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Review. Regulatory mechanisms involved in cold acclimation response

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Abstract

Freezing temperatures are one of the environmental stresses with more impact in plant development in temperate regions. By means of cold acclimation, many plant species are able to increase their freezing tolerance in response to low-nonfreezing temperatures. In the last years, an important effort has been made to characterize the molecular mechanisms that control this adaptive process. Transcriptome analyses indicate that cold acclimation is mainly regulated by low temperatures through changes in gene expression. In this regard, it has been reported that 3.4% of Arabidopsis genes are regulated by low temperature, including 95 genes annotated as transcription factors. The control of cold-inducible gene expression is very complex and involves many different signaling pathways that, in some cases, seem to share common intermediates. Interestingly, recent reports have shown that RNA processing and export, as well as post-translational modifications, including ubiquitylation and SUMOylation, also play important roles in regulating cold acclimation. Furthermore, histone modifications seem to be relevant in this regulation. In fact, histone acetylation and deacetylation are needed for correct activation of gene expression in response to low temperatures. All these data reveal that post-transcriptional mechanisms should be taken into consideration when analyzing the control of cold acclimation. Following, is a brief overview of the current understanding on the regulatory mechanisms that ensures the accurate development of this adaptive response.

Additional key words: abiotic stress, Arabidopsis, freezing tolerance, low temperature.

Resumen

Revisión. Mecanismos reguladores implicados en la respuesta de aclimatación al frío

Las temperaturas de congelación son uno de los estreses ambientales con mayor impacto en el desarrollo de las plantas en las regiones templadas. Por medio de la respuesta de aclimatación, muchas especies son capaces de incrementar su tolerancia a la congelación en respuesta a temperaturas bajas propiamente dichas. En los últimos años, se ha realizado un gran esfuerzo en la caracterización de los mecanismos moleculares que controlan este proceso adaptativo. Análisis transcriptómicos indican que la aclimatación al frío es regulada principalmente por cambios en la expresión génica. En este sentido, se ha descrito que la expresión de un 3,4% de los genes de Arabidopsis está regulada por las temperaturas bajas, incluyendo 95 genes anotados como factores de transcripción. El control de la expresión génica inducible por frío es muy complejo e implica diferentes rutas de señalización de la señal que, en algunos casos, parecen compartir intermediarios comunes. Trabajos recientes han mostrado que el procesamiento y movilización de RNA, y algunas modificaciones post-traduccionales de proteínas como la ubiquitinación y la SUMOilización también juegan un papel importante en la regulación del proceso de aclimatación a las temperaturas bajas. Más aún, la modificación de histonas parece ser también relevante en esta regulación. De hecho, la acetilación y deacetilación de histonas es necesaria para una correcta activación de la expresión génica en respuesta a las temperaturas bajas. Todos estos datos ponen de manifiesto que a la hora de estudiar el control de proceso de aclimatación se han de tener en cuenta los mecanismos de regulación post-transcripcional. A continuación, repasaremos los conocimientos actuales sobre los mecanismos que aseguran el correcto desarrollo de esta respuesta adaptativa.

Palabras clave adicionales: Arabidopsis, estrés abiótico, temperaturas bajas, tolerancia a la congelación.

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Introduction[1]

Plant development is severely limited by environmental conditions, particularly in temperate regions where important temperature changes occur between winter and summer seasons. Taken into consideration that plants are sessile organisms and cannot control their own temperature, survival to these changes is an important challenge. Many species have evolved an adaptive response that allows them to endure freezing temperatures. By means of this process, named cold acclimation (Levitt, 1980), they increase their freezing tolerance after a period of exposition to low-nonfreezing temperatures. Cold acclimation constitutes a representative example of plant interaction with the environment and how this interaction has conditioned the evolution of some species. Understanding the molecular mechanisms that control this adaptive process is not only interesting from a basic point of view but also has a high biotechnological potential. During the last years, many investigations have revealed that plant responses to low temperature and other abiotic stresses, such as high salt and drought, share several components (Salinas, 2002). The study of cold acclimation should provide essential information about how plants coordinately integrate responses to different adverse environmental conditions.

The process of acclimation is very complex and involves many biochemical and physiological changes, mainly controlled at the gene expression level (Salinas, 2002; Kaplan *et al.*, 2004; Zhu *et al.*, 2007). While some of these changes are implicated in the stability of subcellular structures, essentially membranes (Nishida and Murata, 1996) and cytoskeleton (Mita and Shibaoka, 1984), others are directed to control the synthesis of pigments to protect from photoxidative stress (Schoner and Krause, 1990; Christie *et al.*, 1994; Król *et al.*, 1999), enzymes to reduce oxidative stress (Schoner and Krause, 1990; Prasad, 1997; Llorente *et al.*, 2002), and compatible solutes to protect from osmotic changes (Ristic and Ashworth, 1993; Sauter *et al.*, 1996; Xin and Browse, 1998; Wanner and Junttila, 1999; Gilmour *et al.*, 2000; Garg *et al.*, 2002). In addition, during cold acclimation the expression of genes encoding cryoprotective proteins, known as LEA, and antifreezing proteins (AFPs), is induced (Thomashow, 1999; Smallwood and Bowles, 2002).

Recent reports have revealed that the process of cold acclimation is subjected to a complex regulation, which involves several pathways. The implication of transcriptional regulation as well as RNA mobility and processing in controlling low temperature response will be revised. Furthermore, the role post-translational protein modifications play in cold-acclimation signaling will be discussed.

Transcriptional regulation of cold inducible gene expression

Microarray analysis have shown that 939 Arabidopsis genes are regulated by low temperature (Fowler and Thomashow, 2002; Kreps *et al.*, 2002; Seki *et al.*, 2002; Lee *et al.*, 2005; Vogel *et al.*, 2005). Among these genes, one third is down regulated, with only one encoding a transcription factor, which indicates that cold acclimation is mainly regulated by transcriptional activation (Lee *et al.*, 2005). In addition, many early cold-induced genes encode transcription factors or proteins involved in transcription. Thus 113 genes have been annotated to function in transcription, 95 of them coding for transcription factors (Lee *et al.*, 2005). These data indicates that different pathways are involved in cold-responsive gene regulation.

