Review

How do plants feel the heat?

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In plants, the heat stress response (HSR) is highly conserved and involves multiple pathways, regulatory networks and cellular compartments. At least four putative sensors have recently been proposed to trigger the HSR. They include a plasma membrane channel that initiates an inward calcium flux, a histone sensor in the nucleus, and two unfolded protein sensors in the endoplasmic reticulum and the cytosol. Each of these putative sensors is thought to activate a similar set of HSR genes leading to enhanced thermotolerance, but the relationship between the different pathways and their hierarchical order is unclear. In this review, we explore the possible involvement of different thermosensors in the plant response to warming and heat stress.

Why is it important to study heat stress in plants?

Temperature is one of the key physical parameters affecting life on Earth. As a result, almost all living organisms have evolved signaling pathways to sense mild changes in ambient temperature and adjust their metabolism and cell function to prevent heat-related damage. Indeed, many features of the heat stress response (HSR) pathway are conserved among both prokaryotic and eukaryotic organisms [1,2]. The HSR pathway has been extensively studied in plants [3–8], but many questions remain unanswered.

Heat stress can have an adverse impact on almost all aspects of plant development, growth, reproduction and yield [9–14]. Because plants are sessile organisms that cannot escape heat, they are forced to invest valuable resources in modifying their metabolism to prevent damage caused by heat, in a process generally referred to as acclimation. Alternatively, plants can activate programmed cell death in specific cells or tissues, a process that can lead to the shedding of leaves, the abortion of flower or fruit formation, or even death of the entire plant [15–20] (Figure 1). Testimony to the severity of heat stress effects on plant growth and reproduction lies in the 1980 and 1988 US heat waves, which resulted in overall damages to agricultural production estimated at 55 and 71 billion dollars, respectively, and had a devastating economical and sociological impact [9,11,14]. Over the past three decades (1980–2008), heat stress has caused a decrease of 5.5% and 3.8% in the world yields of wheat and maize, respectively [14]. Although all plant tissues are susceptible to heat stress, the reproductive tissues are especially sensitive to heat waves, and a few degrees elevation in temperature during flowering time can result in the loss of entire grain crop cycles [9,21]. Therefore, dissecting the HSR of plants and identifying key components of the heat stress sensing and signal transduction pathway are a high priority and could result in the development of plants and crops with enhanced tolerance to heat stress [9]. The development of plants with enhanced heat tolerance is also crucial in light of the current and anticipated climate changes, which are predicted to cause a gradual increase in ambient temperature and to enhance the frequency and amplitude of heat episodes [9,10].

How does heat stress affect plants?

Heat stress differentially affects the stability of various proteins, membranes, RNA species and cytoskeleton structures, and alters the efficiency of enzymatic reactions in the cell, causing a state of metabolic imbalance [22–24]. Because most cellular reactions are coupled, disrupting the steady-state flux of metabolites can cause the accumulation of toxic by-products, such as reactive oxygen species (ROS). Indeed, an intimate relationship exists between oxidative stress and the HSR in many organisms [22–27]. To counter the effects of heat stress on cellular metabolism, plants and other organisms respond to changes in their ambient temperature by reprogramming their transcriptome, proteome, metabolome and lipidome; that is, by altering their composition of certain transcripts, proteins, metabolites and lipids (Figure 1). Such changes are aimed at establishing a new steady-state balance of metabolic processes that can enable the organism to

Glossary

Brassinosteroids: a group of plant steroid hormones that regulate growth, development and responses to different environmental stresses.
bZIP: a family of TFs that contain a basic leucine zipper (bZIP) domain and regulate many central developmental and physiological processes in plants, such as photomorphogenesis, energy homeostasis, and abiotic and biotic stress responses.
Calmodulin: a calcium-binding protein family that can bind to and regulate several different protein targets in plants.
DREB: dehydration-responsive element-binding TF is a key regulator of drought and HSRs in plants.
IRE1: a transmembrane serine/threonine-protein kinase/endoribonuclease that transmits the UPR signal across the ER or nuclear membranes.
MBF1c: a transcriptional regulator required for basal thermotolerance that regulates ethylene, salicylic acid and trehalose responses during heat stress.
Phospholipase: an enzyme that hydrolyzes phospholipids into fatty acids and other lipophilic substances and is involved in lipid and calcium signaling in plants.
SWR1: an ATP-dependent multisubunit protein complex involved in chromatin remodeling, histone replacement and gene expression regulation in eukaryotic organisms.
WRKY39: a TF containing the WRKY domain (defined by the conserved amino acid sequence WRKYGQK at its N-terminal end). WRKY39 is involved in the response of plants to heat and other environmental stresses.

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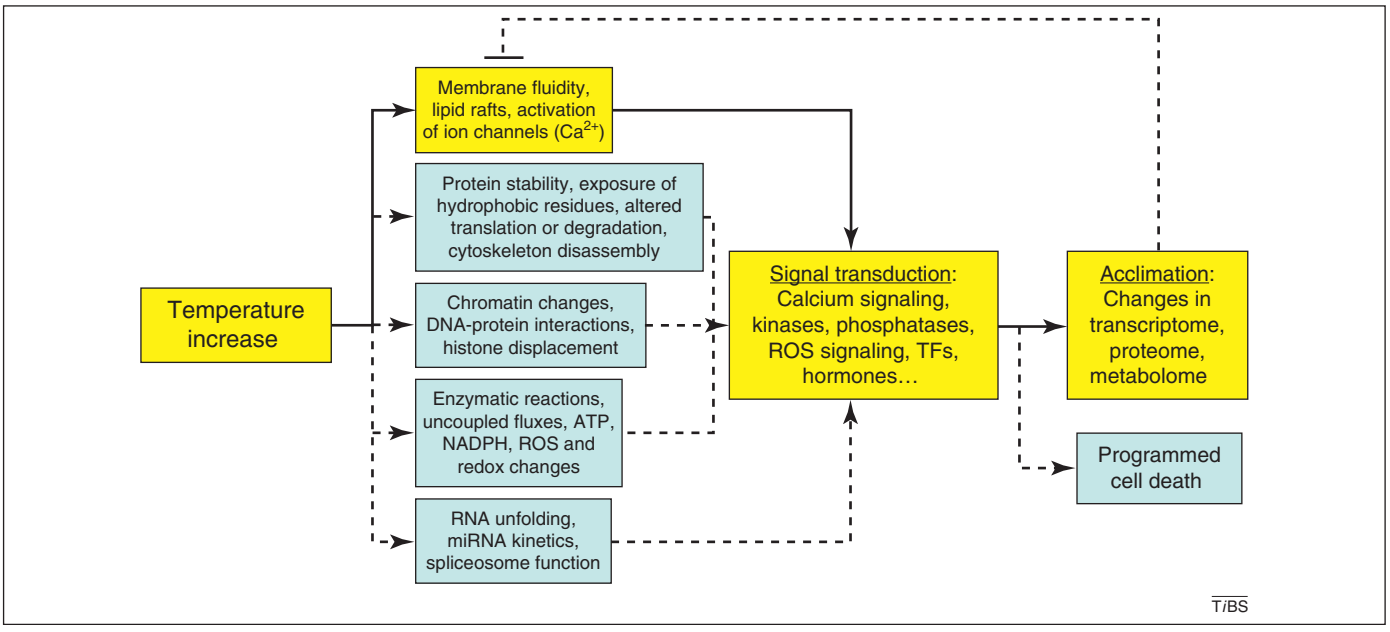

