Plant Signaling & Behavior 9, e28193; March; © 2014 Landes Bioscience

Post-translational regulation of SHORT VEGETATIVE PHASE as a major mechanism for thermoregulation of flowering

Jeong Hwan Lee, Kyung Sook Chung, Soon-Kap Kim, and Ji Hoon Ahn*

Creative Research Initiatives; Department of Life Sciences; Korea University, Seoul, South Korea

Keywords: Alternative splicing, Ambient temperature, Ambient temperature-responsive flowering, *FLM*-β, *FLM*-δ, *SVP*, thermoregulation

Abbreviations: *FLM*, *FLOWERING LOCUS M*; *FT*, *FLOWERING LOCUS T*; GST, Glutathione *S*-transferase; His, Histidine; *SVP*, *SHORT VEGETATIVE PHASE*; *SOC1*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*; *TSF*, *TWIN SISTER OF FT*

In contrast to our extensive knowledge of vernalization, we know relatively little about the regulation of ambient temperature-responsive flowering. Recent reports revealed that *FLOWERING LOCUS M (FLM)* and *SHORT VEGETATIVE PHASE (SVP)* regulate high ambient temperature-responsive flowering through two different mechanisms: degradation of SVP protein and formation of a non-functional SVP-FLM-δ complex. To investigate further the mechanism of thermoregulation of flowering, we performed real-time quantitative polymerase chain reaction (RT-qPCR) and in vitro pull-down assays. We found that *FLM*-β and *FLM*-δ transcripts show similar absolute levels at different temperatures. Also, His-SVP protein bound to the GST-FLM-β or -δ proteins with similar binding intensities. These results suggest that functional SVP-FLM-β and non-functional SVP-FLM-δ complexes form similarly at warmer temperatures, thus indicating that post-translational regulation of SVP functions as a major mechanism for thermoregulation in flowering.

Climate change alters resource availability and growth conditions, essential factors for the survival of all organisms. In evolutionary terms, plants and animals have different survival strategies (plasticity and mobility, respectively). Plants, as sessile organisms, can flexibly adjust their development and thus adapt to continuously fluctuating environments.[1,2] For example, plant reproduction requires the proper seasonal timing of flowering, and plants adjust their flowering time primarily based on day length and temperature.[3-8] Indeed, small changes in ambient growth temperature significantly affect flowering in plants,[9,10] an observation that highlights the potential far-reaching impacts on plant ecosystems due to projected increases in mean global temperature.[11] Although numerous studies have revealed various components that regulate flowering in response to a wide range of temperatures,[12-14] our current knowledge about the regulation of ambient temperature-responsive flowering remains limited.

Recently two papers provided insight into the basic mechanisms controlling ambient temperature-responsive flowering in *Arabidopsis*.[15-17] These reports showed that FLOWERING LOCUS M (FLM) and SHORT VEGETATIVE PHASE (SVP) proteins form a repressor complex to repress flowering at colder temperatures by direct binding to the genomic regions of floral activator genes like *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). However, they also reported different mechanisms for thermoregulation of flowering. We showed that the reduced stability of SVP protein at warmer temperatures leads to decreased levels of the SVP-FLM repressor complex, thereby inducing early flowering at that temperature. By contrast, Posé et al. (2013) showed that increased temperature leads to higher levels of FLM-δ protein; in this model, SVP protein interacts with FLM-δ protein to form a non-functional complex with impaired DNA-binding ability, thereby accelerating flowering.

Here, we examined these two models by measuring the absolute levels of *FLM*-β and *FLM*-δ transcripts at different temperatures, and examining the in vitro interaction between SVP and FLM-β or FLM-δ proteins. SVP-like proteins have conserved functions across plant species,[18-21] and our results suggest that plants may preferentially use post-translational regulation of SVP protein levels at warmer temperatures to regulate ambient temperature-responsive flowering.