The small family of transcription factors named CBFs/DREB1s (Fowler and Thomashow, 2002) is the most characterized component mediating cold-inducible gene expression in Arabidopsis. It is composed by three members (CBF1-3) and regulates the expression of around 12% of the cold-inducible genes in Arabidopsis. CBFs have an AP2 DNA-biding domain, an acidic C-terminal region, that acts as a transcriptional activator motif, and bind to the CRT/DRE *cis*- element (CCGAC) (Stockinger *et al.*, 1997). The expression of the CBF genes seems to be specifically induced by cold in a fast, transient, and specific way. In fact, *CBF* genes

[1] Abbreviations used: ABA (abscisic acid), ABF (ABRE binding factor), ABRE (abscisic acid-responsive element), AFP (antifreezing protein), bHLH (basic helix-loop-helix), CBF (CRT binding factor), CRT/DRE (C-repeat/dehydration responsive element), FRY (fiery), GR-RBP (glycine-rich RNA-binding protein), HAT (histone acetyltransferase), HDAC (histone deacetylase), HOS (high expression of osmotically responsive gene), ICE (inducer of CBF expression), LEA (late embryogenic abundant), LOS (low expression of osmotically responsive gene), LOV (light-oxigene-voltage), PP2A (protein phosphatase 2A), SCOF (soybean cold-inducible factor), STA (stabilized), SUS (sucrose synthase).

are not induced by cold-related stresses such as dehydration or high salt (Gilmour *et al.*, 1998; Liu *et al.*, 1998; Medina *et al.*, 1999). Constitutive overexpression of *CBFs* in Arabidopsis induces the accumulation of mRNAs from genes that contain the CRT/DRE box in their promoters as well as an increase in freezing tolerance (Fowler and Thomashow, 2002). Among the genes induced by CBFs, there are some encoding transcription factors (i.e., *RAP2.1* and *RAP2.6*), indicating that the CBF-regulon is constituted by several subregulons (Fowler and Thomashow, 2002). Furthermore, overexpression of *CBF3* has been shown to induce different metabolic changes associated with cold acclimation (i.e. increase in proline and soluble sugars levels), some of them correlating with the expression levels of related genes, as is the case of *AtP5CS2* (Gilmour *et al.*, 2000). At the morphological level, Arabidopsis transgenic plants constitutively overexpressing individual *CBFs* show dwarf phenotypes, delayed flowering and low number of seeds (Gilmour *et al.*, 2000), which indicates that CBFs are highly detrimental for plant development when present in nonphysiological levels, and suggests that their expression must be tightly regulated.

Recently, Novillo *et al.* (2004) reported the molecular and functional characterization of a *CBF2* null mutant. Interestingly, the absence of CBF2 increases Arabidopsis tolerance to freezing temperatures, before and after cold acclimation. This increase resulted to correlate with a higher accumulation of *CBF1* and *CBF3* transcripts, and, consequently, of CBF-target genes, under both control and low-temperature conditions (Novillo *et al.*, 2004). These results indicate that CBF2 negatively modulates the expression of *CBF1* and *CBF3*, ensuring the adequate expression of the CBF-regulon and, therefore, the proper response of Arabidopsis to cold stress (Novillo *et al.*, 2004). In addition to CBF2, other transcription factors have been shown to control the expression of *CBFs*. A MYC-like bHLH transcription factor, ICE1, binds to the *CBF3* promoter acting as a positive regulator of *CBF3* gene expression (Chinnusamy *et al.*, 2003). Unexpectedly, *ICE1* expression is not induced in response to low temperature, suggesting it should be regulated at the post-transcriptional level (see below). *ice1* mutant plants show low induction of *CBF3* and the CBF-regulon in response to low temperatures, as well as impaired freezing tolerance (Chinnusamy *et al.*, 2003). It has been reported that ICE1 controls the expression of around 40% of cold-regulated genes (Lee *et al.*, 2005). However, it does not seem to control the expression of non-cold-inducible genes (Lee *et al.*, 2005). The comparison of genes regulated by ICE1 with those regulated by CBFs and other transcription factors, revealed that ICE1 is more implicated in regulating CBFs-target genes, particularly CBF3-targets, consistently with its role as *CBF3* activator (Lee *et al.*, 2005). MYB15 is another transcription factor that binds to the promoters of *CBFs*, although in this case to repress their expression (Agarwal *et al.*, 2006). *myb15* null mutants are more tolerant to freezing stress than wild type plants, mainly due to an increase in cold-induction of the *CBFs* and target genes (Agarwal *et al.*, 2006). MYB15 physically interact with ICE1, although the biological meaning of this interaction remains unknown (Agarwal *et al.*, 2006). MYB15 is not the only transcription factor that negatively regulates *CBFs* expression. Vogel *et al.* (2005) reported that the overexpression of an Arabidopsis zinc finger, ZAT12, significantly dumped the induction of the *CBF*s in response to low temperatures. Strikingly, ZAT12 does not seem to affect CBF-targeted gene expression, which suggests a complex role for this protein in the regulation of cold-inducible gene expression and cold acclimation (Vogel *et al.*, 2005). In fact, overexpression of ZAT12 increases the induction of several cold-inducible genes, some of them CBF targets. Furthermore, ZAT12 overexpression enhances the constitutive freezing tolerance of Arabidopsis but does not have any effect on cold acclimation (Vogel *et al.*, 2005). All these results confirm the high complexity to which the regulation of *CBF* expression is subjected, and prompted the importance of an accurate control of this expression for the correct development of the cold acclimation process.

