Gibberellins negatively regulate low temperature-induced anthocyanin accumulation in a HY5/HYH-dependent manner

Yongqiang Zhang, Zhongjuan Liu, Rongzhi Liu, Hualing Hao and Yurong Bi*

School of Life Sciences; Lanzhou University; Lanzhou Gansu, China

Low temperature could significantly induce anthocyanin accumulation in the presence of light. Recently, two bZIP transcription factors LONG HYPOCOTYL 5 (HY5) and HOMOLOG OF HY5 (HYH) were identified to play an important role in the process of low temperature-induced anthocyanin accumulation. However, the mechanism by which HY5/HYH regulates anthocyanin accumulation under low temperature still remains unclear. Here, we found that the gibberellins (GAs) could decrease but PAC (endogenous GAs biosynthesis inhibitor) increases the low temperature-induced anthocyanin accumulation, implying that GAs signaling may involve in this process. Furthermore, the transcript level of *GA2ox1*, encoding a major member of bioactive GAs-deactivating enzymes, was significantly upregulated by low temperature in a HY5/HYH-dependent manner. Moreover, *hy5 hyh* mutant was insensitive to PAC in enhancing anthocyanin accumulation under low temperature. From these data we propose that, together with HY5/HYH, GA signaling may play an important role during low temperature-induced anthocyanin accumulation.

Light, as the most influential environmental factor, not only provides the source of energy for plant life, but also acts as a signal affecting plant growth and development throughout the entire life cycle from germination to flowering.[1,2] Light perceived by photoreceptors will induce activation of genes in photomorphogenesis as strikingly characterized by short hypocotyls, cotyledon expansion and greening.[3] Lots of downstream components of photoreceptors have been identified, such as Long Hypocotyl 5 (HY5) and HY5 Homolog (HYH).[4,5] HY5, a bZIP protein, is the first known and most extensively studied transcription factor involved in promoting photomorphogenesis.[4] Mutation in *HY5* will cause a defect in inhibition of the hypocotyls elongation under all light conditions, suggesting that HY5 acts downstream of all photoreceptors and HY5 is crucial in light signaling.[2,4]

Phytohormone gibberellins (GAs) control a wide range of processes during plant development, including seed germination, leaf expansion, stem and root elongation, flowering time, and flower and fruit development.[6] GA signaling is generally believed to hinge to DELLA proteins, a family of nuclear growth-repressing proteins.[7] The production of active GAs is regulated through modulation of biosynthesis and inactivation. GA 2-oxidases (GA2ox) can inactivate most active GAs.[6,7] Reduced bioactive GA levels will cause an increase of DELLA proteins.[7,8] It has been clearly proven that DELLAs are major components of the adaptively significant mechanism via which light regulates plant growth during photomorphogenesis.[9-12]

Interestingly, recent reports indicated an important function of DELLAs under environmental stresses. It was found that salt stress partially induces DELLA protein stabilization and accumulation and restricts plant growth.[13-15] Moreover, DELLA proteins will accumulate to a relative high level after cold treatment and also will confer higher freezing tolerance in Arabidopsis plants.[16]

