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A 'hot' cocktail: The multiple layers of thermomemory in plants

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Abstract

Heat stress (HS) caused by above-optimal temperatures adversely affects plants' growth and development and diminishes crop yields. In natural and agricultural environments, these stresses are often transient but recurrent and may progressively increase in severity over time. In addition to the inherent ability to cope with a single HS event, plants have evolved mechanisms that enhance their capacity to survive and reproduce under such conditions. This involves the establishment of a molecular 'thermomemory' after moderate HS that allows them to withstand a later — and possibly more extreme — HS event. Here, I summarize the current understanding of the molecular and biochemical mechanisms underlying thermomemory across multiple cellular levels and discuss aspects that require further attention.

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Heat stress, Thermomemory, Plant growth, Development, Molecular mechanisms.

Introduction

In natural and agricultural environments, plants encounter constantly changing biotic and abiotic conditions, including stresses, that are often repetitive and may increase in severity over time [1]. Temperatures above optimal can induce heat stress (HS). This typically impairs growth and development due to cell-damaging effects, such as accumulation of misfolded proteins and/or reactive oxygen species, if not countered by protective mechanisms [2,3]. Amongst other effects, HS impairs the photosynthetic machinery's structural integrity, thereby limiting carbon dioxide fixation [4–6].

However, plants have evolved thermotolerance, involving HS responses encompassing the production of molecular chaperones, including heat shock proteins (HSPs), antioxidants, and other cellular integrity-maintaining mechanisms. HS responses are orchestrated by suites — 20 or more in plants — of DNA-binding heat shock transcription factors (HSFs) [7,8]. In *Arabidopsis thaliana*, HSFA1a, A1b and A1d are master HS response regulators, with essential functions in the activation of HS-responsive transcriptional networks [9].

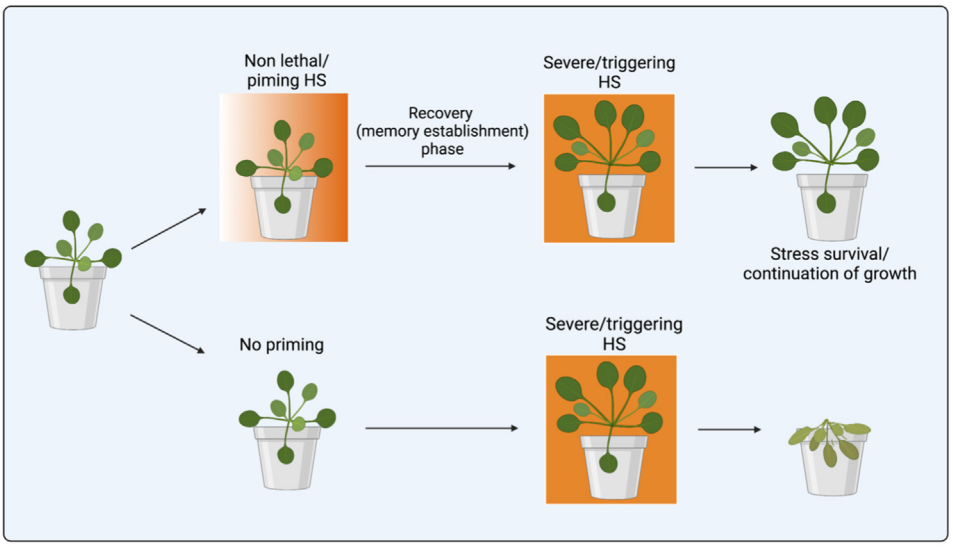
Plants also have 'thermopriming' mechanisms that enable them to respond more effectively to subsequent, and potentially harsher, HS following exposure to moderate HS [10*,11,12**]. Thermopriming-induced molecular changes may persist longer than the priming HS, thereby collectively establishing a molecular 'memory' that can last for several days. Consequently, plants respond more rapidly (or strongly) to a recurring ('triggering') stress before the memory fades (Figure 1). This review focuses on the molecular mechanisms underlying memory of high-temperature stress; processes maneuvering memory to other environmental stresses, including cold, are reviewed elsewhere [13,14].