HOS9 and HOS10 are homeodomain and R2R3-type MYB transcription factors, respectively, that have also been involved in regulating gene expression during cold acclimation (Zhu *et al.*, 2004, 2005). *hos9* mutant plants display reduced freezing tolerance, both before and after cold acclimation (Zhu *et al.*, 2004). Intriguingly, while the cold-induction of *CBFs* in *hos9* is as in the wild type, the cold-induction of some CBF-target genes is higher. Similarly, *hos10-1* mutant also shows high sensitivity to freezing temperatures and is impaired in cold acclimation (Zhu *et al.*, 2005). Moreover, although the cold induction of *CBFs* is not altered in *hos10*, the induction of several CBF targets by low temperature is faster in the mutant than in the wild type. Therefore, HOS9 and HOS10 seem to control cold-acclimation response by ensuring a proper cold-

inducible gene expression through CBF-independent pathways (Zhu *et al.*, 2005). LOV1 is another transcription factor that has been implicated in regulating the expression of CBF-targets in a CBF-independent way (Yoo *et al.*, 2007). *lov1-4* null mutant is hypersensitive to freezing temperatures and has reduced acclimation capability, indicating a positive role for LOV1 during cold acclimation (Yoo *et al.*, 2007). The cold-induction of CBF-target genes is significantly impaired in *lov1-4* when compared to wild type plants, indicating that it acts as a positive regulator of this response. However, the cold-induction of CBFs is identical in *lov1-4* and wild type plants, which demonstrates that LOV1 function is CBF-independent (Yoo *et al.*, 2007). The ABA response element ABRE (PyACGTGCC, Guiltinan *et al.*, 1990) is present in the promoters of numerous cold-inducible genes, including CBF targets (Baker *et al.*, 1994; Yamaguchi-Shinozaki and Shinozaki, 1994; Wang and Cutler, 1995) and could mediate CBF-independent gene expression in response to low temperature. Supporting this conception, a cold- and ABA-inducible gene from Arabidopsis has been described to encode a bZIP transcription factor (ABF1) that binds to the ABRE motif. ABF1 is able to activate gene expression through its interaction with the ABRE element in yeast, suggesting that it could mediate ABRE-dependent cold-induced gene expression (Choi *et al.*, 2000). Another gene which expression is induced by cold and ABA, and encodes a transcription factor is *SCOF1* from soybean (Agarwal *et al.*, 2006). SCOF1 is a zinc finger protein whose overexpression in Arabidopsis originates constitutive expression of cold-inducible genes and increased tolerance to freezing temperatures (Kim *et al.*, 2001). SCOF1 resulted to enhance the binding of SGB1, a bZIP transcription factor, to the ABRE motif (Kim *et al.*, 2001), indicating that it is a positive regulator of cold-acclimation response through the ABRE-dependent pathway. From all these data, a picture can be drawn where gene expression is regulated by low temperature through a sophisticated network of signaling pathways to ensure the correct development of the cold-acclimation process (Fig. 1).

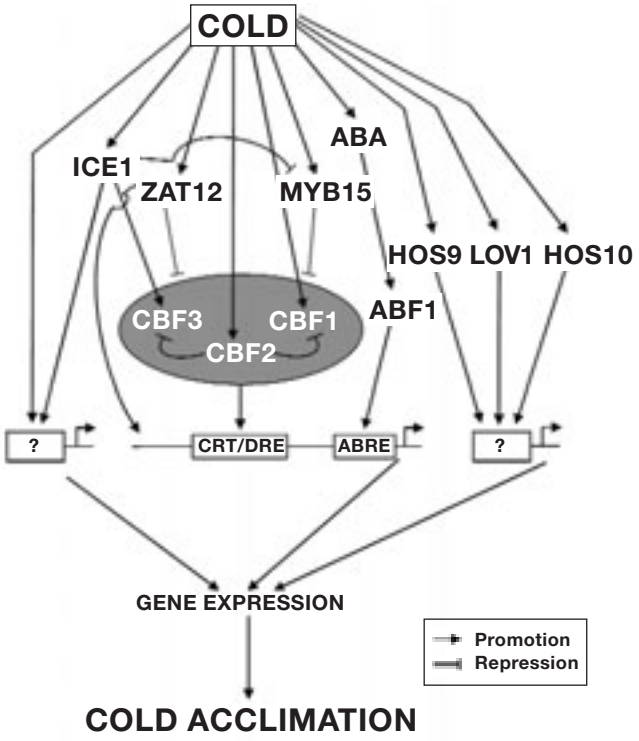

Figure 1. CBFs constitute central intermediates in cold-acclimation signaling by inducing gene expression through CRT/DRE sequence. The induction of *CBFs* in response to low temperature is tightly regulated. ICE1 has been described to positively regulate *CBF3* expression. Positive regulators of *CBF1* and *CBF2* have not been identified yet. ZAT12 and MYB15 negatively regulate *CBF* expression. In addition, CBF2 negatively regulates *CBF1* and *CBF3* expression. ICE1 has also been described to promote cold acclimation by inducing CBF-independent gene expression and to repress MYB15 expression. ABA activates cold acclimation through a CBF-independent pathway. ABA-dependent cold-induced gene expression is mediated by ABF1 via the ABRE *cis*-element. Although still is unknown how HOS9, HOS10 and LOV1 are activated in response to low temperatures they also activate cold-induced gene expression as well as cold acclimation through CBF-independent pathways.

Post-transcriptional regulation of cold acclimation response

mRNA stability and translation yield also play important roles in the proper activation of the cold-acclimation response. In fact, the accumulation of some messengers during cold acclimation seems to be regulated at the post-transcriptional level (Hajela *et al.*, 1990; Wolfraim *et al.*, 1993; Dunn *et al.*, 1994). By using pharmacological experiments, Phillips *et al.* (1997) demonstrated that an unknown nuclear protein actively stabilizes transcripts corresponding to *blt4.0*, a cold-inducible barley gene. On the other hand, Xiong *et al.* (2002) indentified an Arabidopsis gene, *FRY2*, which encodes a protein similar to animal and yeast factors involved in transcription regulation and pre-mRNA processing. Recessive mutations in *FRY2* provokes the superinduction of CBF-target genes in response to cold, suggesting that FRY2 is a negative

