

Acquired tolerance to temperature extremes

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Acquired tolerance to temperature stresses is a major protective mechanism. Recent advances have revealed key components of stress signal transduction pathways that trigger enhanced tolerance, and several determinants of acquired tolerance have been identified. Although high and low temperature stresses impose different metabolic and physical challenges, acquired tolerance appears to involve general as well as stress-specific components. Transcriptome studies and other genomic-scale approaches have accelerated the pace of gene discovery, and will be invaluable in efforts to integrate all the different protective and repair mechanisms that function in concert to confer acquired tolerance.

Temperature affects a broad spectrum of cellular components and metabolism, and temperature extremes impose stresses of variable severity that depend on the rate of temperature change, intensity and duration. What temperature causes stress, and what forms and magnitude of acquired tolerance a given plant possesses depend on the plant, tissue or cell type in question. Generally, four kinds of temperature stress are common among mesophilic plants: sustained high temperature, heat shock, chilling at temperatures above 0°C and freezing at temperatures below 0°C.

Acquired temperature stress tolerance

Acquired stress tolerances to temperature extremes are complex traits dependent on many attributes. Within defined narrow limits, the ability to survive a temperature stress that would be lethal can be conferred by exposure to a mild nonlethal temperature stress. This induced ability to survive a normally lethal stress is known as acquired thermotolerance in the case of heat shock, acquired chilling tolerance in the range of 0° to 15°C and acquired freezing tolerance in the range below 0°C where ice is present inside plant tissues.

Components of induced tolerance

Acquired tolerances to high and/or low temperature stress conditions are complex traits as demonstrated from a long record of plant breeding and crop improvement efforts. More recently, extensive efforts to modify temperature

stress tolerance by transgenic approaches have largely validated that tolerance is a multigenic trait (Table 1). Although numerous studies involving ectopic expression of a single structural gene in transgenic plants have shown some level of benefit to tolerance under highly defined conditions, for the most part, the benefit has been limited and has not led to agronomically improved crops for stress tolerance under field conditions. However, a notable exception involves modifying the expression of transcription factors that activate a range of genes. Here, improvement of freezing tolerance using CBF/DREB (C-repeat binding factor/dehydration-response elements-binding protein) transcription factors has been shown to be highly effective [1,2]. Yet, recent microarray studies have confirmed that not all cold-regulated gene expression is under the direct control of the CBF/DREB family [3,4].

A comparative examination of plant responses to high and low temperature reveals many similarities and parallels as well as differences (Fig. 1). At the level of perception and signal transduction, high and low temperature signals appear to be transduced by nonoverlapping and independent pathway components. However, similar metabolic and physiological responses indicate that some tolerance factors are responsive to both high and low temperature stress. For example, antioxidant metabolism and oxidative stress caused by active oxygen species (AOS) constitute a major part of temperature stress in plants, and recent microarray studies highlight this linkage during cold stress [3]. Other common responses involve compatible solute production, thought to help stabilize proteins, membrane bilayer structure and provide nontoxic osmolytes, dehydrins, proteins of unknown function and heat-shock proteins consisting of a suite of molecular chaperones and agents of protein turnover [5–9] (D-Y. Sung, PhD thesis, University of Florida, 2001). Among heat-shock proteins, Hsp101 appears to play a major and specific role in acquired thermotolerance. A point mutation in *Arabidopsis* Hsp101 (*hot1*) abolished not only basal thermotolerance as measured by hypocotyl elongation, but also blocked or reduced acquired thermotolerance in whole plants [10]. Antisense and co-suppression of Hsp101 expression, and Hsp101 T-DNA insertional mutation in *Arabidopsis* have all confirmed that Hsp101 has an essential role in thermotolerance [11]. Similar to *Arabidopsis* Hsp101, insertional knockouts of Hsp101 in maize were also

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Table 1. Transgenic attempts to enhance plant temperature stress tolerance

