Trends in
Plant Science

CellPress

Spotlight

Post-translational activation of CBF for inducing freezing tolerance

Przemysław Kopeć [1,4,*], Marcin Rapacz [2] and Rajeev Arora [3]

Plants can acquire increased freezing tolerance through cold-acclimation involving the ICE1-CBF-COR pathway. Recently, Lee *et al*. investigated a potential link between the functional activation of CBF and cellular redox state. We propose that redox-mediated CBF activation could be a hub of low temperature as well as light signaling in the cold-acclimation process.

Most plants native to temperate and boreal climates can acquire increased freezing tolerance through prior exposure to low, nonfreezing temperatures. This is an adaptive process termed cold acclimation [1,2]. A well-studied element of this process is the arabidopsis (*Arabidopsis thaliana*) ICE1-CBF-COR pathway (Figure 1). Exposing plants to low temperature results in rapid induction of genes encoding transcriptional activators that belong to the AP2/ERF domain family of DNA-binding proteins, known as C-repeat binding factors (CBF). These genes are under the positive control of the upstream inducer of CBF expression 1 (ICE1). CBF proteins recognize a *cis*-acting regulatory element, C-repeat/dehydration response element (CRT/DRE), present in some of the promoters of cold-regulated (*COR*) genes and induce their expression. Expression of *COR* genes, known as the CBF regulon, boosts plant freezing tolerance [1]. Though the transcriptional control of *CBF* expression *vis-à-vis* cold acclimation has been extensively studied, functional regulation of CBF at a post-translational level remains unclear.

Recently, Lee *et al.* [3], investigated, for the first time, a potential link between the functioning of the CBF system and the cellular redox state in regulating plant freezing tolerance. These authors explored this link by testing the hypothesis that the functional activation of CBF is regulated by a structural shift in the protein brought about by alteration of its redox state (Figure 1). Results of this study fill a long-standing gap in our understanding of the post-translational regulation of CBF proteins. The experimental system used in this study was arabidopsis, which has three CBF family members. Lee *et al.* [3] noted that at warm temperatures, CBF expression remains at a critical threshold since this protein also regulates normal growth besides being involved in plants' response to cold. Under ambient conditions, CBFs exist, predominantly, in inactive form as high-molecular-mass (HMM) oligomers that are formed by intermolecular disulfide bonds. Under low temperature, however, oligomers are dissociated into CBF monomers (Figure 1). Disulfide bonds are also formed inside monomer molecules, which are then biologically inactive. Lee *et al.* [3] hypothesized that the structural switching of CBFs may be controlled by their interacting partners. They identified an h2-type thioredoxin (Trx-h2) as such a partner. Trx-h2 is a cytosolic redox protein belonging to a subgroup II of Trx-hs, which contains two active-site cysteine residues responsible for their disulfide reductase activity. Trx-hs regulates the redox status of target proteins through exchanging disulfide bonds between thioredoxin and the targets [4]. Under warm temperatures Trx-h2 is normally anchored to cytoplasmic endomembranes through fatty acid: myristate attached to the glycine residue at the second amino acid position (G^2) (Figure 1) [3,4]. However, under low temperatures, Trx-h2 is demyristoylated and translocated to the nucleus. Trx-h2, presumably, has a bipartite nuclear localization signal at the C terminus [3]. In the nucleus, Trx-h2 regulates redox status of CBFs. Lee *et al*. [3] focused their study on CBF1 as a representative C repeat binding factor. Their research shows that Trx-h2 reduces all oxidized forms of CBF1, both oligomers present at warm temperature as well as the monomers whose synthesis was induced by cold, rendering them 'functionally active' (Figure 1). Such post-translational activation of CBFs leads to the expression of CBF regulon and attainment of freezing tolerance.

It is worth highlighting that CBFs perform many functions beyond low-temperature response. They are responsible for proper growth and development of plants, which is related to the specialized regulation of their activity. More information on CBFs roles in plant physiology can be found in Wi *et al.* [5].