regulator of the activation of gene expression by cold. In contrast, *fry2* mutants are more sensitive to freezing temperatures than wild type. Taken in consideration the RNA binding domain of FRY2, Xiong *et al.* (2002) proposed that, as in animals, RNA structure could regulate the response of plants to stress and FRY2 could act as a RNA chaperone under low temperature conditions. The glycine-rich RNA-binding proteins (GR-RBPs) have also been proposed to act as RNA chaperones in plants. In this regard, it has been described that the expression of several GR-RBPs is induced by different abiotic stresses including cold (Sachetto-Martins *et al.*, 2000). Functional characterization of one *Arabidopsis* GR-RBP, the atRZ1, provided interesting data about the role of these proteins in cold acclimation (Kim *et al.*, 2005). *atRZ1* mutants are more sensitive to low and freezing temperatures, whereas overexpressing plants are more tolerant to these stresses (Kim *et al.*, 2005). On the basis of these results, Kim *et al.* (2005) concluded that atRZ1 might have an RNA chaperone activity leading to the destabilization of the over-stabilized secondary structures and, thereby, to facilitate an efficient translation at low temperature. Another Arabidopsis protein involved in pre-mRNA processing and cold signaling is STA1. STA1 is similar to the human U5 small ribonucleoprotein-associated 102-kD protein and to the yeast pre-mRNA splicing factors Prp1p and Prp6p, whose corresponding genes, as *STA1*, are induced by low temperature (Lee *et al.*, 2006). Mutations in *STA1* increase Arabidopsis chilling sensitivity, although this mutant do not have any difference with wild type plants in their cold-inducible gene expression. Interestingly, the stability of some mRNAs and the splicing of the cold-induced gene *COR15A* are seriously affected in *sta1-1* mutant plants, suggesting that these processes are important in low temperature response (Lee *et al.*, 2006).

In addition to mRNA processing, the control of mRNA export to the cytoplasm is also influential in plant response to low temperature. In this way, the implication of the Arabidopsis nucleoporin AtNUP160 in cold signaling has been described (Dong *et al.*, 2006a). This protein seems to be involved in mRNA export since a null mutation in the corresponding gene causes mRNA accumulation in the nucleus (Dong *et al.*, 2006a). The *atnup160-1* mutant shows a reduced tolerance to chilling and freezing temperatures, probably due to a decrease in cold-induction of *CBF*s (Dong *et al.*, 2006a). Moreover, transcriptome analysis revealed that the expression of other cold-inducible genes than the *CBFs* is affected in this mutant, which should contribute to its sensitive phenotypes (Dong *et al.*, 2006a). LOS4, a DEAD-box RNA helicase, has also been implicated in cold acclimation (Gong *et al.*, 2005). *los4* mutants display improved cold-induction of *CBF* genes under low temperature conditions and are more sensitive to freezing than wild-type plants (Gong *et al.*, 2005). As in the case of AtNUP160, *in situ* poly(A) hybridizations revealed that LOS4 is needed for a correct partition of mRNAs in the cell (Gong *et al.*, 2005). Taken together, all these results provide a new scenario on the regulation of cold acclimation, where mRNA stability and processing, as well as mobilization, seems to be as important as transcriptional activation.

Translational and post-translational regulation of cold acclimation response

Protein synthesis has been described essential for adequate response to low temperature, indicating that cold acclimation is subjected to translational regulation. Consistently, the translation elongation factor 2-like protein, LOS1, has been shown to be a positive regulator of cold acclimation (Guo *et al.*, 2002). The *los1-1* mutation displays reduced cold-acclimation capability due to a reduced cold induction of the CBF-target genes (Guo *et al.*, 2002). In contrast, the induction of the *CBFs* in response to low temperature is higher in *los1-1* than in wild-type plants (Guo *et al.*, 2002). Additionally, synthesis of new proteins under low temperature is severely impaired in *los1-1*, which could account for the reduction of cold-inducible gene expression (Guo *et al.*, 2002). These results suggest that expression of *CBFs* is independent of new protein synthesis since they are overinduced in *los1-1* mutant under cold conditions. The fact that *CBF* expression is overinduced in *los1-1* indicates that it is subjected to feedback regulation (Guo *et al.*, 2002).

Recent works have revealed that protein stability has important implications in the regulation of cold-acclimation response. In Arabidopsis, Ishitani *et al.* (1998) identified HOS1, a negative regulator of the CBF regulon that contains a RING finger domain characteristic of some plant E3 ubiquitin ligases. In agreement with its role as negative regulator, *HOS1* expression is transiently inhibited in response to cold treatment (Ishitani *et al.,* 1998). Although HOS1 negatively regulates cold-inducible gene expression, *hos1-1* mutant is chilling and freezing sensitive when compared

to wild type, indicating that an accurate expression pattern of *HOS1* is essential for the correct response to low temperature (Ishitani *et al.*, 1998). Subsequently, it has been demonstrated that, in fact, HOS1 has E3 ubiquitin ligase activity and polyubiquitilates ICE1 (see above) mediating its degradation and, therefore, inhibiting the cold-induction of *CBF3* and the CBF regulon (Dong *et al.*, 2006b).

SUMOylation is another post-translational modification that can control protein stability and activity. SUMOylation, as ubiquitilation, requires the sequential action of three enzymes, E1, E2, and E3 (Kurepa *et al.*, 2003; Colby *et al.*, 2006). The Arabidopsis genome only includes one gene homolog to E3 sumo ligases, *SIZ1* (Miura *et al.*, 2007). *SIZ1* expression is not induced by cold although mutations in *SIZ1* decrease Arabidopsis tolerance to freezing and chilling temperatures, indicating that SUMOylation also plays an important role in plant response to low temperature (Miura *et al.*, 2007). SIZ1 catalyzes the SUMOylation of ICE1 which in turn impedes the ubiquitilation of ICE1 (see above) and, thereby, the induction of *CBF3* (Miura *et al.*, 2007). Furthermore, ICE1 SUMOylation inhibits the expression of MYB15, a negative regulator of *CBF* expression (see above) (Miura *et al.*, 2007). Although it has not been reported how SUMOylation affects ICE1 stability, it has been proposed that most probably would induce its stabilization. A hypothetical model that would integrate HOS1, SIZ1 and ICE1 activities in the regulation of cold acclimation is presented in Figure 2. In this model, under control conditions, HOS1 would be targeting ICE1 for its degradation by the proteosome, maintaining the accumulation of the protein at low levels. Temperature decrease would activate, by still unknown pathways, SIZ1. This activation would induce the SUMOylation of ICE1 and, thereby, a decrease in its ubiquitilation levels. The increase in ICE1 accumulation would then induce the expression of the *CBFs*, directly by its interaction with their promoters and indirectly by the repression of *MYB15*, and, therefore, the CBF regulon and the cold-acclimation process.