Figure 1. A schematic model for temperature sensing in plants. Increases in ambient temperature affect many different processes. Mild changes in temperature can alter membrane properties and activate a calcium channel. The inward flux of calcium that follows is thought to activate signal transduction events and alter plant metabolism in a process called acclimation. This pathway (highlighted in yellow and linked by solid arrows) may serve as the primary heat sensing mechanism of plants. Additional temperature-induced changes include alterations in protein stability and exposure of hydrophobic residues of proteins that may trigger the UPR sensors in the cytosol and the ER, histone eviction in the nucleus [which clears the way for TF binding to HSR genes (nuclear sensor)], accumulation of ROS and alteration in cellular energy levels, and unfolding of RNA species that could act as riboswitches or affect spliceosome and microRNAs function. These pathways, (indicated in light turquoise,) are thought to trigger different signal transduction events and contribute to plant heat acclimation. An alternative to acclimation is the activation of programmed cell death, which could be triggered based on a compilation of different signal transduction pathways and conditions. The activation of calcium signaling by the PM channel is thought to allow the triggering of other sensor-derived pathways via the signal transduction box.

function, survive and even reproduce at a higher temperature. Of course, a reverse process of deprogramming is required when the temperature shifts back to normal levels. Although members of the main families of molecular chaperones, such as the heat shock proteins (HSPs) HSP100s, HSP90s, HSP70s, HSP60s, HSP40s and the small HSPs (sHSPs), play a key role in mitigating the effects of heat stress on plant metabolism [28–31], the majority of transcripts upregulated in cells in response to heat do not encode molecular chaperones (Box 1). In fact, the plant HSR is thought to be mediated by multiple pathways, which are orchestrated by a selected group of regulatory proteins, and is triggered by one or more temperature sensors (Figure 1).

What types of heat response exist in plants?

Various treatments have been used to study heat response in plants, the most common of which is to subject plants growing under controlled conditions to an episode of severe heat stress. In *Arabidopsis thaliana*, for example, this entails subjecting plants growing at 21 °C to an abrupt 42–45 °C treatment for a period of 0.5–1 h. The ability

Box 1. What are the components of the HSR in plants?

About 5% of the plant transcriptome is upregulated twofold or more in response to heat stress [3,33,77,80]. Only a fraction of these transcripts encode heat-induced chaperones: 88 out of 1780 in *A. thaliana*, and 117 out of 1509 in wheat [33,80]. The rest of the transcripts encode products involved in calcium signaling, protein phosphorylation, phytohormone signaling, sugar and lipid signaling and metabolism, RNA metabolism, translation, primary and secondary metabolisms, transcription regulation and responses to different biotic and abiotic stresses.

Heat-induced chaperones, although often massively induced by heat stress, are therefore only a minor component of the overall HSR of plants. Nevertheless, mutants lacking different heat-induced chaperones display reduced survival in response to heat stress [28–31]. Heat-induced chaperones belong to the larger family of molecular chaperones that are either constitutively expressed in non-stressed cells or induced at particular developmental stages, or more commonly by different environmental challenges. Under physiological conditions they control cellular signaling, protein folding, translocation and degradation, whereas under heat stress they prevent protein misfolding and aggregation and may protect membranes [81]. One of the regulatory networks that control the expression of chaperones is the HSF network, with 21 members in *A. thaliana* [7]. The regulation can be achieved by a single 'master switch' HSF (e.g. HSFA1 in tomato [82]), or by the collective function of several HSFs (e.g. HSFA1a to HSFA1e in *A. thaliana* [35]). HSF activity can be regulated at the transcriptional, post-transcriptional and post-translational levels, including the dissociation of inhibitory chaperones, various degrees of phosphorylation and other post-translational modifications such as sumoylation. These post-translational modifications can cause HSFs to oligomerize [78], translocate to the nucleus, bind to the promoters of HSR genes [83] and recruit histone acetyltransferase HAC1 [7], thereby indirectly contributing to the decreased occupancy of HSR gene promoters by inhibitory histones.

The importance of HSR pathways outside the chaperone network has been demonstrated in mutants lacking the transcriptional factor MBF1c, in which the ethylene, salicylic acid and trehalose signaling pathways were affected [32,60]. The HSR therefore includes multiple pathways that may be redundant, depending upon the presence of an acclimation period, different environmental conditions and/or the developmental stage of the plant.

