

Molecular Basis of Plant Cold Acclimation: Insights Gained from Studying the CBF Cold Response Pathway¹

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For the 75th Anniversary Issue of *Plant Physiology*, I contributed an article (Thomashow, 2001) in which I highlighted recent advances in the identification of genes with roles in cold acclimation: the process whereby certain plants increase in freezing tolerance in response to low nonfreezing temperatures. At that time, about 20 *Arabidopsis* (*Arabidopsis thaliana*) genes had been identified as cold regulated, and it seemed that it might be relatively easy to model the low-temperature transcriptional regulatory network. Since then, largely through the development and use of microarrays and other genomic tools, we have learned that exposing plants to low temperature triggers a highly complex regulatory program that results in extensive reorganization of the transcriptome. These changes include up- and down-regulation of hundreds of genes occurring in waves with time of incubating plants at low temperature (Fowler and Thomashow, 2002; Maruyama et al., 2004; Hannah et al., 2005; Vogel et al., 2005; Oono et al., 2006; Kilian et al., 2007; Robinson and Parkin, 2008). A hierarchical cluster analysis of the transcriptome data of Kilian et al. (2007) clearly reveals these features (Fig. 1A) and provokes a number of fundamental questions. How do plants sense low temperature; that is, what is the nature of the low-temperature thermometer? How is this information processed to activate the first wave of cold-regulated genes? What is the regulatory logic that underlies the cascading pattern of the low-temperature gene network? And what biological functions can be ascribed to the genes that constitute the various circuits of the network? At present, the answers to these questions are far from complete, but significant insights have been gained. Here, I will highlight a few of the advances that speak to these issues, focusing on the CBF cold response pathway of *Arabidopsis*. Additional information on the topics covered here and other facets of cold acclimation can be found in other recent reviews (Chinnusamy et al., 2007; Guy et al., 2008; Penfield, 2008; Galiba et al., 2009; Hua, 2009).

THE CBF REGULATORY HUB

Determining the overall “wiring diagram” of the low-temperature regulatory network will not be a trivial matter. Indeed, among the more than 1,000 genes that are cold induced, there are more than 170 genes that encode transcription factors (Fig. 1B). Presumably, all of these transcription factors act in concert to reconfigure the transcriptome at low temperature. However, as has been learned in other systems, certain transcription factors can serve as major regulatory “hubs” controlling the expression of a large number of genes and contribute much to the biology under study. The *Arabidopsis* CBF transcription factors constitute such a regulatory hub for cold acclimation.

Arabidopsis encodes three cold-inducible CBF genes, *CBF1*, *CBF2*, and *CBF3* (Stockinger et al., 1997; Gilmour et al., 1998; Medina et al., 1999), also referred to as *DREB1b*, *DREB1c*, and *DREB1a*, respectively (Liu et al., 1998). These genes, which are located in tandem array in the genome, encode closely related transcription factors that are members of the AP2/ERF family of DNA-binding proteins. The CBF proteins bind to the CRT/DRE regulatory element present in the promoters of target genes, referred to as the CBF regulon, and stimulate their transcription. Induction of the CBF genes occurs within about 15 min of transferring plants to low temperature (4°C), followed by induction of the CBF target genes beginning at about 2 to 3 h.

Vogel et al. (2005) found that 85 (28%) of the 302 genes that were determined to be cold induced in wild-type plants were also induced by constitutive overexpression of *CBF2* in transgenic plants grown at warm temperature, indicating that the CBF cold response pathway plays a major role in configuring the low-temperature transcriptome. This may be an underestimate of the size of the CBF regulon, as it assumes that the CBF transcription factors by themselves are sufficient to induce the expression of target genes at warm temperature. This may not be the case, as combinatorial regulation of gene expression is a common theme in gene regulation (Priest et al., 2009). In addition, *CBF2* overexpression was found to cause down-regulation of eight (4%) of the 212 genes that were found to be repressed in response to low temperature (Vogel et al., 2005). The promoters of these did not have the CRT/DRE element and thus would

