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Cold Signal Shuttles from Membrane to Nucleus

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In this issue of *Molecular Cell*, Liu et al. (2017) show that the cold-activated plasma membrane protein kinase CRPK1 phosphorylates 14-3-3 proteins, triggering its nuclear translocation to impair the stabilization of the transcription factor CBFs for a feedback excessive cold defense response during the freezing in *Arabidopsis*.

The ability to adapt to ambient temperatures determinates plant species geographic regions for their survival as well as breeded crop kinds for food production in every country. Cold stress is defined as chilling (0°C–20°C) and freezing (<0°C), which are regulated by distinct mechanisms. Freezing tolerance depends directly on the ability of repression of ice crystallization in cells. Plants acquire increased tolerance to freezing when exposed to a prior non-freezing low temperature, which is called cold acclimation. In nature, vernalization with a prolonged low temperature for winter plants involves a cold acclimation for enhancing the defense response, as well as a developmental response, flowering, and organogenesis (Chinnusamy et al., 2007; Xiao et al., 2014). In the *Arabidopsis* study, in fact, the freezing phenotype always is a result of the prior chilling with cold acclimation, which involves a way to dynamically coordinate defense and growth. But defense is the first response during cold stress. Cold tolerance depends on signaling transduction pathways in cells. As an approbatory temperature-sensing hypothesis, either cold temperature or heat leads to a change in membrane fluidity and the rearrangements of cytoskeleton, followed by the influx of calcium to trigger the downstream responses (such as kinases and transcription factors) for their tolerance in plant (Sangwan et al., 2002; Zhu, 2016). The cold sensor, such as the complex of the transmembrane protein (COLD1) and its partner (RGA1), triggers a calcium influx to lead to activation of key transcription factors, such as DREBs, for tolerance in rice (Ma et al., 2015). In *Arabidopsis*, cold stress could rapidly induce the transcription of C-repeat-binding factors (CBFs), which bind to the promoter of *COR* genes to activate their expression for defense. The response patterns of the key transcription factors (such as CBFs) to freezing in *Arabidopsis* are shared with those of chilling in rice (Ma et al., 2015). ICE1, a bHLH transcription factor, works upstream of CBFs to regulate their expression. Both sumoylation by SIZ1 and ubiquitylation by HOS1 on ICE1 were identified (Chinnusamy et al., 2007; Zhu, 2016). Recent studies reported that OST1/SnRK2.6 could phosphorylate ICE1 to prevent its degradation and positively regulate the CBF-mediated cold response (Ding et al., 2015; Zhu, 2016) (Figure 1). However, less is known about the signaling between the plasma membrane complex and nuclear transcription factors.

In this issue of *Molecular Cell*, Dr. Shuhua Yang's group (Liu et al., 2017) reports that a plasma membrane-located protein kinase, CRPK1 (cold-responsive protein kinase 1), plays a negative role through the classical CBF pathway in regulating the excessive cold response in *Arabidopsis*. In yeast two-hybrid assays, a series of 14-3-3 family members were identified to interact with CRPK1. Further assays either in vitro or in vivo supported that 14-3-3λ interacted with CRPK1 on the plasma membrane.

So how do CRPK1 and 14-3-3λ collaborate to respond to cold stress? The first assumption is that CRPK1 could phosphorylate 14-3-3λ under cold stimuli. Liu et al. (2017) indeed provided strong evidence to verify this assumption through a series of phosphorylation assays. More importantly, the kinase activity is essential for CRPK1 response to cold (0°C–10°C) signal, which was supported by the observation that kinase-dead transformation lines did not complement the phenotype of *crpk1* while the wild-type *CRPK1* did.

How does the plasma membrane protein CRPK1 transduce the signal to the nucleus? Liu et al. (2017) detected the translocation of cytoplasmic 14-3-3λ into the nucleus upon cold stress in a CRPK1-dependent manner. Liu et al. (2017) conclude that CRPK1 phosphorylates 14-3-3λ to trigger its nuclear shuttling under cold stress, thus transducing the signal from the plasma membrane to the nucleus.

Given that there are several 14-3-3s with functional redundancy in *Arabidopsis*, Liu et al. (2017) used a 14-3-3λ and 14-3-3κ double mutant and found that the freezing tolerance of the double mutant was enhanced. This phenotype, together with further genetic complementary tests, demonstrated that 14-3-3s plays negative roles in the freezing tolerance.

What does 14-3-3λ do in the nucleus? Liu et al. (2017) found that the induction of CBFs upon cold stress was slightly affected, but the cold induction of CBF target genes was dramatically upregulated in the 14-3-3κλ double mutant, indicating that it is possible that the function of 14-3-3λ may have an influence on CBFs. In other words, 14-3-3λ is in the same pathway with CBFs in cold signaling. Intriguingly, the interaction between 14-3-3λ and CBFs was identified and confirmed by yeast two-hybrid and co-immunoprecipitation (coIP) assays.

