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# AtHAP5A modulates freezing stress resistance in *Arabidopsis* independent of the CBF pathway

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**Keywords:** HAP5A, CCAAT motif, CBF, freezing stress, XTH21

**Abbreviations:** ABA, abscisic acid; AHK, ARABIDOPSIS HISTIDINE KINASE; ARR, ARABIDOPSIS RESPONSE REGULATOR; bHLH, basic helix-loop-helix; ChIP, chromatin immunoprecipitation; CBF/DREBs, C-repeat/Dehydration responsive element BINDING FACTORs; COR, Cold-regulated; CRT, C-repeat; DRE, Dehydration responsive element; HAPs, heme-associated proteins; HOS1, HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1; ICE, INDUCER OF CBF EXPRESSION; NF-YA/B/C, NUCLEAR FACTOR Y, SUBUNIT A/B/C; ROS, reactive oxygen species; SUMO, SMALL UBIQUITINRELATED MODIFIER

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**The heme-associated proteins (HAPs, also known as NUCLEAR FACTOR Y, SUBUNIT A/B/C (NF-YA/B/C)) have been reported to bind specifically to DNA fragments containing CCAAT-box, however, the physiological functions and direct targets of these HAP proteins remain unclear in plants. In our recent study, we found that *AtHAP5A* and *AtXTH21* positively modulated freezing stress resistance, and chromatin immunoprecipitation (ChIP) assay and genetic evidence indicated that *AtHAP5A* might act in the upstream of *AtXTH21* in freezing stress. Moreover, *AtHAP5A* and *AtXTH21* had significant effects on inhibiting cold stress-induced reactive oxygen species (ROS) accumualtion and activating ABA-related genes' expression. Thus, a possible model that depicting AtHAP5A-mediated cold stress responses was proposed in this study, and we highlighted that AtHAP5A modulates freezing stress resistance in *Arabidopsis* through binding to CCAAT motif of *AtXTH21*, which is independent of the CBF pathway.**

Low temperature (cold stress) is one of the most serious environmental stresses that limits plant growth and crop production.[1-5] To date, several plant hormones and genes especially a number of transcription factors confer improved cold stress resistance in plants.[1-8] In *Arabidopsis*, INDUCER OF CBF EXPRESSION (ICE)-C-repeat (CRT)/Dehydration responsive element (DRE) BINDING FACTORs (CBF/DREBs)-Cold-regulated (COR) genes mediated pathway plays essential roles during plant cold stress response.[1-8] Briefly, ICE1/2, encoding basic helix-loop-helix (bHLH) transcription factors, directly bind to CANNTG box in the promoter regions of *CBF/DREBs* that interact with CRT/DRE of down-stream CORs.[1-8] Additionally, ICE1 is negatively regulated by the ubiquitin E3 ligase HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1), and is positively regulated by the SMALL UBIQUITINRELATED MODIFIER (SUMO) E3 ligase SIZ1 (SAP and Miz) at the post-translational level.[1-8] Recently, Jeon et al. (2010) showed that cytokinin receptors *ARABIDOPSIS* HISTIDINE KINASE2/3 (AHK2/3) and type-A *ARABIDOPSIS* RESPONSE REGULATOR (ARR) proteins mediated plant cold stress signaling through inhibiting ABA signaling.[9] Shi et al. (2012) found that ethylene negatively modulated plant freezing stress responses through repressing CBF/DREBs and type-A ARRs in *Arabidopsis*.[10] Hu et al. (2013) discovered that JA regulated cold resistance through functioning in the upstream signal of the ICE-CBF/DREB1 pathway.[11] Since plant cold stress response is a complex signaling pathway, besides the above pathways, many unknown mechanisms that independent CBF pathays need to be further dissected.

