BMB Reports - Manuscript Submission

Manuscript Draft

Manuscript Number: BMB-17-064

Title: Chaperone surveillance system in plant circadian clock

Article Type: Perspective (Invited Only)

Keywords: Chaperone; Circadian clock; GIGANTEA; HSP90; ZEITLUPE

Corresponding Author: Woe-Yeon Kim

Authors: Joon-Yung Cha[1], Laila Khaleda[1], Hee Jin Park[1], Woe-Yeon Kim[1,*]

Institution: [1]Division of Applied Life Science (BK21Plus), PMBBRC & IALS, Gyeongsang National University, Jinju 52828, Republic of Korea,

Title

Chaperone surveillance system in plant circadian clock

Authors

Joon-Yung Cha[†], Laila Khaleda[†], Hee Jin Park and Woe-Yeon Kim[*]

Affiliation

Division of Applied Life Science (BK21Plus), PMBBRC & IALS, Gyeongsang National University, Jinju 52828, Republic of Korea

[†]Joon-Yung Cha and Laila Khaleda have contributed equally to the work.

[*]Corresponding author

Woe-Yeon Kim

Tel: +82-55-772-1968, Fax: +82-55-772-2631, E-mail: kim1312@gnu.ac.kr

Keywords

Chaperone, Circadian clock, GIGANTEA, HSP90, ZEITLUPE

Abbreviations

FKF1, Flavin-binding kelch repeat F-box protein 1; CDF1, Cyclic DOF factor 1; SVP, Short vegetative phase; TEM1, Tempranillo 1; COP1, Constitutive photomorphogenesis 1; SPY, Spindly; SOS2, Salt overly sensitive 2

Running head (50 characters)

Chaperoning of ZTL by GI associated with HSP90

Prospective to:

Joon-Yung Cha et al (2017), GIGANTEA is a co-chaperone which facilitates maturation of ZEITLUPE in the Arabidopsis circadian clock, Nature Communications, 8(1):3. DOI: 10.1038/s41467-016-0014-9

Abstract

Circadian clock is an internal system synchronized by external stimuli such as light and temperature and influences various physiological and developmental processes in living organisms. In Arabidopsis as a model plant, transcriptional, translational and post-translational processes are interlocked as by feedback loops between morning- and evening-phased genes. In a post-translational loop, plant-specific single-gene encoded GIGANTEA (GI) stabilize the F-box protein ZEITLUPE (ZTL) leading targeted-proteasomal degradation of TIMING OF CAB EXPRESSION 1 (TOC1) and PSEUDO-RESPONSE REGULATOR 5 (PRR5). Here, we showed that a novel biochemical function of GI as a chaperone and/or co-chaperone of Heat-Shock Protein 90 (HSP90). GI itself prevents ZTL degradation as a chaperone and facilitates ZTL maturation together with HSP90/HSP70 enhancing ZTL activity in vitro and in planta. GI has been known to be involved in a wide range of physiology and development as well as abiotic stress responses in plants, and it could interact with diverse client proteins for protein maturation. Our results provide evidence that GI helps proteostasis of ZTL by acting as a chaperone and a co-chaperone of HSP90 for proper running of circadian clock in Arabidopsis.

Circadian clock regulates numerous metabolic, physiological and developmental responses in living organisms due to the light-dark cycle with approximately 24 hours by Earth rotations. In plants, diurnal and seasonal variations are coupled with the changes of day-length and temperature which act as an input signals. It is transduced to the central circadian oscillators, such as *CCA1* (*CIRCADIAN CLOCK-ASSOCIATED 1*), *LHY* (*LATE*

ELONGATED HYPOCOTYL) and *TOC1* (*TIMING OF CAB EXPRESSION 1*) in Arabidopsis. They transcriptionally and post-transcriptionally regulate the expression of various morning-phased and evening-phased genes which are interlocked via feedback loops (Greenham and McClung (2015) Nat Rev Genet 16(10):598-610, DOI:10.1038/nrg3976). In brief, *CCA* and *LHY* peaking at dawn activate the morning-phased genes *PRR9* (*PSEUDO-RESPONSE REGULATOR 9*) and *PRR7* which repress *CCA1* and *LHY*. The PRRs negatively regulate the expression of *RVE8* (*REVEILLE 8*) which activates evening-phased genes, such as *TOC1*, *LUX* (*LUX ARRHYTHMO*) and *ELF4* (*EARLY FLOWERING 4*). Evening complex (EC) including ELF3, ELF4 and LUX represses *PRR9* and *PRR7*. Interestingly, post-translational regulation in plant circadian clock also involves the proteasomal degradation of TOC1 and PRR5 by F-box protein ZTL (ZEITLUPE) possessing blue-light sensing LOV (Light, Oxygen, Voltage) domain. ZTL protein diurnally oscillates with evening-phased peak in the absence of transcriptional changes. ZTL protein abundance is determined by vascular plant-specific single-gene encoded protein GI (GIGANTEA) which displays circadian oscillations in both mRNA and protein levels (Kim et al (2007) Nature 449(7160):356-360, DOI:10.1038/nature06132). Absence of ZTL or GI in Arabidopsis lengthens the period of clock with approximately 2 h. Thus, accumulation of GI protein helps to stabilize ZTL protein for proper running of the circadian clocks.