Initially, thermomemory research largely focused on the control of gene expression by transcription factors (TFs), epigenetic modulators, and post-transcriptional modifications, but recent discoveries have revealed the importance of protein stability control, primary metabolism, and coordination of regulatory networks across different organs for thermomemory. Here, I review current knowledge regarding thermomemory's orchestration in somatic plant tissues. Experimental evidence indicates that offspring may also inherit thermomemory from parents, a phenomenon called transgenerational stress memory [15].

Transcriptional control of thermomemory

HS induces massive changes in gene expression, but only a small fraction of HS-inducible genes' transcriptional changes are persistent. Two types of transcriptional memory have been identified in Arabidopsis: type

Figure 1



Priming-induced thermomemory. Pre-exposure of plants to mild or transient heat stress (HS) increases their resilience to a subsequent severe (triggering) or otherwise lethal HS, a phenomenon called thermopriming or acquired thermotolerance. Thermopriming induces changes at molecular and biochemical levels, some of which are maintained during the recovery phase, in the absence of intervening stress, and form a molecular memory. Thermomemory can last for several days (within a generation), and its establishment enables plants to survive and continue growth following exposure to otherwise lethal subsequent HS events (within taxon-specific limits). The figure was prepared using BioRender (www.biorender.com).

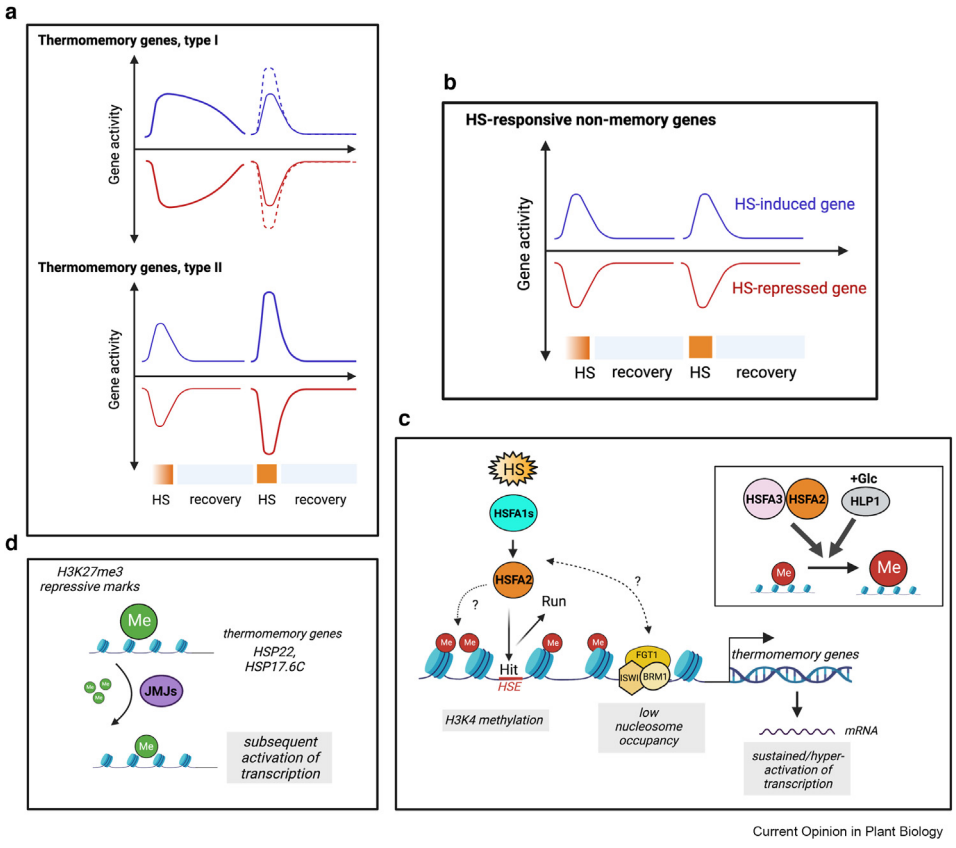
I transcriptional memory refers to the sustained transcriptional activity of genes for a moderately long episode (i.e. a few days) after the recovery from priming HS, while type II memory represents a faster, or stronger, change in transcriptional activity (re-activation or -suppression) upon recurring HS (Figure 2). Transcript analysis identified a number of type I thermomemory genes in Arabidopsis seedlings, such as *HSP18.2*, *HSP21* and *HSP22*, while others, including *APX2*, *HSFA1E*, *MIPS2*, and *XTR6*, are categorized as type II memory genes [11,16].

