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REVIEW

Perception, Transduction, and Networks in Cold Signaling

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Cold temperature is one of the most critical environmental conditions that limit the geographic distribution of plants and account for significant reductions in the yields of agriculturally important crops. As part of their stress-adaptation, plants respond to cold by changing various aspects of their physiological processes, including gene expression. Numerous genes are induced by cold at the transcriptional level, encoding proteins that protect against freezing injury. While the expression of stress-inducible genes is mainly mediated by ABA-dependent and ABA-independent pathways, the C-repeat/dehydration-responsive element (CRT/DRE), containing a core sequence of -CCGAC- in the ABA-independent pathway, is essential for transcriptional activation in response to abiotic stresses, including cold. The CRT/DRE-binding factors (CBFs) and DRE-binding proteins (DREBs) can confer freezing and drought tolerances in transgenic plants by up-regulating the genes involved in enhancing stress tolerance when overexpressed. However, microarray analysis and characterization of various *Arabidopsis* mutants with altered response to cold and freezing temperatures suggest the existence of other cold-signaling pathways that do not involve CBF/DREB transcription factors. This review describes potential mechanisms for cold-perception, the functions of genes induced by cold temperatures in freezing tolerance, and the regulation of cold-responsive gene expression. Genetic approaches, including classical methods and reporter-gene-based screening, have revealed many signaling components in this stress response, suggesting that complicated, multiple pathways and cross-talk are involved. Recent advances in our understanding of the roles for these components as well as calcium-signaling networks are also discussed here.

Keywords: Arabidopsis, CBF/DREB, cold, drought, freezing, signal-transduction

COLD SENSORS AND PERCEPTION MECHANISMS

A potential sensor for cold perception has been suggested, based on experiments with the cyanobacterium *Synechocystis* sp. PCC6803 (Suzuki et al., 2000). Cyanobacteria modulate the composition of membrane lipids in response to temperature shifts from 34 to 22°C, increasing the fluidity of their membranes for adaptation to cold stress by enhancing the expression of three fatty-acid-desaturase genes (*des*): *desA*, *desB*, and *desD* (Los et al., 1997). Reduction in membrane fluidity by catalytic hydrogenation of fatty acids in the plasma membranes of *Synechocystis* resulted in the induction of transcription for *desA* (Vigh et al., 1993). Based on this, Murata and Los (1997) have suggested that the expression of desaturase genes in response to cold stress might be regulated by the degree of membrane rigidity. Murata's group has systematically disrupted putative genes for histidine kinases and randomly mutagenized almost all of the genes in the genome of *Synechocystis* sp. PCC6803. From this, two histidine kinases (Hik), Hik33 and Hik19, and a response regulator, Rer1, have been identified as components for cold-signal perception and transduction (Suzuki et al., 2000). Hik33 contains the strongly conserved histidine kinase domain near the C-terminus and two hydrophobic helices that might span the membrane. A putative leucine-zipper motif and a coiled-coil sequence are located between the second hydrophobic helix and the histidine kinase domain that is known to be involved in the dimerization of histidine kinases. Based on these characteristics, it has been proposed that Hik33 acts as a sensor that can detect a decrease in membrane fluidity due to cold. By contrast, Hik19 is predicted to be a soluble protein in the cytosol and a transducer of cold temperature that might function downstream of Hik33. Subsequent DNA microarray analysis has shown that Hik33 regulates the expression of 28 out of 45 known cold-inducible genes (Suzuki et al., 2001; Mikami et al., 2002). Interestingly, it could also control the expression of osmotic stress-inducible genes, indicating that it is a multifunctional sensor. However, a counterpart for Hik33 has not yet been found in higher plants. Although the molecular identity of those potential cold-temperature sensors remains elusive, a change in membrane fluidity seems to be a possible mechanism for detecting low temperatures in higher plants as well (Örvar et al., 2000; Sangwan et al., 2001; Sharma et al., 2005). Results from analysis of *Arabidopsis* mutants with altered desaturase activity also suggest that membrane rigidification might be a perception mechanism in the activation of the diacylglycerol kinase pathway following a drop in temperature (Vaultier et al., 2006).