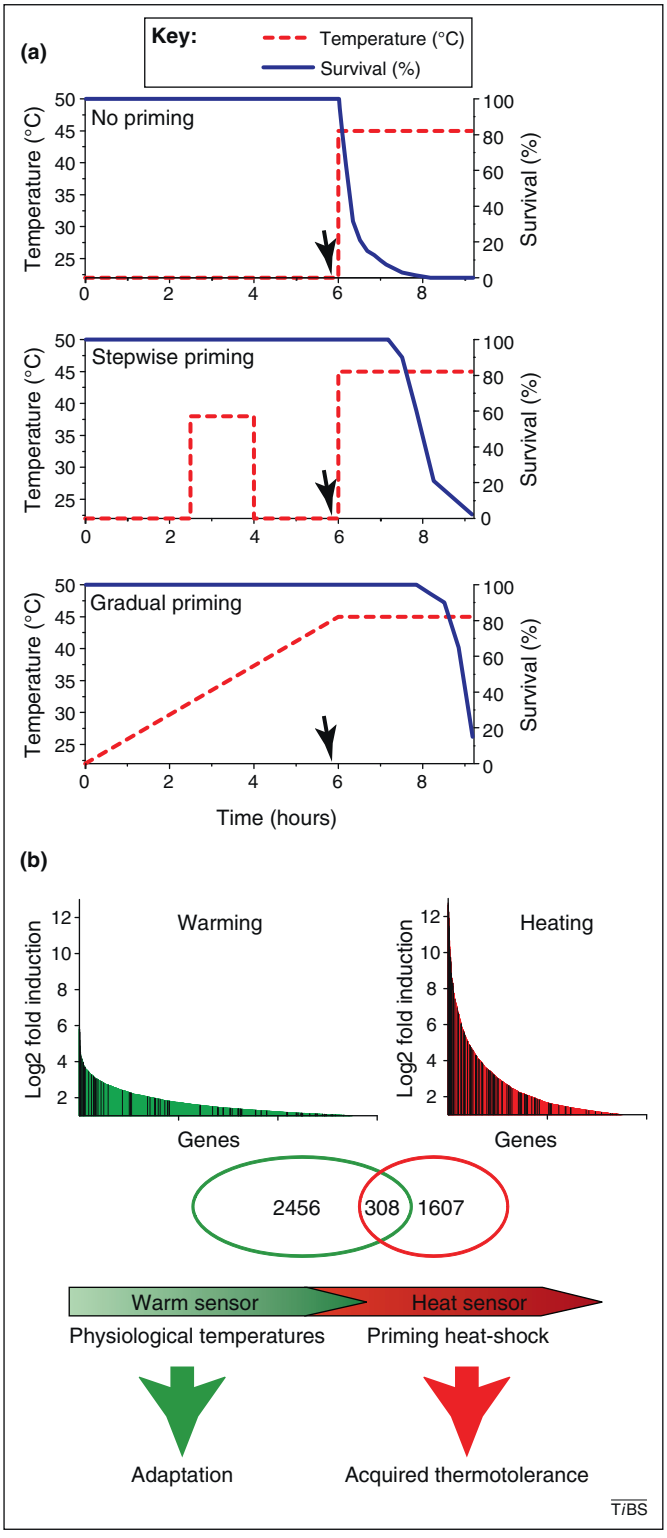

Figure 2. Different warming and heating pretreatments determine the effectiveness of plant thermo-adaptation and acquired thermotolerance. **(a)** Plant survival to a noxious heat stress correlates with a massive upregulation of chaperones and ROS response genes during a prestress heat-priming treatment. A schematic representation (redrawn from [3]) is shown of treatments designed to measure either basal thermotolerance (no priming), acquired thermotolerance (stepwise priming) or acquired thermotolerance by way of a gradual increase in temperature (gradual priming). Red lines are the temperature regimes. Blue lines are the percentage survival of the *Arabidopsis* seedlings measured 5 days after the heat treatments. Arrows indicate the time point before the noxious 45 °C heat stress at which mRNA was collected for microarray analysis. The online Supplementary Table S1 presents a list of the most upregulated transcripts following a stepwise or gradual heat priming, compared to no priming, at $T = 6$ h (indicated by an arrow in panel a). **(b)** *Top*: distribution and fold-expression level of transcripts that accumulate in response to warming (*left*) or heating (*right*) in *A. thaliana*. Microarray probes corresponding to bioinformatically predicted chaperones [77] are indicated in black. Microarray data for warming and heat treatments were extracted from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) under accession numbers GSE18624 [36] and GSE16222 [78], respectively. To obtain transcript fold change for warming, the mean of the absolute units corresponding to the two technical replicates obtained at 27 °C was divided by the mean of the absolute units from two technical replicates at 12 °C. The logarithms of obtained quotients (*y* axis) were plotted against the corresponding genes (*x* axis). Similarly, for the heat transcriptome profile, the mean of the absolute units corresponding to the two technical replicates obtained at 38 °C was divided by the mean of the absolute units from two technical replicates at 23 °C. The logarithms of the obtained quotients (*y* axis) were plotted against corresponding genes (*x* axis) using MS Excel. To obtain black labels for the chaperones in the graph, only the values for upregulated 'chaperome' transcripts were added as separate column in MS Excel and plotted as an overlay. *Middle*: a Venn diagram showing the degree of overlap between warming, left [36] and heat response transcripts, right [79] that are elevated twofold or more in *A. thaliana*. *Bottom*: a schematic model proposing the existence of two independent sensors, one for warming that would work within physiologically relevant temperatures, and one for heat stress that would be involved in acquired thermotolerance.

of plants to respond and successfully acclimate to such treatment is generally referred to as basal thermotolerance, and is assayed by measuring plant survival 5–7 days following the severe heat stress episode (Figure 2a) [3,32]. The second type of treatment involves subjecting plants to a moderate level of heat stress (called 'priming'), letting them recover for a few hours, and then subjecting them to an abrupt episode of severe heat stress. In *A. thaliana*, such treatment involves subjecting plants growing at 21 °C to a 36–38 °C treatment for a period of 1.5 h, letting them recover at 21 °C for 2 h and then subjecting them to increasingly longer periods of severe heat stress (45 °C), as described above (Figure 2a) [3,33]. The ability of plants to survive such treatment is generally referred to as acquired thermotolerance. Priming for acquired thermotolerance can also be induced in *A. thaliana* by a slow and continuous rise in temperature (Figure 2a) [3]. Such a gradual rise in temperature, which best mimics natural conditions, is much more effective in inducing acquired thermotolerance than the artificial and abrupt treatment described above (Figure 2a and Supplementary Table S1). The successful acclimation of *A. thaliana* seedlings to a noxious heat stress at 45 °C correlates with a massive accumulation of certain transcripts during the previous abrupt or gradual priming stages; most of these transcripts encode different molecular chaperones such as the sHSPs and HSP70s, as well as ROS and redox response enzymes, such as ascorbate peroxidase (APX) (Supplementary Table S1). The accumulation of most of these transcripts is much higher during the gradual priming treatment, which translates into a net gain of 1-h survival of the plants following the noxious heat condition (Figure 2a and Supplementary Table S1).

Certain regulatory and acclimation proteins, such as the transcriptional regulator MBF1c (see Glossary) or the ROS detoxifying enzyme catalase, are required for basal thermotolerance but not for acquired thermotolerance [32,34]. By contrast, some heat shock transcription factors (HSFs), as well as the disaggregating chaperone HSP101, seem to be required for both responses [28,35]. Differences between acquired and basal thermotolerance were revealed in a study in wheat that identified 1314 transcripts differentially expressed between heat treatments with or without preacclimation [33].

A different type of heat treatment should be referred to as mere 'warming' (Figure 2b) [36]. In this treatment, plants are kept at a temperature that is lower than the normal growth temperature (e.g. 12 °C instead of 21 °C for *A. thaliana*) and are then transferred to a physiological temperature that is slightly above their normal growth temperature (27 °C instead of 21 °C). Because 27 °C is not a damaging heat stress for most plants and does not trigger the expression of many HSR markers [36], this treatment cannot be considered a heat stress treatment. As shown in Figure 2b, the transcriptome response of *A. thaliana* to warming is remarkably different from that of a simple heat stress treatment (e.g. heating from 22 °C to 37 °C), suggesting that different heat sensors, as well as distinct signaling pathways, are involved in the activation of these two plant responses to a temperature rise. In contrast to HSR priming, which leads to a relatively short-term acclimation and acquired thermotolerance [3], warming seems to result in longer-term adaptation of plants to warmer growth conditions and can include developmental reprogramming, such as shedding leaves or early flowering [36]. The differences in transcriptome footprints between warming and heat stress (Figure 2b), as well as the differences in protein requirements and transcriptome expression between basal and acquired thermotolerance [32,33], strongly suggest that different pathways and, importantly, different thermosensors are involved in triggering the different thermal responses in plants.