Gene	Plant	Function	Modification	Phenotypes	Remarks	Refs
Heat stress						
HsfA1	Tomato	TF	COE or CS	HS tolerant; HS sensitive	Reduced expression of HS genes in CS lines	[59]
Hsf3	<i>Arabidopsis</i>	TF	COE	HS tolerant	Increase in APX activity at HT	[60]
Hsp70	<i>Arabidopsis</i>	HSP	AS	HT sensitive	Delayed repression of HS response	[61]
Hot1–Hot4	<i>Arabidopsis</i>	HSP	EMS	Abolished acquired thermotolerance	Hot1 is Hsp101	[10]
Hsp101	Maize	HSP	Knockout	Abolished acquired thermotolerance	Faster primary root growth in mutants	[12]
Hsp17.7	Carrot	HSP	COE or AS	HT tolerant; HT sensitive	Reduced expression of other HS genes	[62]
Fad7	Tobacco	Fatty acid desaturation	cOE in Cp	Increased levels of 16:3 and 18:3 fatty acids	Low-temperature chlorosis was reduced and young leaf growth rate improved	[63]
Fad7	Tobacco	Fatty acid desaturation	CS	HT tolerant	Enhanced growth at long-term HT, oxygen evolution assay for heat tolerance	[20]
Fad7, Fad8	<i>Arabidopsis</i>	Fatty acid desaturation	EMS	HT tolerant	Enhanced growth at long-term HT, oxygen evolution assay for heat tolerance	[20]
Hvapx1	Barley	AOS metabolism	cOE	HS tolerant	Marginal increase in thermotolerance	[64]
Chilling stress						
SCOF-1	<i>Arabidopsis</i>	TF	cOE	Tolerant to chilling and freezing	Chilling assay on calli, no quantitative data on freezing	[65]
OsCDPK7	Rice	Signal transduction	cOE	Enhanced chilling tolerance	CDPK, positive regulator of acclimation, Ps electron transport preserved	[66]
frs1	<i>Arabidopsis</i>	ABA biosynthesis	EMS	ABA deficient, wilted, loses excessive water, freezing sensitive	Limited acclimation at low temperature	[67]
Glutamine synthetase	Rice	Amino acid metabolism	cOE	Enhanced recovery of PSII after chilling	Salt-stress tolerant	[68]
betA/betB	Tobacco	Betaine biosynthesis	cOE	Enhanced tolerance of PSII to chilling	Marginal increase of tolerance	[69]
ALA1	<i>Arabidopsis</i>	Phospholipid metabolism	AS	LT sensitive	Reduced plant height and altered leaf-shape at LT	[24]
Glycerol-3-P-acyl transferase	Rice	Phospholipid metabolism	cOE	Higher content of unsaturated fatty acids	Slightly improved net photosynthesis at moderate temperature	[70]
Glycerol-3-P-acyl transferase	Tobacco	Phospholipid metabolism	cOE in Cp	Decrease lipid unsaturation, repair of damaged PSII enhanced	Transgenic plants more sensitive to chilling-induced photoinhibition. No difference in PSII sensitivity at high and low temperature compared with wild type	[71]
pho1-2 pho2-1	<i>Arabidopsis</i>	Phosphate metabolism	EMS	Increased and decreased shoot phosphate	Low phosphate triggers cold acclimation	[72]
Nonspecific $\Delta 9$ -desaturase	Tobacco	Phospholipid metabolism	cOE in Cp	Increased fatty acid unsaturation	Increased chilling resistance	[73]
Glycerol-3-P-acyl transferase	<i>Arabidopsis</i> , tobacco	Phospholipid metabolism	cOE in Cp	Decrease in lipid unsaturation, in PG	Transgenic plants sensitive to chilling	[74]
Glycerol-3-P-acyl transferase	Rice	Phospholipid metabolism	cOE	Improved rate of photosynthesis and growth at low temperature	Linear relationship between degree of PG unsaturation and PS and growth at low temperature	[75]
MnSOD	Maize	AOS metabolism	cOE in Cp	Improved growth at lower temperature	Showed enhanced antioxidant metabolism	[76,77]
Catalase	Tobacco	AOS metabolism	EMS	Increased susceptibility to paraquat, ozone and salt stress	Not more sensitive to chilling than wild type is	[78]
Fe-SOD	Tobacco	AOS metabolism	cOE in Cp	Chilling sensitivity not different from wild type	Fe-SOD provides better protection against methyl viologen than Mn-SOD does	[79]
Cu/Zn-SOD	Tobacco	AOS metabolism	cOE in Cp	Enhanced stability of photosynthetic capacity during chilling	Enhanced recovery of O ₂ evolution following chilling	[80]

Table 1 (continued)