Although several heme-associated proteins (HAPs, also known as NUCLEAR FACTOR Y, SUBUNIT A/B/C (NF-YA/B/C)) have been reported to bind specifically to DNA fragments containing CCAAT-box, the physiological functions and direct

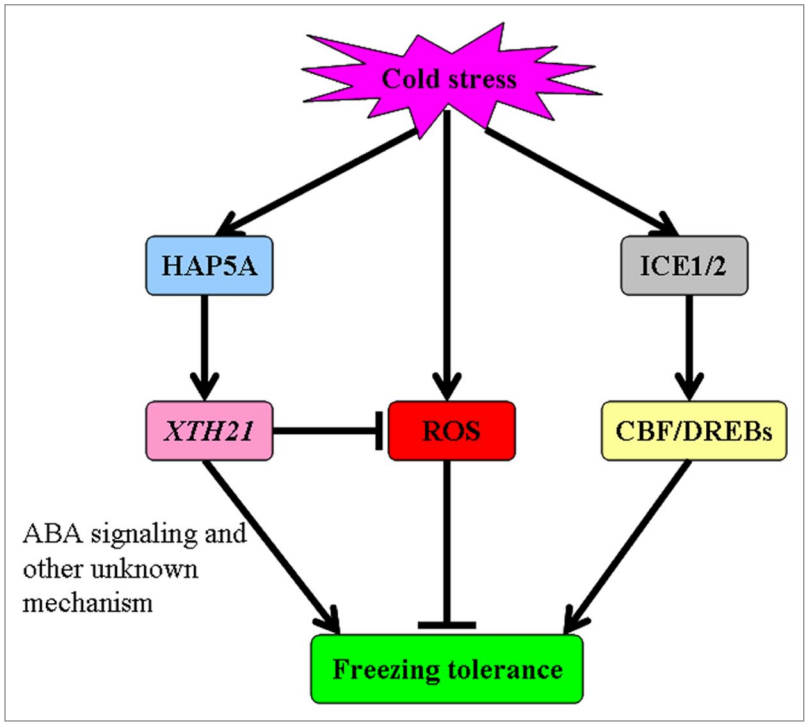


**Figure 1.** A possible model that depicting AtHAP5A-mediated cold stress responses.

targets of these HAP proteins remain unclear in plants.[12-15] Recently, Petroni et al. (2012) reviewed the possible involvement of plant HAPs in plant stress responses. However, no direct stress-related target of HAPs has been revealed, and the direct link between CCAAT element and plant-environment interaction is also unknown.[15]

In our recent study, we found that expression of *HAP5A* was induced by different abiotic stress treatments, and AtHAP5A as a transcription factor interacted with CCAAT motif in vivo.[16] Through genome sequence analysis, CCAAT motif was found in promoters of many genes, and five CCAAT motifs were enriched in *AtXTH21* promoter region.[16] Based on the phenotype data of *AtHAP5A* modulated plants, we focused on the involvement of *AtHAP5A* in freezing stress.[16] The direct link of expression between *AtHAP5A* and *AtXTH21* under control and stress conditions made *AtXTH21* as a potential target.[16] Moreover, the genetic evidence suggested that *AtXTH21* as a direct target of AtHAP5A was largely contributed to *AtHAP5A*-mediated freezing stress response.[16] Notably, *AtXTH21* might not be the only target of AtHAP5A, and the dissection of other targets of AtHAP5A will shed more light on the in vivo role of *AtHAP5A* (**Fig. 1**).[16]

Based on our observations, a possible model that depicting AtHAP5A-mediated cold stress responses was proposed.[16] When cold stress was applied, the expression of *HAP5A* was largely induced, and the activation of *HAP5A* increased the expressions of *XTH21* through directly binding to the CCAAT elements of in the promoters of *XTH21*. Moreover, upregulation of *XTH21* led to improved freezing stress resistance in *Arabidopsis*, via inhibiting cold stress-induced reactive oxygen species (ROS) accumulation, ABA-related genes' expression, and other unknown pathways. Although ICE1/2-CBF/DREBs mediated cold stress responses were activated by cold stress treatment, the expressions of three core genes (*CBF1/2/3*) were not significantly affected by *HAP5A* and *XTH21* expression, and no appropriate motif that is responsible for CBFs binding was found in the promoter of *AtXTH21*. Thus *AtHAP5A* and *AtXTH21* might mediate cold stress resistance independent of the CBF pathway.

## Conclusions

Taken together, our experiments highlight that AtHAP5A modulates freezing stress resistance in *Arabidopsis* through binding to CCAAT motif of *AtXTH21*, which is independent of the CBF pathway.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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