FUNCTION OF COLD-REGULATED GENES IN FREEZING TOLERANCE

Low temperatures induce the expression of numerous genes that encode proteins involved in a wide range of physiological processes associated with cold acclimation (Hughes and Dunn, 1996; Thomashow, 1999; Xin and Browse, 2000; Browse and Xin, 2001). Some of these proteins have known enzymatic functions, and their gene transcript levels increase by only 2~5-fold when temperatures decrease from 24 to 4°C. Some of cold-inducible genes also encode a group of proteins involved in the dehydration response; these include dehydrins or late embryogenesis

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abundant (LEA)-like proteins, antifreeze proteins, osmotin, mRNA-binding proteins, and heat shock proteins. Some encode kinases, such as MAP kinases and calcium-dependent protein kinases. One class of cold-regulated genes is strongly induced, typically 50- to 100-fold, during cold treatment. These are *COLD REGULATED* (*COR*), *LOW-TEMPERATURE INDUCED* (*LTI*), *COLD-ACCLIMATION-SPECIFIC* (*CAS*), *RESPONSIVE TO DEHYDRATION* (*RD*), *EARLY RESPONSIVE TO DEHYDRATION* (*ERD*), and *COLD-INDUCIBLE* (*KIN*). Although their encoded proteins have no enzymatic activity, many are strongly hydrophilic and remain stable even upon boiling, suggesting their function as cryoprotectants (Thomashow, 1998). Freezing temperatures cause ice to form in the intercellular spaces of plant tissues, leading to severe cellular dehydration (Thomashow, 1998). This decreases water potential outside of the cell, and consequently, frozen water moves down a chemical potential gradient from the cell interior to the intercellular spaces. Cellular dehydration caused by such movement of water molecules causes most plant injuries because of membrane damage. Hydrophilic polypeptides encoded by the *COR* genes help stabilize cell and organelle membranes against this freeze-induced injury (Artus et al., 1996; Steponkus et al., 1998).

Microarray technology has enabled genome-wide analysis of the cold-regulated transcriptome in *Arabidopsis* (Fowler and Thomashow, 2002; Seki et al., 2002; Lee et al., 2005; Oono et al., 2006). Expression-profiling via Affymetrix GeneChips that represented approximately 24000 genes showed that the *Arabidopsis* genome contains 939 cold-regulated genes, with 655 being up-regulated and 284 down-regulated (Lee et al., 2005). Among the up-regulated genes, 113 are involved in transcription and 63 are associated with signal-transduction. Those components of the latter type are Ca^{2+} binding proteins, a finding consistent with a role proposed for calcium in early cold-signal transduction, as well as a large number of protein kinases and phosphatases (including receptor-like kinases), pseudoresponse regulators, and phospholipid signaling components. Among those involved in transcription, the two major families are APETALA 2/ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR (AP2/ERF) and the Zn finger domains, which account for 21 and 27 genes, respectively. Others include basic leucine zipper transcription factors, e.g., ABA-responsive element binding factors (ABFs), WRKY transcription factors, and MYB transcription factors, as well as plant-development transcription factors, such as ARF, GRAS, MADS, and NAC.

CIS-ACTING ELEMENTS AND *TRANS*-ACTING FACTORS

A novel *cis*-acting element responsive to cold temperature, drought, and high salt has been identified from *Arabidopsis RD29A* (Yamaguchi-Shinozaki and Shinozaki, 1994). This element contains -TACCGACAT-, and is named dehydration-responsive element (DRE). Promoter analysis of *Arabidopsis COR15A* has revealed a similar *cis*-acting element that responds to cold and drought (Baker et al., 1994). As a core sequence, these two elements contain the -CCGAC- sequence, i.e., a C-repeat/dehydration-responsive element; both elements are found in the promoter regions of most cold-inducible genes in *Arabidopsis*, *Brassica*, and monocot plants (Hughes and Dunn, 1996; Jaglo et al., 2001). The CBF/DREB transcription factors that bind to CRT/DRE possess a DNA binding motif found in the ERF and AP2 proteins (Stockinger et al., 1997; Liu et al., 1998). Overexpression of *CBF1* (=*DREB1B*) and *DREB1A* (=*CBF3*) in transgenic *Arabidopsis* enhances freezing and drought tolerances, with concomitant expression of various *COR* genes (Jaglo-Ottosen et al., 1998; Liu et al., 1998). Likewise, overexpressing *CBF2* (=*DREB1C*) in transgenic *Arabidopsis* also increases freezing tolerance, suggesting that the CBF1, 2, and 3 transcriptional activators all have redundant functional activities in improving tolerance against cold (Gilmour et al., 2004). However, CBF4, an apparent homolog of the CBF/DREB1 proteins, is unique in that *CBF4* expression is up-regulated by drought stress and ABA, but not by cold temperatures, even though its overexpression renders transgenic *Arabidopsis* tolerant of both freezing and drought (Haake et al., 2002). Compared with the wild type, *cbf2 Arabidopsis* mutants exhibit greater tolerance to freezing, high salinity, and dehydration, and also show increased expression of *CBF1* and *CBF3* as well as stronger and more sustained expression of CBF/DREB-regulated genes, thereby suggesting that CBF2 is a negative regulator of CBF1 and CBF3 expression (Novillo et al., 2004). Thirty-eight genes have been identified as DREB1a downstream genes, including 20 previously unreported that contain a consensus CRT/DRE in the promoter regions (Maruyama et al., 2004).

