levels of heat-related genes were similar in Col-0 and *ztl-105* plants under identical conditions (Fig. 1B). These observations indicate that ZTL is not related with the expression of heat-responsive genes. Heat stress response is interrelated with light quality in plants. It is known that the interactive effects of heat and light quality influence floral bud development in cowpea.[8] It has been reported that phytochrome B (phyB), the red and far-red light-sensing photoreceptor functioning in photomorphogenic growth under red light, mediates heat stress response in *Arabidopsis*.[9] Accordingly, absence of phyB increases thermal tolerance, supporting that the role of

**CONTACT** Chung-Mo Park cmpark@snu.ac.kr Department of Chemistry, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, 08826, Korea.

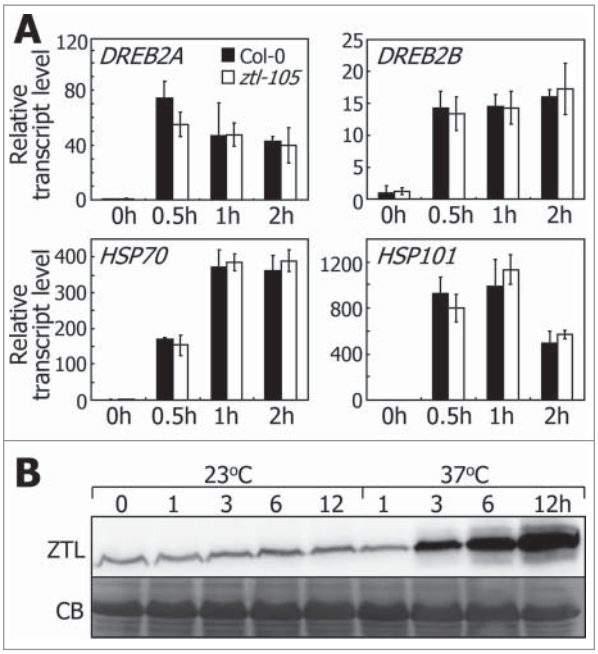


**Figure 1.** ZTL protein stability at high temperatures. (A) Expression of heat-responsive genes. Eight-day-old seedlings grown on MS-agar plates were exposed to 37°C in the light. Transcript levels were examined by RT-qPCR. Biological triplicates were averaged. Bars indicate standard error of the mean. (B) Levels of ZTL protein. The 35S:*ZTL-MYC* transgenic seedlings were grown and heat-treated as described in (A) for the indicated time durations before harvesting whole seedlings for total protein extraction. ZTL-MYC proteins were immunologically detected using an anti-MYC antibody. CB, Coomassie blue-stained membrane.

phyB in coordinating light and temperature signals.[10] ZTL is a blue light receptor.[11] It is possible that ZTL also manipulates the coordinated actions of light and temperature signals (Fig. 2).

There are several questions to be addressed on molecular schemes of the ZTL-HSP90 module in heat responses. It is currently unclear how ZTL perceives heat signals. It is also unclear whether ZTL directly or indirectly removes protein aggregates at high temperatures. In the proposed direct route, the ZTL-HSP90 complex directly detects the exposed hydrophobic residues of denatured proteins and degrades them via an ubiquitin-mediated pathway. An alternative route would be that ZTL alleviates heat stress perhaps by recruiting other as-yet-unidentified E3 ubiquitin ligases that directly target denature proteins.

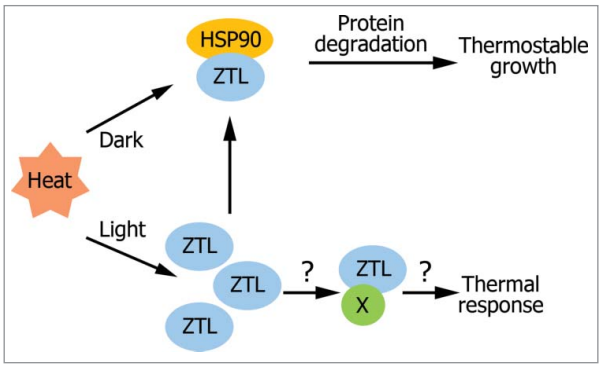


**Figure 2.** Schematic model for ZTL-mediated thermal responses. The ZTL-HSP90 complex directs the degradation of protein aggregates at high temperatures in darkness. Under this condition, ZTL protein abundance is unaltered. Upon exposure to heat stress in the light, ZTL protein abundance is elevated and modulates thermal response. It is likely that an additional factor X is involved in this process.