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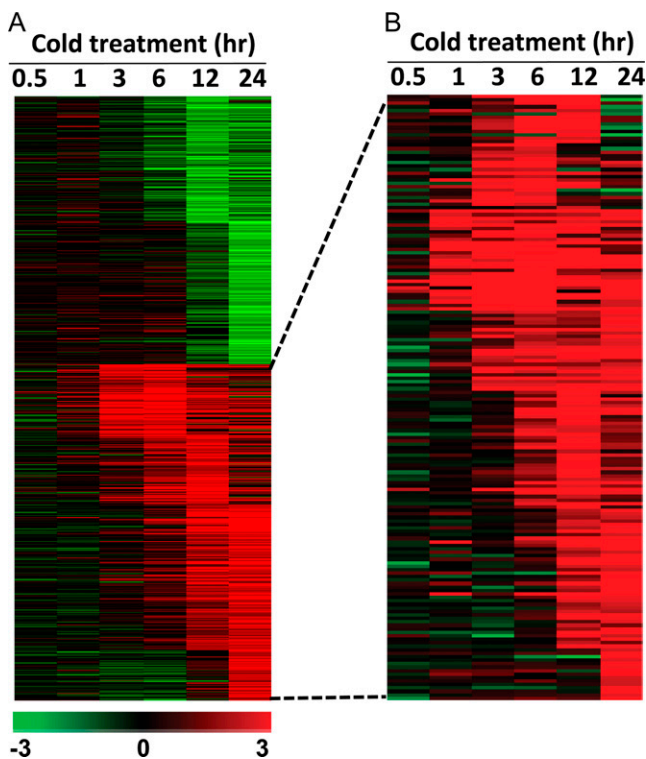


Figure 1. Waves of cold-regulated genes produced upon exposing Arabidopsis plants to low temperature. The AtGenExpress global stress expression data set (Kilian et al., 2007) was analyzed using the Bioconductor LIMMA package to identify genes that were up- or down-regulated at least 4-fold (false discovery rate < 0.01) at any time point of the cold treatment. This resulted in the identification of 1,335 up-regulated and 1,061 down-regulated genes. A, Genes were grouped using k-means cluster analysis. Log₂-transformed expression levels are represented by the color gradient included at the bottom of the cluster diagram. The extreme values given are -3 to +3 or higher in expression level. B, The patterns for up-regulated genes encoding transcription factors (174 total) were identified according to Arabidopsis Gene Ontology (Berardini et al., 2004) and grouped using k-means cluster analysis.

not appear to be direct targets of CBF, but they might be regulated by transcriptional repressors that are induced by CBF expression and thus be part of the CBF regulatory network.

ROLE OF THE CBF REGULATORY HUB IN FREEZING TOLERANCE

The CBF regulon has a major role in freezing tolerance. The initial evidence for this was the finding that transgenic Arabidopsis plants constitutively expressing *CBF1*, *CBF2*, or *CBF3* resulted in an increase in freezing tolerance without exposing plants to low temperature (Jaglo-Ottosen et al., 1998; Liu et al., 1998; Gilmour et al., 2004). Subsequently, Novillo et al. (2007) used RNA interference and antisense constructs to down-regulate the expression of *CBF1*

and *CBF3* and found that this resulted in about a 25% to 50% decrease in freezing tolerance in cold-treated plants. In addition, Hannah et al. (2006) examined six accessions of Arabidopsis that differed in freezing tolerance and found that the levels of *CBF1* and *CBF2* expression, as well as genes that constitute the CBF2 regulon, positively correlated with freezing tolerance. Finally, Alonso-Blanco et al. (2005) mapped quantitative trait loci (QTLs) for freezing tolerance in recombinant inbred lines produced from a cross between two accessions that differ in their abilities to cold acclimate, Cape Verde Islands (Cvi) and Landsberg erecta (*Ler*); Cvi is less freezing tolerant than *Ler*. A total of seven QTLs were identified, one of which, *FTQ4*, mapped to the CBF locus and accounted for about 20% of the variation in freezing tolerance. The recombinant inbred lines carrying the *FTQ4* locus from Cvi were those that were less freezing tolerant. It was found that cold induction of the *CBF2* gene (and three known CBF regulon genes) was much less in Cvi plants than in *Ler* plants and that the promoter region of the Cvi *CBF2* gene had a 1.6-kb deletion compared with the promoter of the *Ler* *CBF2* gene. From these and other results, it was concluded that the promoter deletion was likely to account for the poor induction of the Cvi *CBF2* gene and that this was the underlying molecular basis for the freezing tolerance QTL.