Alternative splicing of *FLM* is temperature-dependent, and *FLM*-β and *FLM*-δ transcripts are highly expressed at 16 °C and 27 °C, respectively[15,16]; therefore, we measured absolute

*Corresponding author: Ji Hoon Ahn; Email: jahn@korea.ac.kr
Submitted: 02/03/2014; Accepted: 02/12/2014; Published Online: 03/10/2014
Citation: Hwan Lee J, Sook Chung K, Kim SK, Ahn JH. Post-translational regulation of SHORT VEGETATIVE PHASE as a major mechanism for thermoregulation of flowering. Plant Signaling & Behavior 2014; 9:e28193; PMID: 24614351; http://dx.doi.org/10.4161/psb.28193

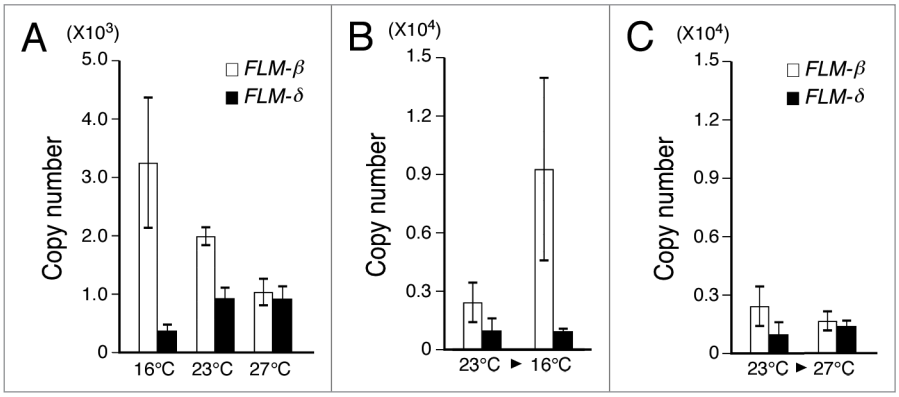

Figure 1. Absolute quantification of *FLM*-β and *FLM*-δ transcripts at different temperatures. (**A**) Copy numbers of *FLM*-β and *FLM*-δ transcripts in 8-d-old Col seedlings grown at 16 °C, 23 °C, and 27 °C under long-day (LD) conditions. (**B** and **C**) Copy numbers of *FLM*-β and *FLM*-δ transcripts in temperature-shifted Col seedlings under LD conditions. Error bars indicate standard deviation of one biological replicate with three technical replicates.

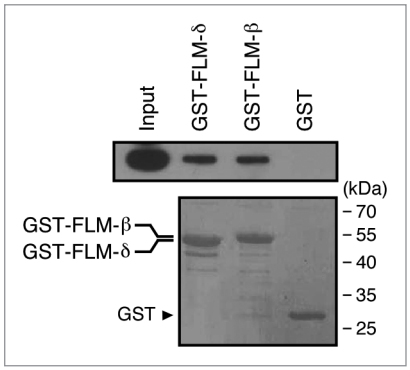

Figure 2. In vitro pull-down assay between His-SVP protein and GST-FLM-β or GST-FLM-δ proteins. Anti-GST antibody was used for pull-down. The eluates were separated by 12.5% SDS-PAGE, transferred to polyvinylidene difluoride membranes, and probed with anti-His antibody. About 10% of His-SVP protein was loaded as an input control. The amounts and qualities of the GST-tagged proteins tested are shown below.

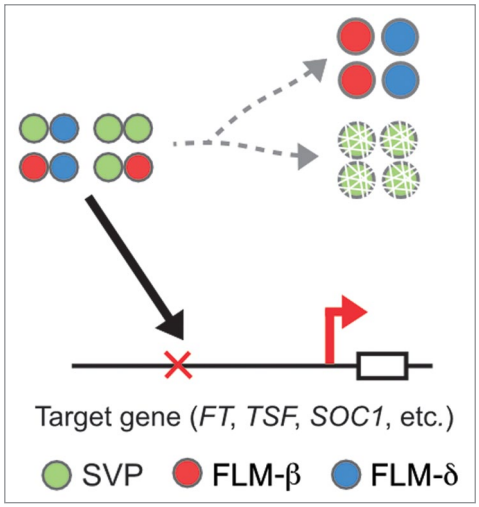

Figure 3. A proposed model to explain the thermal induction of flowering at warmer temperatures. When temperature increases, the absolute expression levels of two different *FLM* transcripts are almost identical and two different FLM proteins have similar binding affinities for SVP protein. Ultimately, this leads to formation of similar amounts of functional SVP-FLM-β and non-functional SVP-FLM-δ complexes. Because SVP protein levels are rapidly reduced through post-translational regulation,[15] the amounts of functional SVP-FLM-β complex are also reduced, which would alleviate the repression of the transcription of downstream target genes, thereby accelerating flowering at that temperature.

transcripts at different temperatures. We calculated their absolute copy numbers using standard curves of *FLM*-β and *FLM*-δ transcripts, as previously reported.[22] At 16 °C, the copy numbers of *FLM*-β transcripts were higher than those of *FLM*-δ transcripts; by contrast, at 27 °C the copy numbers of *FLM*-β and *FLM*-δ transcripts were nearly identical (**Fig. 1A**). Also, after a shift from 23 °C to 16 °C, the copy numbers of *FLM*-β transcripts increased within 1 d (**Fig. 1B**). However, after a shift from 23 °C to 27 °C, the copy numbers of *FLM*-β and *FLM*-δ transcripts were almost the same (**Fig. 1C**). This suggested that the plants produce similar amounts of *FLM*-β and *FLM*-δ transcripts at higher temperatures.