Gene	Plant	Function	Modification	Phenotypes	Remarks	Refs
GST, GPX	Tobacco	AOS metabolism	cOE	Improved growth during chilling	Showed increased levels of oxidized glutathione	[81]
SOD, GR, APX	Cotton	AOS metabolism	cOE	Rapid recovery of photosynthesis following chilling	Findings limited to short chilling duration	[82]
FeSOD	Maize	AOS metabolism	cOE	Enhanced tolerance to methyl viologen	Increased growth rate	[77]
CAX1	<i>Arabidopsis</i>	Ion transport	COE or AS	cOE more sensitive to cold shock, but AS not different from wild type	Over-expression of CAX1 (vacuolar Ca ²⁺ -H ⁺ antiporter) reduced growth and altered morphology	[36]
Freezing stress						
esk1	<i>Arabidopsis</i>	Unknown	EMS	Constitutive freezing tolerance	Proline over-accumulator	[83]
CBF1	<i>Arabidopsis</i>	TF	cOE	Strongly enhanced freezing tolerance	Constitutive expression of cor genes	[1]
CBF1	Tomato	TF	cOE	Freezing tolerant	CAT1 activity increased, tolerant to oxidative stress	[84]
CBF3	<i>Arabidopsis</i>	TF	cOE	Freezing tolerant	Increase in proline and total soluble sugars	[85]
CBF4	<i>Arabidopsis</i>	TF	cOE	Enhanced freezing tolerance, constitutive COR expression	OE plants show varying degrees of dwarfing correlated with enhanced freezing tolerance	[48]
DREB1A	<i>Arabidopsis</i>	TF	cOE	Freezing tolerant	Induced the expression of multiple COR genes	[2]
DREB1A, DREB2A	<i>Arabidopsis</i>	TF	cOE	Enhanced freezing tolerance	Dwarfed, marked enhanced freezing tolerance correlated with degree of dwarfing	[52]
HOS2	<i>Arabidopsis</i>	TF	EMS	Freezing sensitive	Ion leakage assay for freezing tolerance	[86]
ABI3	<i>Arabidopsis</i>	TF	cOE	Enhanced development of freezing tolerance	No difference in basal tolerance	[87]
AtPP2CA	<i>Arabidopsis</i>	Signal transduction	AS	Accelerated development of freezing tolerance	No difference in basal tolerance	[88]
Mn-SOD	Alfalfa	AOS metabolism	cOE	Cu/Zn-SOD lower in transgenic plants	Modest increase on freezing tolerance	[89]
Fe-SOD	Alfalfa	AOS metabolism	cOE in Cp	Increased winter survival, but no difference in shoot dry-matter yield	Rare field test of transgenic plants	[90]
SbwAFP	Tobacco	AFP	cOE	Freezing tolerant	sbwAFP is targeted to apoplastic space	[91]
Type II Fish AFP	Tobacco	AFP	cOE	No difference in frost tolerance from wild type	Protein found in apoplast	[92]
Synthetic AFP	Potato	AFP	cOE	Freezing tolerant	Marginal increase of tolerance	[93]
Coda	<i>Arabidopsis</i>	Betaine biosynthesis	cOE in Cp	Enhanced freezing tolerance	Modest enhanced tolerance	[94,95]
Proline dehydrogenase	<i>Arabidopsis</i>	Amino acid metabolism	AS	Enhanced freezing tolerance	No data on proline content reported	[96]
CAP 160 and CAP 85	Tobacco	Dehydrin and Lea-like	cOE	Slightly enhanced freezing tolerance	Marginal increase of tolerance	[97]
HiC6	Tobacco	Group III LEA	cOE		No significant change in freezing tolerance	[98]

Abbreviations: ABA, abscisic acid; AFP, antifreeze protein; AOS, active oxygen species; APX, ascorbate peroxidase; AS, antisense; CBF, C-repeat binding factor; CDPK, calcium-dependant protein kinase; cOE, constitutive over-expression; COR, cold-responsive gene; Cp, chloroplast; CS, co-suppression; DRE/CRT, dehydration responsive element/C-repeat; DREB, dehydration-responsive element binding protein; EMS, ethyl metanesulfonate; HAMK, heat shock-activated MAPK, His kinase, histidine kinase; HOS, high expression of osmotically responsive genes; HS, heat shock; HSE, heat-shock element; HSF, heat-shock factor; HSP, heat-shock protein; HT, high temperature; LEA, late-embryogenesis abundant protein; LT, low temperature; MAPK, mitogen-associated protein kinase; OE, over-expression; PG, phosphatidylglycerol; Ps, photosynthesis; PS, phosphatidylserine; PSII, photosystem II; SAMK, stress-activated MAPK; sbwAFP, spruce budworm antifreeze protein; SFR, sensitive to freezing; SOD, superoxide dismutase; TF, transcription factor.

found to be defective in basal and acquired thermotolerance [12]. Together these findings establish a central role for Hsp101 in the development of acquired thermotolerance in plants.

