Nature Reviews Molecular Cell Biology | Published online 5 Apr 2017; doi:10.1038/nrm.2017.38

STRESS RESPONSES

Membrane-to-nucleus signals modulate plant cold tolerance

Plants have developed sophisticated mechanisms to adapt to low temperatures. A transcriptional network responsible for cold acclimation has been identified, but how cold signals are transduced from the plasma membrane to the nucleus to fine-tune cold responses has remained elusive. Now, Yang and colleagues report in *Molecular Cell* that, in *Arabidopsis thaliana*, phosphorylation of 14-3-3 proteins by plasma membrane-located cold-responsive protein kinase 1 (CRPK1; also known as CRLK1) promotes their translocation to the nucleus, where they negatively regulate key cold-responsive transcription factors.

CRPK1 was identified in a genetic screen for *A. thaliana* protein kinase mutants that altered freezing tolerance. *crpk1* null mutants were substantially more resistant to exposure to freezing temperatures compared to the wild-type plants.

Previous studies have identified CRT-binding factors (CBFs; also known as DREBs) as key transcription factors that are rapidly induced by cold stress. CBFs promote the expression of cold-regulated (COR) genes, which enables plants to tolerate freezing temperatures. The authors found that COR activation was markedly increased in *crpk1* mutants, indicating that CRPK1 reduces cold tolerance by regulating COR genes.

> “cold stress activates plasma membrane-localized CRPK1 … promoting the degradation of CBF transcription factors”

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Expression of CRPK1–GFP in wild-type protoplasts and plants revealed that CRPK1 localizes at the plasma membrane, and that this subcellular localization remains unchanged following cold exposure. Yeast two-hybrid screens and co-immunoprecipitation assays identified a member of the 14-3-3 protein family (14-3-3λ) that interacts with CRPK1 at the plasma membrane. CRPK1 phosphorylated 14-3-3λ *in vivo* following cold treatment. Moreover, a kinase-dead mutant version of CRPK1 was unable to suppress the freezing tolerance and cold induction of COR genes seen in *crpk1* mutants, suggesting that the CRPK1-mediated phosphorylation of 14-3-3λ is essential for the modulation of freezing tolerance.

14-3-3λ–GFP fusion proteins were detected predominantly in the cytoplasm at 22 °C, but 14-3-3λ also accumulated in the nucleus after cold treatment. Translocation to the nucleus did not occur in *crpk1* mutants or when the phosphorylation sites on 14-3-3λ that are targeted by CRPK1 were mutated. Thus, CRPK1 phosphorylates 14-3-3 proteins to facilitate their nuclear translocation during cold stress. As in *crpk1* mutants, COR genes were upregulated in mutants lacking 14-3-3 proteins, indicating that 14-3-3 proteins function in the same pathway as CRPK1 to reduce cold tolerance.

So how do 14-3-3 proteins function? Yeast two-hybrid and co-immunoprecipitation assays revealed that 14-3-3 proteins interact with CBF1 and CBF3 *in vitro* and *in vivo*, and that this interaction decreases CBF stability. Overexpression of 14-3-3λ, but not of phosphorylation-resistant 14-3-3λ, promoted the degradation of CBF proteins by the 26S proteasome pathway. However, CBF3 protein levels were unchanged in *crpk1* protoplasts under cold stress, indicating that phosphorylation of 14-3-3λ by CRPK1 is required to promote the degradation of CBF proteins. In plants, the basal levels of CBF1 were higher in 14-3-3 mutants than in wild-type plants; induction of CBF3 expression upon cold exposure was stronger in 14-3-3 mutants than in the wild type, and it remained high for longer than in wild-type plants.

Together, these findings indicate that cold stress activates plasma membrane-localized CRPK1, which leads to phosphorylated 14-3-3 proteins entering the nucleus and promoting the degradation of CBF transcription factors, thus attenuating the cold-induced response. This negative feedback is consistent with previous studies showing that negative feedback regulation is important for plant adaptation and optimization of growth. Further studies are required to elucidate how cold is perceived at the plasma membrane to activate CRPK1.

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ORIGINAL ARTICLE Liu, Z. *et al.* Plasma membrane CRPK1-mediated phosphorylation of 14-3-3 proteins induces their nuclear import to fine-tune CBF signaling during cold response. *Mol. Cell* http://dx.doi.org/10.1016/j.molcel.2017.02.016 (2017)