An upstream transcription factor, ICE1 (INDUCER OF CBF EXPRESSION 1), that regulates the expression of *CBF3* in the cold has been isolated by a screen for mutations that impair cold-induced expression of a *CBF3* promoter-luciferase reporter gene (Chinnusamy et al., 2003). ICE1, a basic helix-loop-helix transcriptional activator, binds to the MYC recognition sequences in the *CBF3* promoter. *Arabidopsis* transcriptome analysis has shown that the expression of 369 out of 939 cold-regulated genes is affected, including cold-responsive genes known to be crucial to cold tolerance. This suggests that ICE1 has an important role in plant cold responses (Lee et al., 2005).

Overexpression of stress-inducible *Arabidopsis DREB1A* in tobacco results in the concomitant expression of CBF/DREB target genes, leading to enhanced tolerance against drought stress and low temperatures (Kasuga et al., 2004). Likewise, the overexpression of either rice or maize CBF/DREB homologs in transgenic *Arabidopsis* causes the CBF/DREB target genes to be induced, again conferring increased tolerance to drought, freezing stress, and high salinity (Dubouzet et al., 2003; Qin et al., 2004). Furthermore, overexpression of *Brassica* or rice *CBF/DREB*-like genes in either transgenic *Brassica* (Savitch et al., 2005) or rice (Ito et al., 2006) also results in improved freezing tolerance. These reports suggest that the CBF/DREB cold-responsive signaling pathway is conserved in *Brassica* and rice and, likely, also in maize. Conservation of this pathway has also been found in other plant species, such as barley (Skinner et al., 2005), *Populus* (Benedict et al., 2006), and perennial ryegrass (Xiong and Fei, 2006). In the case of tomato, which has no native capacity for cold acclimation, three *CBF* homologs, *LeCBF1*, *LeCBF2*, and *LeCBF-3*, are encoded, but only *LeCBF1* is cold-inducible (Zhang et al., 2004). Overexpression of either *LeCBF1* or *CBF3* in transgenic tomato plants does not increase freezing tolerance. Only a few apparent CBF target genes were found to be induced. However, the overexpression of *CBF1* leads to elevated tolerance against chilling and water-deficit stresses (Hsieh et al., 2002a, b). Therefore, these results indicate that, although tomato has a functional CBF cold-response pathway, its CBF regulon differs from that of *Arabidopsis*, and appears to be considerably narrower in its functioning.

GENETIC APPROACH IS REVEALING VARIOUS COLD-SIGNALING COMPONENTS

To understand the cold-acclimation mechanism, Warren et al. (1996) have isolated *Arabidopsis* mutants that exhibit sensitivity to freezing tolerance after they become adjusted to low temperatures. Plants of *sensitive to freezing 6* (*sfr6*) mutant exhibit a deficiency in their cold-inducible expression of *KIN1*, *COR15a*, and *LTI78*, but show normal induction of *CBF1*, *CBF2*, *CBF3*, and *ATP5CS1*, which lack CRT/DRE. This indicates that the SFR6 protein is a critical component for potentiating the action of CBF/DREBs (Knight et al., 1999). Microarray analysis has further demonstrated that the *sfr6* mutation affects the cold-induced expression of genes regulated via CRT/DRE (Boyce et al., 2003). Even in the absence of cold acclimation, *eskimo1* (*esk1*) *Arabidopsis* mutants exhibit constitutive tolerance to freezing (Xin and Browse, 1998). This mutation affects the transcript levels of genes associated with proline synthesis, including Δ^1-pyrroline-5-carboxylate synthetase, and with degradation, proline oxidase, but they do not constitutively express several *COR* genes. This suggests that ESK1 functions in a different pathway that does not involve CBF/DREBs. Recent map-based cloning of *esk1* indicates that ESK1 is a novel negative regulator of cold acclimation (Xin et al., 2007). Indeed, a comparison study of the *Arabidopsis* transcriptome has revealed that at least 28% of the cold-responsive genes identified from ~8000 transcripts are not regulated by CBF transcription factors, indicating the existence of multiple cold-responsive pathways (Fowler and Thomashow, 2002).