Transcriptome studies

Recent transcriptome studies have helped to provide a better understanding of plant stress responses [3,4,13–15].

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Through these studies, numerous novel stress-responsive genes have been discovered [3,4,13–15]. Overlaps of genes induced by various stress conditions suggest extensive cross-talk between the signaling pathways [3,4,13–15]. Identification of cold-inducible transcription factors, such as RAV2.1, RAV2.6, RAV1 and ZAT12, have begun to shed light on additional down-stream components of the low-temperature signaling pathway [3]. Subsequent induction

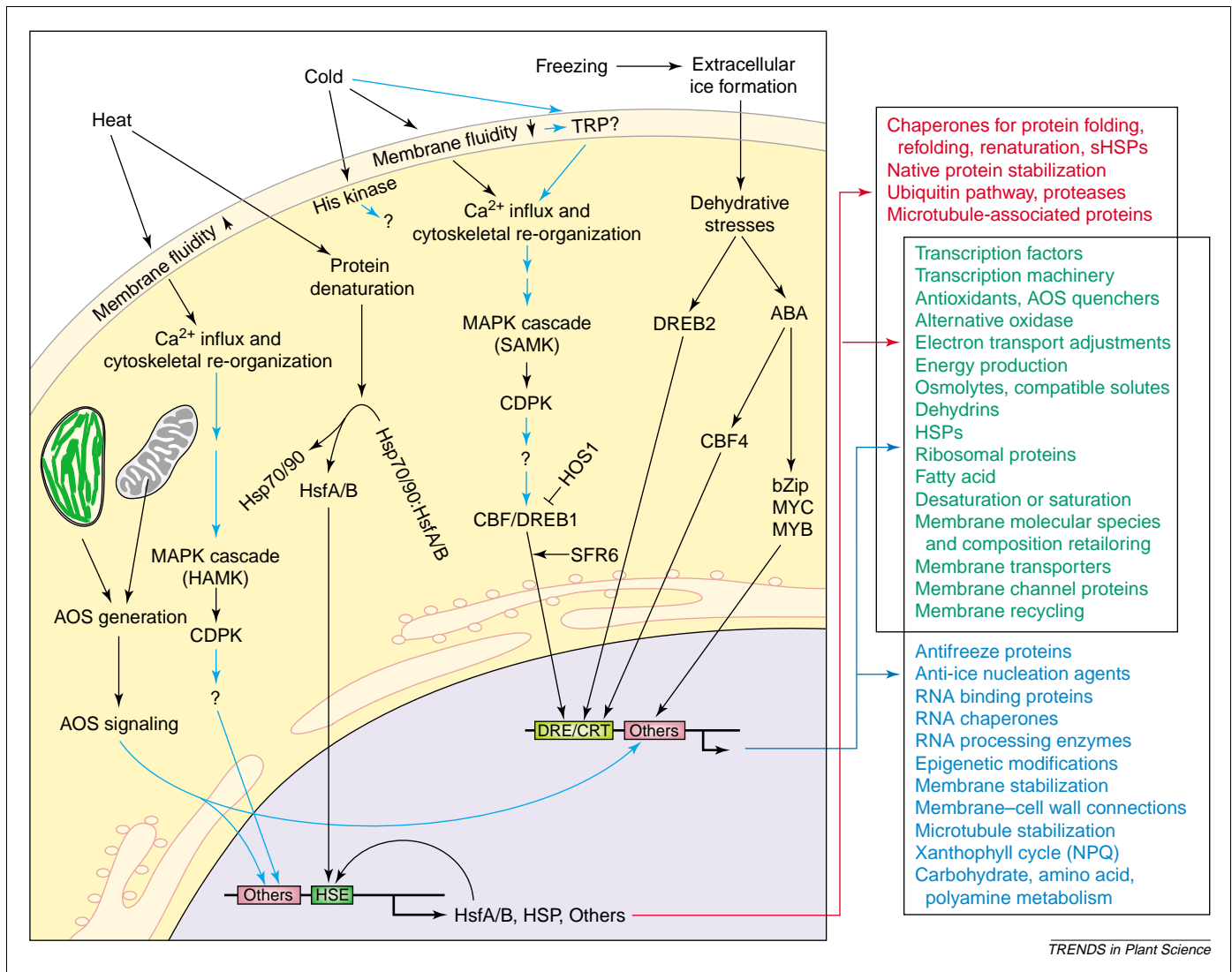


Fig. 1. Integrated model depicting temperature perception, signal transduction, transcriptional activation and components of temperature stress tolerance. Temperature stresses provoke either a rise of cytoplasmic calcium ions or dehydrative stresses inside the cell that conveys stress-induced signals to responding genes through respective pathways. These pathways often converge and/or diverge from one another. However, for the sake of clarity, the pathways for cross-talks are not indicated. Black arrows indicate pathways that have been confirmed in the literature and light-blue arrows depict pathways with circumstantial evidence. HSE- and DRE/CRT-containing genes represent aggregates of heat and cold/freezing induced genes, respectively. The contribution of these gene products is listed in the boxes. Red and blue lettering indicate heat and cold responsive components, respectively. Green lettering indicates components responsive to heat and cold. This model does not show temperature controlled events that are under post-transcriptional, translational or post-translational control or that involve repression and down-regulation of expression and function, which surely are major contributors to tolerance and cell survival as evidenced in a recent study of LOS1 [58]. Abbreviations: ABA, abscisic acid; AOS, active oxygen species; CBF, C-repeat binding factor; CDPK, calcium-dependant protein kinase; DRE/CRT, dehydration responsive element/C-repeat; DREB, dehydration responsive element binding protein; HAMK, heat shock-activated MAPK, His kinase, histidine kinase; HSE, heat-shock element; Hsf, heat-shock factor; HSP, heat-shock protein; MAPK, mitogen-associated protein kinase; NPQ, nonphotochemical quenching; sHSPs, small heat-shock proteins; SFR, sensitive to freezing.

of RAV2.1 and RAV2.6 following CBF induction and up-regulation of RAV2.1 and RAV2.6 in CBF-over-expressing transgenic plants suggest that they regulate expression of subgroups of CBF regulon [3]. Furthermore, CBF-independent expression of RAV1 and ZAT12 indicates the presence of a signaling pathway that is parallel or independent of CBF-mediated pathways [3]. This notion is also supported by findings that ~30% of cold-responsive genes are not directly regulated by CBFs [3], and that some cold-inducible genes do not have CRT/DRE *cis*-elements in their promoter regions [3,14]. Transcriptome studies with multiple time points suggest that (i) plants experience oxidative stresses during the initial adjustment period to a cold shock [3] and (ii) plant responses progress from general responses to specific responses [4]. Specific