The studies summarized above point to the CBF regulatory hub having a major role in configuring the low-temperature transcriptome and in conditioning freezing tolerance. But, do all three CBF genes have the same function? To address this question, Gilmour et al. (2004) compared the transcriptomes of transgenic Arabidopsis plants (ecotype Wassilewskija-2) constitutively overexpressing *CBF1*, *CBF2*, or *CBF3* and found that each transcription factor affected the expression of the same set of cold-regulated genes; no evidence for specialized function was obtained. In contrast, Novillo et al. (2004) concluded that the function of *CBF2* is distinct from that of *CBF1* and *CBF3*. In particular, they found that Arabidopsis plants (ecotype Columbia) carrying a T-DNA insertion that inactivated the expression of *CBF2* had higher levels of *CBF1* and *CBF3* transcripts and greater freezing tolerance than did wild-type plants; this was true for comparisons of both nonacclimated and cold-acclimated plants. In addition, they reported that *CBF1* and *CBF3* were induced a little earlier than *CBF2*. From these and other results, the investigators proposed that *CBF2* is a negative regulator of *CBF1* and *CBF3* expression and a negative regulator of freezing tolerance. The apparent conflict in conclusions reached in the Gilmour et al. (2004) and Novillo et al. (2004) studies may only be apparent due to the different ecotypes or approaches used. In regard to the latter, overexpression studies (in these cases using the cauliflower mosaic virus 35S promoter) not only eliminate potential tissue specificity issues but may also eliminate potential subtle differences in CRT/DRE binding af-

finities between the three CBF proteins. A bit more difficult to understand is why the natural variation study of Alonso-Blanco et al. (2005) indicated that down-regulation of *CBF2* results in a decrease in freezing tolerance, whereas Novillo et al. (2004) concluded that down-regulation of *CBF2* results in an increase in freezing tolerance; but again, the systems are different. Clearly, additional work is needed to resolve this fundamental issue about the CBF regulatory hub.

Another fundamental question is how does the CBF regulon bring about an increase in freezing tolerance? A complete answer to this question will require additional study, but it is clear that multiple mechanisms are involved. As has been reviewed elsewhere (Thomashow, 1999; Sung et al., 2003; Penfield, 2008) and thus will only be mentioned here, certain CBF regulon genes encode enzymes involved in the biosynthesis of low- M_r cryoprotectants such as Suc, raffinose, and Pro, and others encode hydrophilic cryoprotective polypeptides. All of these cryoprotectants appear to function, at least in part, by protecting membranes and proteins against the severe dehydration stress that occurs with freezing.

The function of the CBF regulatory hub, however, involves mechanisms that go beyond the production of cryoprotectants. Arabidopsis plants constitutively overexpressing *CBF1*, *CBF2*, or *CBF3* grow slowly, have a dwarf stature, and are delayed in flowering (Liu et al., 1998; Gilmour et al., 2004). It is not surprising that CBF-overexpressing transgenic plants grow poorly at normal growth temperature, as they are forced to grow with a transcriptome (Maruyama et al., 2004; Vogel et al., 2005) and metabolome (Cook et al., 2004; Kaplan et al., 2007; Usadel et al., 2008) designed for life at low temperature. However, a more profound reason for the observed effects of CBF expression was discovered by Achard et al. (2008a). What these investigators found was that constitutive overexpression of *CBF1* results in the accumulation of DELLA proteins, a small family of growth-restraining proteins that are part of the gibberellin (GA) signaling pathway (Harberd et al., 2009). In plants with normal levels of biologically active GAs, the DELLA proteins are degraded through the ubiquitin-proteasome pathway. However, when biologically active GA levels are low, DELLA proteins accumulate, restrain growth, and cause a dwarf stature and delayed flowering. Achard et al. (2008a) found that plants overexpressing *CBF1* had reduced levels of biologically active GAs and that this was due to increased expression of two genes encoding GA 2-oxidases, enzymes that convert biologically active GAs to inactive GAs. The decrease in active GAs results in an increase in DELLA proteins, which in turn causes the dwarf and delayed flowering phenotypes. Indeed, constitutive overexpression of *CBF1* did not result in dwarf and delayed flowering phenotypes in plants that carried the *gai-t6* and *rga-24* mutations, which inactivate two major DELLA proteins, GAI and RGA, respectively.

In addition to affecting plant growth, DELLA protein activity was found to be required for plants to attain full freezing tolerance (Achard et al., 2008a). The increase in freezing tolerance brought about by constitutive overexpression of *CBF1* in transgenic plants grown at warm temperature was reduced about 50% by introduction of the *gai-t6* and *rga-24* mutations. Moreover, the increase in freezing tolerance observed in cold-treated wild-type plants was reduced about 50% by introduction of the *gai-t6* and *rga-24* mutations. These results, along with others reported by Achard et al. (2008a), indicate that a function of the CBF regulatory hub is to restrain plant growth through affecting the activity of the GA signaling pathway, and this contributes to the ability of plants to survive freezing. The mechanism by which the DELLA proteins contribute to freezing tolerance is not known but may involve reducing the levels of reactive oxygen species (Achard et al., 2008b). In addition, DELLA-mediated change in plant architecture could potentially contribute to survival in the field. For instance, it has been observed (Roberts, 1990) that varieties of wheat (*Triticum aestivum*) with greater winter survival have a more prostrate growth habit, which results in a greater chance of the plants being covered by snow and insulated against harsh air temperatures. Finally, DELLA-induced restraint of growth may have a broad role in abiotic stress tolerance, as mutations inactivating the *DELLA* genes also reduce tolerance to high salinity stress (Achard et al., 2006).