Screening with a reporter gene system has been very useful for yielding a number of *Arabidopsis* mutants that are altered in their abiotic stress (e.g., cold)-signal transduction pathways (Ishitani et al., 1997). This system makes use of firefly luciferase (*LUC*) or β-glucuronidase (*GUS*) as a reporter gene fused to the promoter of a specifically regulated or inducible gene (Eckardt, 2001). Seeds of *Arabidopsis* plants transformed with the *LUC* gene under the control of the stress-responsive *RD29A* promoter were mutagenized by treatment with ethyl methane sulfonate, then screened for their altered *LUC* expression under defined conditions using a high-throughput luminescence imaging system. Such an approach yielded a large number of *constitutive expression of osmotically responsive* (*cos*), *low expression of osmotically responsive* (*los*), and *high expression of osmotically responsive* (*hos*) mutants (Ishitani et al., 1997). As an example, the *hos1-1* mutation causes super-induction of cold-responsive genes, such as *RD29A*, *COR47*, *COR15a*, *KIN1*, and *ADH*, which are correlated with the increased induction of *CBF* genes in response to cold; their expression is enhanced only under cold conditions (Ishitani et al., 1998; Lee et al., 2001). The *HOS1* gene encodes a novel protein containing a C_3HC_4-type RING finger motif, i.e., a small zinc-binding domain found in many proteins with diverse functions that include cell-signaling (Lee et al., 2001). Although cold treatment causes a nuclear accumulation of the HOS1-GFP fusion protein at normal temperatures, the fusion protein disappears in the nucleus, indicative of cold-regulated nuclear-cytoplasmic shuttling of the HOS1 protein. Furthermore, HOS1 interacts with ICE1, a transcriptional activator of *CBF3*, and mediates the ubiquitination of ICE1 both *in vitro* and *in vivo* (Dong et al., 2006a). Cold induces degradation of HOS1. These results suggest that the ubiquitination/proteasome degradation pathway, in which HOS1 mediates the degradation of ICE1, is involved in the cold response.

The induction of several stress-responsive genes in *los5* mutant plants can be somewhat reduced by exposure to cold, but is severely diminished or completely blocked by high-salt or dehydration conditions (Xiong et al., 2001a). *los5* is allelic to *aba3*, having a mutation in the ABA-biosynthetic pathway. *LOS5/ABA3* encodes a molybdenum cofactor sulfurase, an enzyme involved in ABA biosynthesis. These findings emphasize the necessity for abscisic acid in osmotic stress-signaling, but that hormone seems to play a minor role in cold-stress signaling. Two other mutants, *fiery1* (*fry1*) and *supersensitive to ABA and drought 1* (*sad1*), also have been characterized, and have revealed the components in ABA-signal transduction (Xiong et al., 2001b, c). *FRY2* mutation results in a significant increase in the expression of stress-responsive genes with CRT/DRE (Xiong et al., 2002). Although *fry2* mutants show greater tolerance to salt stress and to ABA during seed germination, they are more sensitive to freezing, indicating a complex role for FRY2 in the cold response. *FRY2* encodes a novel transcriptional repressor harboring double-stranded RNA-binding domains and a region homologous to the catalytic domain of RNA polymerase II C-terminal domain phosphatase in yeasts and animals. LOS4, a nuclear-localized RNA helicase, functions in the cold-regulated expression of *CBF* genes and their downstream target genes (Gong et al., 2002). Plants of *los4* mutant are chilling-sensitive, thereby demonstrating the importance of CBF transcriptional activators to chilling tolerance. LOS2 is proposed as a bi-functional enolase that can act as a transcriptional repressor for *SALT TOLERANCE ZINC FINGER/ZINC TRANSPORTER FROM ARABIDOPSIS THALIANA 10* (*STZ/ZAT10*), which encodes a zinc finger transcriptional repressor by binding to the *STZ/ZAT10* promoter (Lee et al., 2002). Consistent with this, cold-induced expression of *STZ/ZAT10* is stronger and more sustained in *los2* mutants than in wild type. Moreover, transient gene expression assays from *Arabidopsis* leaves have shown that expression of the *RD29A-LUC* reporter gene is repressed by STZ/ZAT10. *HOS10* encodes an R2R3-type MYB transcription factor essential for cold acclimation in *Arabidopsis* (Zhu et al., 2005). The *hos10-1* mutant plants are extremely sensitive to freezing temperatures, completely unable to acclimate, and exhibit such developmental alterations as smaller size, early flowering, and reduced fertility. This *hos10-1* mutation results in the enhanced expression of CBF regulon genes, but does not affect the expression of the *CBF* genes themselves. One explanation for this apparent conflict may be that the *hos10-1* mutant response of ABA biosynthesis/accumulation to dehydration is impaired. Freezing-sensitivity and the lack of cold acclimation in those mutants is thought to be due to an inability to adjust to dehydration stress (Zhu et al., 2005). A homeodomain transcription factor, HOS9, mediates the cold response through a CBF-independent pathway (Zhu et al., 2004). Even though *CBF* expression is unaffected, the *HOS9* mutation causes a hyperactive response by *RD29A* and other stress-inducible genes to cold, resulting in freezing sensitivity, both before and after cold acclimation. Microarray analysis has shown that none of the genes regulated by CBFs is influenced by this *hos9-1* mutation. These results implicate the complex nature of cold-stress signaling and networks. *GUS* gene-trapped system has been used in rice to isolate a cold-responsive gene, showing that *OsPTR1* might be a gene regulated by CBF/DREB cold signaling pathway (Kim et al., 2004).

