The ICE1 transcription factor, also known as SCREAM (SCRM), is thought to be a master regulator of genes that impart freezing tolerance (Yamaguchi-Shinozaki and Shinozaki, 2006; Knight and Knight, 2012; Guo et al., 2018; Shi et al., 2018). In this issue of The Plant Cell, Kidokoro et al. (2020) present results that challenge this thinking. In this commentary, invited by the journal editors, we first provide background information establishing a context for the Kidokoro et al. (2020) study and then summarize their findings and present our thoughts on fundamental questions raised by the results.

Plants from cold environments increase in freezing tolerance in response to low nonfreezing temperatures, a phenomenon known as cold acclimation (Xin and Browse, 2000). The CBF/DREB1 transcription factors, which are highly conserved in higher plants, have a major role in regulating cold acclimation. Arabidopsis (Arabidopsis thaliana) has three CBF/DREB1 genes—CBF1/DREB1B, CBF2/DREB1C, and CBF3/DREB1A—that are located in tandem array and rapidly induced upon exposing plants to low temperature (~4°C; Thomashow, 2010). Induction of the CBF/DREB1 genes is followed by the induction of CBF/DREB1-targeted COR (cold-regulated) genes, some of which contribute to an increase in freezing tolerance. Arabidopsis mutants in which all three CBF/DREB1 genes are inactivated are severely impaired in freezing tolerance (Jia et al., 2016; Zhao et al., 2016; Park et al., 2018).

Given the importance of the CBF/DREB1 genes in cold acclimation, it is not surprising that considerable effort has been directed at determining how they are induced in response to low temperature. A major breakthrough was reported by Chinusuamy et al. (2003), who identified ICE1 (Inducer of CBF Expression1) as a positive regulator of CBF3/DREB1A expression and freezing tolerance. In particular, the investigators generated a transgenic line of Arabidopsis that carried a luciferase (LUC) reporter under the control of the CBF3/DREB1A promoter and screened for mutants that showed reduced expression of the CBF3/DREB1A::LUC reporter gene in response to low temperature. This led to identification of the ice1 mutation, now designated ice1-1, a dominant mutation that caused reduced cold induction of both the CBF3/DREB1A::LUC reporter gene and the endogenous CBF3/DREB1A gene and caused a reduction in freezing and chilling tolerance. Further analysis indicated that the ICE1 gene encoded a MYC-like basic helix-loop-helix transcription factor and that the ice1-1 mutation resulted in an R236H amino acid substitution in ICE1. Transforming the wild-type CBF3/DREB1A::Luc reporter line with the ice1-1 gene led to reduced cold induction of the reporter gene, consistent with the ice1-1 allele having a dominant negative effect on CBF3/DREB1A expression (expression of the endogenous CBF3/DREB1A gene was not reported). Additionally, overexpression of wild-type ICE1 resulted in a transient upregulation of CBF3/DREB1A and CBF2/DREB1C in response to low temperature and an increase in freezing tolerance. Taken together, these results provided a strong case that ICE1 had an important role in regulating the CBF/DREB1 genes and freezing tolerance.

In a subsequent unrelated study that is germane here, ICE1 was identified as a regulator of stomatal development (Kanaoka et al., 2008). The gene, named SCRM, and its paralog SCRM2/ICE2 were shown to promote stomatal differentiation via forming heterodimers with core stomatal transcription factors, including SPEECHLESS (SPCH; Kanaoka et al., 2008). Consistently, the ice1-1 scrm-2 double knockout mutant developed an epidermis devoid of stomata, identical to the spch mutant phenotype. By contrast, the gain-of-function mutant scrm-D elevated expression of the stomatal gene EFP1 and produced a “screaming” epidermis solely composed of stomata (Pillitteri et al., 2011; Putarjunan et al., 2019). Of importance here is that the molecular lesion of scrm-D was found to be the same ICE1(R236H) substitution found in ice1-1. Thus, as would be expected, the ice1-1
mutant produces overwhelming stomata on the epidermis (see figure).

