Cold Tolerance, SFR2, and the Legacy of Gary Warren

The beauty of a genetic approach to biological questions is that it can lead to the discovery of completely unexpected processes. This was a guiding precept for the research of Gary Warren, a molecular geneticist and prominent researcher in the field of cold tolerance at Royal Holloway College of the University of London until his untimely death earlier this year. The power of genetics to reveal new mechanisms involved in how plants acclimate to tolerate freezing conditions is finely illustrated in this issue of The Plant Cell with the characterization of SENSITIVE TO FREEZING2 (SFR2) by Thorlby et al. (pages 2192–2203), the last paper of senior author Gary Warren to be submitted for publication before his death.

Significant progress has been made over the past 10 years in understanding the molecular basis of cold acclimation, initiated largely from the study of induced genes (Thomashow, 1999). These studies have led to the identification of the CBF transcription factors that are responsible for activating expression of many of the genes induced during cold acclimation in Arabidopsis (Jaglo-Ottosen et al., 1998; Kasuga et al., 1999; Gilmour et al., 2000). Characterization of the closely related (or in some cases identical) DREB transcription factors has led to a similar understanding of gene regulation in response to drought stress and also to an appreciation of the mechanistic links between higher plant responses to cold and drought (Liu et al., 1998; Shinozaki and Yamaguchi-Shinozaki, 2000; Seki et al., 2003). The target genes of the CBF transcription factors (cold-induced genes; CORs) provide some clues to the metabolic processes and cellular changes that are important components of acclimation. For example, COR15a is thought to decrease the rate at which the membranes of the chloroplast inner envelope undergo phase transition at low temperatures (Steponkus et al., 1998). Its activity emphasizes that impairment of membranes is one of the most damaging effects of exposure to freezing, and many cellular processes induced during cold acclimation are associated with membrane stabilization. However, it has proved difficult to evaluate the effective contribution of each of the CORs to freezing tolerance after acclimation (Xin and Browse, 2000). Where such studies have been attempted, the general conclusion seems to be that the individual contributions of CORs to freezing tolerance are relatively modest, with each COR gene making a small contribution.

Plant breeders would like to identify single genes that make large contributions to the tolerance of freezing conditions and the ability of plants to acclimate to cold. The reasons are obvious: high impact traits are easier to select and breed for and likely confer greater returns. The transcriptional regulators of COR gene expression have provided some potential candidate genes. The analysis of quantitative trait loci associated with cold tolerance is another approach. Gary Warren opted for a mutational strategy designed to complement the other approaches being taken to investigate molecular mechanisms underlying freezing tolerance in higher plants and to identify genes conferring large effects on freezing tolerance (Warren et al., 1996). In work initiated at the now sadly defunct DNA Plant Technology (Oakland, CA), Gary established a genetic screen to identify mutants of Arabidopsis with impaired ability to tolerate freezing after acclimation. His logic was that genetic screens might reveal something new that could not be revealed by other strategies. For example, quantitative trait loci can be difficult to map with high resolution, and genes affecting cold tolerance might not be polymorphic within the recombinant inbred populations available. A limitation of studying COR genes is that some of the genes that confer freezing tolerance might not themselves be induced by low temperature, and if they are constitutively expressed they would be missed in the COR strategy. Gary’s mutational approach led to the identification several sfr mutants (Warren et al., 1996).

Thorlby et al. show that SFR2 is more or less constitutively expressed in aerial tissues of Arabidopsis. Despite its expression during growth under normal conditions, sfr2 mutant plants fail to show an abnormal phenotype when grown under optimal conditions or after 2 weeks acclimation at 4°C. It is only after exposure of sfr2 mutants to freezing conditions that the essential function of SFR2 is revealed because the mutants fail to resume growth, unlike acclimated controls. Freezing sensitivity is observed all over the aerial parts of the plant and is classified as severe relative to other mutants, such as those affecting the CBF induction of COR genes. Most freezing-sensitive mutants exhibit significant membrane damage after exposure to freezing conditions—measured by electrolyte leakage after cell death (McKown et al., 1996). Significantly, although sfr2 mutants are severely affected by exposure to freezing conditions, the inhibition of regrowth does not result from massive tissue damage after impairment of membrane function because sfr2 mutants show only low levels of electrolyte leakage after freezing conditions. Because other mutations that affect the CBF pathway and COR gene expression do result in high levels of electrolyte leakage, the role of SFR2 in freezing tolerance after acclimation is clearly distinct from the CBF pathway of response to cold.