The importance of RNA metabolism in cold-stress response has recently been highlighted by the molecular cloning and characterization of new mutants that are altered in their mRNA export, splicing, and turnover (Gong et al., 2005; Dong et al., 2006b; Lee et al., 2006). Mutations in *CRYOPHYTE/LOS4* encoding a DEAD box RNA helicase either block or weaken the export of poly(A)$^+$ RNAs and impact on cold response (Gong et al., 2005). The *stabilized1-1* (*sta1-1*) mutants, with enhanced stability of the normally unstable luciferase transcripts, have developmental and stress-response phenotypes that include chilling sensitivity, and are defective in their splicing of the cold-induced *COR15A* gene. This suggests that STA1 functions as a pre-mRNA splicing factor for splicing and mRNA turnover, and has an important role in plant responses to abiotic stresses (Lee et al., 2006). A mutant with impaired cold-induced expression of the *CBF3-LUC* reporter gene has a mutation in the gene encoding AtNUP160, a homolog of animal nucleoporin NUP160, and is also defective in its mRNA export (Dong et al., 2006b).

ROLE OF CALCIUM SIGNALING

Calcium signaling is widely used in plants for regulating a variety of cellular processes such as stomatal closure, osmoadaption, and thermotolerance (Sanders et al., 1999). This involvement of calcium was initially proposed based on observations that levels of cytosolic calcium change transiently in response to cold shock, and that calcium channel activity is modulated by low temperatures (Knight et al., 1991). Calcium influx is correlated with the accumulation of transcripts for two *CAS* genes from alfalfa, again indicating a role for calcium under such circumstances (Monroy and Dhindsa, 1995). When the temperature drops below 15°C, the influx of calcium increases. The addition of calcium chelators or calcium channel blockers inhibits the influx of $^{45}Ca^{2+}$ as well as the expression of *CAS* genes at 4°C. Furthermore, the addition of calcium ionophore or a calcium channel agonist to non-acclimated cells causes an influx of extracellular $^{45}Ca^{2+}$ and induces the expression of *CAS* genes at 25°C. These results suggest that cold-induced calcium influx plays an important role in cold acclimation by regulating gene expression. Similar study with calcium inhibitors has also shown that the cold-induction of *KIN* genes from *Arabidopsis* is regulated by calcium-signaling (Tähtiharju et al., 1997).

An inhibitor of calcium-dependent protein kinases (CDPKs) and calmodulin prevents cold acclimation as well as the cold-induction of *KIN* genes. Independent experiments with protoplast transfection assays have demonstrated that two related protein kinases, CDPK1 and CDPK1a, are positive regulators that control stress-signal transduction in plants (Sheen, 1996). The Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin has been proposed as a regulatory component that mediates calcium-signaling under stress conditions, including cold (Kudla et al., 1999). However, direct evidence is still lacking as to whether calcineurin is involved as well. Cold-activation of the *Brassica napus BN115* promoter requires Ca^{2+} influx, and is mediated by changes in membrane fluidity (Sangwan et al., 2001). Recent research has now provided direct evidence for a cold-activated calcium-permeable channel in plants; this was accomplished by applying the patch-clamp technique to *Arabidopsis* mesophyll protoplasts (Carpaneto et al., 2007).