Transcriptional memory arises from a coordinated action of TFs and chromatin regulators that affect histone methylation and nucleosome remodeling (Figure 2) [10*,17*], [18-20]. Initially, only HSFA2 was identified as a TF that specifically functions in thermomemory [10*], but recently, a requirement for HSFA3 for extending the duration of thermomemory was detected. Loss-of-function alleles of either *HSFA2* or *HSFA3* substantially impair thermomemory, whereas immediate HS responses are unaffected in those mutants [10*,18**]. While HSFA2 regulates both type I and type II transcriptional memory, the disruption of *HSFA3* specifically compromises only type I memory [18**]. The partly complementary functions of the two TFs may be due to their different transcriptional induction dynamics after priming HS; while *HSFA2* expression is rapidly induced and peaks right after priming HS, the induction of *HSFA3* transcripts occurs at a slower pace [18**].

HSFA2 controls the expression of HS memory genes by binding to *Heat Shock Elements* in target promoters [17*,19**,20]. In some cases, this binding occurs transiently, 1–4 h after HS ends. A hit-and-run mechanism [21] of HSF2A's action has been proposed [17*] and corroborated by data showing enrichment of histone H3 lysine 4 tri-methylation marks (H3K4me3) at actively transcribed memory loci (Figure 2). The marks reportedly persist after a priming HS and outlast the period of active transcription, suggesting their importance for the hyper-induction of memory genes upon recurring HS [17*]. Thus, HSFA2 triggers sustained changes in chromatin modifications, which then act in support of HSFA2-induced transcriptional memory. Of note, HSFA2 can form a heterodimer with HSFA3; the binding of HSFA3 to HSFA2 enhances histone H3K4 methylation at memory loci. Accordingly, nonfunctional alleles of *HSFA2/3* impair thermomemory and limit sustained induction of type I memory genes [18**]. However, how HSFA2 and HSFA3 recruit chromatin-modifying factors to the target loci (directly or indirectly) remains to be investigated.

Another chromatin modification that plays a role in establishing thermomemory has recently been reported. Yamaguchi et al. found that multiple Jumonji C

Figure 2



Transcriptional and epigenetic control of thermomemory. (**a**) and (**b**), schematic representation of thermomemory and heat stress (HS)-responsive non-memory genes. HS triggers changes (induction or repression) in transcriptional activity. Genes that participate in transcriptional thermomemory (a) are grouped into two categories, genes whose transcriptional change (induction or repression) by HS continues for some time (days) after recovery from stress (type I, upper panel) and genes whose transcriptional change (re-activation or -suppression) upon subsequent HS (following an intervening period of no/reduced activity) is faster or stronger (type II, lower panel) [24*]. The two categories may partially overlap depending on the duration of the recovery phase, for example, both sustained induction after priming (up to at least 52 h into the recovery phase) and hyper-activation after a second HS of *ASCORBATE PEROXIDASE 2* (*APX2*) transcription has been observed [17*]. (b) HS-responsive non-memory genes include those whose transcriptional activity is similar in response to each HS. (**c**) HSFA1 isoforms (a, b and d) are key players in the immediate response to HS, including transcriptional induction of *HSFA2* [65]. HSFA2 transiently binds to (hits) *Heat Shock Elements* (*HSEs*) in promoters of thermomemory genes initiating their transcription, then enrichment of histone H3K4me3 marks (Me) at the memory loci mediates their continued active transcription without binding of HSFA2 to those promoters (run) [17*]. However, it remains unclear how HSFA2 recruits chromatin-modifying factors (directly or indirectly) to the target loci. In addition, FGT1 binds to the chromatin of thermomemory genes near their transcription start sites, where it interacts with catalytic components of ISWI (CHR11 and CHR17) and SWI/SNF (BRAHAMA) chromatin remodelers. The complex formation leads to maintenance of low nucleosome occupancy at the memory loci after HS and thus sustained active transcription of thermomemory genes [26**]. The mechanism whereby the FGT1-chromatin remodeling complex targets the memory genes and whether HSFA2 (or HSFA1s) participates in this process remains to be determined. Inset: sustained accumulation of H3K4me3 at memory loci is promoted by binding of an HSFA2/HSFA3 heteromeric complex [18**], as well as glucose-induced HLP1 to those loci [57*]. (**d**) In addition to active chromatin marks, a decreased occupancy of repressive histone marks such as H3K27me3 at the gene body of thermomemory-associated genes plays a role in establishing memory. JMJ-mediated removal of H3K27me3 marks (Me) from the *HSP22* and *HSP17.6C* loci during the recovery from HS priming (acclimation) contributes to their reactivation upon exposure to subsequent HS [22**]. The figure was prepared using BioRender (www.biorender.com).