SFR2 encodes a novel type of β-glycosidase. Thorlby et al. demonstrate that the preferred substrates for the enzyme are probably glucosides and that the SFR2 protein has a signal or transit targeting sequence at its N terminus that is probably cleaved to give the mature enzyme. This implies that the activity of SFR2 is either extracellular or targeted to a subcellular organelle. Although Thorlby and colleagues...
favor an extracellular location because SFR2 is secreted when expressed in yeast, there remains a possibility that it is targeted to plastids or mitochondria. An association with plastid function would explain the high levels of SFR2 expression in tissues involved in photosynthetic carbon fixation.

Analysis of the primary structure of SFR2 revealed, intriguingly, that the protein is most closely related to β-glycosidases from thermophilic and halophilic archaeabacteria. Is SFR2 performing a function also associated with stress tolerance in Archaea? Although such speculation is tempting, sfr2 mutants are not more sensitive to abscisic acid treatment or to water deficit. Consequently, the structural link between SFR2 and the β-glycosidases from Archaea does not seem to reflect a functional link in stress tolerance.

SFR2 is a novel protein, with a previously unrecognized function in cold acclimation and freezing tolerance in Arabidopsis. The details of what is known about SFR2 are intriguing because they point the way to as yet undiscovered mechanisms in stress response. The work of Thorlby et al. invites many new questions and further lines of investigation. Unfortunately, Gary’s untimely death means that he will not have the satisfaction of elucidating the mechanism by which SFR2 confers freezing tolerance after cold acclimation. He has left us with some tantalizing suggestions: perhaps SFR2 is involved in the turnover of polysaccharides in the cell wall, or perhaps it is involved in protecting chloroplasts from freezing damage. There is circumstantial evidence to support both ideas, but the immediate need is for these suggestions to be tested. Subcellular localization of the SFR2 protein should go part of the way toward discerning the biochemical function of SFR2. Studies of the tolerance of mutants affecting wall composition or turnover to freezing after cold acclimation could address the possibility of SFR2 affecting cell wall metabolism. Such studies could be augmented by studying the tolerance of double mutants affecting the wall in combination with sfr2. Similarly, investigation of mutants affecting chloroplast development and nucleus-plastid signaling for tolerance to freezing might address whether SFR2 plays a role in protecting the chloroplasts. Perhaps the most exciting possibility at this stage is to identify the full biochemical function of SFR2. Strategies based on high-throughput analysis of possible substrates may offer the best possibility for rapid functional identification.

The work of Thorlby et al. is a fine example of top quality science. The experimentation is complete and satisfyingly rigorous. The article is beautifully and concisely written; it casts no aspersions on the other authors to state that these were the trademarks of Gary Warren’s work. Gary’s legacy in this article and in his foundation work on freezing tolerance in plants will contribute significantly to the identification of new processes in cold acclimation and to the expansion of our understanding of those processes already established in acclimation. Gary’s other legacy comprises his careful and thoughtful approach to scientific methodology, interpretation, and presentation, which should serve as a shining example for plant scientists in the future.

Gary studied genetics as an undergraduate at Cambridge University and was a graduate student with Dave Sherratt at the University of Sussex and a postdoctoral researcher at Berkeley with John Clark. After using his skills with plasmids to generate vectors to manipulate Agrobacterium with Marc van Montagu in Gent, he took a position in late 1982 at the modestly named Advanced Genetic Sciences (later to merge with DNA Plant Technology). There he ran the bacterial molecular genetics group and made some beautiful contributions to our understanding of the ice nucleation proteins of Pseudomonas syringae. The repetitive motifs within the ice protein created numerous difficulties with the analysis that Gary overcame with aplomb. He also created the first genetically modified organism ever to be released into a field: the famous ice minus Pseudomonas strain in which the ice nucleation gene had been precisely deleted. His team also looked into the properties of certain Pseudomonas strains that are plant growth–promoting rhizobacteria, trying to analyze the genes and chemicals that underpinned this trait.

When it became clear that selling genetically modified bacteria would never be a business for Advanced Genetic Sciences, Gary embraced the burgeoning field of plant molecular genetics to continue his interest in the biology of freezing and freezing tolerance. After a period studying the expression of arctic flounder antifreeze protein in plants (yes, the experiment was done, but it didn’t work), he embarked in the early 1990s on the program that lead to the research presented by Thorlby et al. His former colleagues at Advanced Genetic Sciences and his many friends at Royal Holloway College, Imperial College, and in Dundee remember him with great affection and a sincere sadness that he did not live to see the full blooming of the scientific field to which he contributed so significantly.

**REFERENCES**