The Ca^{2+}/H^+ antiporter *CAX1* (*CALCIUM EXCHANGER1*) is important in the adaptation to cold shock (Hirschi, 1999). *Arabidopsis CAX1* has been identified by its ability to suppress mutants of yeast defective in vacuolar Ca^{2+} transport. In the case of yeast, its CAX appears to be responsible for maintaining Ca^{2+} homeostasis, and may attenuate the propagation of Ca^{2+} signals. Transgenic tobacco overexpressing *CAX1* has altered Ca^{2+} homeostasis and displays hypersensitivity to that shock. A major function of the vacuolar Ca^{2+}/H^+ antiporter seems to reduce cytosolic Ca^{2+} concentrations after they have been increased as a result of external stimuli such as cold shock (Hirschi, 1999). Constitutive expression of *CAX1* in transgenic plants may perturb these transient concentrations of cytosolic Ca^{2+} during their cold-adaptative response. Although further studies are needed to understand how CAX1 functions in calcium signaling, calcium homeostasis apparently is critical to the cold-adaptation process.

The calcineurin B-like protein (CBL) calcium sensors and CBL-interacting protein kinases (CIPK) are emerging as components of a new signaling system to mediate a complex array of environmental stimuli (Batistic and Kudla, 2004; Kolukisaoglu et al., 2004). CBL-type calcium sensors were originally identified because of their similarity to calcineurin B (CNB) and neuronal calcium sensors from animals, and were designated as calcineurin B-like proteins (Batistic and Kudla, 2004). The CBL proteins are folded into two globular domains that are separated by a short linker region (Nagae et al., 2003). These harbor four EF-hands, the helix-loop-helix motifs that serve as calcium-binding sites, with varying degrees of conservation (Kolukisaoglu et al., 2004). CBLs can interact with a specific group of protein kinases, the CBL-interacting protein kinases (CIPKs) (Shi et al., 1999; Halfter et al., 2003). In the *Arabidopsis* genome, 10 CBLs and 25 CIPKs are encoded, while 10 CBLs and 30 CIPKs are encoded in rice genome (Kolukisaoglu et al., 2004). Fundamentally, the CBL/CIPK network could provide mechanisms for signaling specificity and diversity. Expression of *CBL1* is strongly and transiently induced by drought, salt, wounding, and cold, but not by ABA treatment (Albrecht et al., 2003; Cheong et al., 2003). Loss-of-function and the overexpression of *CBL1* alter plant responses to abiotic stresses, including cold temperatures, thus implying a potential role for CBL proteins in cold-stress signal-transduction. Analysis of loss-of-function for *CIPK3* also suggests a signaling component for ABA and cold-signal transduction pathways (Kim et al., 2003). However, a discrepancy has been noted between overexpression and loss-of-function, in terms of tolerance and stress-regulated marker gene expression, indicating that the CBL/CIPK network system has a complex nature. This issue may be clarified by increasing the number of loss-of-function studies. Isolation and functional characterization of the genes directly regulated by CBL/CIPK proteins are also necessary to elucidate the role of CBL proteins in the cold response.

SIGNALING NETWORKS IN THE COLD RESPONSE

A large number of evidences have been accumulated to support the notion that signaling pathways are interconnected to constitute the networks that lead to various plant responses (Knight and Knight, 2001; Shinozaki et al., 2003; Chen and Zhu, 2004). Results from the analyses of *Arabidopsis* transcriptome and *Arabidopsis* mutants altered in their cold response indicated that crosstalk exists in stress-responsive gene expression, including during cold as well as between the stress response and plant growth and development. A signaling network that occurs through interactions among Ca^{2+}, Ca^{2+}-regulated proteins, MAP kinases, and numerous transcription factors allows plants to exhibit a variety of responses, enabling them to adapt to adverse environmental conditions such as freezing temperatures.

Interactive effects of temperature, osmotic stress, and ABA indicate that both positive and negative interactions exist among these stress factors in regulating gene expression (Xiong et al., 1999). CBF4 mediates drought tolerance via an ABA-dependent pathway (Haake et al., 2002), demonstrating crosstalk between the ABA-dependent and -independent pathways. However, it is unclear whether the cold-responsive ABA-independent pathway is connected to changes in ABA accumulation. Analysis of transgenic plants containing four copies of CRT/DRE fused to the *GUS* reporter gene (*4C/DRE-GUS*) has implicated phytochrome as a photoreceptor in mediating light-signaling to activate cold-induced gene expression through CRT/DRE, thereby suggesting that cross-talk occurs between the cold response and light-signaling (Kim et al., 2002). Dark incubation of *los4-1* mutants at a low temperature enhances their chilling sensitivity compared with the wild type, while illumination alleviates such sensitivity in the mutant. This also indicates that light has role in the cold response (Gong et al., 2002).