Molecular chaperones not only inhibit the protein denaturation and aggregation but also facilitate the protein folding and refolding. HSP90 (HEAT-SHOCK PROTEIN 90)-associated heteromeric chaperone machinery is well characterized in animal cells, and composed by HSP90, HSP70, HOP (HSP90/HSP70 ORGANIZING PROTEIN), HSP40 and p23 from capture the denatured substrates to release of refolded substrates with correctly folded states activating enzyme activity. We had been reported that diurnal rhythmicity of circadian clock in Arabidopsis was lengthened and the level of ZTL protein was diminished when cytosolic HSP90s are absent or are inactivated by Hsp90 inhibitor geldanamycin (Kim et al (2011) Proc Natl Acad Sci USA 108(40):16843-16848, DOI:10.1073/pnas.1110406108). The result showed that molecular chaperone activity of HSP90 is necessary for proper folding of ZTL protein. Based on the findings, the possible function of GI as a chaperone has been questioned to protect ZTL protein or to enhance the ZTL folding together with HSP90 to maintain the circadian clock in Arabidopsis.

Our study has suggested that GI exhibits novel chaperone activity to protect and to

enhance the refolding of a model substrate (such as malate dehydrogenase and glucose-6-phosphate dehydrogenase, respectively) as well as a real substrate ZTL in vitro (Fig. 1). Diurnal oscillation of ZTL protein without transcriptional regulation is essential to accumulate certain amount of ZTL protein and to function as an E3 ligase degrading TOC1 and PRR5 for proper running of circadian clock. The protein abundance and its enzyme activity of ZTL are regulated by the proteostasis. HSP90-based chaperone machinery is composed with various co-chaperones such as HOP, AHA (ACTIVATOR OF HSP90 ATPASE), SGT (SUPPRESSOR OF G2 ALLELE of SKP1) and p23 regulating ATPase activity of HSP90, positively or negatively. We also found that GI synergistically enhances the activity of HSP90/HSP70 for the maturation of ZTL , indicating that GI as a co-chaperone positively regulates the HSP90 chaperone and promotes ZTL maturation to activate as a F-box E3 ligase for the degradation of TOC1 and PRR5. It is consistent with the findings in planta that the accumulation or the activity of ZTL-LUC is determined by GI protein abundance. Taken together, oscillation of ZTL protein is regulated by both GI and HSP90, indicating that GI may protect ZTL protein as a chaperone and/or help the maturation of ZTL protein as a co-chaperone together with a major molecular chaperone HSP90.

GI is a large and unique plant protein without any known conserved domain, but involves in diverse physiological and developmental processes such as flowering (FKF1, CDF1, SVP, TEM1, TEM2, COP1 and ELF3 as partner proteins), circadian clock (ZTL and TOC1), light signaling, hypocotyl elongation (SPY), sucrose signaling, starch accumulation, chlorophyll accumulation, transpiration, abiotic stress response (SOS2) (Mishra and Paniqrahi (2015) Front Plant Sci 6:8, DOI:10.3389/fpls.2015.00008). However, it still needs to elucidate how GI regulates these wide range of physiological processes in plants. Our finding revealed novel biochemical function of GI as a chaperone and/or co-chaperone that could effect the maturation of large number of client proteins to regulate diverse physiological processes.