domain-containing (JMJ) histone demethylases, including JMJ11 (also called EARLY FLOWERING 6, ELF6), JMJ12 (RELATIVE OF EARLY FLOWERING 6, REF6), JMJ30, and JMJ32, contribute to removing repressive histone H3 lysine 27 tri-methylation (H3K27me3) marks from thermomemory-associated small *HSP22* and *HSP17.6C* [22**] (Figure 2d). Importantly, REF6 also affects transgenerational thermomemory *via* a heritable feedback loop that involves HSFA2 [15].

Furthermore, JMJ histone demethylases influence the expression of *HSP21*, which encodes a plastidic HSP crucial for maintaining thermomemory [12**] by balancing H3K27me3 and H3K4me3 levels at the *HSP21* locus [23]. The mechanisms involved in

recruiting JMJs to the memory loci and their possible interactions with other histone modifiers and TFs remain to be explored. A similarly important question that needs to be addressed in the future is how the activity of the histone demethylases is balanced with methylases acting on H3K27 to control thermomemory.

Nucleosome organization affects thermomemory

In addition to histone methylation, nucleosome organization affects thermomemory, as recently reviewed [24*]. Briefly, FORGETTER1 (FGT1), a functional Arabidopsis ortholog of metazoan Strawberry notch (Sno) [25], binds to chromatin of HS-induced memory genes at nucleosome-free regions near transcription start sites, and interacts with catalytic components of evolutionarily conserved ISWI (CHR11 and CHR17) and SWI/SNF (BRAHAMA) chromatin remodelers. This leads to maintenance of low nucleosome occupancy at memory loci after HS, thereby supporting sustained transcription of memory genes (Figure 2) [26**].

Another chromatin regulator of thermomemory is BRUSHY1 (BRU1) [27]. Early studies demonstrated the role of BRU1, also known as TONSOKU or MGOUN3, in DNA damage responses, epigenetic inheritance of gene silencing, and meristem organization [28–30]. *Bru1* mutants show reduced thermomemory and sustained activation of memory genes. As the mammalian ortholog TONSL functions in DNA replication [31], BRU1 might play a key role in the inheritance of thermomemory-related chromatin modifications across cell divisions during growth in the memory phase [27].

Post-transcriptional regulation of thermomemory

Through profiling transcriptome changes and alternative splicing events, a form of splicing memory has been discovered in Arabidopsis (Figure 3a). Alternative splicing is suppressed in seedlings subjected to a priming HS [32*], mostly leading to enhanced intron retention [33]. Repression of splicing under HS has been previously reported for multiple organisms and appears to be an evolutionarily conserved phenomenon in HS adaptation [34–39]. Intriguingly, results showed that HS-primed plants returned to efficient splicing following relief from a second HS, while non-primed plants exposed to the same HS tended to accumulate intron-retaining transcripts [32*]. Affected genes included those controlling the HS response. Thus, HS priming establishes memory, enabling a rapid return to constitutive/correct splicing once the stress has subsided, which is particularly important for the maintenance of adequate levels of transcript and protein isoforms that support stress survival and continuation of growth under repeated stress conditions.