Anthocyanins are often induced by many environmental factors, such as light, low temperature and so on.[17] It has been shown that HY5/HYH play an important role during low temperature-induced anthocyanin accumulation.[18] However, how HY5/HYH function in this process still remains elusive. Given that GAs are involved in both light singling and low temperature response,[9,10,16] whether GA signaling is involved in low temperature-induced anthocyanin accumulation becomes a question to be addressed. Very interestingly, it was found that exogenous GA_3 (an active form of GAs) significantly reduced the level of low temperature-induced anthocyanin, whereas PAC conversely increased anthocyanin level (**Fig. 1B**). As a control, GA_3 and PAC only slightly affect the anthocyanin levels at normal temperature (23°C) (**Fig. 1A**). This result at least suggests that GAs could negatively regulate low temperature-induced anthocyanin accumulation, although it's still unknown whether GAs regulates this process directly or indirectly through deregulation of DELLAs. However, Achard et al. recently indicated that RGA, a main DELLA member in Arabidopsis, accumulated after low temperature treatment,[16] implying that the negative regulation
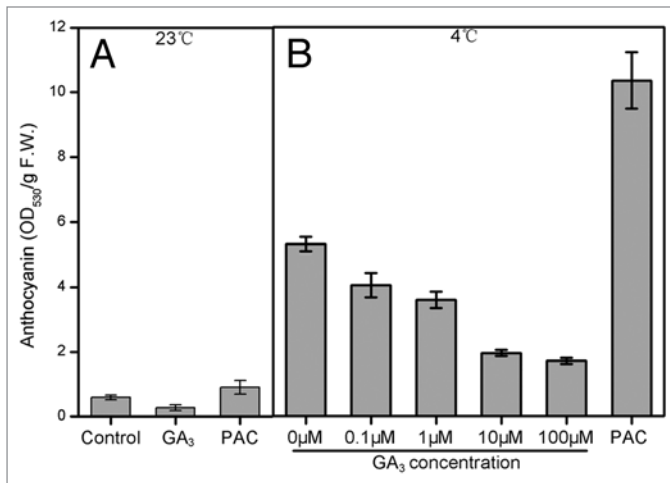
Figure 1. Effects of exogenous GA$_3$ and PAC on anthocyanin accumulation. Col-0 seedlings grown at control condition for four days were treated by exogenously supplied 10 μM GA$_3$ and 1 μM PAC at 23°C (A), or different concentration of GA$_3$ and 10 μM PAC at 4°C (B) for 3 days. Data are mean values ± SE of at least four independent experiments.
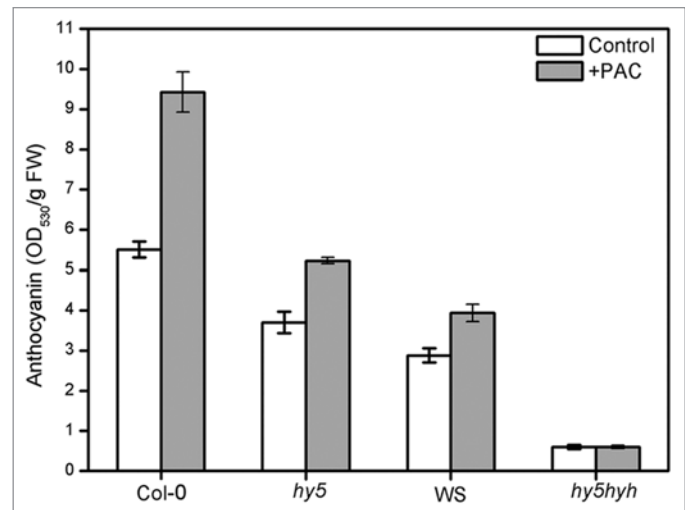

Figure 3. Effect of PAC (1 μM) on anthocyanin contents in Col-0, *hy5*, WS and *hy5 hyh* at 4°C. Four-day-old seedlings were treated at 4°C in the presence of constant white light for three days, and anthocyanin contents of the seedlings were determined. Data are mean values ± SE of at least four independent experiments.
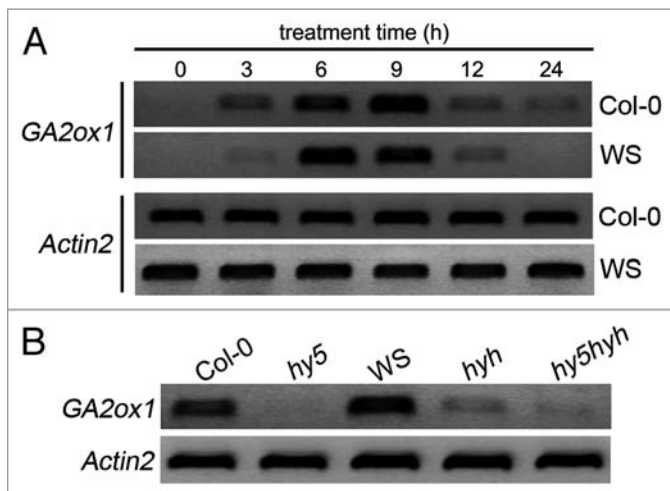

Figure 2. Effect of low temperature (4°C) on the expression of *GA2ox1*. (A) Col-0 and WS plants grown for four days in the control condition were treated under low temperature in the presence of constant white light for indicated time. (B) Low temperature induced upregulation of *GA2ox1* is impaired in *hy5*, *hyh* and *hy5 hyh* mutants. Four-day-old seedlings of Col-0, WS, *hy5*, *hyh* and *hy5 hyh* were treated under low temperature (4°C) in the presence of constant white light for 6 h. The gel images shown here are representative of up to three independent experiments.

of low temperature-induced anthocyanin accumulation by GAs is likely achieved by DELLAs degradation. Low temperature was able to stimulate *GA2ox1* expression, as a result, endogenous GAs decreased and DELLAs accumulated.[16] We also found that low temperature markedly upregulated the *GA2ox1* expression (**Fig. 2A**). Moreover, it was intriguing that this upregulation was strongly impaired in *hy5* and *hy5 hyh* mutants (**Fig. 2B**), indicating that the effect of low temperature treatment is HY5/HYH-dependent.