responses require sustained expression of several genes involved in processes specific to individual stresses [3,4]. Transcriptome studies are proving to be useful for identifying novel *cis*-elements governing the expression of a group of genes under specific stress conditions, as evidenced in the identification of discreet regulons containing high-occurrence *cis*-elements in temperature-stress-inducible genes [15].

Membranes: a target of temperature extremes

Membrane fluidity is largely dictated by the composition of lipid molecular species, the degree of membrane saturation and temperature environments. Temperature-induced change in membrane fluidity is one of the immediate consequences in plants during temperature

stresses and might represent a potential site of perception and/or injury [16,17]. The importance of proper membrane fluidity in temperature tolerance has been delineated by mutation analysis, transgenic and physiological studies. For example, a soybean mutant deficient in fatty acid unsaturation showed strong tolerance to high temperature [18]. Also, the thylakoid membranes of two *Arabidopsis* mutants deficient in fatty acid unsaturation (*fad5* and *fad6*) showed increased stability to high temperature [19] and increased lipid saturation in tobacco caused by silencing a ω -3 desaturase gene also rendered the plants more tolerant to high temperature [20]. Yet at low temperature, greater membrane lipid unsaturation appears to be crucial for optimum membrane function. An *Arabidopsis fab1* (fatty acid biosynthesis) mutant with more saturated membranes showed decreased quantum efficiency of photosystem II (PSII), chlorophyll content and the amount of chloroplast glycerolipids after prolonged exposure to low temperature [21]. A triple mutant (*fad3-2 fad7-2 fad8*; fatty acid desaturation) devoid of trienoic fatty acids (18:3 or 16:3) produced a phenotype similar to *fab1* when plants were subjected to prolonged low temperature exposure [22]. Similarly, *fad5* and *fad6* mutants with more saturated membranes became chlorotic and showed growth retardation during low temperature incubation [23]. In addition to membrane unsaturation, it appears that lipid asymmetry in the membrane also contributes to membrane physical structure at low temperature [24]. When overexpressed in yeast, ALA1 (aminophospholipid ATPase1), a putative aminophospholipid translocase in *Arabidopsis*, restored phosphatidylserine internalization from the outer leaflet of the plasma membrane. This is supported by the finding that internalization of phosphatidylserine was tightly linked to the rescue of a cold sensitivity phenotype of the yeast *drs2* mutant [24].