How do plants sense heat?

When an *A. thaliana* leaf is exposed to a rise in ambient temperature, its large surface-to-volume ratio ensures that almost all macromolecules in the cells, such as protein complexes, membranes and nucleic acid polymers, 'perceive' the heat simultaneously. The increased kinetic movement of these macromolecules is expected to concomitantly cause reversible physical changes, such as increased membrane fluidity, partial melting of DNA and RNA strands, protein subunit dissociation and exposure of protein hydrophobic cores (Figure 1). Therefore, all macromolecules might, in principle, serve as thermosensors by providing an output in the form, for example, of a transient loss of function [4]. Yet, to properly answer the question 'How do plants feel the heat?', we need to identify which among the many heat-responsive macromolecules in the plant cell acts as a primary heat sensor. This sensor must be able to not only precisely perceive and differentially react to various temperature increments but also differentially trigger a unique signaling pathway that can specifically upregulate hundreds of HSR genes (Figures 1 and 2, and Supplementary Table S1).

The sensing of heat stress in plants could be mediated by different routes (Figure 1). The specific sensor molecule could be directly affected by heat, for example due to temperature-induced changes in its quaternary and tertiary structures. Alternatively, the sensor molecule could be indirectly affected by heat due to the effects of heat on other components of the cell. For example, a membrane protein might be affected by heat via temperature-induced changes in membrane fluidity. Heat-related changes in plant metabolism, such as altered metabolic fluxes, accumulation of ROS, release of ATP from cells, or reduced energy levels could also activate the heat sensor molecule. Energy depletion in *A. thaliana* is sensed via a pathway that involves SNF1-related protein kinase (SnRK1) [37]. Sensing of heat stress could also be mediated by the detection of unfolded proteins or RNAs in the cell [4,22,23]. In the classical model for heat stress activation, the presence of heat stress-induced unfolded proteins in the cell causes molecular chaperones to be released from their constitutive inhibitory association with HSF monomers [38] and to bind the unfolded proteins, while the free HSFs subunits trimerize, undergo phosphorylation, bind to HSR promoters and activate the HSR [7]. Although this model may function in certain cells or organisms, heat stress sensing in plants, especially at temperatures that do not unfold proteins, is much more complicated and could involve multiple pathways present in different compartments of the cell. In the next section, we summarize some of the new findings as they pertain to the sensing of heat in plants.

Sensing heat at the plasma membrane

The primary sensing event of heat stress in the moss *Physcomitrella patens* occurs at the plasma membrane (PM) [39,40]. A combination of electrophysiology, reporter gene assays and biochemical measurements [39] revealed that mild increases in temperature are sensed at the PM, lead to the opening of a specific calcium channel that triggers an influx of calcium into the cell, and activate the HSR. The suppression of this pathway by calcium channel blockers or chelators indicates that the calcium channel that triggers the heat-induced calcium influx serves as one of the primary heat sensors of plants (Figure 1). Because membrane properties are altered by heat stress and can adapt to different growth temperatures [41–44], and because chemicals that fluidize the membrane can induce the PM calcium-dependent HSR in the absence of a change in temperature [39,40], the membrane calcium channel is probably activated by a temperature-induced increase in fluidity of the PM [39]. Although the identity of the PM heat stress sensor in plants is unknown, studies in animal cells have indicated that some ion channels – such as stromal interaction molecule (STIM), members of the transient receptor potential cation channel subfamily V (TRPV) and cyclic nucleotide-gated channels (CNGCs) – might function as temperature sensors [45–48]. The *A. thaliana* genome encodes over 40 putative calcium channels, many of which are probably located in the PM and might serve as heat sensors [49]. Interestingly, many candidate channels display a cytosolic C-terminus with a putative calmodulin-binding domain, raising the possibility that a specific calmodulin may be involved in the ensuing steps of the heat stress signaling pathway. The suppression of HSR pathways in higher plants by calcium channel blockers and chelators is an additional indication that PM calcium signaling plays a key role in heat stress signaling [50–52].

A heat stress-induced inward calcium flux can regulate multiple signaling pathways in plants (Figure 3) [53]. In *A. thaliana*, the calmodulin AtCaM3 is required for heat stress signaling [54,55] and may be involved in the activation of different transcription factors (TFs) such as