UPSTREAM OF THE CBF REGULATORY HUB: SENSING LOW TEMPERATURE AND INDUCING CBF EXPRESSION

As alluded to earlier, a fundamental question regarding cold acclimation is how do plants sense low temperature and initiate the regulatory program that results in enhanced freezing tolerance; that is, what is that nature of the low temperature thermometer? One approach to address this issue is to identify the transcription factors involved in expression of the first wave of cold-regulated genes and to work back upstream into the temperature-sensing system by understanding how the activities of the transcription factors are affected by low temperature. Such an approach has provided clues about the nature of two cold-sensing pathways that control *CBF* expression, one of which involves posttranslational protein modification and the other calcium signaling.

A major positive regulator of *CBF3* is ICE1, a basic helix-loop-helix transcription factor that binds to multiple Myc DNA regulatory elements present in the *CBF3* promoter and stimulates *CBF3* transcription (Chinnusamy et al., 2003). The role of ICE1 has been extensively reviewed (Chinnusamy et al., 2007; Hua, 2009), so only key points regarding two forms of posttranscriptional regulation will be mentioned here. The *ICE1* gene is constitutively expressed, sug-

gesting that cold induction of *CBF3* involves low temperature-induced activation of the ICE1 protein. The results of Miura et al. (2007) provide strong evidence that this is the case and that this is accomplished through low temperature-induced sumoylation of ICE1 mediated by the SIZ1 protein, a SUMO E3 ligase. This activation process, however, is countered by HOS1, a RING finger E3 ligase that mediates ubiquitination and degradation of ICE1 (Lee et al., 2001; Dong et al., 2006). At normal warm growth temperature, the *HOS1* gene is expressed and the HOS1 protein is located in the cytoplasm, but upon exposing plants to low temperature, HOS1 locates to the nucleus. Thus, low temperature appears to initiate a cycle of activation and inactivation of ICE1: a rapid activation of ICE1 due to SIZ1-mediated sumoylation followed by ICE1 inactivation due to HOS1-mediated ubiquitination and degradation. These events are consistent with the "transient" nature of CBF expression; low temperature induces a rapid dramatic increase (greater than 10-fold) in *CBF* transcript levels, reaching a maximum at about 2 to 3 h followed by a decrease to levels that are only a few fold over those found in warm-grown plants. Thus, the SIZ1-HOS1 system appears to contribute to a fine tuning of CBF gene expression that enables up-regulation of the system but also prevents "runaway" induction with potential deleterious effects. Such fine tuning, however, includes factors in addition to the SIZ1-HOS1 system, as the loss-of-function *hos1* mutation does not eliminate the transient nature of CBF induction; peak levels of *CBF3* transcripts are greater and remain higher for a longer period of time in plants carrying the *hos1* mutation but still return to a few fold over those found in nonacclimated plants (Lee et al., 2001). Major questions that remain to be answered regarding the SIZ1-HOS1 system and relate to low-temperature thermometers are how does low temperature control the cellular location of HOS1, how does it affect the ability of SIZ1 to mediate sumoylation of ICE1, and how does sumoylation increase the activity of ICE1?

Another cold-sensing pathway involves calcium. Nearly 20 years ago, Knight et al. (1991) established that exposure of plants to low temperature results in a rapid transient spike in cytoplasmic calcium levels. Subsequent studies linked the cold-induced calcium spikes to cold-regulated gene expression in *Arabidopsis* (Knight et al., 1996; Tahtiharju et al., 1997; Henriksson and Trewavas, 2003) and alfalfa (*Medicago sativa*; Monroy et al., 1993; Monroy and Dhindsa, 1995). For instance, the studies with *Arabidopsis* demonstrated that chemical agents that reduce calcium influx into the cytoplasm impaired cold induction of certain CBF regulon genes. The calcium channel(s) involved in cold-induced calcium influx has not yet been determined, but a mechanism whereby calcium levels may be sensed and the information processed to induce *CBF* gene expression is suggested by the findings of Doherty et al. (2009). These investigators identified a 27-bp region of the *CBF2* promoter that can impart

