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Cold-responsive gene regulation during cold acclimation in plants

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Regulation of the transcriptome is necessary for plants to acquire cold tolerance, and cold induces several genes via a cold signaling pathway. The transcription factors CBF/DREB1 (C-repeat binding factor/dehydration responsive element binding1) and ICE1 (inducer of CBF expression1) have important roles in the regulation of cold-responsive gene expression. ICE1 is post-translationally regulated by ubiquitylation-mediated proteolysis and sumoylation. This mini-review highlights some recent studies on plant cold signaling. The relationships among cold signaling, salicylic acid accumulation and stomatal development are also discussed.

Introduction

Low temperature is one of the environmental stresses affecting plant productivity. Cold triggers cell death by cytoplasmic dehydration and ice formation in the cell wall. Plants from temperate regions become more tolerant to freezing when they are exposed to non-freezing low temperatures, a process known as cold acclimation.[1] Numerous physiological, biochemical and molecular changes occur during cold acclimation, including upregulation of antioxidative mechanisms, synthesis and accumulation of cryoprotectant solutes and proteins, and changes that protect and stabilize cellular membranes.[1,2]

To reduce rigidification and thereby stabilize membranes, the composition of membrane lipids is modified to increase desaturated phospholipids.[3] Cells also accumulate osmolytes and antifreezing proteins because sucrose and proline-rich proteins trap water by creating hydrogen links. Gas chromatography-mass spectrometry analysis has revealed the existence of 311 cold-responsive metabolites.[4] The *eskimo1* mutant over-accumulates sugar and proline, resulting in cold tolerance.[5] Some pathogen-related proteins are also induced to function in cold tolerance because the proteins interact with ice to prevent damage from ice crystal growth.[1]

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Many of these physiological, biochemical and molecular changes are brought about by changes in gene expression, and the transcriptional activation and repression of genes by low temperature are of central importance.[6] This review summarizes recent work on the cold signaling cascade. In addition, we discuss the involvement of stomatal development and SA (salicylic acid) in cold-responsive gene regulation.

Cold Signaling

A prevalent view is that a thermosensor either directly or indirectly perceives low temperatures to initiate cold-induced signaling pathways.[7] Cold may be sensed through changes in the physical properties of membranes, as low temperatures are known to reduce fluidity and increase rigidity.[8] Cold-induced Ca^{2+} increases in the cytosol can also be mediated through membrane rigidification-activated mechano-sensitive or ligand-activated Ca^{2+} channels.[2,9]

Transient increases in cytosolic Ca^{2+} levels regulate *COR* (cold responsive) gene expression. Certain Ca^{2+}-dependent protein kinases act as positive regulators.[10] Calmodulin3 and protein phosphatase 2C play negative roles in the regulation of *COR* gene expression. Ca^{2+}-dependent freezing tolerance has been observed.[11,12] SYT1, a homolog of synaptotagmin (which is a Ca^{2+} sensor that initiates exocytosis), is likely to function in resealing of membranes punctured by ice crystals.[13]

The MAP (mitogen-activated protein) kinase (MPK) cascade is involved in the regulation of cold signaling and cold tolerance. MKK2 (MAP kinase kinase2) phosphorylates and activates MPK4 and MPK6 in response to cold.[14] Because the expression level of *CBF2/DREB1C* [C-repeat (CRT)/dehydration responsive element (DRE) binding protein] was upregulated in *MKK2* overexpressing plants,[14] the cold signaling pathway is likely to be activated via protein phosphorylation.