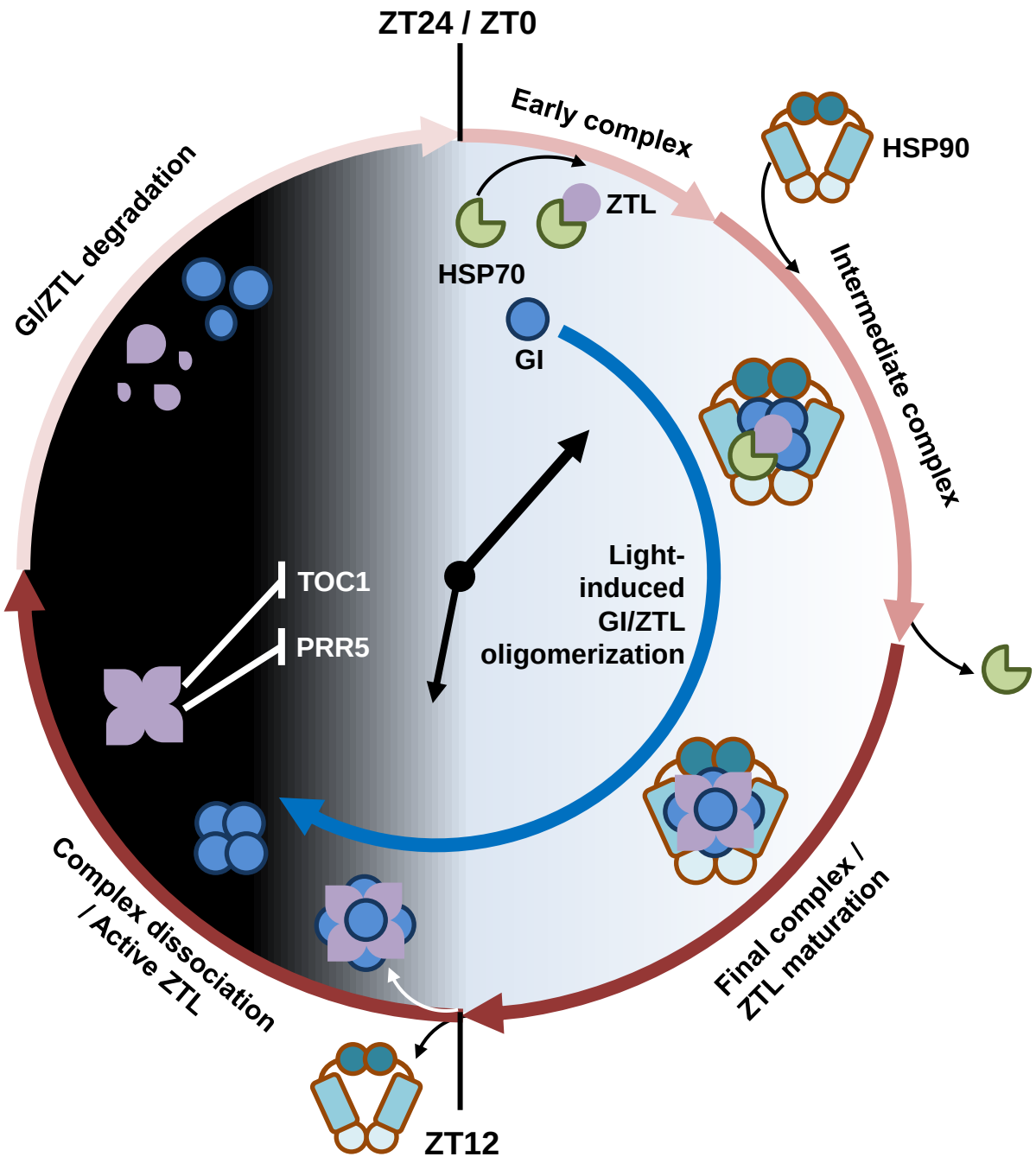
ACKNOWLEDGEMENTS

This work was supported by a grant from the Cooperative Research Program for Agriculture Science & Technology Development (project no. PJ010953042017), Rural Development Administration, Republic of Korea (WYK).

Figure legend

Fig. 1. Chaperone machinery composed of HSP90/HSP70/GI/ZTL in plant circadian clock. Blue-light sensing F-box protein ZTL oscillates with the accumulation during day and degradation during night in a post-translational loop. Nascent ZTL polypeptide may be captured by HSP70 in an early complex and then transferred to HSP90-GI to form an intermediate complex. GI as a co-chaperone of HSP90 helps ZTL maturation to activate proteasomal degradation of PRR5 and TOC1 in the night.

Fig. 1.


ZT24 / ZT0
Early complex
HSP90
ZTL
HSP70
GI
Intermediate complex
GI/ZTL degradation
TOC1
PRR5
Light-
induced
GI/ZTL
oligomerization
Complex dissociation
/ Active ZTL
Final complex /
ZTL maturation
ZT12