Because FLM-β and FLM-δ proteins both interact with SVP protein,[15,16] we also compared the binding affinity of SVP to FLM-β or FLM-δ proteins in vitro. We used affinity-purified histidine (His)-SVP, Glutathione *S*-transferase (GST)-FLM-β, and GST-FLM-δ proteins expressed in *Escherichia coli*. To test binding, we incubated His-SVP and GST-FLM-β or GST-FLM-δ proteins, used anti-GST antibody to pull down the FLM proteins, then used anti-His antibody to detect SVP by western blot. This in vitro assay revealed that the SVP protein bound to the GST-FLM-β or GST-FLM-δ proteins with similar intensities (**Fig. 2**, lanes 2 and 3); however, GST protein did not bind to SVP protein (lane 4). This suggested that the binding strengths of SVP protein to FLM-β or FLM-δ proteins are substantially similar.

Based on these results, we propose that decrease in functional SVP-FLM-β complex caused by rapid degradation of SVP protein at warmer temperatures functions as a more important

mechanism for thermoregulation in flowering than alterations in *FLM*-β and *FLM*-δ transcripts (**Fig. 3**). At warmer temperatures, the absolute levels of *FLM*-β and *FLM*-δ transcripts are almost identical, and two different FLM proteins produced by two spliced transcripts have similar binding affinities for SVP protein. This results in the formation of functional SVP-FLM-β and non-functional SVP-FLM-δ complexes in similar amounts. However, rapid degradation of SVP proteins at high temperatures reduces the abundance of the functional SVP-FLM-β complex, which can bind to the genomic regions of downstream target genes like *FT*, *TWIN SISTER OF FT* (*TSF*), and *SOC1*, thereby inducing flowering at that temperature. However, we cannot exclude several possibilities, including that formation of non-functional SVP-FLM-δ complex facilitates the degradation of SVP protein, and that FLM-β or FLM-δ proteins also could be subject to degradation. Thus, it will be interesting to investigate which conditions, such as the formation of SVP-FLM-δ complex, facilitate the degradation of SVP protein, and how degradation of FLM proteins contribute to ambient temperature-responsive flowering.

Disclosure of potential conflicts of interests

No potential conflicts of interests were disclosed.

Acknowledgments

This work was supported by a National Research Foundation of Korea grant funded by the Korea government (Ministry of Science, ICT, and Future Planning) (2008-0061988) to J.H.A.

