Brassinosteroids (BRs) are steroid plant hormones with important roles in plant growth and development through the regulation of processes such as cell division and expansion. In addition to their role as growth promoters, BRs can also protect plants from a variety of abiotic stresses, including drought, salt, heat, and cold stress. Nevertheless, the molecular determinants underlying this protective effect remain largely uncharacterized. Two very recent works have shed light on the molecular mechanisms by which BRs contribute to increase plant tolerance to freezing temperatures (Eremina et al., 2016a; Li et al., 2017). They present solid genetic evidence of BR involvement in Arabidopsis constitutive freezing tolerance and cold acclimation, an adaptive response whereby plants increase their constitutive freezing tolerance after exposure to low non-freezing temperatures. Interestingly, exogenous BR application increases the expression of C-repeat binding factor (CBF) genes, which encode a small family of transcription factors that control a significant portion of cold-regulated genes (COR) and are required for the adequate development of cold acclimation response (Eremina et al., 2016a). Indeed, gene expression analysis in diverse BR-signaling mutants indicates that BRs enhance freezing tolerance at least in part through activation of the CBF-COR pathway (Figure 1) (Eremina et al., 2016a; Li et al., 2017). Furthermore, two BR-responsive transcription factors, Brassinazole-resistant 1 (BZR1) and CESTA (CES), are characterized as direct regulators of CBF expression through their binding to the promoters of these genes (Eremina et al., 2016a; Li et al., 2017) (Figure 1).

Attaining a clear picture of the way BRs signal the perception of low temperature and elicit a response still requires, however, the elucidation of a number of issues. For instance, the dynamics of BRs levels during the cold acclimation response remains unknown. This is a relevant question since it has been reported that some important genes involved in BR biosynthesis are quickly downregulated after cold exposure, which has been interpreted as a negative regulatory feedback loop triggered by the activation of the BR-signaling pathway (Eremina et al., 2016a). A detailed time-course, and perhaps tissue-specific, study on BR biosynthesis all along the cold response is essential. To better understand the role of BRs in low temperature signaling, the clarification of some discrepancies existing between the two works discussed here is also needed. While Eremina et al. (2016a) reported that freezing tolerance under non-acclimated conditions was either not affected or even decreased in constitutive BR-signaling mutants, Li et al. (2017) describe that these type of mutants are more tolerant than the wild-type in both non-acclimated and cold-acclimated conditions. These differences may arise from the fact that they used plants from different developmental stages and/or grown under distinct experimental conditions. In this sense, growth conditions have been described to be decisive for correct hormone signaling in cold acclimation (Catalá et al., 2014). Future research on the function of BRs in freezing tolerance and cold acclimation should also address the possible involvement of additional elements of BR signaling in the elicitation of a cold response. For example, it has been shown that disruption of BR perception at the plasma membrane level decreases freezing tolerance (Eremina et al., 2016a), yet how the BR signal is transduced during cold response is unknown. In addition, Li et al. (2017) report that ARABIDOPSIS SHAGGY-LIKE KINASES (ASKs), negative regulators of the BR signal, determine the levels of activated BZR1 through their kinase activity (Figure 1). However, while the levels of active dephosphorylated BZR1 are increased by low temperature, the levels of ASK21/BIN2 are unchanged (Li et al., 2017). Interestingly, ASK21/BIN2 activity has been shown to be inhibited by the deacetylase HDA6 (Hao et al., 2016), which has also been involved in cold acclimation (To et al., 2011) (Figure 1). Thus, an inviting hypothesis is that HDA6 might be inhibiting ASK21/BIN2 activity after cold exposure. Finally, the relative contribution of BR signaling to the activation of the CBF-COR pathway requires further study. The reason for this is the observation that the enhancement of constitutive freezing tolerance caused by the gain-of-function bsr1-1D mutation seems independent of the function of CBFs (Li et al., 2017). Moreover, the cold upregulation of the CBF-COR pathway does not appear to be severely compromised in a quadruple mutant cesta bee1 bee2 bee3 lacking four BR-responsive transcription factors (Eremina et al., 2016a). Nonetheless, the evidence that both BZR1 and CESTA bind to CBF promoters is conclusive, which indicates a direct role of these transcription factors in the induction of the pathway. Perhaps functional redundancy among additional BR-signaling transcription factors may account for the relatively mild effect of these mutations on CBF-COR cold activation. On the other hand, the use of whole plants in both laboratories for these analyses may have masked a tissue-specific regulation of the CBF-COR pathway by BRs.

Regardless of the issues that require further research, it is noteworthy to highlight the importance of the discovery of two novel CBF regulators that respond to BR signaling. To date, a dozen factors have been described, including ICE1, ICE2, MYB15, CAMTA1, CAMTA3, EIN3, SOC1, PIF4, PIF7, CCA1 (Seo et al., 2009; Dong et al., 2011; Lee and Thomashow, 2012; Eremina et al., 2016b), and now BZR1 and CESTA (Eremina et al., 2016a; Li et al., 2017), with CBF promoter-binding activity. This circumstance raises several interesting questions: how do these factors interact with each other? How do these interactions condition the expression of CBFs and the subsequent development of cold acclimation? In the case of ICE1 and MYB15, an interaction has been reported and its effects on the cold acclimation
process have been studied (Eremina et al., 2016b), but for the most part these interactions remain unexplored and, definitively, deserve more attention in the future. For instance, it has been reported that BZR1 and PIF4 may interact in vivo to act synergistically to promote cell elongation (Oh et al., 2012). It is tempting to speculate that this interaction may also occur at the CBF promoters during the cold response. A model of the fascinatingly complex regulation of the CBF locus will only arise from an analysis of expression patterns, protein interactions, and synergistic and antagonistic effects of all these regulators. This model should also take into account tissue- and developmental-stage effects on the cold activation of CBF genes.

Another relevant consideration to be made from the results described in the papers from Poppenberger and Yang laboratories (Eremina et al., 2016a; Li et al., 2017) is that, with the addition of BRs as specific regulators of the CBF-COR pathway, it becomes clear that the CBF genes constitute a central node of hormone cross-talk during cold stress response. Indeed, so far it has been demonstrated that CBF expression is modulated by gibberellins, jasmonate, ABA, ethylene, and BRs (Eremina et al., 2016b). Although in most cases the molecular mechanisms underlying this regulation require further analysis, current evidence shows that multiple hormone signaling mediators can either directly bind to CBF promoters or interact with key regulators of CBF expression (Figure 1). The picture that emerges is one in which different hormone signaling pathways coalesce at the CBF promoter level, and the output of this hormone cross-talk consists of fine-tuned transcript levels for each CBF, ensuring correct plant development and cold acclimation response (Figure 1). One may ask why such complex regulation for the expression of these transcription factors? The answer may lie in the fact that when plants are challenged by abiotic stress, they are faced with a dilemma: to grow or to resist stress. It has been proposed that CBFs may be at the bottom of this dilemma. In this way, cold induces retardation in growth triggered by a decrease of gibberellin biosynthesis, which is mediated by CBF transcription factors (Achard et al., 2008). As a result, growth stops, and more resources can be allocated to stress defense. Coherently, plants overexpressing CBFs usually display dwarfism. In this context, it is intriguing that plants with constitutive BR response display higher CBF expression but no signs of growth retardation. This observation suggests that, in certain conditions, the outcome of hormone signaling cross-talk may result in increased tolerance to cold stress without affecting growth. The molecular mechanisms underlying such hormone cross-talk and its control of CBF cold upregulation remain to be described. These studies are of the utmost relevance to achieve the long-sought goal of engineering crops with higher tolerance to abiotic stress without losing yield.

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