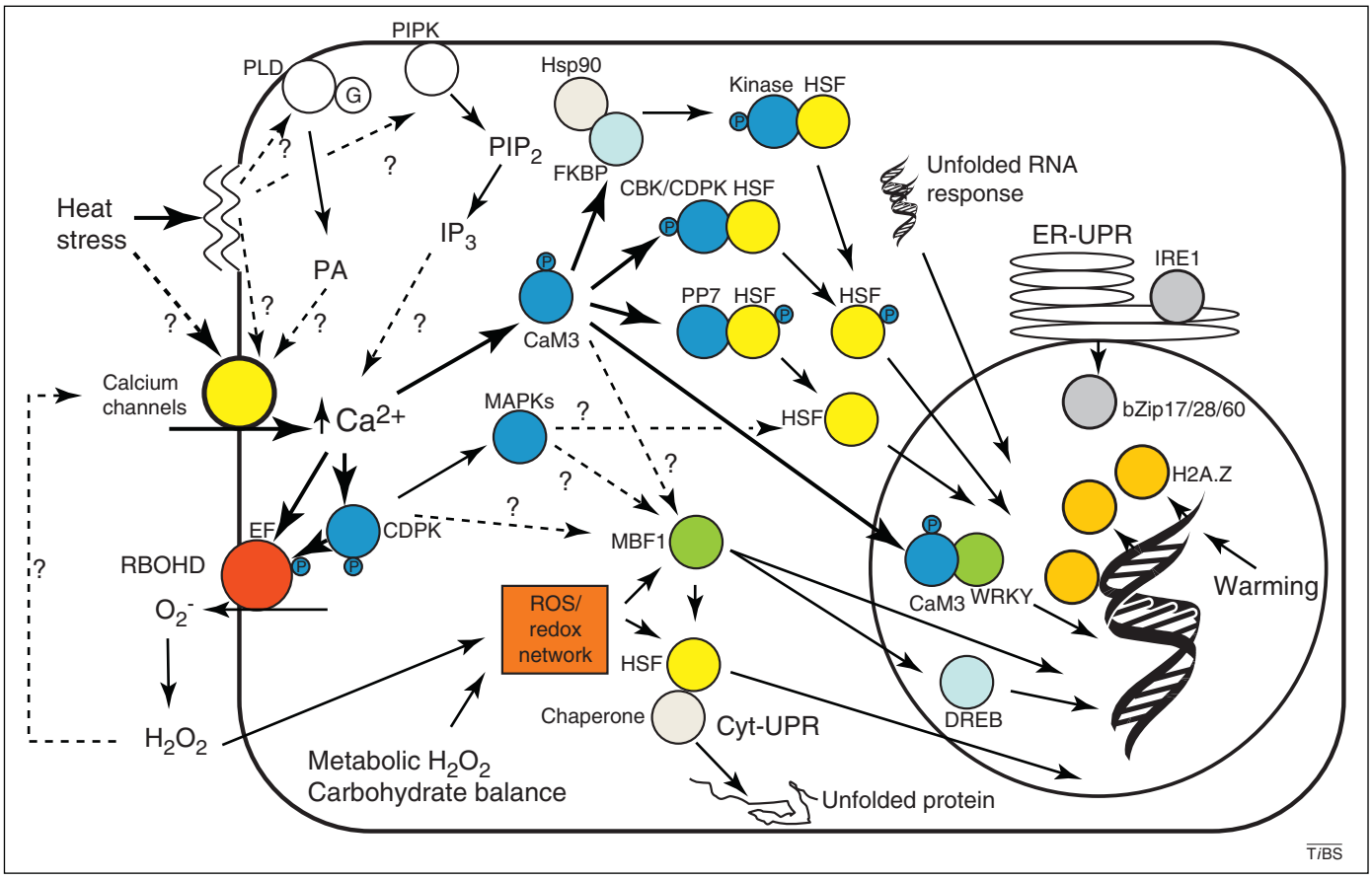

Figure 3. Signal transduction pathways activated in response to heat stress in plants. Heat stress affects membrane stability and activates a PM calcium channel, resulting in an inward flux of calcium (*left*). Changes in membrane stability could also trigger lipid signaling through the lipid-modifying enzymes PLD and PIPK. Calcium binds the calmodulin CaM3 and activates multiple kinases as well as transcriptional regulators of the HSR, such as HSFs, MBF1c, WRKY and DREB. Increased levels of cytosolic calcium also activate the ROS-producing enzyme RBOHD, located at the PM, by direct interaction or through activation of a CDPK that phosphorylates RBOHD (*lower left*). RBOHD-derived ROS [i.e. superoxide (O_2^-), which is converted to hydrogen peroxide H_2O_2] can cause membrane depolarization, as well as enter cells and trigger the ROS/redox signaling network, which would in turn activate MBF1c and HSFs. ROS may also accumulate in cells during heat stress because of metabolic imbalances, and trigger the same network. Two UPR pathways are shown in the model, one in the cytosol (Cyt-UPR) and one in the ER (ER-UPR). The Cyt-UPR functions through HSFs whereas the ER-UPR functions through the sensor molecule IRE1 and the transcription factors bZip17, bZIP28 and bZIP60. In the nucleus, warming decreases H2A.Z histone occupancy at HSP promoters (*right*).

WRKY39 [56] and HSFs [35]. In addition, an inward flux of calcium activates several calcium-dependent protein kinases (CDPKs), which can, in turn, activate multiple mitogen-activated protein kinases (MAPKs) [57] or the ROS-producing enzyme NADPH oxidase [58]. AtCaM3 can also activate calcium/calmodulin-binding protein kinase (CBK), which phosphorylates HSFA1a, a member of the HSF family [55], whereas the phosphatase PP7 dephosphorylates HSFA1a [53]. An HSP90/FKBP-dependent kinase (ROF1, also known as FKBP62) can also mediate HSF phosphorylation resulting from calcium binding to calmodulin [59]. Phosphorylation of the key transcriptional regulator of basal thermotolerance, MBF1c, which functions upstream to the dehydration-responsive element-binding (DREB) transcriptional activator and certain HSFs, may be a direct or indirect result of CDPK activation [60,61].

In addition to activating PM ion channels, heat-induced changes in membrane fluidity might trigger lipid signaling. Heat stress results in the activation of phospholipase D (PLD) and phosphatidylinositol-4-phosphate 5-kinase (PIPK) and the accumulation of various lipid signaling molecules such as phosphatidic acid, phosphatidylinositol-4,5-bisphosphate (PIP_2) and D-myo-inositol-1,4,5-trisphosphate (IP_3) [62]. Moreover, a reduction of phospholipase C9 activity correlates directly with reduced IP_3 concentration, downregulation of sHSPs, and reduced thermotolerance [63]. The accumulation of lipid signaling molecules could in turn cause the opening of channels and the triggering of a calcium influx. However, the relationship, if any, between the PM channels that are directly or indirectly activated by heat and the channels that are activated by lipid signaling in plants is unknown. In addition, the order of events in the heat stress sensing and signaling response is poorly understood. Some of the signaling pathways that probably function downstream to heat stress sensing at the PM are summarized in Figure 3.

Unfolded protein response (UPR) in the ER and the cytosol

The UPR is a signaling pathway activated in cells in response to stresses that impair protein stability in the endoplasmatic reticulum (ER) [15,64]. In plants, there seem to be at least two UPR pathways, one in the ER and the other in the cytosol [65–67]. The UPR can be activated by heat stress, by chemicals that cause the unfolding of proteins, or by different abiotic stresses such as changes in salinity or osmotic stress [15]. The activation of the ER UPR pathway in plants involves the proteolytic cleavage and release of different bZIP TFs from the ER membrane [66,67]. The displaced TFs enter

the nuclei and activate the transcription of specific genes, which leads to the accumulation of ER chaperone transcripts and the activation of brassinosteroid signaling [67]. An ER chaperone (binding immunoglobulin protein, BiP) and the sensor molecule IRE1 regulate the cleavage of the different bZIP proteins in response to the accumulation of unfolded proteins in the ER [66]. In contrast to the ER UPR, the cytosolic UPR, which is triggered by the presence of unfolded proteins in the cytosol, is primarily regulated by an HSF, HSFA2, which binds to HSF-binding elements in the promoters of HSR genes (Box 1) [65].

The UPR may not be as sensitive as the calcium channel PM response because few unfolded proteins are expected to accumulate at low and mild heat stresses. Moreover, HSR chaperones can accumulate in plant cells under non-denaturing temperatures and in the absence of heat stress [68], and activation of the UPR seems to require specific calcium signals from the PM [39], suggesting that the UPR is not the primary heat sensor in plants.