cold-induced gene expression and showed that this activity was dependent on a DNA sequence known as the CG-1 element, the binding site for the six CAMTA (for calmodulin-binding transcription activator) proteins encoded by *Arabidopsis* (Finkler et al., 2007). Moreover, they showed that the activity of the 27-bp promoter fragment required a functional *CAMTA3* gene. The possibility raised is that low temperature induces a rapid increase in calcium that leads to the formation of a calcium-calmodulin (or calmodulin-like protein)-CAMTA3 complex that stimulates the transcription of target genes. *Arabidopsis* plants carrying a *camta3* mutation were found to be impaired in the cold induction of *CBF1*, *CBF2*, and *ZAT12* (cold-induced transcript accumulation is reduced by about 50%), all of which have the CAMTA CG-1 DNA-binding site in their promoters, suggesting that these genes are direct targets of CAMTA3. Plants carrying a single *camta3* mutation were not impaired in freezing tolerance, but those carrying a *camta1/camta3* double mutation were, indicating an important role for the CAMTA transcription factors in cold acclimation. Additional study will be required to firmly establish the connection between low-temperature-induced calcium influx and CAMTA-mediated changes in gene expression, including a determination of the extent to which cold-regulated gene expression involves the CAMTA regulatory proteins.

CIRCADIAN GATING OF THE CBF REGULATORY HUB

Many studies on cold acclimation in *Arabidopsis*, including most of those conducted in our laboratory, have used plants grown under constant light. The thinking was that low temperature induces changes in gene expression and increases freezing tolerance under constant light and that determining the nature of the low-temperature regulatory network that accounts for these changes would be difficult enough without adding in potentially "complicating" effects caused by diurnal, circadian, and photoperiodic regulation; these inputs could be considered later. However, such a reductionist approach can lead to reduced appreciation for the complexity of the biological process under study, and factors important to the system can be missed. This is the case with cold-regulated gene expression.

The first indication that constant-light experiments were potentially missing important aspects of cold-regulated gene expression was the finding that *CBF3* and certain CBF regulon genes were subject to circadian regulation at normal growth temperatures (Harmer et al., 2000). This raised the question of whether low-temperature input into the CBF regulatory hub might be influenced by the clock; indeed, it is. If *Arabidopsis* plants are grown under a 12-h photoperiod and then transferred to constant light, the cold induction of *CBF1*, *CBF2*, and *CBF3* is much greater if

the temperature is dropped at ZT4 (4 h after dawn) than at ZT16 (Fowler et al., 2005; i.e. cold-regulated expression of the CBF genes is “gated” by the clock). Significantly, circadian gating of CBF expression also occurs in tomato (*Solanum lycopersicum*; Pennycook et al., 2008), suggesting that this is a highly conserved form of regulation and of fundamental importance. Determining how the clock and low-temperature regulatory pathways are integrated to affect CBF gene expression and how this affects the ability of plants to cope with cold temperatures must now be determined. An important step in this regard was recently made by Kidokoro et al. (2009), who showed that the basic helix-loop-helix protein PIF7 is a transcriptional repressor involved in circadian regulation of CBF1 and CBF2 and that this regulation involves interactions of PIF7 with TOC1, a component of the central circadian oscillator, and PHYB, a red light photoreceptor. In addition, it should be noted that the level of cytosolic free calcium oscillates during the day under control of the circadian clock (Dodd et al., 2005) and that the spike in cytosolic calcium produced in response to low temperature is gated by the clock (Dodd et al., 2006). As mentioned above, the regulation of CBF1 and CBF2 involves action of the calmodulin-binding CAMTA transcription factors (Doherty et al., 2009). Perhaps the CAMTA proteins participate in decoding the circadian oscillations and low-temperature gating of calcium and contribute to CBF gene regulation and freezing tolerance.