Genetic evidence has provided a link between cold responses and flowering-time control (Kim et al., 2004). Using a targeted-genetic approach with *4C/DRE-GUS*, they have identified an *Arabidopsis* mutant, *acg1* that have a higher level of *GUS* as well as *COR15A*, with and without cold treatment compared with wild type. *acg1* mutants exhibit late flowering with elevated expression of FLOWERING LOCUS C (FLC), a flowering repressor, and the mutation has been identified as a null allele of *fve*. FVE encodes a homolog of the mammalian retinoblastoma-associated protein, a component of the histone deacetylase (HDAC) complex, and is involved in the de-acetylation of *FLC* chromatin for transcriptional repression (Ausín et al., 2004; Kim et al., 2004). The CBF1 proteins can interact with *Arabidopsis* GCN5 and ADA2 proteins that have histone acetylase (HAT) activity (Stockinger et al., 2001), such that mutations in *ADA2b* and *GCN5* result in the reduced expression of *COR* genes without affecting *CBF* (Vlachonasios et al., 2003). These observations support the involvement of chromatin modification in the CBF regulon genes. Intermittent cold treatment substantially delays flowering with increasing *FLC* expression, thus reducing the expression of *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*) in wild-type plants. However, cold has no effect on *acg1* and *fve* mutants, suggesting that FVE plays a role in sensing intermittent cold for regulating flowering time. Because the level of *FVE* mRNA remains constant during this response (Kim et al., 2004), cold regulation of flowering-time by FVE might occur at the post-transcriptional level, like decrease in FVE protein levels or activity. Alternatively, but not mutually exclusively, FVE may exist as part of an HDAC complex for constitutive repression, so that low temperature induces CBFs that recruit the HAT chromatin remodeling complex to the CBF regulon genes (Amasino, 2004). In either case, it will be interesting to examine the acetylation state of the chromatin of the CBF regulon genes in both wild type and *acg1* mutants, before and after cold treatment, and also to test whether the overexpression of *FVE* in those mutants can reduce the acetylation of chromatins of *FLC* and the CBF regulon genes, including *COR15A*.

Transgenic *Arabidopsis* plants overexpressing *DREB1A*, *CBF1*, and *CBF2* exhibit slow growth rates and dwarfism at a high level of expression (Liu et al., 1998; Gilmour et al., 2004). *STZ*, which encodes the zinc-finger transcriptional repressor, is a downstream target gene of DREB1A (Maruyama et al., 2004). Overexpression of the *STZ* gene in transgenic *Arabidopsis* results in growth retardation and drought tolerance (Sakamoto et al., 2004). These reports indicate that CBF/DREB-signaling is linked to plant growth, contributing to the acquisition of stress tolerance. The restraint of growth conferred by DELLA proteins, key negative regulators in the gibberellin-signal transduction pathway, is advantageous and promotes survival under adverse environmental conditions, such as high salinity (Achard et al., 2006), suggesting a potential link between plant development and abiotic stress via phytohormones. Fig. 1 depicts schematic model showing regulatory networks of cold-signaling pathways.