Metabolic changes and ROS signaling

Because different metabolic pathways probably depend on enzymes with different sensitivities to excessive heat, it has been suggested that heat stress might uncouple some metabolic pathways and cause the accumulation of unwanted by-products, such as ROS, that could act as signals to trigger the HSR [69,70]. Nevertheless, ROS accumulation during heat stress is also an active response that is mediated by specific ROS-producing enzymes [25]. ROS accumulation can be triggered in tobacco cells by chemicals that alter membrane fluidity, and heat stress-induced ROS accumulation can be blocked by an inhibitor of the ROS-producing enzyme NADPH oxidase [25]. Moreover, heat stress survival and heat stress signal transduction require respiratory burst oxidase homolog D (RBOHD), a ROS-generating NADPH oxidase located in the PM [26,71]. The activities of this protein are regulated by phosphorylation via protein kinases such as CDPKs [58], and by direct binding of calcium to certain RBOHD domains that face the cytosol [58] (Figure 3). Therefore, an influx of calcium mediated by calcium channels at the PM could activate RBOHD and result in the accumulation of ROS. The accumulated ROS might in turn activate downstream pathways via MBF1c, certain HSFs, MAPKs and/or SnRKs, and might alter the redox state of the cell [27,69]. Moreover, because ROS accumulation at the PM outer surface can cause membrane depolarization [27], calcium-induced RBOHD activation and ROS accumulation could amplify the HSR by opening additional calcium channels at the PM (Figure 3). RBOHD is required for rapid sensing of heat stress in *A. thaliana*, which suggests that ROS and calcium signaling are highly interlinked in the activation of the HSR [27,72]. In addition, ROS accumulation in cells can trigger programmed cell death [16–20,58] and may explain some aspects of HSR-induced programmed cell death in plants (Figures 1 and 3).

Temperature-induced changes in histone occupancy

A screen of *A. thaliana* mutants impaired in heat sensing identified the gene *ARP6* as involved in mediating responses to temperature change [36]. *ARP6* encodes a subunit of the SWR1 complex, which is necessary for inserting the alternative histone H2A.Z into nucleosomes, instead of H2A, and could be involved in temperature sensing [73–75]. Mutants lacking *ARP6* have a reduced content of H2A.Z bound to their chromosomes. Interestingly, the transcriptome of *arp6* null mutants grown at 12 °C is similar to that of wild-type plants grown at 12 °C and warmed to 27 °C, suggesting that H2A.Z-containing nucleosomes can modulate transcription in a temperature-dependent manner [36]. Accordingly, in wild-type plants, warming induces a dramatic decrease in H2A.Z occupancy in nucleosomes located at the transcription start site of warming-induced genes, a process expected to allow for enhanced transcription of these genes. However, it is unclear whether this mechanism is also responsible for heat sensing during more classical HSR treatments that lead to acquired thermotolerance. In addition, as suggested by the authors [36], the decreased occupancy of H2A.Z at certain HSP promoters in the *arp6* mutant might also result in alteration in the expression or DNA binding of a specific TF or other regulatory proteins, which would trigger the transcriptome response. It would be interesting to examine whether heat still decreases the occupancy of H2A.Z at promoters of HSP-encoding genes during heat stress when PM-controlled calcium entry is suppressed (for example by EGTA).

Concluding remarks

In plants, changes in ambient temperature seem to be sensed via a complex network of molecular sensors located in different cell compartments (Figures 1 and 3). The sensors include a rapid PM sensing mechanism that triggers a specific inward calcium flux, UPR sensors in the ER and the cytosol, and histone decreased occupancy in the nuclei (Figure 3). The signals generated by these different sensors are probably integrated by a signal transduction network that involves calcium fluxes, calmodulin, CDPKs, MAPKs, phosphatases and transcriptional regulators. Known transcriptional regulators include the HSF network (with 21 members in *A. thaliana*) [7], MBF1c [32] and different TFs of the WRKY, DREB and bZIP families [56,66,76] (Figure 3). The activation of the different pathways may be tissue specific and, in particular, may differ between reproductive and vegetative tissues.

Despite recent advances in our understanding of the molecular mechanisms involved in heat stress sensing in plants, many questions remain unanswered (Box 2). Perhaps the most important question is: how are the different heat-sensing pathways integrated? It is unknown if the triggering of one pathway is required for the activation of the others. The finding that blocking the inward calcium flux in the moss *P. patens* results in blockage of the HSR suggests that other heat stress sensors of plants, such as the UPR pathways, may depend on the triggering of the PM pathway [39]. Is calcium signaling at the PM a master sensor for the HSR? According to such a model, the initial sensing of heat stress would occur at the PM, and the calcium signaling events that follow would enable the activation of the UPR and/or the histone decreased occupancy response. It is also possible that additional pathways await discovery.

Box 2. Questions for future research

- What is the identity of the thermoresponsive calcium channels? Do they resemble animal TRPVs and/or CNGCs? Do they assemble into various homo-oligomers and hetero-oligomers to form different warm and heat sensors?
- By which precise mechanism do small temperature increments translate into the transient opening of specific calcium channels?
- What is the mechanism of heat-induced opening of the calcium channel? Is it voltage-gated, ligand-gated or otherwise?
- How does lipid composition and organization at the PM affect the HSR? For example, is the ratio between saturated and non-saturated lipids or the formation of lipid rafts important for the HSR?
- What is the particular contribution of specific calmodulins, phospholipases and kinases to the specificity of the HSR in comparison with the warming signals?
- During heat shock, is chromatin remodeling at HSP promoters a direct response to heat or merely an indirect consequence of the specific binding of heat-activated HSFs?
- How can heat-induced changes in RNA structure translate into a specific HSR signal?
- How are the different heat-sensing pathways integrated in plant cells?
- How is heat stress sensing integrated with the sensing of other stresses?

Because different biotic and abiotic signaling pathways use calcium for signaling [53], it would also be interesting to find out how heat stress sensing is integrated with the sensing of other stresses in plants [9,11] and how it maintains its exquisite specificity. Additional studies using genetic screens and protein structure–function analysis, as well as advanced RNA, protein and lipid labeling and microscopy tools, are needed to resolve some of these and other questions pertaining to the sensing of heat stress in plants (Box 2).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tibs.2011.11.007.