AtCHIP also encodes an Arabidopsis E3 ubiquitin ligase which expression is cold induced (Yan *et al.*, 2003). AtCHIP seems to be a negative regulator of freezing tolerance since overexpression of the corresponding gene increases Arabidopsis sensitivity to freezing temperatures (Yan *et al.*, 2003). The A subunit of protein phosphatase 2A, PP2A, has been identified as one of the AtCHIP substrates (Luo *et al.*, 2006).

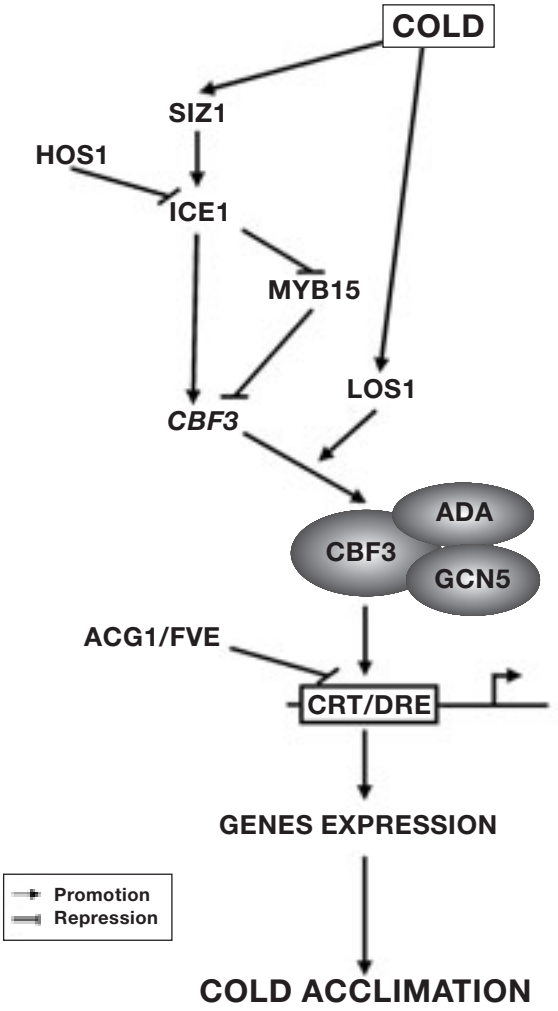

Figure 2. Hypothetical model for post-transcriptional regulation in cold acclimation. Protein modification seems to have an important role in the transduction of the cold signal. Under control conditions, HOS1 ubiquitylates ICE1 leading to its degradation by the proteasome. Low temperature would activate, by still unknown mechanisms, SIZ1, which, in turn, would label ICE1 with SUMO to stabilize the protein. The stabilization of ICE1 would increase the expression of several genes, including *CBF3*. In addition, ICE1 SUMOylation seems to repress MYB15 expression, which is inhibiting *CBF3* expression. The synthesis of CBF3 depends on the activation of LOS1, a translation elongation factor 2-like protein. On the one hand, CBF3 binding to the CRT/DRE element to activate gene expression would be positively regulated by its interaction with different components of the HAT complex, such as ADA2 and CGN5. HAT activity would remove inhibitory chromatin structures by acetylating histones near to the CRT/DRE box. On the other hand, FVE, a component of the HDAC complex, would inhibit the activation of gene expression by CBF3 binding to the CRT/DRE box through the deacetylation of the same histones. HDAC inhibition of gene expression would be required to ensure an accurate expression pattern of the cold inducible genes, and the correct cold-acclimation response.

PP2A is a negative regulator of sucrose synthase (SUS), and therefore of sugar biosynthesis, that has an important role in tolerance to low temperatures, including freezing ones. AtCHIP-overexpressing plants show an increased PP2A activity under cold, which could account for the freezing sensitivity of these plants (Luo *et al.*, 2006). AtCHIP seems to monoubiquitylate PP2A that would not lead to PP2A degradation (Luo *et al.*, 2006). It has been proposed that PP2A monoubiquitylation could alter its interaction with another subunit of PP2A which would increase its phosphatase activity, provoking a decrease in freezing tolerance (Luo *et al.*, 2006). F-box proteins are the degradation substrate receptor subunits of SCF-type E3 ligases (Jin *et al.*, 2005). *AtFBP7* encodes an F-box protein which expression is induced by low temperature (Calderón-Villalobos *et al.*, 2007). *fbp7* mutants have an impaired protein synthesis under low temperature conditions although they do not show any significant difference in growth and cell integrity compared to wild-type plants (Calderón-Villalobos *et al.*, 2007). Therefore, it seems that, although AtFBP7 has a role in the regulation of protein synthesis under cold conditions, its function is not essential for plant tolerance to low temperature stress.

Histone acetylation mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) is another post-translational mechanism that has been related to cold acclimation. Several reports have involved HATs in transcriptional regulation by remodeling repressive chromatin structures (Sterner and Berger, 2000; Roth *et al.*, 2001). In Arabidopsis, different components of the HATs complex have been identified (ADA2a, ADA2b and GCN5) which interact *in vitro* with CBF1 and seems to be needed for CBF1 function (Stockinger *et al.*, 2001). Functional characterization of ADA2b and GCN5 showed that these proteins control cold-inducible gene expression (Vlachonasios *et al.*, 2003). *ada2b* and *gcn5* mutant plants display wild-type *CBF* expression, although the cold induction of their targets seems to be reduced (Vlachonasios *et al.*, 2003). Interestingly, only the induction of the CBF-regulon is affected in *ada2b* and *gcn5* mutants, suggesting that the activity of the HATs complex in response to cold is highly specific (Vlachonasios *et al.*, 2003). Reversibility of histone acetylation seems also to have a role in regulating cold-inducible gene expression. Thus, Kim *et al.* (2004) identified the Arabidopsis mutant *acg1* as a negative regulator of the CBF-regulon. The *acg1* mutant has similar constitutive freezing tolerance as the wild type but increased cold-acclimation capability, which correlates with a higher cold induction of *CBFs* and their target genes (Kim *et al.*, 2004). *ACG1* resulted to encode FVE, a component of the HDAC complex in Arabidopsis homolog to the mammalian retinoblastoma associated protein (Kim *et*