References

1. Anderson JT, Inouye DW, McKinney AM, Colautti RI, Mitchell-Olds T. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proc Biol Sci 2012; 279:3843-52; PMID:22787021; http://dx.doi.org/10.1098/rspb.2012.1051
2. Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, et al. Plant phenotypic plasticity in a changing climate. Trends Plant Sci 2010; 15:684-92; PMID:20970368; http://dx.doi.org/10.1016/j.tplants.2010.09.008
3. Lee JH, Lee JS, Ahn JH. Ambient temperature signaling in plants: an emerging field in the regulation of flowering time. J Plant Biol 2008; 51:321-6; http://dx.doi.org/10.1007/BF03036133
4. McClung CR, Davis SJ. Ambient thermometers in plants: from physiological outputs towards mechanisms of thermal sensing. Curr Biol 2010; 20:R1086-92; PMID:21172632; http://dx.doi.org/10.1016/j.cub.2010.10.035
5. Samach A, Wigge PA. Ambient temperature perception in plants. Curr Opin Plant Biol 2005; 8:483-6; PMID:16054430; http://dx.doi.org/10.1016/j.pbi.2005.07.011
6. Penfield S. Temperature perception and signal transduction in plants. New Phytol 2008; 179:615-28; PMID:18466219; http://dx.doi.org/10.1111/j.1469-8137.2008.02478.x
7. Song YH, Ito S, Imaizumi T. Flowering time regulation: photoperiod- and temperature-sensing in leaves. Trends Plant Sci 2013; 18:575-83; PMID:23790253; http://dx.doi.org/10.1016/j.tplants.2013.05.003
8. Kobayashi Y, Weigel D. Move on up, it's time for change--mobile signals controlling photoperiod-dependent flowering. Genes Dev 2007; 21:2371-84; PMID:17908925; http://dx.doi.org/10.1101/gad.1589007
9. Craufurd PQ, Wheeler TR. Climate change and the flowering time of annual crops. J Exp Bot 2009; 60:2529-39; PMID:19505929; http://dx.doi.org/10.1093/jxb/erp196
10. Fitter AH, Fitter RS. Rapid changes in flowering time in British plants. Science 2002; 296:1689-91; PMID:12040195; http://dx.doi.org/10.1126/science.1071617
11. Lenoir J, Gégout JC, Marquet PA, de Ruffray P, Brisse H. A significant upward shift in plant species optimum elevation during the 20th century. Science 2008; 320:1768-71; PMID:18583610; http://dx.doi.org/10.1126/science.1156831
12. Sung S, Amasino RM. Remembering winter: toward a molecular understanding of vernalization. Annu Rev Plant Biol 2005; 56:491-508; PMID:15862105; http://dx.doi.org/10.1146/annurev.arplant.56.032604.144307
13. Delk NA, Johnson KA, Chowdhury NI, Braam J. CML24, regulated in expression by diverse stimuli, encodes a potential Ca2+ sensor that functions in responses to abscisic acid, daylength, and ion stress. Plant Physiol 2005; 139:240-53; PMID:16113225; http://dx.doi.org/10.1104/pp.105.062612
14. Kim HJ, Hyun Y, Park JY, Park MJ, Park MK, Kim MD, Kim HJ, Lee MH, Moon J, Lee I, et al. A genetic link between cold responses and flowering time through *FVE* in *Arabidopsis thaliana*. Nat Genet 2004; 36:167-71; PMID:14745450; http://dx.doi.org/10.1038/ng1298
15. Lee JH, Ryu HS, Chung KS, Posé D, Kim S, Schmid M, Ahn JH. Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. Science 2013; 342:628-32; PMID:24030492; http://dx.doi.org/10.1126/science.1241097
16. Posé D, Verhage L, Ott F, Yant L, Mathieu J, Angenent GC, Immink RG, Schmid M. Temperature-dependent regulation of flowering by antagonistic FLM variants. Nature 2013; 503:414-7; PMID:24067612; http://dx.doi.org/10.1038/nature12633
17. Nilsson O. Plant science. A pathway to flowering--why staying cool matters. Science 2013; 342:566-7; PMID:24179209; http://dx.doi.org/10.1126/science.1245861
18. Wu RM, Walton EF, Richardson AC, Wood M, Hellens RP, Varkonyi-Gasic E. Conservation and divergence of four kiwifruit SVP-like MADS-box genes suggest distinct roles in kiwifruit bud dormancy and flowering. J Exp Bot 2012; 63:797-807; PMID:22071267; http://dx.doi.org/10.1093/jxb/err304
19. Trevaskis B, Tadege M, Hemming MN, Peacock WJ, Dennis ES, Sheldon C. Short vegetative phase-like MADS-box genes inhibit floral meristem identity in barley. Plant Physiol 2007; 143:225-35; PMID:17114273; http://dx.doi.org/10.1104/pp.106.090860
20. Lee JH, Park SH, Ahn JH. Functional conservation and diversification between rice OsMADS22/OsMADS55 and Arabidopsis SVP proteins. Plant Sci 2012; 185-186:97-104; PMID:22325870; http://dx.doi.org/10.1016/j.plantsci.2011.09.003
21. Ramamoorthy R, Phua EE, Lim SH, Tan HT, Kumar PP. Identification and characterization of RcMADS1, an AGL24 ortholog from the holoparasitic plant Rafflesia cantleyi Solms-Laubach (Rafflesiaceae). PLoS One 2013; 8:e67243; PMID:23840638; http://dx.doi.org/10.1371/journal.pone.0067243
22. Yoo SJ, Chung KS, Jung SH, Yoo SY, Lee JS, Ahn JH. BROTHER OF FT AND TFL1 (BFT) has TFL1-like activity and functions redundantly with TFL1 in inflorescence meristem development in Arabidopsis. Plant J 2010; 63:241-53; PMID:20409005; http://dx.doi.org/10.1111/j.1365-313X.2010.04234.x