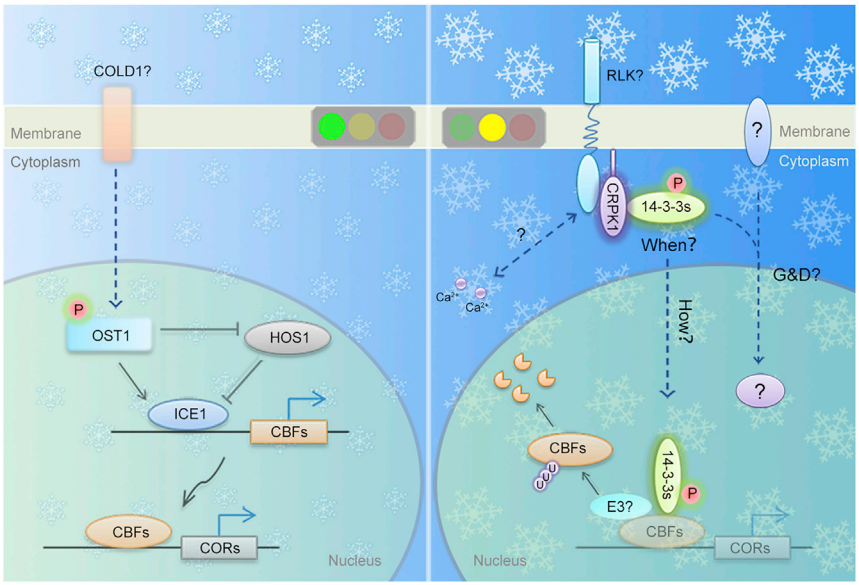

Figure 1. Working Model of CRPK1 and 14-3-3s Function for an Excessive Cold Response in *Arabidopsis*

The mechanism (on the right) is a negative feedback for an excessive cold response in *Arabidopsis*, while cold induces activated CBFs working for the defense (on the left). Light blue background represents the initial cold stress, and deep blue background means middle or later periods of cold stress that initiate the braking system for defense response. The green light indicates the pulse-on of the defense to cold stress, and the yellow light represents braking defense strength for an excessive response. Dotted arrows represent predicted connections. G&D stands for the targets of growth and development.

As reported, CBFs are degraded through the 26S proteasome-mediated ubiquitin pathway. The phosphorylated 14-3-3λ by CRPK1 could promote the degradation of CBF1 and CBF3 upon cold stress. Consistent with the molecular data, loss-of-function mutant *crpk1* or double mutant *14-3-3κλ* showed an enhanced freezing tolerance. Genetic analysis showed that CRPK1 and 14-3-3s are upstream of CBFs, which plays a negative role in the tolerance. Physiologically, it may be a novel mechanism to regulate the duration of excessive cold defense response to dynamically balance the stress and growth in *Arabidopsis*.

This is the first report of how plants transmit a cold signal from the plasma membrane to the nucleus. In addition, CRPK1 acts as a fine regulator and coordinates with 14-3-3s to play negative roles to prevent excessive cold defense responses. For exploring a mechanism of plant adaption to cold environment, this elegant work evokes a series of attractive questions to be addressed in future studies (Figure 1). For example, which pathway of growth and development is directly coordinated by the CRPK1-14-3-3s cold signaling? In what physiological condition is CRPK1-14-3-3κλ-CBFs signaling axis important? How does CRPK1 sense the cold signal to be activated? And what is the relation between calcium signaling and CRPK1-14-3-3 s during cold stress? All these interesting points will help to get a more complete understanding of the mechanism of cold adaptation in plants.

REFERENCES

Chinnusamy, V., Zhu, J., and Zhu, J.K. (2007). Trends Plant Sci. *12*, 444–451.

Ding, Y., Li, H., Zhang, X., Xie, Q., Gong, Z., and Yang, S. (2015). Dev. Cell *32*, 278–289.

Liu, Z., Jia, Y., Ding, Y., Shi, Y., Li, Z., Guo, Y., Gong, Z., and Yang, S. (2017). Mol. Cell *66*, this issue, 117–128.

Ma, Y., Dai, X., Xu, Y., Luo, W., Zheng, X., Zeng, D., Pan, Y., Lin, X., Liu, H., Zhang, D., et al. (2015). Cell *160*, 1209–1221.

Sangwan, V., Orvar, B.L., Beyerly, J., Hirt, H., and Dhindsa, R.S. (2002). Plant J. *31*, 629–638.

Xiao, J., Xu, S., Li, C., Xu, Y., Xing, L., Niu, Y., Huan, Q., Tang, Y., Zhao, C., Wagner, D., et al. (2014). Nat. Commun. *5*, 4572–4585.

Zhu, J.K. (2016). Cell *167*, 313–324.