So, it was proposed that the DELLAs accumulation under low temperature might also be HY5/HYH-dependent. Accordingly, PAC enhanced anthocyanin level under low temperature was impaired in *hy5* and *hy5 hyh* mutants (**Fig. 3**), implying that the effect of DELLAs on anthocyanin accumulation is HY5/HYH-dependent. In other words, the reduction of GAs enhanced anthocyanin accumulation under low temperature may depend on HY5/HYH. Interestingly, it was recently found that GAs reduction in Arabidopsis seedlings could enhance HY5/HYH activity.[10] We assume that the accumulation of DELLAs under low temperature could enhance the anthocyanin accumulation through enhancing the HY5/HYH transcription activity. In a word, HY5/HYH plays a central role during this process. **Figure 4** represents a proposed model of how HY5/HYH and GA signaling regulate low temperature-induced anthocyanin accumulation: in the presence of light, low temperature elevates *GA2ox1* expressions in the presence of HY5/HYH; increased *GA2ox1* expression results in decrease of endogenous active GAs and induction of DELLA protein accumulation, which in turn enhance the transcription activity of HY5 and HYH on the expression of *DFR*. However, more investigation is still needed to clearly identify the role of both HY5/HYH and GA signaling in low temperature inducing anthocyanin accumulation.
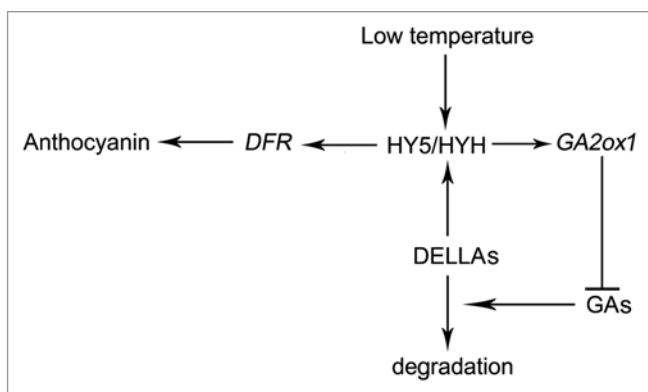
Figure 4. A flow scheme based on the results obtained from our studies and others showing components involved in signal transduction under low temperature inducing anthocyanin accumulation. Arrowheads and tees indicate positive and negative regulation, respectively.

References

1. Jiao Y, Lau OS, Deng XW. Light-regulated transcriptional networks in higher plants. Nat Rev Genet 2007; 8:217-30.
2. Lee J, He K, Stolc V, Lee H, Figueroa P, Gao Y, et al. Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell 2007; 19:731-49.
3. Lorrain S, Genoud T, Fankhauser C. Let there be light in the nucleus! Curr Opin Plant Biol 2006; 9:509-14.
4. Oyama T, Shimura Y, Okada K. The Arabidopsis HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. Genes Dev 1997; 11:2983-95.
5. Holm M, Ma LG, Qu LJ, Deng XW. Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in Arabidopsis. Genes Dev 2002; 16:1247-59.
6. Yamaguchi S. Gibberellin metabolism and its regulation. Annu Rev Plant Biol 2008; 59:225-51.
7. Hartweck LM. Gibberellin signaling. Planta 2008; 229:1-13.
8. Achard P, Genschik P. Releasing the brakes of plant growth: How GAs shutdown DELLA proteins. J Exp Bot 2009; 60:1085-92.
9. Achard P, Liao L, Jiang C, Desnos T, Bartlett J, Fu X, et al. DELLAs contribute to plant photomorphogenesis. Plant Physiol 2007; 143:1163-72.
10. Alabadí D, Gallego-Bartolomé J, Orlando L, Gárcia-Carcel L, Rubio V, Martínez C, et al. Gibberellins modulate light signaling pathways to prevent Arabidopsis seedling de-etiolation in darkness. Plant J 2008; 53:324-35.
11. de Lucas M, Davière JM, Rodríguez-Falcón M, Pontin M, Iglesias-Pedraz JM, Lorrain S, et al. A molecular framework for light and gibberellin control of cell elongation. Nature 2008; 451:480-4.
12. Feng S, Martinez C, Gusmaroli G, Wang Y, Zhou J, Wang F, et al. Coordinated regulation of *Arabidopsis thaliana* development by light and gibberellins. Nature 2008; 451:475-9.
13. Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, et al. Integration of plant responses to environmentally activated phytohormonal signals. Science 2006; 311:91-4.
14. Achard P, Renou JP, Berthomé R, Harberd NP, Genschik P. Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Curr Biol 2008; 18:656-60.
15. Magome H, Yamaguchi S, Hanada A, Kamiya Y, Oda K. The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, *GA2ox7*, under high-salinity stress in Arabidopsis. Plant J 2008; 56:613-26.
16. Achard P, Gong F, Cheminant S, Alioua M, Hedden P, Genschik P. The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. Plant Cell 2008; 20:2117-29.
17. Hatier JH, Gould KS. Foliar anthocyanins as modulators of stress signals. J Theor Biol 2008; 253:625-7.
18. Zhang Y, Zheng S, Liu Z, Wang L, Bi Y. Both HY5 and HYH are necessary regulators for low temperature-induced anthocyanin accumulation in Arabidopsis seedlings. J Plant Physiol 2011; 168:367-74.