The CBF/DREB1 Pathway and Cold-Responsive Gene Regulation

The *CBF/DREB1*-dependent cold signaling pathway is the best understood regulatory pathway involved in cold acclimation.[6,15] The CBF/DREB1 protein activates the transcription of *COR* (cold response) genes, whose promoters contain a *CRT/DRE* cis-element.[16,17] Overexpression of *CBF/DREB1A* induces *COR*

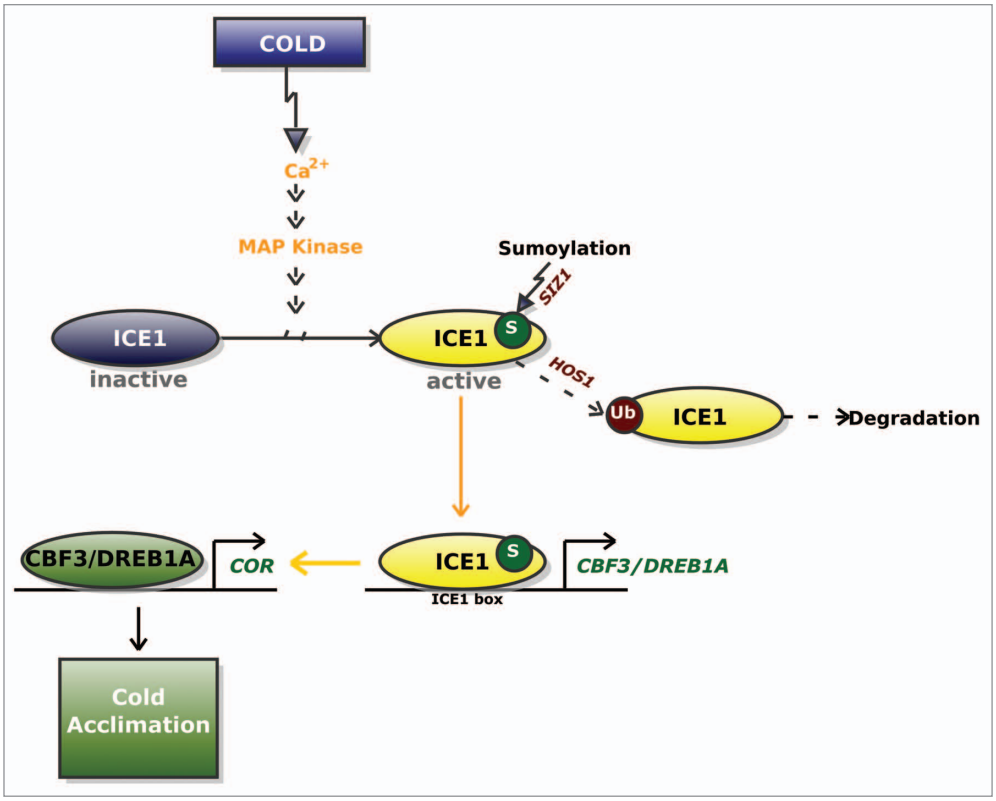

Figure 1. The cold signaling pathway, which involves ICE1 and CBF3/DREB1A. Cold stress transiently increases Ca^{2+} levels and activates protein kinases, including MAP kinases, necessary for cold acclimation. ICE1 is activated by cold stress. SIZ1 mediates sumoylation of ICE1 to stabilize it and to control *CBF3/DREB1A* gene expression. Phosphorylation may be involved in the activation of ICE1, but no clear evidence for this has been demonstrated. HOS1 ubiquitylates ICE1 for proteasome-dependent degradation. SIZ1 mediates sumoylation of ICE1 for stabilization. The CBF3/DREB1A transcription factor controls cold-responsive genes and cold acclimation. Abbreviations: CBF, C-repeat binding factor (an AP2-type transcription factor); COR, cold-responsive genes; HOS1, high expression of osmotically responsive genes1 (a RING finger ubiquitin E3 ligase); ICE1, inducer of CBF expression1 [a MYC-type bHLH (basic helix-loop-helix) transcription factor]; SIZ1, SAP and Miz1 [a SUMO (small ubiquitin-related modifier) E3 ligase]; P, phosphorylation; S, sumoylation; Ub, ubiquitylation.

genes and cold tolerance in Arabidopsis,[18-21] Brassica,[22] tomato[23] and rice.[24]