A major insight into the interaction of low temperature and the clock and the effects that this has on identifying cold-regulated genes has come from the work of Bieniawska et al. (2008). These investigators found that low temperature disrupts the function of the circadian clock in *Arabidopsis* and that this accounts for much of the variation in the composition of the low-temperature transcriptome reported in different studies. For instance, when plants that are grown under a 16-h photoperiod are transferred to low temperature, the transcript levels for certain genes that are components of the circadian clock, such as *PRR7*, and certain clock-output genes, such as *CAB2*, are greatly impaired in cycling (i.e. the circadian oscillations are greatly dampened or eliminated upon transferring plants to low temperature). Thus, if one compares the transcript level for one of these genes at a given time point of cold treatment with the transcript level of the gene at the same time point in “control” warm-treated plants, under which conditions the transcript level for the gene is cycling, the gene will be scored as either cold induced, cold repressed, or unaffected, depending on the time point. That the cycling of certain circadian-regulated genes can be affected by low temperature was not a completely new observation, as normal cycling of certain circadian-regulated genes had been observed to be impaired in cold-treated tomato (Martino-Catt and Ort, 1992) and chestnut (*Castanea sativa*; Ramos et al., 2005). However, the extent to which circadian-regulated gene expression is

disrupted by low temperature and the impact that this has on deciphering the low-temperature regulatory network were not appreciated prior to the study by Bieniawska et al. (2008).

CONCLUDING REMARKS

In the review that I contributed to the 75th Anniversary Issue of *Plant Physiology* (Thomashow, 2001), I concluded with a speculation: that additional study incorporating the new powerful approaches of genomics would likely soon produce a core set of “first principles” that would enable the rational design of plants with increased freezing tolerance. The ensuing 10 years has not produced the blueprints required to convert freezing-sensitive plants into freezing-tolerant plants, so depending on how one defines “soon,” my speculation might be judged as being overly optimistic. However, what the past 10 years has produced is a body of work that has greatly expanded our understanding of the cold acclimation phenomenon and has significantly advanced us toward answering the fundamental questions raised at the outset of this article. The coming 10 years, along with the further development and incorporation of “omics” tools and computational approaches, will bring an even deeper understanding of the cold acclimation phenomenon that may well include intelligible “systems”-level knowledge that, at a minimum, will advance us significantly toward the aspired-to first principles.

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LITERATURE CITED

- Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. *Science* 311: 91–94
- Achard P, Gong F, Cheminant S, Alioua M, Hedden P, Genschik P (2008a) 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* 20: 2117–2129
- Achard P, Renou JP, Berthome R, Harberd NP, Genschik P (2008b) Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. *Curr Biol* 18: 656–660
- Alonso-Blanco C, Gomez-Mena C, Llorente F, Koornneef M, Salinas J, Martinez-Zapater JM (2005) Genetic and molecular analyses of natural variation indicate CBF2 as a candidate gene for underlying a freezing tolerance quantitative trait locus in *Arabidopsis*. *Plant Physiol* 139: 1304–1312
- Berardini TZ, Mundodi S, Reiser R, Huala E, Garcia-Hernandez M, Zhang P, Mueller LM, Yoon J, Doyle A, Lander G, et al (2004) Functional annotation of the *Arabidopsis* genome using controlled vocabularies. *Plant Physiol* 135: 1–11
- Bieniawska Z, Espinoza C, Schlereth A, Sulpice R, Hinch DK, Hannah MA (2008) Disruption of the *Arabidopsis* circadian clock is responsible