The molecular mechanisms underpinning splicing memory are still unclear, but changes in chromatin accessibility and DNA methylation have known involvement in intron retention regulation [40,41]. As activation of thermomemory-related genes is affected by chromatin status, it seems plausible that priming-induced changes in splicing are influenced by the chromatin environment [32*], but this possibility requires further research.

MiRNA-dependent gene silencing also participates in thermomemory, as shown for Argonaute 1, an effector protein involved in small RNA-based gene silencing [42], and heat-responsive *miR156* [11]. Heat-induced activation of *miR156* sustains expression of thermomemory genes by inhibiting two squamosa promoter-binding-like TFs, *SPL2*, and *SPL11* (Figure 3b). Accordingly, thermomemory is compromised in mutants with reduced Argonaute 1 activity or *miR156* expression. Similarly, enhanced accumulation of *miR156*-resistant *SPL2* and *SPL11* weakens thermomemory and expression of memory genes, including *HSFA2* and *HSPs* [11].

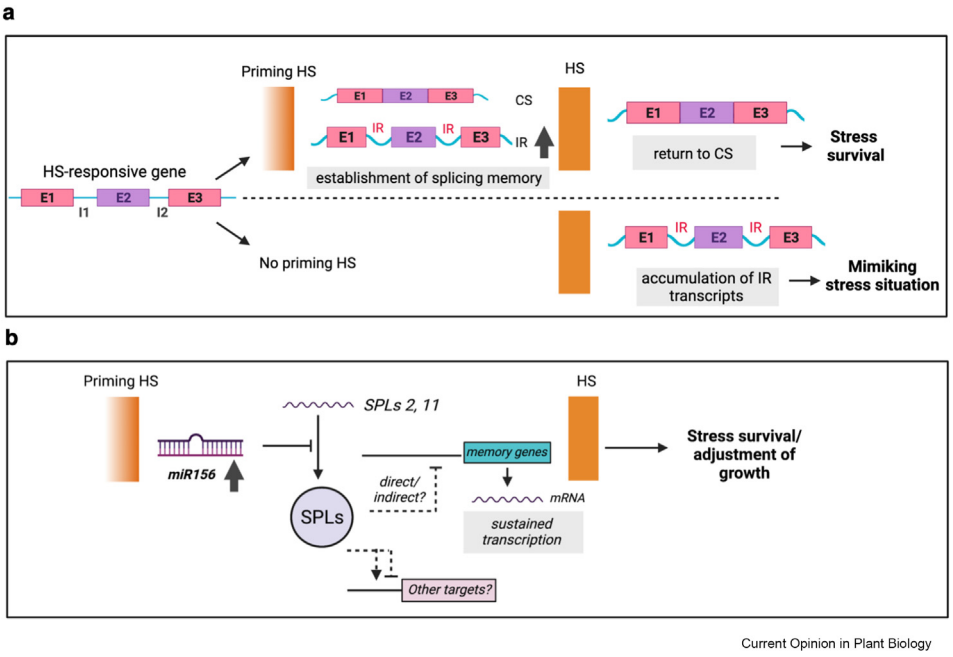
MiR156-regulated *SPLs* promote developmental phase transitions but inhibit the formation of new leaves at the shoot apical meristem (SAM) [43–45]. Thus, inhibition of *SPLs* by *miR156* after HS is apparently involved in mechanisms that counter negative effects of HS and reinitiate growth after stress dissipation [11]. The developmental function of *miR156* and its responsiveness to HS are conserved in plants, including Arabidopsis, *Brassica rapa* and wheat [46,47], highlighting its importance in the integration of thermomemory with developmental processes.