Now, in this issue of *The Plant Cell*, Kidokoro et al. (2020) present results that challenge the thinking that ICE1 has a major role in regulating the CBF/DREB1 genes. This challenge comes via two approaches, the first involving a genetic analysis of the *ice1-1* mutant. Kidokoro et al. (2020) crossed the *ice1-1* mutant with a newly made transgenic line carrying the emerald luciferase (ELUC) reporter fused to a portion of the *CBF3/DREB1A* promoter; the construct was designated 1AR::ELUC. Analysis of the F2 population showed that the *ice1-1/scrm-D* phenotypes of increased numbers of stomata and the induction of *EPF1* did not cosegregate with impaired cold induction of the 1AR::ELUC reporter or *CBF3/DREB1A*. Additionally, it was found that transformation of plants with the *ice1-1* gene resulted in the induction of *EPF1* but did not reduce cold induction of the endogenous *CBF3/DREB1A* gene. These results indicate that the ICE1(R236H) protein encoded by *ice1-1/scrm-D* is not responsible for the impaired cold induction of *CBF3/DREB1A*, as initially reported for the *ice1-1* mutation (Chinnusamy et al., 2003). If *ice1-1/scrm-D* was not responsible, what was? Additional analysis showed that it was due to transgene-induced DNA methylation: a *CBF3/DREB1A::LUC* transgene present in the *ice1-1* mutant caused an increase in DNA methylation of the endogenous *CBF3/DREB1A* locus, resulting in reduced cold induction of its expression.

The genetic analysis of Kidokoro et al. (2020) is thorough but includes a troublesome mystery. As the investigators note, their results indicate that the *CBF3/DREB1A::LUC* transgene responsible for DNA methylation: a *CBF3/DREB1A::LUC* transgene present in the *ice1-1* mutant caused an increase in DNA methylation of the endogenous *CBF3/DREB1A* locus, resulting in reduced cold induction of its expression.

Consequently, it is unclear whether transgene-induced methylation was an issue in the Chinnusamy et al. (2003) analysis. Nevertheless, the genetic analysis presented by Kidokoro et al. (2020), in conjunction with their *ice1-1* transformation experiment, provides a compelling case that the ICE1(R236H) protein encoded by *ice1-1/scrm-D* does not impair the cold induction of *CBF3/DREB1A*.

But what about the wild-type ICE1 protein? Does it have a role in regulating *CBF1/DREB1B* genes? In a second approach, Kidokoro et al. (2020) addressed this question by testing the effects that upregulation and downregulation of ICE1 activity had on cold induction of the *CBF/DREB1* genes. What they found is that ICE1 overexpression had no effect on cold induction of any of the three *CBF/DREB1* genes. Additionally, the authors found that the null T-DNA insertion mutations **ice1-2** and **scrm2-1** had no effect on cold induction of any of the three *CBF/DREB1* genes. Likewise, an **ice1-2 scrm2-1** double mutation had no effect on cold induction of *CBF1/DREB1B* and *CBF2/DREB1C* and only a small transient effect on the induction of *CBF3/DREB1A*.

So, where do the Kidokoro et al. (2020) results leave us regarding the role of ICE1 in regulating the *CBF/DREB1* genes? If their results were the only results available on the topic, it would be reasonable to conclude that ICE1 has little if any role in cold induction of the *CBF/DREB1* genes. However, their results are not the only results on the topic, and other studies lead to a different conclusion. For instance, whereas the results of Kidokoro et al. (2020) indicate that the *ice1-2* mutation has no effect on cold induction of the *CBF/DREB1* genes, the results of Ding et al. (2015) and Kim et al. (2015) indicate that the *ice1-2* mutation reduces peak cold induction of the *CBF/DREB1* genes by 15 to 55%, depending on the gene and study. Also, whereas Kidokoro et al. (2020) found that overexpression of ICE1 had no effect on cold induction of the *CBF/DREB1* genes, the results of Ding et al. (2015) and Kim et al. (2015) indicate that the overexpression of ICE1 had no effect on cold induction of the *CBF/DREB1* genes, Miura et al. (2007, 2011). Moreover, overexpression of ICE1 variants that stabilize the ICE1 protein at low temperature has been shown to increase the freezing tolerance of cold-acclimated plants (Miura et al., 2007, 2011). However, overexpression of ICE1 variants that stabilize the ICE1 protein at low temperature has been shown to increase the freezing tolerance of cold-acclimated plants (Miura et al., 2011; Li et al., 2017). Do such results indicate that ICE1 acts as a positive regulator of the *CBF/DREB1* pathway in all of these experiments? In short, the answer is “no.” This is because the *CBF/DREB1*
pathway is not the only regulatory pathway that contributes to freezing tolerance. This is evident from the finding that Arabidopsis plants carrying null mutations in all three CBF/DREB1 genes, although severely impaired in freezing tolerance, still increase in freezing tolerance in response to low temperature (Jia et al., 2016; Zhao et al., 2016; Park et al., 2018). These results suggest that other transcription factors contribute to freezing tolerance independent of the CBF/DREB1 pathway. Indeed, there is direct evidence for this: for instance, overexpression of ZAT12 (Vogel et al., 2005) and HSFC1 (Park et al., 2015) has been shown to increase plant freezing tolerance and upregulate the expression of many COR genes, including some that are coregulated by CBF/DREB1. Thus, even if ICE1 does not have a major role in regulating the CBF/DREB1 pathway under a given set of conditions, it might contribute to freezing tolerance by affecting the expression of other transcription factors that contribute to freezing tolerance.