Concluding remarks and future perspectives

Notably, cold acclimation is triggered not only by plant exposure to low temperatures but also by a combination of decreasing temperature and light signals. Light can be considered in a variety of contexts, both in terms of quality (wavelength) and the quantity (intensity and/or photoperiod). Integration of cold and light signaling pathways determines plant survival under freezing temperatures [6,7] but still remains poorly understood. One factor that is postulated to play a role in the crosstalk between light and cold signals is hydrogen peroxide (H_2O_2), a relatively stable molecule in biological systems compared with its usual precursor superoxide [8]. Low/freezing temperature disrupts the balance between light-harvesting and the subsequent processing of this energy through dark reaction biochemistry, which is down-regulated under cold. Thus, the unused excess excitation energy can lead to the

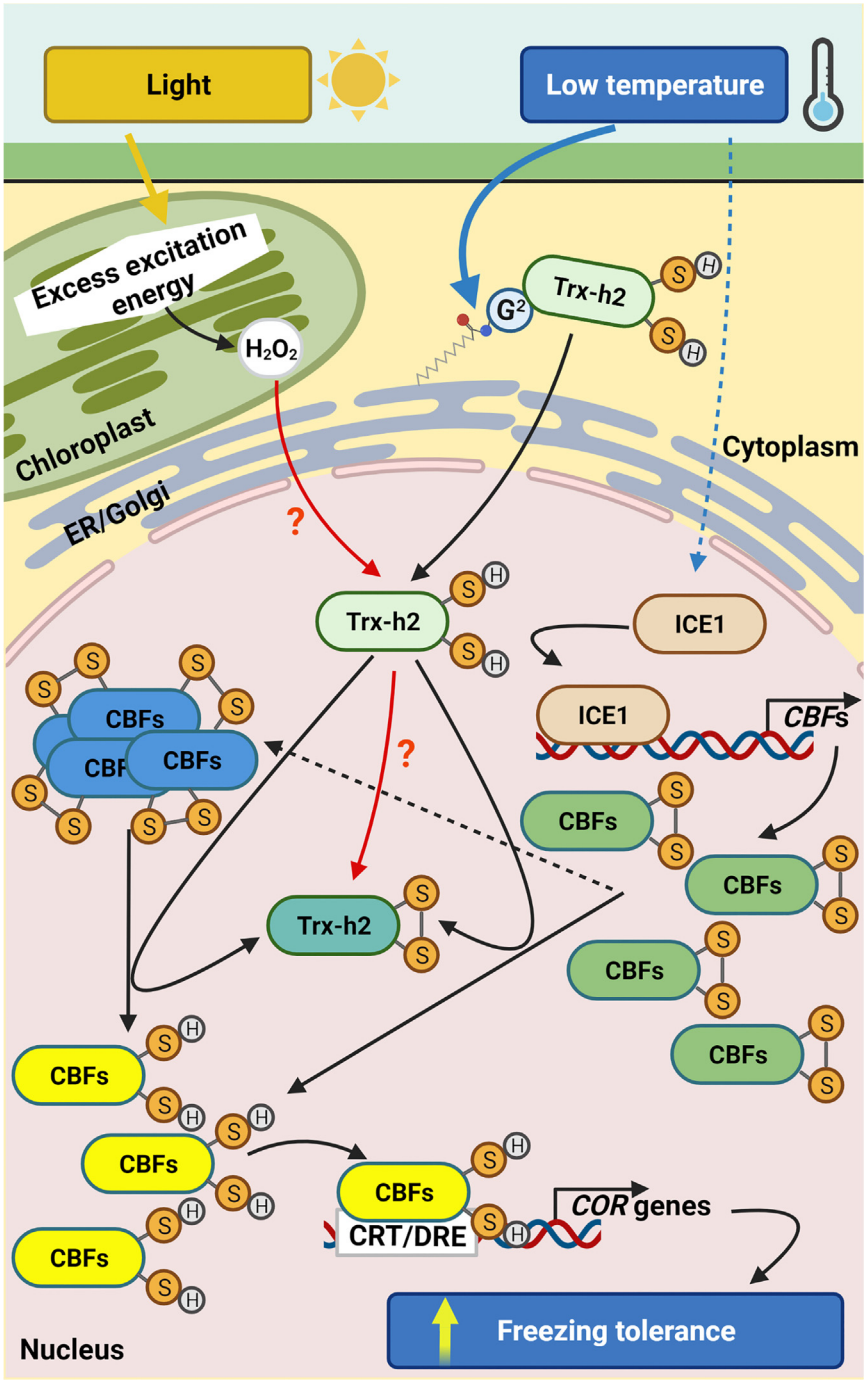

Figure 1. Proposed integration of low temperature and light signaling in redox-dependent functional activation of the CBF pathway leading to increased freezing tolerance in plants. Cold acclimation involves the action of the ICE1-CBF-COR pathway, thus far the best-understood regulatory process. Low temperature causes binding of inducer of CBF expression 1 (ICE1) to the promoters of *C-repeat binding factors* (*CBF*) genes, thereby promoting their expression. ICE1 is constitutively expressed in plant tissues and its function depends on its post-translational modification [1]. The model proposed by Lee *et al.* [3] assumes that

(Figure legend continued at the bottom of the next page.)

formation of reactive oxygen species (ROS) (Figure 1) [7]. In chloroplasts, H_2O_2 is mainly formed from the dismutation of superoxide radical anion. The reaction may be spontaneous or catalyzed by superoxide dismutase. Furthermore, H_2O_2 production can result from partial oxidation of H_2O at the PSII electron donor side or reduction of O_2^- by plastohydroquinone (PQH2) [9]. Generally, the consequence of ROS production in plants is twofold: at lower concentrations they could serve as signaling molecules, whereas at higher accumulation they are toxic for cellular elements. Additionally, the level of their abundance also depends upon the efficiency of the plant`s detoxification systems [10]. It is suspected that chloroplast-sourced H_2O_2 may be driven directly to the nucleus. Chloroplasts and nuclei are closely associated and the peri-nuclear endoplasmic reticulum has been noted to form a layer between the two organelles [8,11]. In the context of the above discussion, the results of Lee *et al.