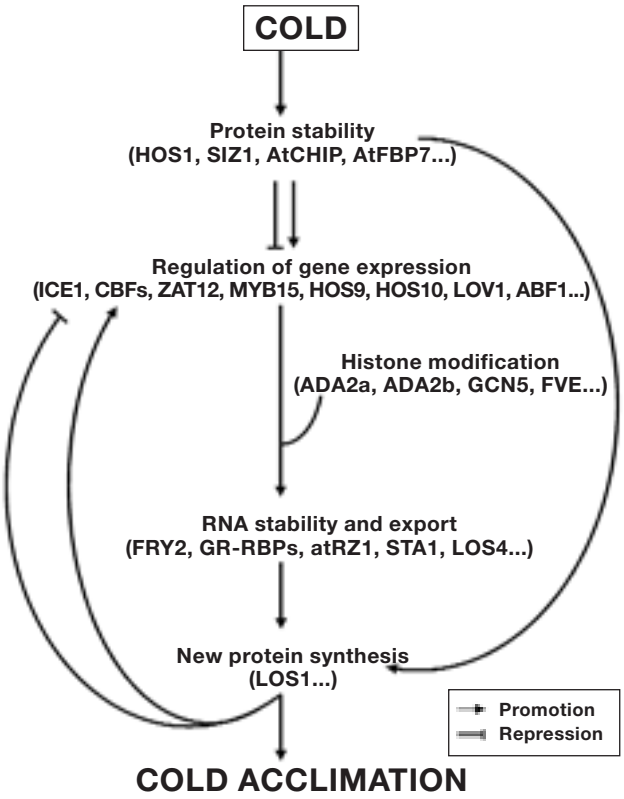

Figure 3. General scheme of the different regulatory levels acting in cold acclimation. With the data available so far, the first level of regulation seems to imply changes in protein stability, mediated by ubiquitylation and SUMOylation. At this level, we would find the E3 ubiquitin ligases HOS1, AtCHIP and AtFBP7, as well as the E3 SUMO ligase AtSIZ1. These proteins would act on a second level of regulation, by modifying transcription factors such as ICE1, that, in turn, would regulate the cold induction of genes encoding other transcription factors (i.e., *CBFs*), that would activate gene expression response to low temperatures. A third level of regulation involves histone modifications, which are also necessary for the activation of gene expression. Components of the HATs (i.e. ADA2a, ADA2b and GCN5) and HDACs (i.e. FVE) participate at this regulating level. Once gene expression is activated, mRNA mobility, stability and structure would be the subsequent level of regulation, involving FRY2, GR-RBPs and atRZ proteins as RNA-chaperones, and STA1 and LOS4 in RNA mobilization. Finally, at the fifth level, activation of protein synthesis at low temperature by proteins such as LOS1 would be essential for the induction of gene expression and the cold-acclimation response. Besides, new protein synthesis would negatively regulate cold-inducible gene expression. This regulatory level would also be controlled by protein stability mechanisms as it has been demonstrated in *atfbp7* mutants.

al., 2004). All these results indicate that histone acetylation is important for the interaction of CBFs with the CRT/DRE element, and the subsequent activation of gene expression under low temperatures, in all likelihood to avoid inhibitory chromatin structures.

Future directions

In this review, recent results that have contributed to extend our knowledge on how the cold-acclimation response is regulated have been summarized (Fig. 3). It has been shown that regulation of gene expression during cold acclimation involves different pathways. At this point, a raising question that needs to be answered is how plants precisely coordinate these signaling pathways and whether they share common components. Regarding the post-transcriptional regulation, the data reported so far evidence the essential role of this kind of regulation for the correct development of cold acclimation. The identification of intermediates in the regulation of mRNA transport and stability will be essential in order to have a complete scenario on how plant response to low temperatures is controlled. Finally, protein stability plays also a role in low temperatures response, involving, at least, ubiquitylation and SUMOylation. The identification and characterization of new ubiquitin and SUMO E3 ligases involved in the regulation of the cold-acclimation process should constitute an important challenge for the future.

References

AGARWAL M., HAO Y., KAPOOR A., DONG C.H., FUJII H., ZHENG X., ZHU J.K., 2006. A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. J Biol Chem 281, 37636-37645.

BAKER S.S., WILHELM K.S., THOMASHOW M.F., 1994. The 5'-region of *Arabidopsis thaliana cor15a* has *cis*-acting elements that confer cold-, drought- and ABA-regulated gene expression. Plant Mol Biol 24, 701-713.

CALDERÓN-VILLALOBOS L.I., NILL C., MARROCCO K., KRETSCH T., SCHWECHHEIMER C., 2007. The evolutionarily conserved *Arabidopsis thaliana* F-box protein AtFBP7 is required for efficient translation during temperature stress. Gene 392, 106-116.

CHINNUSAMY V., OHTA M., KANRAR S., LEE B.H., HONG X., AGARWAL M., ZHU J.K., 2003. ICE1: a regulator of cold-induced transcriptome and freezing tolerance in Arabidopsis. Genes Dev 17, 1043-1054.

CHOI H., HONG J., HA J., KANG J., KIM S.Y., 2000. ABFs, a family of ABA-responsive element binding factors. J Biol Chem 275, 1723-1730.

CHRISTIE P.J., ALFENITO M.R., WALBOT W., 1994. Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. Planta 194, 541-549.

COLBY T., MATTHAI A., BOECKELMANN A., STUIBLE H.P., 2006. SUMO-conjugating and SUMO-deconjugating enzymes from Arabidopsis. Plant Physiol 142, 318-332.

DONG C.H., HU X., TANG W., ZHENG X., KIM Y.S., LEE B.H., ZHU J.K., 2006a. A putative Arabidopsis nucleoporin, AtNUP160, is critical for RNA export and required for plant tolerance to cold stress. Mol Cell Biol 26, 9533-9543.

DONG C.H., AGARWAL M., ZHANG Y., XIE Q., ZHU J.K., 2006b. 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.