Control of thermomemory by affecting protein stability

Findings that transcriptional memory involves a small fraction of HS-inducible genes strongly suggest that thermomemory involves additional regulatory mechanisms, and there are growing indications that they include selective accumulation and stabilization of stress proteins and regulated protein degradation (Figure 4) [12**,48-51]. For instance, transcription patterns of heat-inducible HSP90.1, its co-chaperone ROF1 (a plant homolog of FK506-binding proteins), and HSP101 are not memory-associated, but elevated levels of proteins they encode during the memory phase are essential for extending thermomemory [48-50,52].

HS-induced formation of ROF1-HSP90-HSFA2 complexes in the cytosol and subsequent nuclear import enhances the transcriptional activity of *HSFA2* and secure continuity of expression of *HSP*s [52]. HSP90 and ROF1 protein stability during the recovery phase are at least partly regulated through selective autophagic

Figure 3



Splicing and miRNA-mediated regulation of thermomemory. (**a**) Primed plants can establish a splicing memory that enables them to respond differently to an upcoming HS event [32*]. Subjecting Arabidopsis seedlings to a priming HS and later to a severe heat shock leads to repression of AS, as shown by higher levels of intron retention (IR), the most prevalent form of splicing in plants [33]. After relief from the second HS exposure, primed plants return to efficient/correct splicing (CS) ensuring rapid adjustment of the abundance (and functions) of stress-response components. In contrast, non-primed plants continue to accumulate intron-retaining transcripts, mimicking those of plants under HS conditions. (**b**) The heat-induced *miR156* enhances plant thermomemory and survival after exposure to a second HS by downregulating SPL family TFs (*SPL2* and *SPL11*) and thus suppressing their inhibitory effect on expression of thermomemory genes. It is not yet known how SPLs regulate expression of thermomemory genes (directly or indirectly) and what the identities of their other (stress-or developmental-related) target genes during the recovery phase are. The figure was prepared using BioRender (www.biorender.com). AS, alternate splicing; HS, heat stress.

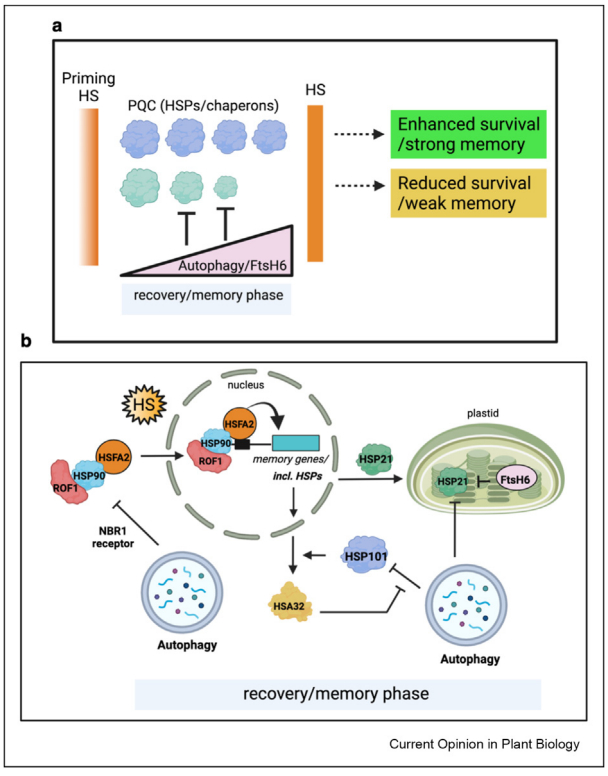
degradation mediated by NBR1 (Next-to-BRCA1), a plant homolog of the mammalian autophagic cargo receptor p62 [49**,53]. HSP101 stability is also regulated by autophagy [48,50], although NBR1's involvement remains to be investigated. Autophagic degradation of HSPs could participate in reversion of the cellular proteome to a pre-stress state and restoration of growth during between-stress periods.

Continued accumulation of HSP101 during the recovery/memory phase is also crucial for the high abundance of the HS-associated 32-kD protein (HSA32), another essential component of thermomemory [50]. In return, HSFA32 increases HSP101 stability by retarding its degradation. Although details of the interplay between the proteins are not yet known, a conserved positive HSP101-HSA32 feedback loop has been found in Arabidopsis and rice (*Oryza sativa*), indicating the importance of this regulatory mechanism for thermomemory evolution [54].