We found that the ZTL-HSP90 module constitutes a protein quality control system that assure thermostable growth and circadian clock.[6] More works are required to identify functional mechanisms by which the module sustains the clock function at high temperatures. Another critical issue is as to the signaling crosstalks between light quality and temperature signals. Considering the structural and functional traits of ZTL as photoreceptor and E3 ligase,[6,7,11-13] it seems that ZTL has additional roles in thermal responses in plants, depending on light signals. Addressing these questions would contribute to the functional elucidation of the signaling networks consisting of light, temperature, and circadian clock.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Acknowledgments

This work was supported by the Leaping Research (NRF-2015R1A2A1A05001636) and Global Research Lab (NRF-2012K1A1A2055546) Programs provided by the National Research Foundation of Korea and the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center No. PJ0111532015) provided by the Rural Development Administration of Korea. K.-E.G. was partially supported by the Global PH.D Fellowship Program through the National Research Foundation of Korea (NRF-2015H1A2A1034250).

## References

1. McClellan AJ, Tam S, Kaganovich D, Frydman J. Protein quality control: chaperones culling corrupt conformations. Nat Cell Biol. 2005;7(8):736–41. https://doi.org/10.1038/ncb0805-736. doi:10.1038/ncb0805-736.
2. Wickner S, Maurizi MR, Gottesman S. Posttranslational quality control: Folding, refolding, and degrading proteins. Science. 1999;286(5446):1888–93. https://doi.org/10.1126/science.286.5446.1888. doi:10.1126/science.286.5446.1888.
3. Arndt V, Rogon C, Hohfeld J. To be, or not to be – molecular chaperones in protein degradation. Cell Mol Life Sci. 2007;64(19–20):2525–41. https://doi.org/10.1007/s00018-007-7188-6. doi:10.1007/s00018-007-7188-6.
4. Samant RS, Clarke PA, Workman P. E3 ubiquitin ligase Cullin-5 modulates multiple molecular and cellular responses to heat shock protein 90 inhibition in human cancer cells. Proc Natl Acad Sci USA. 2014;111(18):6834–9. https://doi.org/10.1073/pnas.1322412111. doi:10.1073/pnas.1322412111.
5. Ehrlich ES, Wang T, Luo K, Xiao Z, Niewiadomska AM, Martinez T, Xu W, Neckers L, Yu XF. Regulation of Hsp90 client proteins by a Cullin5-RING E3 ubiquitin ligase. Proc Natl Acad Sci USA. 2009;106(48):20330–5. https://doi.org/10.1073/pnas.0810571106. doi:10.1073/pnas.0810571106.
6. Gil KE, Kim WY, Lee HJ, Faisal M, Saquib Q, Alatar A, Park CM. ZEITLUPE contributes to a thermoresponsive protein quality control system in *Arabidopsis*. Plant Cell. 2017;. https://doi.org/10.1105/tpc.17.00612. doi:10.1105/tpc.17.00612.
7. 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. 2011;108(40):16843–8. https://doi.org/10.1073/pnas.1110406108. doi:10.1073/pnas.1110406108.
8. Ahmed FE, Mutters RG, Hall AE. Interactive effects of high temperature and light quality on floral bud development in cowpea. Aust J

Plant Physiol. 1993;20(6):661–7. https://doi.org/10.1071/PP9930661. doi:10.1071/PP9930661.
9. Song J, Liu Q, Hu B, Wu W. Photoreceptor PhyB involved in *Arabidopsis* temperature perception and heat-tolerance formation. Int J Mol Sci. 2017;18(6). https://doi.org/10.3390/ijms18061194. doi:10.3390/ijms18061194.
10. Jung JH, Domijan M, Klose C, Biswas S, Ezer D, Gao M, Khattak AK, Box MS, Charoensawan V, Cortijo S, et al. Phytochromes function as thermosensors in *Arabidopsis*. Science. 2016;354(6314):886–889. https://doi.org/10.1126/science.aaf6005. doi:10.1126/science.aaf6005.
11. Liu H, Wang Q, Liu Y, Zhao X, Imaizumi T, Somers DE, Tobin EM, Lin C. *Arabidopsis* CRY2 and ZTL mediate blue-light regulation of the transcription factor CIB1 by distinct mechanisms. Proc Natl Acad Sci U S A. 2013;110(43):17582–7. https://doi.org/10.1073/pnas.1308987110. doi:10.1073/pnas.1308987110.
12. Más P, Kim WY, Somers DE, Kay SA. Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. Nature. 2003;426(6966):567–70. https://doi.org/10.1038/nature02163. doi:10.1038/nature02163.
13. Kiba T, Henriques R, Sakakibara H, Chua NH. Targeted degradation of PSEUDO-RESPONSE REGULATOR5 by an $SCF^{ZTL}$ complex regulates clock function and photomorphogenesis in *Arabidopsis thaliana*. Plant Cell. 2007;19(8):2516–30. https://doi.org/10.1105/tpc.107.053033. doi:10.1105/tpc.107.053033.