More-extreme low temperature conditions resulting in freezing injury also target membranes. Freezing-induced dehydration destabilizes plasma membrane resulting in the formation of inverted hexagonal phase membrane structure [25]. In a series of elegant studies, Peter Steponkus and colleagues have shown that the formation of the inverted hexagonal phase membrane structure can be prevented by the cold acclimation process, and in part by overexpressing *Cor15a* [26,27]. During cold acclimation, the membrane lipid molecular species composition undergoes rather complex changes that help to enhance membrane cryo-tolerance and stability [27,28].

Temperature sensing

The identity of a plant thermometer has not been established, but findings with microbial and mammalian cells suggest some possible targets. Modifying membrane fluidity can influence gene expression. Membrane fluidization of heat-acclimated *Synechocystis* by benzyl alcohol lowered the threshold temperature for the induction of heat-shock-responsive genes [16]. By contrast, membrane rigidification by DMSO at 25°C induced a cold-acclimation marker gene and development of freezing tolerance [17]. Such studies indicate that membrane fluidity plays a central role in sensing both high and low temperature.

Rigidification of thylakoid membrane, but not the plasma membrane, appears to invoke altered expression profiles of heat-shock genes suggesting that the temperature-sensing mechanism could reside in the thylakoid membrane [16]. The prospect of the thylakoid membrane acting as a heat sensor is physiologically pertinent because it is susceptible to a temperature up-shift owing to its highly unsaturated character and the presence of photosystems that are fragile to temperature changes.

Recently, two groups of possible cold sensors have been identified. They are a membrane-associated histidine kinase (Hik33) [29] and TRP (for transient receptor potential) channel proteins [30,31]. By systematic disruption of all possible genes for histidine kinases and random mutagenesis of nearly all other genes in the genome of *Synechocystis* sp. PCC 6803, HIK33 and HIK19 and a response regulator (RER1) were found to act as components of the pathway for perception and transduction of low-temperature signals. Inactivation, by targeted mutagenesis, of each of these genes prevented the transcriptional activation of several cold-inducible genes. HIK33 contains putative membrane-spanning domains and, hence, was proposed as a membrane-associated cold sensor that transduces a cold-elicited signal to a down-stream transducer, possibly HIK19 [29]. However, HIK33 seems only able to transduce a cold signal to a subset of cold-regulated genes represented by *desB*, but not to those represented by *desD* and *desA*, suggesting the presence of other cold sensors [29] or signal pathways.

A member of the TRP family of ion channels was recently identified in rat (CMR1) and mouse (TRPM8) as cold sensors of the mammalian peripheral nervous system [30,31]. CMR1/TRPM8 is activated by menthol, a long-known elicitor of the 'cool sensation', and by cold treatment. Upon activation, CMR1/TRPM8 rapidly develops membrane currents by opening non-selective monovalent cation channels highly permeable for calcium ions [30,31]. This is intriguing because plants also show rapid cold-induced cytosolic Ca^{2+} influx that mainly comes from extracellular calcium ions entering through calcium channels [32]. One impasse in making a connection between CMR1/TRPM8 and a corresponding homolog in plants is the threshold temperature for activation of CMR1/TRPM8 (22°C to 26°C). Plants are poikilothermic and are constantly exposed to a broad range of temperature changes. Thus, it is unlikely that they would have narrow threshold temperature limits for cold sensing like that of CMR1/TRPM8. Instead, plants sense quantitative changes in temperature such as rate of cooling rather than an absolute temperature [33]. A plant homolog of CMR1/TRPM8 therefore could possess a different activation mechanism and might have little sequence conservation with CMR1/TRPM8. Sequence-based searches have identified possible homologs of CMR1/TRPM8 in human, fruit fly and nematode, but none in plants. Whether menthol treatment elicits a similar response in plants as that elicited in the mammalian nerve system has yet to be tested. However, if it does, one could isolate a mutant(s) lacking menthol response to try to discover a plant homolog of CMR1/TRPM8.

