https://doi.org/10.1093/plcell/koad148

THE PLANT CELL

THE PLANT CELL 2023: 35: 2711–2712

In the heat of the moment: The miR165/166-*PHB* module mediates thermotolerance in Arabidopsis

Sophie Hendrix [1,2,*]

1 Assistant Features Editor, The Plant Cell, American Society of Plant Biologists, USA
2 Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium

*Author for correspondence: sophie.hendrix@uhasselt.be

In Brief

The increasing occurrence of heat waves due to climate change forms a major threat to global food security because high temperatures negatively impact plant growth and crop yield (Bailey-Serres et al. 2019). Hence, it is crucial to increase our knowledge of the molecular mechanisms underlying plant responses to heat stress to hasten the development of strategies to enhance plant thermotolerance. In *Arabidopsis thaliana*, class A1 heat stress transcription factors (HSFA1s) function as master regulators of heat stress responses. HSFA1s transcriptionally induce *HSFA2*, which in turn controls the expression of many heat-responsive genes, including those encoding heat shock proteins (HSPs) (Yoshida et al. 2011).

In this issue, **Jie Li and colleagues (Li et al. 2023)** identify the role of a miR165/166-*PHABULOSA* (*PHB*) module in transcriptional and posttranslational regulation of HSFA1s (see Fig.). The highly conserved miRNAs miR165/166 regulate a variety of cellular processes by controlling transcript levels of Class III HD-ZIP transcription factors, such as *PHB* and *PHAVOLUTA* (*PHV*), which are important regulators of radial patterning in shoots (McConnell et al. 2001).

In the first set of experiments, the authors showed that heat stress strongly induced the expression of miR165/166, leading to decreased transcript levels of *PHB* and *PHV*. To further explore the role of these 4 genes in heat stress responses, several transgenic lines were grown on agar plates or soil at ambient temperature and then subjected to a temperature of 37 °C. Whereas transgenic lines overexpressing miR165/166 and a *phb phv* double mutant displayed a higher thermotolerance compared with wild-type plants, the opposite was observed for miR165/166 knockdown lines and plants expressing a miR165/166 cleavage-resistant form of *PHB*.

Chromatin immunoprecipitation and electrophoretic mobility shift assays revealed that both *HSFA1a* and *HSFA1b* are direct PHB targets, and a dual-luciferase assay showed that their expression is negatively regulated by PHB. Together, these data suggest an important role of the miR165/166-*PHB* module in transcriptional control of plant heat stress responses. RNA-sequencing experiments then confirmed that many heat stress–responsive genes, including *HSFA2*, were downregulated in plants expressing a miR165/166 cleavage-resistant form of *PHB* and in miR165/166 knockdown lines and were upregulated in miR165/166-overexpressing lines. Interestingly, PHB was shown to directly bind to the *HSFA2* promoter and negatively affect *HSFA2* expression, indicating that *HSFA2* is a common target of both PHB and HSFA1s.

In the next part of the study, the authors demonstrated that PHB also controls HSFA1s at the posttranslational level. Using a combination of in vitro and in vivo approaches, they showed that PHB physically interacts with both HSFA1a and HSFA1b. PHB had an antagonistic effect on HSFA1b transcriptional activity by interfering with its DNA binding ability. Transcriptional responses to heat stress were similar between seedlings expressing a miR165/166 cleavage-resistant form of *PHB* and an *hsfa1* quadruple mutant, indicating that PHB and HSFA1s coregulate transcriptional reprogramming in response to heat stress. A final set of experiments revealed that the miR165/166-PHB module functions upstream of HSFA1s because the enhanced thermotolerance of transgenic lines overexpressing miR165/166 was suppressed in a *hsfa1a hsfa1b hsfa1d* triple mutant.

In conclusion, this study established the importance of the miR165/166-*PHB* module in plant heat stress responses and identified miRNAs as potential targets to enhance plant thermotolerance. However, the molecular mechanisms functioning upstream of the miR165/166-*PHB* module remain to be identified. In future work, it would be interesting to assess whether

Received May 17, 2023. Accepted May 18, 2023. Advance access publication May 24, 2023

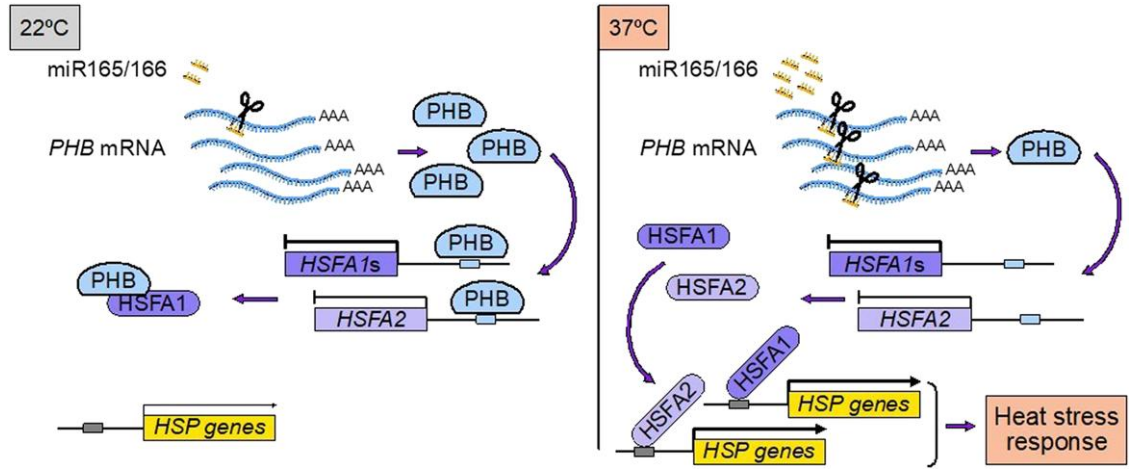

Figure. Schematic overview of the involvement of the miR165/166-*PHB* module in heat stress–induced transcriptional reprogramming in Arabidopsis. Reprinted from Li et al. (2023), Figure 10.

this regulatory module also contributes to thermomemory, the phenomenon of acquired thermotolerance after exposure to mild or transient heat stress (Balazadeh 2022).

References

Bailey-Serres J, Parker JE, Ainsworth EA, Oldroyd GE, Schroeder JI. Genetic strategies for improving crop yields. Nature. 2019: **575**(7781):109–118. https://doi.org/10.1038/s41586-019-1679-0

Balazadeh S. A 'hot' cocktail: the multiple layers of thermomemory in plants. Curr Opin Plant Biol. 2022:**65**:102147. https://doi.org/10.1016/j.pbi.2021.102147

Li Y, Cao Y, Zhang J, Zhu C, Tang G, Yan J. The miR165/166-*PHABULOSA* module promotes thermotolerance by transcriptionally and post-translationally regulating HSFA1. Plant Cell. 2023; **35**(8):2952–2971. https://doi.org/10.1093/plcell/koad121

McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK. Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots. Nature. 2001:**411**(6838):709–713. https://doi.org/10.1038/35079635

Yoshida T, Ohama N, Nakajima J, Kidokoro S, Mizoi J, Nakashima K, Maruyama K, Kim JM, Seki M, Todaka D, et al. Arabidopsis HsfA1 transcription factors function as the main positive regulators in heat shock-responsive gene expression. Mol Genet Genom. 2011:**286**(5-6): 321–332. https://doi.org/10.1007/s00438-011-0647-7