DUNN M.A., GODDARD N.J., ZHANG L., PEARCE R.S., HUGHES M.A., 1994. Low-temperature-responsive barley genes have different control mechanisms. Plant Mol Biol 24, 879-888.

FOWLER S., THOMASHOW M.F., 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.

GARG A.K., KIM J.K., OWENS T.G., RANWALA A.P., CHOI Y.D., KOCHIAN L.V., WU R.J., 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc Natl Acad Sci USA 99, 15898-15903.

GILMOUR S.J., ZARKA D.G., STOCKINGER E.J., SALAZAR M.P., HOUGHTON J.M., THOMASHOW M.F., 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.

GILMOUR S.J., SEBOLT A.M., SALAZAR M.P., EVERARD J.D., THOMASHOW M.F., 2000. Overexpression of the Arabidopsis *CBF3* transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiol 124, 1854-1865.

GONG Z., DONG C.H., LEE H., ZHU J., XIONG L., GONG D., STEVENSON B., ZHU J.K., 2005. A DEAD box RNA helicase is essential for mRNA export and important for development and stress responses in Arabidopsis. Plant Cell 17, 256-267.

GUILTINAN M.J., MARCOTTE W.R. Jr., QUATRANO R.S., 1990. A plant leucine zipper protein that recognizes an abscisic acid response element. Science 250, 267-271.

GUO Y., XIONG L., ISHITANI M., ZHU J.K., 2002. An Arabidopsis mutation in translation elongation factor 2 causes superinduction of CBF/DREB1 transcription factor genes but blocks the induction of their downstream targets under low temperatures. Proc Natl Acad Sci USA. 99, 7786-7791.

HAJELA R.K., HORVATH D.P., GILMOUR S.J., THOMASHOW M.F., 1990. Molecular-cloning and expression of *cor* (cold-regulated) genes in *Arabidopsis thaliana*. Plant Physiol 93, 1246-1252.

ISHITANI M., XIONG L., LEE H., STEVENSON B., ZHU J.K., 1998. HOS1, a genetic locus involved in cold-responsive gene expression in Arabidopsis. Plant Cell 10, 1151-1161.

JIN J., ANG X.L., SHIROGANE T., WADE HARPER J., 2005. Identification of substrates for F-box proteins. Methods Enzymol 399, 287-309.

KAPLAN F., KOPKA J., HASKELL D.W., ZHAO W., SCHILLER K.C., GATZKE N., SUNG D.Y., GUY C.L., 2004. Exploring the temperature-stress metabolome of Arabidopsis. Plant Physiol 136, 4159-4168.

KIM J.C., LEE S.H., CHEONG Y.H., YOO C.M., LEE S.I., CHUN H.J., YUN D.J., HONG J.C., LEE S.Y., LIM C.O., CHO M.J., 2001. A novel cold-inducible zinc finger protein from soybean, SCOF-1, enhances cold tolerance in transgenic plants. Plant J 25, 247-259.

KIM H.J., HYUN Y., PARK J.Y., PARK M.J., PARK M.K., KIM M.D., KIM H.J., LEE M.H., MOON J., LEE I., KIM J., 2004. A genetic link between cold responses and flowering time through FVE in *Arabidopsis thaliana*. Nat Genet 36, 167-171.

KIM Y.O., KIM J.S., KANG H., 2005. Cold-inducible zinc finger-containing glycine-rich RNA-binding protein contributes to the enhancement of freezing tolerance in *Arabidopsis thaliana*. Plant J 42, 890-900.

KREPS J.A., WU Y., CHANG H.S., ZHU T., WANG X., HARPER J.F., 2002. Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol 130, 2129-2141.

KRÓL M., IVANOV A.G., JANSSON S., KLOPPSTECH K., HUNER N.P.A., 1999. Greening under high light or cold temperature affects the level of xanthophyll-cycle pigments, early light-inducible proteins, and light-harvesting polypeptides in wild-type barley and the Chlorina f2 mutant. Plant Physiol 120, 193-203.

KUREPA J., WALKER J.M., SMALLE J., GOSINK M.M., DAVIS S.J., DURHAM T.L., SUNG D.Y., VIERSTRA R.D., 2003. The small ubiquitin-like modifier (SUMO) protein modification system in Arabidopsis. Accumulation of SUMO1 and -2 conjugates is increased by stress. J Biol Chem 278, 6862-6872.

LEE B.H., HENDERSON D.A., ZHU J.K., 2005. The Arabidopsis cold-responsive transcriptome and its regulation by ICE1. Plant Cell 17, 3155-3175.

LEE B.H., KAPOOR A., ZHU J., ZHU J.K., 2006. STABILIZED1, a stress-upregulated nuclear protein, is required for pre-mRNA splicing, mRNA turnover, and stress tolerance in Arabidopsis. Plant Cell 18, 1736-1749.

LEVITT J., 1980. Responses of plants to environmental stress: chilling, freezing and high temperatures stresses. Academic Press, NY.

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.

LUO J., SHEN G., YAN J., HE C., ZHANG H., 2006. AtCHIP functions as an E3 ubiquitin ligase of protein phosphatase 2A subunits and alters plant response to abscisic acid treatment. Plant J 46, 649-657.

LLORENTE F., LÓPEZ-COBOLLO R.M., CATALÁ R., MARTÍNEZ-ZAPATER J.M., SALINAS J., 2002. A novel cold-inducible gene from Arabidopsis, RCI3, encodes a peroxidase that constitutes a component for stress tolerance. Plant J 32, 13-24.

MEDINA J., BARGUES M., TEROL J., PÉREZ-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.

MITA T., SHIBAOKA H., 1984. Gibberellin stabilizes microtubules in onion leaf sheath cells. Proteoplasma 119, 100-109.

MIURA K., JIN J.B., LEE J., YOO C.Y., STIRM V., MIURA T., ASHWORTH E.N., BRESSAN R.A., YUN D.J., HASEGAWA P.M., 2007. SIZ1-mediated sumoylation of ICE1 controls CBF3/DREB1A expression and freezing tolerance in Arabidopsis. Plant Cell 19, 1403-1414.