Role of calcium in temperature stress signaling

Profiles of calcium influx during temperature stresses are distinct and calcium-mediated signal transduction of temperature stresses involves protein phosphorylation and activation of various transcriptional factors [34]. Cytosolic $[Ca^{2+}]$ sharply rises upon a steep temperature drop [32] or a heat shock [35]. Perturbations of cytosolic Ca^{2+} fluxes can make plants more sensitive to temperature stresses. Reduced cytosolic $[Ca^{2+}]$ owing to enhanced sequestration of calcium into vacuoles by overexpression of *CAX1* (calcium exchanger 1) rendered transgenic plants more chilling susceptible [36]. Similarly, heat-induced oxidative damage was exacerbated in *Arabidopsis* when cytosolic Ca^{2+} signaling is perturbed by calcium channel blockers and calmodulin inhibitors [35]. One intriguing aspect of cytosolic Ca^{2+} flux is that the kinetics of cytosolic Ca^{2+} flux are different under heat and cold shock. Cytosolic Ca^{2+} rise takes place within minutes after cold shock whereas it is initiated in the recovery phase after heat shock [32,33].

Cytosolic $[Ca^{2+}]$ also seems to be linked to the acquisition of tolerance to temperature stresses. Mild heat treatment that elicits the development of acquired thermotolerance fortifies the cytosolic Ca^{2+} rise after heat stress [35]. Similarly, incubation of plants at low temperature that promotes cold acclimation enhances the second peak of bi-modal cytosolic Ca^{2+} rise that is manifested by cold shock [37].

Cytosolic Ca^{2+} might transduce temperature-induced signals to mitogen-activated protein kinases (MAPK). MAPK cascades are an important part of signal transduction pathways in plants and thought to function ubiquitously in many responses to external signals. In plants, MAPKs are activated by hormone treatment, pathogens, cold, drought and salt stress [38–40]. Recently, a MAPK that was activated by heat shock (HAMK) [40] was identified, and was found to be different from the cold-activated MAPK (SAMK) [41]. Interestingly, activation of HAMK and SAMK were triggered by apparent opposite changes in membrane fluidity coupled with cytoskeletal remodeling, Ca^{2+} influx and the action of Ca^{2+} -dependent protein kinases (CDPK) [40]. Through these studies, calcium-mediated pathways of signal perception, transduction and plant responses have emerged. However, large gaps are still remaining and immediate attention is required to identify components that relay calcium signals to MAPK cascades and transcription factors downstream of CDPK.

Transcriptional regulation by temperature

High temperature

Induction of many heat-inducible genes is attributed to the conserved heat-shock element (HSE) in the promoter. HSE consists of alternating units of pentameric nucleotides (5'-nGAAn-3') that serve as the binding site for heat-shock factor (HSF). Efficient HSF binding requires at least three alternating units (5'-nGAAnnTTCnnGAAn-3') [42]. In spite of their conserved transactivation function upon heat shock, HSFs show differences in induction threshold [43] and regulation of the heat response [44], which could

provide diverse induction profiles for target genes under various stress conditions.

Plant HSFs are distinct compared with HSFs from other organisms. Regulation of plant HSF activity involves DNA binding and nuclear localization, but not a heat-inducible negative regulatory domain interaction analogous to human hHSF1 [45]. The multiplicity and diversity of HSF in plants is much greater than in other organisms [46]. For example, *Arabidopsis* has 21 HSF genes belonging to three major classes; HsfA, HsfB and HsfC based on structural differences [46]. HsfAs appear to be the major factor(s) responsible for heat-induced activation of heat-shock genes. HsfBs apparently lack the heat-inducible transactivation function in spite of having normal DNA binding function, and might act as co-activators of transcription with HsfAs [45]. In spite of extensive studies on HSFs, no immediate upstream factors to HSF in heat signal transduction have been identified. Several HSF genes are heat inducible, indicating the presence of transcriptional activators for HSF genes. Whether they are HSFs themselves or other novel transcriptional factors awaits further investigation.

Low temperature

Induction of many, but not all, cold responsive genes is mediated by CBF/DREB [47]. CBF/DREB transcription factors belong to a small gene family in *Arabidopsis* consisting of three sub-groups [48–52]. Of the three, the group of CBF/DREB1 members was specifically induced by cold. By contrast, DREB2A was induced by drought, NaCl and abscisic acid, but not by cold [52]. Expression of CBF3/DREB1A was followed by the induction of RD29A/COR78 gene, whereas expression of DREB2A did not precede the induction of RD29A/COR78 gene upon cold treatment [52]. This suggests that the synthesis of CBF3/DREB1A is necessary for the induction of RD29A/COR78, whereas modification of DREB2A rather than the direct synthesis of the protein is required [52]. The phenotype of the *sfr6* (sensitive to freezing) mutant suggests that SFR6 is involved in such a modification process. In *sfr6* mutant plants, induction of COR (cold regulated) genes possessing CRT/DRE elements was significantly reduced, whereas the expression of CBF genes remained unchanged, suggesting SFR6 protein might augment transactivation of COR genes by modifying CBFs [53]. In contrast to *sfr6*, the *hos1* (high expression of osmotically responsive genes) mutant showed enhanced induction of CBF2, CBF3 and downstream cold-responsive genes upon low-temperature exposure, indicating HOS1 might be a negative regulator of CBF2 and CBF3 [54].