The *COR* genes affect metabolism, protein stability and cell structure. Microarray analyses have revealed that 655 and 284 of 24,000 genes were up and downregulated by cold treatment, respectively.[25] These include genes for metabolism, signal transduction, defense against pathogens, and transcription factors.[25] The majority of the most highly induced genes are in the *CBF* regulon.[26]

The levels of metabolites also change after cold treatment. The pool of amino acids derived from pyruvate and oxaloacetate, polyamine precursors, and compatible solutes such as sucrose, maltose, glucose and fructose all increase during cold stress.[4] *CBF3* overexpression enhanced 79% of the cold-inducible metabolites.[27]

In Arabidopsis, the three *CBF/DREB1* genes are likely to have different functions, even though overexpression of each gene activates the expression of a similar gene set.[21] Molecular analysis of a *cbf2* null mutant in Arabidopsis suggested that *CBF2/DREB1C* negatively regulates the expression of *CBF1DREB1B* and *CBF3/DREB1A* during cold acclimation.[28] Based on an analysis of *CBF1* and *CBF3* RNAi lines, *CBF1/DREB1B* and *CBF3/DREB1A* are not involved in regulating other *CBF/DREB1* genes.[29] Furthermore, plants, which reduced both *CBF1* and *CBF3* transcripts by RNAi, exhibit lower cold acclimation capacity, suggesting that CBF1/DREB1B and CBF3/DREB1A coordinately induce *CBF/DREB1*-regulon genes and cold acclimation.[29]

Regulation of CBF/DREB1 Transcription Factors

CBF/DREB1 genes are induced by cold stress; thus, regulatory factors are required to control their expression. ICE1 (inducer of *CBF* expression1), a MYC-type bHLH (basic helix-loop-helix) transcription factor, has been identified as a regulator of *CBF3/DREB1A* expression by binding to a MYC *cis*-element in its promoter (**Fig. 1**).[30] The *ice1* mutant shows impaired induction of *CBF3/DREB1A* and other cold-inducible genes, as well as reduced chilling tolerance and cold acclimation.[30] The *ice1* mutation alters the expression of 83 of 933 cold-regulated genes.[25] ICE2 is another MYC-type bHLH transcription factor with high similarity to ICE1. ICE2 activates the expression of *CBF1/DREB1B* and promotes freezing tolerance.[31]

ABA also induces the expression of *CBF/DREB1* genes, but to a significantly lower level does cold induction.[32] ABA-induced

expression of *CBF3/DREB1A* in the *ice1* mutant was less than that in wild-type,[7] implying that ICE1 may regulate ABA-mediated expression of *CBF3/DREB1A*.

Two post-translational modifications, ubiquitylation and sumoylation, control ICE1-dependent cold signaling. The RING-type ubiquitin E3 ligase HOS1 (high expression of osmotically responsive gene1) mediates polyubiquitylation of ICE1 for proteasome-dependent degradation (**Fig. 1**).[33,34] Overexpression of HOS1 dramatically decreases cold tolerance. Another post-translational modification of ICE1, sumoylation at K393, is important for the regulation of *CBF3/DREB1A*, cold-inducible genes and cold tolerance (**Fig. 1**).[35-37] This sumoylation is mediated by the SUMO (small ubiquitin-related modifier) E3 ligase SIZ1 (SAP and Miz1), which is localized to nuclear speckles.[35,38,39] In vitro analysis demonstrated that sumoylation of ICE1 blocks ubiquitylation of the transcription factor,[35] suggesting that the two modifications are functionally competitive.

MYB15 binds to MYB recognition *cis*-elements in the promoters of *CBF/DREB1s* to negatively regulate their expression. The *myb* mutation enhances the expression of *CBF/DREB1s* and freezing tolerance, whereas plants overexpressing *MYB15* are sensitive to cold stress.[40] Furthermore, *MYB15* expression is negatively regulated by ICE1.[40] The cold-inducible C_2H_2 zinc finger transcription factor gene, *ZAT12*, functions as a negative regulator of *CBF/DREB1s*, because transgenic *ZAT12*-overexpressing plants showed reduced expression of *CBF/DREB1s*.[26]

Promoter fusion experiments have revealed that calmodulin binding transcription activator (CAMTA) factors bind to a regulatory element (CG-1 element, vCGCGb) in the promoter of *CBF2/DREB1C* and that CAMTA3 is a positive regulator of *CBF2/DREB1C* expression.[41] Furthermore, double *camta1 camta3* mutant plants are sensitive to freezing temperatures. The expression of *CBF3/DREB1A* is not regulated by CAMTA because there is no CG-1 element in its promoter.[41] These results suggest that CAMTA may function to integrate cold-inducible calcium signaling with gene expression.