References

1 Richter, K. *et al.* (2010) The heat shock response: life on the verge of death. *J. Mol. Cell* 40, 253–266
2 Meyer, A.S. and Baker, T.A. (2011) Proteolysis in the *Escherichia coli* heat shock response: a player at many levels. *Curr. Opin. Microbiol.* 14, 194–209
3 Larkindale, J. and Vierling, E. (2008) Core genome responses involved in acclimation to high temperature. *Plant Physiol.* 146, 748–761
4 Saidi, Y. *et al.* (2010) Heat perception and signalling in plants: a tortuous path to thermotolerance. *New Phytologist* 190, 556–565
5 Vierling, E. (1991) The roles of heat-shock proteins in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42, 579–620
6 Kotak, S. *et al.* (2007) Complexity of the heat stress response in plants. *Curr. Opin. Plant Biol.* 10, 310–316
7 von Koskull-Döring, P. *et al.* (2007) The diversity of plant heat stress transcription factors. *Trends Plant Sci.* 12, 452–457
8 Lu, X.Y. and Huang, X.L. (2008) Plant miRNAs and abiotic stress responses. *Biochem. Biophys. Res. Commun.* 368, 458–462
9 Mittler, R. and Blumwald, E. (2010) Genetic engineering for modern agriculture: challenges and perspectives. *Ann. Rev. Plant Biol.* 61, 443–462
10 Ahuja, I. *et al.* (2010) Plant molecular stress responses face climate change. *Trends Plant Sci.* 15, 664–674
11 Mittler, R. (2006) Abiotic stress, the field environment and stress combination. *Trends Plant Sci.* 11, 15–19
12 Wheeler, T.R. *et al.* (2000) Temperature variability and the yield of annual crops. *Agr. Ecosys. Environ.* 82, 159–167
13 Tubiello, F.N. *et al.* (2007) Crop and pasture response to climate change. *Proc. Natl. Acad. Sci. U.S.A.* 104, 18686–18690
14 Lobell, D.B. *et al.* (2011) Climate trends and global crop production since 1980. *Science* 333, 616–620
15 Moreno, A.A. and Orellana, A. (2011) The physiological role of the unfolded protein response in plants. *Biol. Res.* 44, 75–80
16 Doyle, S.M. *et al.* (2010) Chloroplast and reactive oxygen species involvement in apoptotic-like programmed cell death in *Arabidopsis* suspension cultures. *J. Exp. Bot.* 61, 473–482
17 Watanabe, N. and Lam, E. (2008) *Arabidopsis* Bax inhibitor-1: a rheostat for ER stress-induced programmed cell death. *Plant Signal. Behav.* 3, 564–566
18 Blanvillain, R. *et al.* (2011) The Arabidopsis peptide kiss of death is an inducer of programmed cell death. *EMBO J.* 30, 1173–1183
19 Qi, Y. *et al.* (2010) Over-expression of mitochondrial heat shock protein 70 suppresses programmed cell death in rice. *FEBS Lett.* 585, 231–239
20 Qu, G.Q. *et al.* (2009) Evidence for programmed cell death and activation of specific caspase-like enzymes in the tomato fruit heat stress response. *Planta* 229, 1269–1279
21 Zinn, K.E. *et al.* (2010) Temperature stress and plant sexual reproduction: uncovering the weakest links. *J. Exp. Bot.* 61, 1959–1968
22 McClung, C.R. and Davis, S.J. (2010) Ambient thermometers in plants: from physiological outputs towards mechanisms of thermal sensing. *Curr. Biol.* 20, 1086–1092
23 Ruelland, E. and Zachowski, A. (2010) How plants sense temperature. *Environ. Exp. Bot.* 69, 225–232
24 Suzuki, N. *et al.* (2011) ROS and redox signalling in the response of plants to abiotic stress. *Plant Cell Environ.* DOI: 10.1111/j.1365-3040.2011.02336.x
25 Königshofer, H. *et al.* (2008) Early events in signalling high-temperature stress in tobacco BY2 cells involve alterations in membrane fluidity and enhanced hydrogen peroxide production. *Plant Cell Environ.* 31, 1771–1780
26 Miller, G. *et al.* (2007) Double mutants deficient in cytosolic and thylakoid ascorbate peroxidase reveal a complex mode of interaction between reactive oxygen species, plant development and response to abiotic stresses. *Plant Physiol.* 144, 1777–1785
27 Miller, G. *et al.* (2009) The plant NADPH oxidase RbohD mediates rapid, systemic signaling in response to diverse stimuli. *Sci. Signal.* 2, ra45
28 Queitsch, C. *et al.* (2000) Heat shock protein 101 plays a crucial role in thermotolerance in *Arabidopsis*. *Plant Cell* 12, 479–492
29 Su, P.H. and Li, H.M. (2008) *Arabidopsis* stromal 70-kD heat shock proteins are essential for plant development and important for thermotolerance of germinating seeds. *Plant Physiol.* 146, 1231–1241
30 Yamada, K. *et al.* (2007) Cytosolic HSP90 regulates the heat shock response that is responsible for heat acclimation in *Arabidopsis thaliana*. *J. Biol. Chem.* 282, 37794–37804
31 Dafny-Yelin, M. *et al.* (2008) Non-redundant functions of sHSP-CIs in acquired thermotolerance and their role in early seed development in *Arabidopsis*. *Plant Mol. Biol.* 67, 363–373
32 Suzuki, N. *et al.* (2008) The transcriptional co-activator MBF1c is a key regulator of thermotolerance in *Arabidopsis thaliana*. *J. Biol. Chem.* 283, 9269–9275
33 Qin, D. *et al.* (2008) Heat stress-responsive transcriptome analysis in heat susceptible and tolerant wheat (*Triticum aestivum* L.) by using Wheat Genome Array. *BMC Genomics* 9, 432
34 Vanderauwera, S. *et al.* (2011) Extranuclear protection of chromosomal DNA from oxidative stress. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1711–1716