Another recent finding of importance is that several translation-associated/ribosomal proteins, including the 60S ribosomal proteins RPL5A and RPL10A/SAC52 and the 40S ribosomal proteins RP40 and RPS10B, are high-confidence interactors of NBR1 during recovery from HS [49**]. Thus, NBR1 appears to act as a receptor for selective autophagy of ribosomes (called ribophagy) in this process. Analyzing the details of ribophagy during thermomemory as an important mechanism contributing to protein quality control remains an important task of future research.

Strikingly, an ability to maintain an active protein quality control system in organelles such as plastids appears to be crucial for establishing thermomemory. A recent study demonstrated that a sustained high level of plastidial small HSP21 is essential for the maintenance of the primed state and the duration of thermomemory. A heat-induced metalloprotease, FstH6, located in the same organelle, regulates HSP21's *in vivo* stability by degrading it and thus limiting plants' thermomemory capacity. Notably, natural variation in the FtsH6-HSP21 regulatory module underlies differences in thermomemory, corroborating this organellar mechanism's importance in the HS response [12**]. In addition to FtsH6, autophagy controls HSP21 abundance during the thermomemory phase [51].

Figure 4



Regulation of the *in vivo* stability of stress proteins is important for establishing thermomemory. (**a**) Conceptual scheme: priming HS triggers the accumulation of stress proteins such as HSPs (or molecular chaperones in general). Sustained high levels of a subset of molecular chaperones during the recovery phase enhance the plant's thermomemory capacity and protection against an upcoming severe HS, whereas their degradation by thermomemory-associated protein degradation systems (by autophagy or FtsH6 metalloprotease) weakens thermomemory, and thus the response to the next HS. (**b**) Simplified working model summarizing current knowledge on the control of thermomemory by regulation of protein stability [12**,48-50,52–54]. Blunt-ending lines indicate inhibition or degradation. Lines with arrows indicate activation or transport. The figure was prepared using BioRender (www.biorender.com). HS, heat stress; HSP, heat shock protein.

Metabolic control of thermomemory

Establishing memory enhances plants' capability not only to survive harsher HS but also to recover growth after stress dissipation [19**], so it is not surprising that complex metabolic adjustments are involved. A mass spectrometric analysis recently detected substantial differences in metabolic states between HS-primed and non-primed Arabidopsis plants [55], including markedly higher levels of sucrose and raffinose family oligosaccharides after HS priming. The priming generated additional lasting metabolic imprints, some of which (such as changes in galactinol, δ-tocopherol, stachyose, and raffinose levels) were found to persist during the memory phase and are likely crucial for optimal responses in upcoming HS [55]. However, another recent report did not find an increase of δ-tocopherol level in Arabidopsis leaves upon prolonged (1—4 days) HS at 37 °C [56], suggesting different metabolic programs in primed versus constantly heat-stressed plants. Moreover, levels of several HS-induced metabolites (including sucrose) decreased to basal levels after release from the second HS in primed plants, but remained high in non-primed plants, resembling those in HS-stressed plants. Collectively, these results suggest that HS priming triggers the formation of a metabolic memory that promotes survival and faster recovery from stress.

Two other recent studies [19**,57*] support the importance of carbohydrates in the establishment of thermomemory. Sharma et al. [57*] showed that a lack of glucose impairs maintenance of high expression of memory genes during the recovery phase. The effect on thermomemory appears to be due to enhanced deposition of H3K4me3 marks at memory loci through glucose-induced HIKESHI-LIKE PROTEIN1 (HLP1), an Arabidopsis ortholog of the human nuclear transport receptor Hikeshi [58]. Like *HSFA2*, *HLP1* expression is directly regulated by HSFA1 [57*], suggesting the cooperation of the two proteins in modulating the epigenetic landscape of thermomemory genes.