Transcriptional Regulation and SA (Salicylic Acid)

During chilling conditions, free SA and glucosyl SA become accumulated in Arabidopsis shoots.[42] Reduction of SA by introduction of bacterial SA hydroxylase gene *nahG*[43] or SA-deficient *eds5* (enhanced disease susceptibility) mutation[44] allows plant grow much larger than wild-type plants at chilling temperature.[42] In contrast, SA-accumulation mutation such as *cpr1* (constitutive expresser of pathogenesis-related gene)[45] causes a strong reduction in growth under low temperature,[42] suggesting that SA is one of phytohormones to control growth inhibition at low temperature. The other SA accumulating mutations, *siz1*,[46-48] and *acd6* (accelerated cell death),[49] also reduces ability of cold tolerance and the sensitivity is restored by reduction of SA level by introduction of *nahG*.[50] Furthermore, downregulation of *CBF3/DREB1A* and cold-inducible gene expression in *siz1* or *acd6* is also recovered by *nahG* expression,[50] suggesting that SA is involved in regulation of cold signaling. Interestingly, several SA-inducible genes are upregulated in the *ice1* mutant.[50] SA accumulation also affects cold tolerance in different organisms. Hydroponic application of SA to winter and spring wheat reduces freezing tolerance.[51]

Cold Signaling and Stomata Development

Genetic screening for mutations affecting stomata development identified a dominant mutation with constitutive stomatal differentiation (the *scrm-D* mutation) that is identical to the *ice1* mutation.[52] ICE1/SCRM and ICE2/SCRM2 directly interact with three closely related bHLH transcription factors, SPCH, MUTA and FAMA.[52] SPCH, MUTA and FAMA are positive regulators that direct entry into the stomatal cell lineage, the transition from meristemoid to guard mother cells, and the terminal differentiation of guard cells, respectively.[53-55] Based on the phenotypes of *ice1/scrm* and *scrm2* mutants, these two proteins are redundant and promote the sequential steps of the stomatal cell development program: entry, proliferation and terminal differentiation.[52] These results demonstrate a linkage between environmental responses and the formation of stomata.

MPK4 and MPK6 are phosphorylated by MKK2 in response to cold,[14] implicating that MPK6 is involved in the regulation of cold signaling. In addition, MPK3 and MPK6 are also phosphorylated by MKK4/5 to block entry into the stomatal pathway, and loss of MPK3/MPK6 function results in the formation of clustered stomata.[56,57] Thus, it is thought that cold signaling and signaling for stomata development may be integrated.

Conclusion and Perspectives

A cold sensor has not yet been identified, but current evidence suggests that plant cells may perceive changes in plasma membrane fluidity in response to cold. Cold transiently increases Ca^{2+} concentrations in the cytosol, but how Ca^{2+} signaling is decoded and transduced by Ca^{2+} sensory proteins remains unclear. Cold stress induces the MAP kinase cascade, but the molecular link between the kinases and the transcription factors is still unknown.

The ICE1-CBF3/DREB1A transcriptional cascade is an important cascade for the regulation of cold signaling and cold acclimation. This pathway is conserved in diverse plant species. ICE1 is posttranslationally regulated by SIZ1-mediated sumoylation and HOS1-mediated ubiquitylation. However, it is still unclear how ICE1 is activated. Understanding the cold signaling mechanism will help in the transcriptome engineering of crop plants for enhanced tolerance to cold and other abiotic stresses.

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