NISHIDA I., MURATA N., 1996. Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. Annu Rev Plant Physiol Plant Mol Biol 47, 541-568.

NOVILLO F., ALONSO J.M., ECKER J.R., 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.

PHILLIPS J.R., DUNN M.A., HUGHES M.A., 1997. mRNA stability and localisation of the low-temperature-responsive barley gene family blt14. Plant Mol Biol 33, 1013-1023.

PRASAD T.K., 1997. Role of catalase in inducing chilling tolerance in pre-emergent maize seedlings. Plant Physiol 114, 1369-1376.

RISTIC Z., ASHWORTH E.N., 1993. Ultrastructural evidence that intracellular ice formation and possibly cavitation are the sources of freezing injury in supercooling wood tissue of *Cornus florida* L. Plant Physiol 103, 753-761.

ROTH S.Y., DENU J.M., ALLIS C.D., 2001. Histone acetyltransferases. Annu Rev Biochem 70, 81-120.

SACHETTO-MARTINS G., FRANCO L.O., DE OLIVEIRA D.E., 2000. Plant glycine-rich proteins: a family or just proteins with a common motif? Biochim Biophys Acta 1492, 1-14.

SALINAS J., 2002 Molecular mechanism of signal transduction in cold acclimation. Frontiers in Molecular Biology (Hames B.D., Glover D.M, eds). Oxford Univ Press. pp. 116-139.

SAUTER J.J., WISNIEWSKI M., WITT W., 1996. Interrelationships between ultrastructure, sugar levels, and frost hardiness of ray parenchyma cells during frost acclimation

and deacclimation in poplar (*Populus × canaiensis* Moench «robusta») wood. J Plant Physiol 149, 451-461.

SCHONER S., KRAUSE G.H., 1990. Protective systems against active oxygen species in spinach: responses to cold acclimation in excess light. Planta 180, 383-389.

SEKI M., NARUSAKA M., ISHIDA J., NANJO T., FUJITA M., OONO Y., KAMIYA A., NAKAJIMA M., ENJU A., SAKURAI T. *et al.*, 2002. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J 31, 279-292.

SMALLWOOD M., BOWLES D.J., 2002. Plants in a cold climate. Philos Trans R Soc Lond B Biol Sci 357, 831-847.

STERNER D.E., BERGER S.L., 2000. Acetylation of histones and transcription-related factors. Microbiol Mol Biol Rev 64, 435-459.

STOCKINGER E.J., GILMOUR S.J., THOMASHOW M.F., 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.

STOCKINGER E.J., MAO Y., REGIER M.K., TRIEZENBERG S.J., THOMASHOW M.F., 2001. Transcriptional adaptor and histone acetyltransferase proteins in Arabidopsis and their interactions with CBF1, a transcriptional activator involved in cold-regulated gene expression. Nucleic Acids Res 29, 1524-1533.

THOMASHOW M.F., 1999. Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol 50, 571-599.

VLACHONASIOS K.E., THOMASHOW M.F., TRIEZENBERG S.J., 2003. Disruption mutations of ADA2b and GCN5 transcriptional adaptor genes dramatically affect Arabidopsis growth, development, and gene expression. Plant Cell 15, 626-638.

VOGEL J.T., ZARKA D.G., VAN BUSKIRK H.A., FOWLER S.G., THOMASHOW M.F., 2005. Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis. Plant J 41, 195-211.

WANG H., CUTLER A.J., 1995. Promoters from *kin1* and *cor6.6*, two *Arabidopsis thaliana* low- temperature- and ABA-inducible genes, direct strong beta-glucuronidase expression in guard cells, pollen and young developing seeds. Plant Mol Biol 28, 619-634.

WANNER L.A., JUNTTILA O., 1999. Cold-induced freezing tolerance in Arabidopsis. Plant Physiol 120, 391-400.

WOLFRAIM L.A., LANGIS R., TYSON H., DHINDSA R.S., 1993. cDNA sequence, expression, and transcript stability of a cold acclimation-specific gene, *cas18*, of alfalfa (*Medicago falcata*) cells. Plant Physiol 101, 1275-1282.

XIN Z., BROWSE J., 1998. Eskimo1 mutants of Arabidopsis are constitutively freezing-tolerant. Proc Natl Acad Sci USA 95, 7799-7804.

XIONG L., LEE H., ISHITANI M., TANAKA Y., STEVENSON B., KOIWA H., BRESSAN R.A., HASEGAWA P.M., ZHU J.K., 2002. Repression of stress-responsive genes by FIERY2, a novel transcriptional regulator in Arabidopsis. Proc Natl Acad Sci USA 99, 10899-108904.

YAMAGUCHI-SHINOZAKI K., SHINOZAKI K., 1994. A novel *cis*-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6, 251-264.

YAN J., WANG J., LI Q., HWANG J.R., PATTERSON C., ZHANG H., 2003. AtCHIP, a U-box-containing E3 ubiquitin ligase, plays a critical role in temperature stress tolerance in Arabidopsis. Plant Physiol 132, 861-869.

YOO S.Y., KIM Y., KIM S.Y., LEE J.S., AHN J.H., 2007. Control of flowering time and cold response by a NAC-domain protein in Arabidopsis. PLoS ONE 2, e642. doi:10.1371/journal.pone.0000642.

ZHU J., SHI H., LEE B.H., DAMSZ B., CHENG S., STIRM V., ZHU J.K., HASEGAWA P.M., BRESSAN R.A., 2004. An Arabidopsis homeodomain transcription factor gene, HOS9, mediates cold tolerance through a CBF-independent pathway. Proc Natl Acad Sci USA 101, 9873-9878.

ZHU J., VERSLUES P.E., ZHENG X., LEE B.H., ZHAN X., MANABE Y., SOKOLCHIK I., ZHU Y., DONG C.H., ZHU J.K., HASEGAWA P.M., BRESSAN R.A., 2005. HOS10 encodes an R2R3-type MYB transcription factor essential for cold acclimation in plants. Proc Natl Acad Sci USA 102, 9966-9971.

ZHU J., DONG C.H., ZHU J.K., 2007. Interplay between cold-responsive gene regulation, metabolism and RNA processing during plant cold acclimation. Curr Opin Plant Biol 10, 290-295.