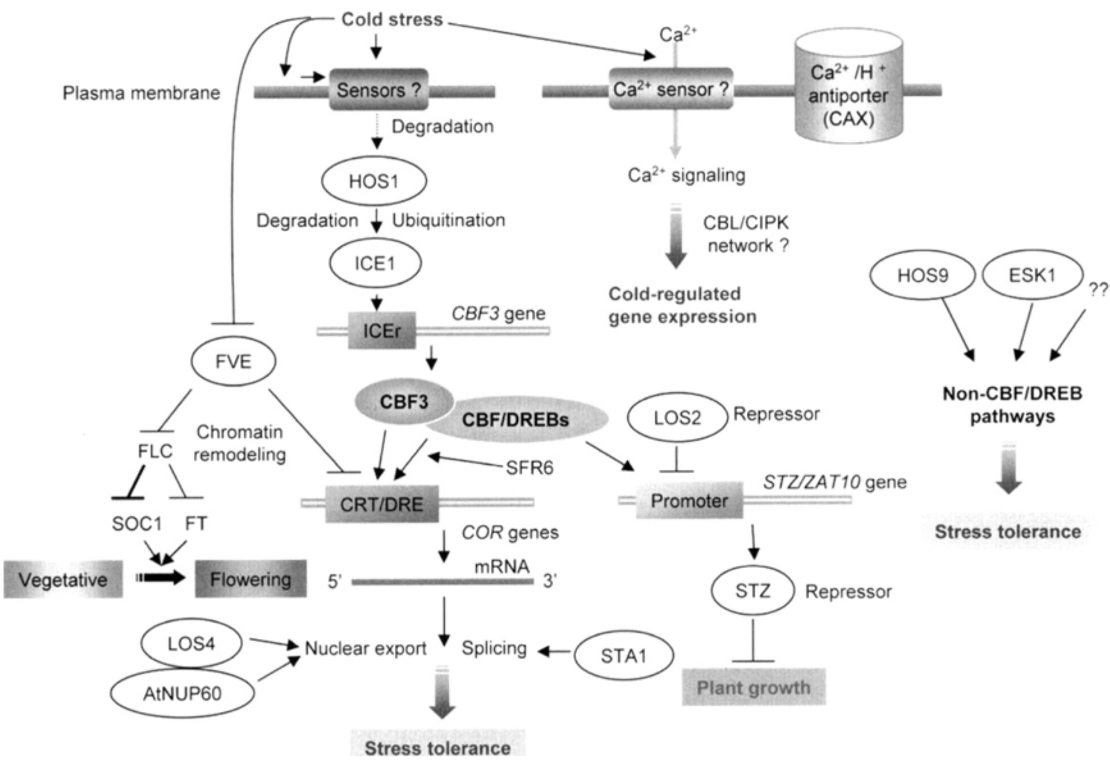

Figure 1. Regulatory networks of signaling pathways for cold-regulated gene expression and stress tolerance. Components identified as being involved in cold-regulated gene expression and signaling are depicted as ellipses. ICEr (Zarka et al., 2003) and CRT/DRE are *cis*-acting elements involved in cold-regulated gene expression. ICE1 and CBF/DREBs function as transcriptional activators, whereas LOS2 and STZ act as transcriptional repressors. *SFR6* has not been cloned yet. LOS4, AtNUP, and STA1 are newly identified components involved in mRNA metabolism for stress responses, including cold and chilling tolerances. Arrows indicate signaling pathways for cold-regulated gene expression and stress response. Refer to text for detailed descriptions. AtNUP, *Arabidopsis thaliana* nucleoporin; CBF/DREB, C-repeat binding factors/DRE-binding proteins; CBL/CIPK, calcineurin B-like protein/CBL-interacting protein kinase; *COR*, *cold-regulated*; CRT/DRE, C-repeat/dehydration-responsive element; ESK, eskimo; FLC, flowering locus C; FT, flowering locus T; HOS, high expression of osmotically responsive gene; ICE1, inducer of CBF expression 1; ICEr, region identified as required for cold induction; LOS, low expression of osmotically responsive gene; SFR6, sensitive to freezing6; SOC1, suppressor of overexpression of CO1; STA, stabilized; STZ/ZAT, salt tolerance zinc finger/zinc transporter from *A. thaliana*.

CONCLUDING REMARKS

Exposure to low temperatures induces a large number of genes encoding the proteins that function as plant cryoprotectants against freezing injury. Expression of such stress-inducible genes is mainly mediated by ABA-dependent and ABA-independent pathways. Cold-signaling that leads to the expression of a vast array of genes is mediated by pathway that does not require ABA action. The CBF/DREB proteins have been identified as key players in cold acclimation in *Arabidopsis*, and many homologs are also functionally conserved in other plant species, including monocot plants such as rice. Genetic data and transcriptome analysis have revealed various cold-signaling pathways other than the CBF/DREB pathway. Calcium signaling plays an important role in early stage of cold response. The CBL/CIPK network is emerging as a new signaling system, but clear evidence is still required to confirm its role in cold tolerance. Forward- and targeted-genetics approaches with *Arabidopsis* have allowed researchers to isolate a large number of mutants, thus revealing the cold stress-signaling components. HOS1-ICE1-CBF/DREB-signaling for cold-regulated gene expression is a good example of the successful elucidation of a cold-signaling pathway using these genetics tools. A cold sensor in cyanobacteria, Hik33, is a transmembrane histidine kinase. Although no such cold sensors have yet been found in plants, a change in membrane fluidity is proposed as one mechanism for the perception of cold temperatures in plants. It will be a future challenge to unveil molecular mechanisms by which multiple pathways for this cold response and cross-talk among other signaling pathways (e.g., plant development and flowering-time control) are integrated and coordinated in response to cold to improve survival rates.

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