- for extensive variation in the cold-responsive transcriptome. *Plant Physiol* **147**: 263–279
- Chinnusamy V, Ohta M, Kanrar S, Lee BH, Hong X, Agarwal M, Zhu JK** (2003) ICE1: a regulator of cold-induced transcriptome and freezing tolerance in *Arabidopsis*. *Genes Dev* **17**: 1043–1054
- Chinnusamy V, Zhu J, Zhu JK** (2007) Cold stress regulation of gene expression in plants. *Trends Plant Sci* **12**: 444–451
- Cook D, Fowler S, Fiehn O, Thomashow MF** (2004) A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of *Arabidopsis*. *Proc Natl Acad Sci USA* **101**: 15243–15248
- Dodd AN, Jakobsen MK, Baker AJ, Telzerow A, Hou SW, Laplaze L, Barrot L, Poethig RS, Haseloff J, Webb AA** (2006) Time of day modulates low-temperature Ca signals in *Arabidopsis*. *Plant J* **48**: 962–973
- Dodd AN, Love J, Webb AA** (2005) The plant clock shows its metal: circadian regulation of cytosolic free Ca²⁺. *Trends Plant Sci* **10**: 15–21
- Doherty CJ, Van Buskirk HA, Myers SJ, Thomashow MF** (2009) Roles for *Arabidopsis* CAMTA transcription factors in cold-regulated gene expression and freezing tolerance. *Plant Cell* **21**: 972–984
- Dong CH, Agarwal M, Zhang Y, Xie Q, Zhu JK** (2006) The negative regulator of plant cold responses, HOS1, is a RING E3 ligase that mediates the ubiquitination and degradation of ICE1. *Proc Natl Acad Sci USA* **103**: 8281–8286
- Finkler A, Ashery-Padan R, Fromm H** (2007) CAMTAs: calmodulin-binding transcription activators from plants to human. *FEBS Lett* **581**: 3893–3898
- Fowler S, Thomashow MF** (2002) *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. *Plant Cell* **14**: 1675–1690
- Fowler SG, Cook D, Thomashow MF** (2005) Low temperature induction of *Arabidopsis* CBF1, 2, and 3 is gated by the circadian clock. *Plant Physiol* **137**: 961–968
- Galiba G, Vagujfalvi A, Li CX, Soltesz A, Dubcovsky J** (2009) Regulatory genes involved in the determination of frost tolerance in temperate cereals. *Plant Sci* **176**: 12–19
- Gilmour SJ, Fowler SG, Thomashow MF** (2004) *Arabidopsis* transcriptional activators CBF1, CBF2, and CBF3 have matching functional activities. *Plant Mol Biol* **54**: 767–781
- Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, Houghton JM, Thomashow MF** (1998) Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression. *Plant J* **16**: 433–442
- Guy C, Kaplan F, Kopka J, Selbig J, Hinch DK** (2008) Metabolomics of temperature stress. *Physiol Plant* **132**: 220–235
- Hannah MA, Heyer AG, Hinch DK** (2005) A global survey of gene regulation during cold acclimation in *Arabidopsis thaliana*. *PLoS Genet* **1**: e26
- Hannah MA, Wiese D, Freund S, Fiehn O, Heyer AG, Hinch DK** (2006) Natural genetic variation of freezing tolerance in *Arabidopsis*. *Plant Physiol* **142**: 98–112
- Harberd NP, Belfield E, Yasumura Y** (2009) The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: how an “inhibitor of an inhibitor” enables flexible response to fluctuating environments. *Plant Cell* **21**: 1328–1339
- Harmer SL, Hogenesch LB, Straume M, Chang HS, Han B, Zhu T, Wang X, Kreps JA, Kay SA** (2000) Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science* **290**: 2110–2113
- Henriksson KN, Trewavas AJ** (2003) The effect of short-term low-temperature treatments on gene expression in *Arabidopsis* correlates with changes in intracellular Ca²⁺ levels. *Plant Cell Environ* **26**: 485–496
- Hua J** (2009) From freezing to scorching, transcriptional responses to temperature variations in plants. *Curr Opin Plant Biol* **12**: 568–573
- Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF** (1998) *Arabidopsis* CBF1 overexpression induces COR genes and enhances freezing tolerance. *Science* **280**: 104–106
- Kaplan F, Kopka J, Sung DY, Zhao W, Popp M, Porat R, Guy CL** (2007) Transcript and metabolite profiling during cold acclimation of *Arabidopsis* reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. *Plant J* **50**: 967–981
- Kidokoro S, Maruyama K, Nakashima K, Imura Y, Narusaka Y, Shinwari ZK, Osakabe Y, Fujita Y, Mizoi J, Shinozaki K, et al** (2009) The phytochrome-interacting factor PIF7 negatively regulates DREB1 expression under circadian control in *Arabidopsis*. *Plant Physiol* **151**: 2046–2057
- Kilian J, Whitehead D, Horak J, Wanke D, Weinl S, Batistic O, D’Angelo C, Bornberg-Bauer E, Kudla J, Harter K** (2007) The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. *Plant J* **50**: 347–363
- Knight H, Trewavas AJ, Knight MR** (1996) Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signature after acclimation. *Plant Cell* **8**: 489–503
- Knight MR, Campbell AK, Smith SM, Trewavas AJ** (1991) Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. *Nature* **352**: 524–526
- Lee H, Xiong L, Gong Z, Ishitani M, Stevenson B, Zhu JK** (2001) The *Arabidopsis* HOS1 gene negatively regulates cold signal transduction and encodes a RING finger protein that displays cold-regulated nucleocytoplasmic partitioning. *Genes Dev* **15**: 912–924
- Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K** (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell* **10**: 1391–1406
- Martino-Catt S, Ort DR** (1992) Low-temperature interrupts circadian regulation of transcriptional activity in chilling-sensitive plants. *Proc Natl Acad Sci USA* **89**: 3731–3735
- Maruyama K, Sakuma Y, Kasuga M, Ito Y, Seki M, Goda H, Shimada Y, Yoshida S, Shinozaki K, Yamaguchi-Shinozaki K** (2004) Identification of cold-inducible downstream genes of the *Arabidopsis* DREB1A/CBF3 transcriptional factor using two microarray systems. *Plant J* **38**: 982–993
- Medina J, Bargas M, Terol J, Perez-Alonso M, Salinas J** (1999) The *Arabidopsis* CBF gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. *Plant Physiol* **119**: 463–470
- Miura K, Jin JB, Lee J, Yoo CY, Stirn V, Miura T, Ashworth EN, Bressan RA, Yun DJ, Hasegawa PM** (2007) SIZ1-mediated sumoylation of ICE1 controls CBF3/DREB1A expression and freezing tolerance in *Arabidopsis*. *Plant Cell* **19**: 1403–1414
- Monroy AF, Dhindsa RS** (1995) Low-temperature signal-transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25°C. *Plant Cell* **7**: 321–331
- Monroy AF, Sarhan F, Dhindsa RS** (1993) Cold-induced changes in freezing tolerance, protein phosphorylation, and gene expression: evidence for a role of calcium. *Plant Physiol* **102**: 1227–1235
- Novillo F, Alonso JM, Ecker JR, Salinas J** (2004) CBF2/DREB1C is a negative regulator of CBF1/DREB1B and CBF3/DREB1A expression and plays a central role in stress tolerance in *Arabidopsis*. *Proc Natl Acad Sci USA* **101**: 3985–3990
- Novillo F, Medina J, Salinas J** (2007) *Arabidopsis* CBF1 and CBF3 have a different function than CBF2 in cold acclimation and define different gene classes in the CBF regulon. *Proc Natl Acad Sci USA* **104**: 21002–21007
- Oono Y, Seki M, Satou M, Iida K, Akiyama K, Sakurai T, Fujita M, Yamaguchi-Shinozaki K, Shinozaki K** (2006) Monitoring expression profiles of *Arabidopsis* genes during cold acclimation and deacclimation using DNA microarrays. *Funct Integr Genomics* **6**: 212–234
- Penfield S** (2008) Temperature perception and signal transduction in plants. *New Phytol* **179**: 615–628
- Pennycooke JC, Cheng HM, Roberts SM, Yang QF, Rhee SY, Stockinger EJ** (2008) The low temperature-responsive, *Solanum* CBF1 genes maintain high identity in their upstream regions in a genomic environment undergoing gene duplications, deletions, and rearrangements. *Plant Mol Biol* **67**: 483–497
- Priest HD, Filichkin SA, Mockler TC** (2009) Cis-regulatory elements in plant cell signaling. *Curr Opin Plant Biol* **12**: 643–649
- Ramos A, Perez-Solis E, Ibanez C, Casado R, Collada C, Gomez L, Aragoncillo C, Allona I** (2005) Winter disruption of the circadian clock in chestnut. *Proc Natl Acad Sci USA* **102**: 7037–7042
- Roberts DWA** (1990) Identification of loci on chromosome 5A of wheat involved in control of cold hardiness, vernalization, leaf length, rosette growth habit, and height of hardened plants. *Genome* **33**: 247–259
- Robinson SJ, Parkin IA** (2008) Differential SAGE analysis in *Arabidopsis* uncovers increased transcriptome complexity in response to low temperature. *BMC Genomics* **9**: 434
- Stockinger EJ, Gilmour SJ, Thomashow MF** (1997) *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that

- binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proc Natl Acad Sci USA* **94**: 1035–1040
- Sung DY, Kaplan F, Lee KJ, Guy CL** (2003) Acquired tolerance to temperature extremes. *Trends Plant Sci* **8**: 179–187
- Tahtiharju S, Sangwan V, Monroy AF, Dhindsa RS, Borg M** (1997) The induction of *kin* genes in cold-acclimating *Arabidopsis thaliana*: evidence of a role for calcium. *Planta* **203**: 442–447
- Thomashow MF** (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Annu Rev Plant Physiol Plant Mol Biol* **50**: 571–599
- Thomashow MF** (2001) So what's new in the field of plant cold acclimation? Lots! *Plant Physiol* **125**: 89–93
- Usadel B, Blasing OE, Gibon Y, Poree F, Hohne M, Gunter M, Trethewey R, Kamlage B, Poorter H, Stitt M** (2008) Multilevel genomic analysis of the response of transcripts, enzyme activities and metabolites in *Arabidopsis* rosettes to a progressive decrease of temperature in the non-freezing range. *Plant Cell Environ* **31**: 518–547
- Vogel JT, Zarka DG, Van Buskirk HA, Fowler SG, Thomashow MF** (2005) Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of *Arabidopsis*. *Plant J* **41**: 195–211