Induction of the CBF family by low temperature has prompted the search for the presence of an earlier signal transducer than CBF, namely ICE (inducer of CBF expression) [49]. The presence of multiple motifs related to MYB or MYC recognition in the promoter regions of CBF genes [51] and high relative occurrence of G-box and ABRE-like *cis*-elements in a cold-inducible cluster of genes [15] suggest that proteins interacting with these *cis*-elements might be involved in low-temperature signal cascade.

Using bioluminescence imaging-aided genetic screening, Jian-Kang Zhu and colleagues have identified numerous mutants involved in low-temperature transcriptional regulation. Of these, FIERY2 and LOS2 provided information on new aspects of low-temperature transcriptional regulation [55,56]. FRY2 encodes a novel transcriptional repressor with two dsRNA-binding domains and a domain similar to RNA polymerase II-CTD phosphatase. Mutation in this locus resulted in significant increases in DRE/CRT-containing genes. Regulation of DRE/CRT-containing genes by FRY2 appeared to be through repression of stress induction upstream of the CBF/DREB transcription factor genes. By contrast, mutation in LOS2 locus resulted in repression of cold-responsive genes such as COR, KIN, RD and LTI genes [56]. The binding of LOS2 to the promoter of a transcriptional repressor gene (STZ/ZAT10) and consequent repression of STZ/ZAT10, confirm that LOS2 acts as a positive regulator of cold-responsive genes [56]. Intriguingly, LOS2 encodes an enolase in the glycolytic pathway. Whether perturbations of metabolic flux in the glycolytic pathway by cold resulted in increase of LOS2 activity awaits further investigation. However, this study identified a novel mode of gene regulation that is linked to the metabolic pathway.

Future prospects

Applications of genomic approaches and gene knockout strategies are beginning to accelerate efforts to assess systematically and understand complex quantitative traits such as acquired tolerance to temperature extremes. Major advances have already been made in identifying many components of stress-signal transduction pathways using mutant analysis, and with genomic methods such as expression profiling, a better picture of signal transduction networks and cross-talk should rapidly emerge (Box 1). A major question that needs to be addressed is whether sensing is through a primary thermostat or if it results from temperature sensing that is a function of a network of cellular signals that arise from within as a physical and/or chemical consequence of less optimal temperature conditions on cell biochemistry and physiology. Although

Box 1. Future challenges to understanding acquired tolerance to temperature extremes

- Identify primary temperature sensor(s) or thermostat(s) and/or an array of sensing signals leading to an interlaced network.
- Better understand the role of signal transduction pathway cross-talk.
- Identify *cis*-elements and all transcription factors that regulate temperature stress responses.
- Better understand post-transcriptional and post-translational regulatory mechanisms in stress responses.
- Identify the genes and mechanisms of general stress processes as well as stress-specific responses.
- Identify the function of genes whose function is presently unknown and determine role in tolerance.
- Understand nature of cross-protection.
- Identify all molecular and macromolecular targets of injury.
- Integrate disparate injury mechanisms and stress responses into a unifying perspective of tolerance.

transcriptome studies will be great for gene-discovery purposes, transcriptional control mechanisms are likely to provide only part of the story. Significant regulatory control of gene expression resides at post-transcriptional levels of RNA stability, translatability, protein turnover and catalytic activation–deactivation processes and awaits further studies. Out of the current gene-discovery phase, much future effort needs to be devoted to identifying exactly what the purpose is of the 20–40% of genes of unknown function in stress tolerance. One example of how important this will be is the recent discovery that a maize dehydrin (Dnh1) can physically interact and bind with unilamellar vesicles and undergo secondary structure changes that would be consistent with a role in membrane structure stabilization [57]. Ultimately, detailed molecular, biochemical and physiological information will be necessary for perhaps hundreds of genes and their products for a clear understanding of the basis of acquired tolerance to temperature extremes.

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