Another important finding is that sugar availability at the SAM is essential for the expression of memory genes, plant survival, and growth recovery following a subsequent HS [19**]. The shortage of sugar reservoirs, triggered by removing cotyledons, lowering sucrose supply, or knocking out the primary carbohydrate metabolism gene *FRUCTOSE-BISPHOSPHATE ALDOLASE 6* (*FBA6*), impairs plants' survival and growth recovery following recurring HS. *FBA6* expression is directly controlled by HSFA2 [19**].

Levels of phospholipid glycerol backbone precursors also increase during priming and remain higher after recovery and even after HS in primed plants than in non-primed plants, suggesting that membrane phospholipids are important for thermomemory [55]. Accordingly, phospholipase Dα2, an enzyme involved in membrane phospholipid metabolism, is essential for thermomemory [59].

Effects of HS on the SAM

The SAM, including its stem cells, plays crucial roles in aboveground plant development, so it must be well protected from potential damage by abiotic stresses. Recent RNA-seq analysis has elucidated important aspects of the control of SAM development under recurrent HS, including a demonstration that priming enhances a key response to a triggering HS, down-regulation of the key stem cell regulators *CLAVATA1* (*CLV1*) and *CLV3* [19**]. This shows that both developmental control genes are *bona fide* thermomemory

genes. Moreover, transcriptional downregulation of the *CLV* genes mostly vanished within 24 h following a triggering HS in primed plants but persisted in non-primed plants. These observations clearly demonstrate that priming protects stem cells in the SAM from the potentially damaging effects of harsher HS [19**].

Several *HSF* genes are induced by priming and triggering treatments at the SAM of Arabidopsis plants, including *HSFA1e*, *HSFA2*, *HSFA3*, *HSFA7a*, *HSFA7b*, *HSFB1*, *HSFB2a*, and *HSFB2b* [19**]. In the light of the recent finding that HSFA3, together with HSFA2, prolongs thermomemory [18**], it will be important to identify HSFA3′s target genes at the SAM and establish if it interacts with any of the other HSFs to exert its function in this organ.

Concluding remarks and future perspectives

Global warming and the increasing frequencies of heatwaves are adversely affecting crop yields and food supplies. A potentially powerful strategy for enhancing heat tolerance is to enhance thermomemory. Recent research has greatly increased our mechanistic understanding of thermomemory and revealed crucial regulators acting at various levels [12**,18**,19**,22**,32*,49**,57*]. However, despite considerable progress, many unanswered questions remain regarding the coordination of regulators in different cells and organs in the establishment of coherent plant-wide thermomemory; which mechanisms act in specific cells, specific tissues and diverse organs; the evolution of thermomemory signaling pathways; and strategies for exploiting knowledge of thermomemory in breeding or editing genomes of agricultural crops.

Much current understanding of thermomemory stems from research on vegetative Arabidopsis seedlings. Processes controlling thermomemory in mature plants, during reproductive growth, and in crops have received surprisingly little attention so far [60,61]; although, for example, exposure of flowers to a high temperature significantly decreases pollen viability, fruit set, and yield [62,63]. An elegant recent study detected differences in the orchestration of thermomemory at the SAM and whole seedlings [19**], suggesting that organ-specific mechanisms are involved in the establishment of thermomemory. Thus, given the importance of reproductive growth and development for agricultural yields, it is crucial to elucidate thermomemory's establishment and control in reproductive organs and identify alleles that enhance thermomemory during flowering. Once a better understanding of thermomemory is available for crops, precision genome editing (e.g. using CRISPR/Cas9-based methods) can be used to enhance their field performance in anticipated climatic conditions.

Another interesting concept for improving thermotolerance in crops in a sustainable manner comes from recent studies of plant-interacting microbes, that is, root endophytes, that benefit plant growth at high temperature by impacting the expression of thermomemory genes [64*]. In the future, it might be possible to use synthetic-biology approaches to optimize such beneficial interactions by modifying the microbial or crop genomes. Finally, the identification and optimization of small molecules that enhance plants' priming capacity might be useful for stress-memory establishment in the field.

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Declaration of competing interest

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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* of special interest
** of outstanding interest

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