* [3] could prompt new questions: Does Trx-h2 receive signals from chloroplasts in the form of ROS? Or how might disulfide reductase activity of Trx-h2 be regulated in the nucleus by ROS generated in the chloroplast? (Figure 1). The reductase function of Trx-h2 is induced through the demyristoylation process as a result of the accumulation of low temperature-induced ROS [5]. But what (if any) role could H_2O_2-generated under cold together with light and moving from the chloroplasts directly into the nucleus, play in such induction? Thioredoxins are potential H_2O_2 sensors [10]. ROS can oxidize the thiolate anion of protein cysteine residues to the sulfenic form (Cys-SOH), which can also react with another thiolate to form a disulfide bond [12].

Integration and the crosstalk of cold and light signals may also be crucial for overwintering plants under the climate change scenario characterized by an increased frequency of erratic and extreme temperature swings. Unseasonal warm spells in

the spring can cause premature deacclimation (loss of acquired freezing tolerance during cold acclimation), rendering the plants vulnerable to returning frigid temperatures. Depending upon the extent, and thereby the reversibility or irreversibility of deacclimation, plants may be able to regain some of the freezing tolerance during reacclimation when temperatures drop again [2]. Warming climate is also causing growing seasons to become warmer/longer, decreasing the effectiveness of cold acclimation. This raises an important question: how might the CBF-COR pathway be involved in regulating deacclimation and reacclimation processes? The work by Lee *et al*. [3] appears to be a promising step in the direction of answering such questions. They have proposed a mechanism of structural switching and functional activation of CBF proteins in which the h2-type of thioredoxin plays a key role. Still, much work remains to be done to establish the detailed networks involved in cold acclimation and acquiring of freezing tolerance by plants. Understanding the mechanisms regulating the response of plants to unfavorable environmental conditions will allow adjusting the strategy of winter crops in a changing climate.

Declaration of interests

No interests are declared.