35 Liu, H.C. *et al.* (2011) The role of class A1 heat shock factors (HSFA1s) in response to heat and other stresses in *Arabidopsis*. *Plant Cell Environ.* 34, 738–751
36 Kumar, S.V. and Wigge, P.A. (2010) H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*. *Cell* 140, 136–147
37 Baena-González, E. and Sheen, J. (2008) Convergent energy and stress signaling. *Trends Plant Sci.* 13, 474–482
38 Voellmy, R. and Boellmann, F. (2007) Chaperone regulation of the heat shock protein response. *Adv. Exp. Med. Biol.* 594, 89–99
39 Saidi, Y. *et al.* (2009) The heat shock response in moss plants is regulated by specific calcium-permeable channels in the plasma membrane. *Plant Cell* 21, 2829–2843
40 Saidi, Y. *et al.* (2010) Membrane lipid composition affects plant heat sensing and modulates Ca^{2+}-dependent heat shock response. *Plant Signal. Behav.* 5, 1530–1533
41 Murata, N. and Los, D.A. (1997) Membrane fluidity and temperature perception. *Plant Physiol.* 115, 875–879
42 Vigh, L. *et al.* (2007) Membrane regulation of the stress response from prokaryotic models to mammalian cells. *Ann. N. Y. Acad. Sci.* 1113, 40–51
43 Carratu, L. *et al.* (1996) Membrane lipid perturbation modifies the set point of the temperature of heat shock response in yeast. *Proc. Natl. Acad. Sci. U.S.A.* 93, 3870–3875
44 Horváth, I. *et al.* (1998) Membrane physical state controls the signaling mechanism of the heat shock response in *Synechocystis* PCC 6803: identification of *hsp17* as a 'fluidity gene'. *Proc. Natl. Acad. Sci. U.S.A.* 95, 3513–3518
45 Ramot, D. *et al.* (2008) Bidirectional temperature-sensing by a single thermosensory neuron in *C. elegans*. *Nat. Neurosci.* 11, 908–915
46 Yao, J. *et al.* (2011) Modular thermal sensors in temperature-gated transient receptor potential (TRP) channels. *Proc. Natl. Acad. Sci. U.S.A.* 108, 11109–11114
47 Xiao, B. *et al.* (2011) Temperature-dependent STIM1 activation induces Ca^{2+} influx and modulates gene expression. *Nat. Chem. Biol.* 7, 351–358
48 Sokabe, T. *et al.* (2008) Drosophila painless is a Ca^{2+}-requiring channel activated by noxious heat. *J. Neurosci.* 28, 9929–9938
49 Ward, J.M. *et al.* (2009) Plant ion channels: gene families, physiology, and functional genomics analyses. *Annu. Rev. Physiol.* 71, 59–82
50 Braam, J. (1992) Regulated expression of the calmodulin-related TCH genes in cultured Arabidopsis cells: induction by calcium and heat shock. *Proc. Natl. Acad. Sci. U.S.A.* 89, 3213–3216
51 Larkindale, J. and Knight, M.R. (2002) Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. *Plant Physiol.* 128, 682–695
52 Suri, S.S. and Dhindsa, R.S. (2008) A heat-activated MAP kinase (HAMK) as a mediator of heat shock response in tobacco cells. *Plant Cell Environ.* 31, 218–226
53 Reddy, A.S. *et al.* (2011) Coping with stresses: roles of calcium- and calcium/calmodulin-regulated gene expression. *Plant Cell* 23, 2010–2032
54 Zhang, W. *et al.* (2009) Molecular and genetic evidence for the key role of AtCaM3 in heat-shock signal transduction in *Arabidopsis*. *Plant Physiol.* 149, 1773–1784
55 Liu, H.T. *et al.* (2008) The calmodulin-binding protein kinase 3 is part of heat-shock signal transduction in *Arabidopsis thaliana*. *Plant J.* 55, 760–773
56 Li, S. *et al.* (2010) Functional characterization of *Arabidopsis thaliana* WRKY39 in heat stress. *Mol. Cells* 29, 475–483
57 Sangwan, V. *et al.* (2002) Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways. *Plant J.* 31, 629–638
58 Suzuki, N. *et al.* (2011) Respiratory burst oxidases: the engines of ROS signaling. *Curr. Opin. Plant Biol.* DOI: 10.1016/j.pbi.2011.07.014
59 Meiri, D. and Breiman, A. (2009) *Arabidopsis* ROF1 (FKBP62) modulates thermotolerance by interacting with HSP90.1 and affecting the accumulation of HsfA2-regulated sHSPs. *Plant J.* 59, 387–399
60 Suzuki, N. *et al.* (2011) Identification of the MBF1 heat-response regulon of *Arabidopsis thaliana*. *Plant J.* 66, 844–851
61 Zanetti, M.E. *et al.* (2003) Phosphorylation of a member of the MBF1 transcriptional co-activator family, StMBF1, is stimulated in potato cell suspensions upon fungal elicitor challenge. *J. Exp. Bot.* 54, 623–632
62 Mishkind, M. *et al.* (2009) Heat stress activates phospholipase D and triggers PIP2 accumulation at the plasma membrane and nucleus. *Plant J.* 60, 10–21
63 Zheng, S.Z. *et al.* (2011) Phosphoinositide-specific phospholipase C9 is involved in the thermotolerance in *Arabidopsis*. *Plant J.* DOI: 10.1111/j.1365-313X.2011.04823.x
64 Pincus, D. *et al.* (2010) BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response. *PLoS Biol.* 8, e1000415
65 Sugio, A. *et al.* (2009) The cytosolic protein response as a subcomponent of the wider heat shock response in *Arabidopsis*. *Plant Cell* 21, 642–654
66 Deng, Y. *et al.* (2011) Heat induces the splicing by IRE1 of a mRNA encoding a transcription factor involved in the unfolded protein response in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7247–7252
67 Che, P. *et al.* (2010) Signaling from the endoplasmic reticulum activates brassinosteroid signaling and promotes acclimation to stress in *Arabidopsis*. *Sci. Signal.* 3, ra69
68 Saidi, Y. *et al.* (2007) Activation of the heat shock response in plants by chlorophenols: transgenic *Physcomitrella patens* as a sensitive biosensor for organic pollutants. *Plant Cell Environ.* 30, 753–763
69 Mittler, R. *et al.* (2004) The reactive oxygen gene network of plants. *Trends Plant Sci.* 9, 490–498
70 Vasseur, F. *et al.* (2011) Changes in light intensity reveal a major role for carbon balance in *Arabidopsis* responses to high temperature. *Plant Cell Environ.* 34, 1563–1576
71 Larkindale, J. *et al.* (2005) Heat stress phenotypes of *Arabidopsis* mutants implicate multiple signaling pathways in the acquisition of thermotolerance. *Plant Physiol.* 138, 882–897
72 Mittler, R. *et al.* (2011) ROS signaling: the new wave? *Trends Plant Sci.* 16, 300–309
73 Clapier, C.R. and Cairns, B.R. (2009) The biology of chromatin remodeling complexes. *Annu. Rev. Biochem.* 78, 273–304
74 Erkina, T.Y. *et al.* (2008) Different requirements of the SWI/SNF complex for robust nucleosome displacement at promoters of heat shock factor and Msn2- and Msn4-regulated heat shock genes. *Mol. Cell. Biol.* 28, 1207–1217
75 Erkina, T.Y. *et al.* (2010) Functional interplay between chromatin remodeling complexes RSC, SWI/SNF and ISWI in regulation of yeast heat shock genes. *Nucleic Acids Res.* 38, 1441–1449
76 Schramm, F. *et al.* (2007) A cascade of transcription factor DREB2A and heat stress transcription factor HsfA3 regulates the heat stress response of *Arabidopsis*. *Plant J.* 53, 264–274
77 Finka, A. *et al.* (2011) Meta-analysis of heat- and chemically upregulated chaperone genes in plant and human cells. *Cell Stress Chap.* 16, 15–31
78 Chan-Schaminet, K.Y. *et al.* (2009) Specific interaction between tomato HsfA1 and HsfA2 creates hetero-oligomeric superactivator complexes for synergistic activation of heat stress gene expression. *J. Biol. Chem.* 284, 20848–20857
79 Török, Z. *et al.* (2001) *Synechocystis* HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone-mediated refolding. *Proc. Natl. Acad. Sci. U.S.A.* 98, 3098–3103
80 Rizhsky, L. *et al.* (2004) When defense pathways collide: the response of *Arabidopsis* to a combination of drought and heat stress. *Plant Physiol.* 134, 1683–1696
81 Banti, V. *et al.* (2010) The heat-inducible transcription factor HsfA2 enhances anoxia tolerance in *Arabidopsis*. *Plant Physiol.* 152, 1471–1483
82 Mishra, S.K. *et al.* (2002) In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermotolerance in tomato. *Genes Dev.* 16, 1555–1567
83 Li, M. *et al.* (2010) Promoter specificity and interactions between early and late *Arabidopsis* heat shock factors. *Plant Mol. Biol.* 73, 559–567