One additional note: it is known that dysfunction of ICE1 confers a severe developmental phenotype that could impact cold tolerance indirectly. The surface of the ice1-1/scrm-2 mutant plants is covered with stomata (see figure; Kanaoka et al., 2008). These mutant plants lack a means to shield their bodies from the external environment. On the other hand, the ice1-2 scrm2-1 mutant produces an epidermis solely composed of pavement cells (Kanaoka et al., 2008) and thus is unable to do gas exchange or transpiration via stomatal pores. With stomata serving a critical interface between a plant and the atmosphere, it is possible that such epidermal defects may impact cold tolerance in a given growth condition.

The discovery of ICE1 by Chinnusamy et al. (2003) some 17 years ago launched the current wisdom, which holds ICE1 as a major regulator of the CBF/DREB1 pathway and that the results of Kidokoro et al. (2020) must be placed. One take-home lesson is that conclusions from previous work using the ice1-1 mutant may have to be reinterpreted given the seemingly clear results of Kidokoro et al. (2020) indicating that the ice1-1/scrm-2 mutation — encoding the ICE1(R236H) protein — does not impair cold induction of the CBF/DREB1 genes. A second lesson is that ICE1 may not be an indispensable master regulator of the CBF/DREB1 genes. Certainly, the results presented by Kidokoro et al. (2020) provide evidence that, under a given set of experimental conditions, overexpression of ICE1 or lack of ICE1 function (or even a lack of both ICE1/SCRM and SCRM2/ICE2 function) has little effect on cold induction of the CBF/DREB1 genes. Whether ICE1 contributes significantly to CBF/DREB1 expression under some conditions and not others and whether ICE1 contributes significantly to freezing tolerance independently of CBF/DREB1 are questions for future study. Indeed, it would seem prudent to think, as Kidokoro et al. (2020) propose, that “the current ICE1-DREB1 regulatory model should be reevaluated without the previous assumptions.”

Michael F. Thomashow
Michigan State University
Department of Energy Plant Research Laboratory and
Department of Plant, Soil, and
Microbial Sciences
East Lansing, Michigan 48824
thomash@msu.edu
ORCID ID: 0000-0002-7832-6989

Keiko U. Torii
University of Texas at Austin
Department of Molecular Biosciences and Howard Hughes
Medical Institute
Austin, Texas 78712
ktorii@utexas.edu
ORCID ID: 0000-0002-6168-427X

REFERENCES


Miura, K., Jin, J.B., Lee, J., Yoo, C.Y., Stirm, V., Miura, T., Ashworth, E.N., Bressan,


SCREAMing Twist on the Role of ICE1 in Freezing Tolerance
Michael F. Thomashow and Keiko U. Torii

Plant Cell 2020;32;816-819; originally published online February 19, 2020;
DOI 10.1105/tpc.20.00124

This information is current as of February 3, 2021

| References | This article cites 24 articles, 9 of which can be accessed free at: /content/32/4/816.full.html#ref-list-1 |
| eTOCs | Sign up for eTOCs at: http://www.plantcell.org/cgi/alerts/ctmain |
| CiteTrack Alerts | Sign up for CiteTrack Alerts at: http://www.plantcell.org/cgi/alerts/ctmain |
| Subscription Information | Subscription Information for The Plant Cell and Plant Physiology is available at: http://www.asp.org/publications/subscriptions.cfm |