[1]The Franciszek Górski of Plant Physiology, Polish Academy of Sciences, Kraków 30-239, Poland
[2]Department of Plant Breeding, Physiology and Seed Science, University of Agriculture, Kraków 31-120, Poland
[3]Department of Horticulture, Iowa State University, Ames, IA 50010, USA
[4]http://en.ifr-pan.edu.pl/about-the-institute/staff/147-przemyslaw-kopec

*Correspondence:
p.kopec@ifr-pan.edu.pl (P. Kopeć).

https://doi.org/10.1016/j.tplants.2022.01.003

© 2022 Elsevier Ltd. All rights reserved.

References

1. Shi, Y. *et al.* (2018) Molecular regulation of CBF signaling in cold acclimation. *Trends Plant Sci.* 23, 623–637
2. Vyse, K. *et al.* (2019) Deacclimation after cold acclimation-a crucial, but widely neglected part of plant winter survival. *J. Exp. Bot.* 70, 4595–4604
3. Lee, E.S. *et al.* (2021) Redox-dependent structural switch and CBF activation confer freezing tolerance in plants. *Nat. Plants* 7, 914–922
4. Park, J.H. *et al.* (2021) Disulfide reductase activity of thioredoxin-h2 imparts cold tolerance in *Arabidopsis*. *Biochem. Biophys. Res. Commun.* 568, 124–130
5. Wi, S.D. *et al.* (2021) Redox-mediated structural and functional switching of C-repeat binding factors enhances plant cold tolerance. *New Phytol.* 233, 1067–1073
6. Krasensky-Wrzaczek, J. and Kangasjärvi, J. (2018) The role of reactive oxygen species in the integration of temperature and light signals. *J. Exp. Bot.* 69, 3347–3358
7. Chang, C.Y.Y. *et al.* (2021) Champions of winter survival: cold acclimation and molecular regulation of cold hardiness in evergreen conifers. *New Phytol.* 229, 675–691
8. Exposito-Rodriguez, M. *et al.* (2017) Photosynthesis-dependent H_2O_2 transfer from chloroplasts to nuclei provides a high-light signalling mechanism. *Nat. Commun.* 8, 1–10
9. Mignolet-Spruyt, L. *et al.* (2016) Spreading the news: subcellular and organellar reactive oxygen species production and signalling. *J. Exp. Bot.* 67, 3831–3844
10. Smirnoff, N. and Arnaud, D. (2019) Hydrogen peroxide metabolism and functions in plants. *New Phytol.* 221, 1197–1214
11. Mullineaux, P.M. *et al.* (2020) Spatial chloroplast-to-nucleus signalling involving plastid–nuclear complexes and stromules. *Philos. Trans. R. Soc. B: Biol. Sci.* 375, 20190405
12. Sevilla, F. *et al.* (2015) The thioredoxin/peroxiredoxin/sulfiredoxin system: current overview on its redox function in plants and regulation by reactive oxygen and nitrogen species. *J. Exp. Bot.* 66, 2945–2955

low temperature induces nuclear translocation of Trx-h2, earlier anchored to cytoplasmic endomembranes thorough myristate linked to the glycine residue at the second amino acid position (G^2). Nucleus-localized Trx-h2 reduces all oxidized forms of CBFs (functionally inactive), both pre-existing oligomers (marked in blue) as well as the monomers (marked in green) whose synthesis was induced by cold, rendering them 'functionally active' (marked in yellow). The reduced and active CBF monomers bind to the CRT/DRE motif in some of the promoters of *cold-regulated* (*COR*) genes and induce their expression leading to increased freezing tolerance in plants. The mechanism of CBF activation proposed by Lee *et al*. [3] could also apply to light signaling (during cold acclimation) through oxidation of the thiolate anion of protein cysteine residues of Trx-h2 by chloroplast-sourced hydrogen peroxide (H_2O_2). Unbroken and dashed lines indicate direct and indirect activation of signaling pathways, respectively. This figure was created using BioRender (https://biorender.com). Abbreviations: ER, endoplasmic reticulum.