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POINT-OF-VIEW

HSFA2 orchestrates transcriptional dynamics after heat stress in *Arabidopsis thaliana*

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ABSTRACT
In nature, stress is typically chronic or recurring and stress exposure can prime modified responses to recurring stress. Such stress priming may occur at the level of transcription. Here, we discuss the connection between plant stress memory, transcription, and chromatin modifications using the example of recurring heat stress.

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As sessile organisms plants need to cope with various stresses "on the spot" and hence need to adapt to these in order to ensure survival and reproductive success. Plants face a number of different stresses, ranging from biotic stress such as herbivory to abiotic stress such as extreme temperatures or drought. Most of these stresses are not singular events, but are recurring more or less regularly. This suggests that it may be beneficial for a plant to store information about a past stress event as this may predict a series of similar stress events in the future. Indeed, plants can be primed by a stress exposure to respond more efficiently to a recurring stress event.[1,2] This is, however, a classical trade-off situation as the maintenance of constitutively high levels of molecular defenses consumes energy and interferes with growth and thus is beneficial when stress is constitutive, but disadvantageous if not.[3] Hence, plants may benefit from storing information about previous stress exposure in a way that requires little resources, but that allows the plant to respond more efficiently to a second stress exposure. It has been hypothesized that chromatin modifications may provide such a lasting memory mark that can be maintained with relatively few resources. In this commentary, we discuss recent findings and mechanistic insights into transcriptional regulation during adaptation to recurring heat stress (HS).

Whereas the immediate responses to a single HS were analyzed in great detail (reviewed in[4-6]), much less is known about how plants cope with recurring HS and how they store and use the information conveyed by the first HS to modulate the response to recurring stress. Plants can acquire thermotolerance by exposure to a moderate HS; this acquired thermotolerance allows plants to then withstand a HS that is lethal to a naive plant.[7] Interestingly, plants actively maintain this HS memory for several days and it was demonstrated that this memory is genetically separable from the mere acquisition.[8,9] HS memory specifically requires HEAT SHOCK TRANSCRIPTION FACTOR A2 (HSFA2).[8] *HSFA2* is one of 21 *HSF* genes identified in *Arabidopsis thaliana*;[5] *HSFA2* is strongly heat inducible and its expression depends on the four constitutively expressed HSFA1 isoforms.[10,11] *In vitro*, HSFA2 was shown to interact with the promoters of different HS-dependent genes such as *HEAT SHOCK PROTEIN 22.0* (*HSP22.0*)[12] but no *in vivo* data on binding kinetics to potential target loci were available until very recently, thus precluding inferences about the molecular role of this transcription factor during HS memory.

One component of HS memory is the sustained induction of a subset of HS-responsive genes (memory-associated genes); this is in contrast to other HS-induced genes that are activated quickly but whose expression strongly drops within hours after the end of heat exposure.[13] Interestingly, the initial induction of

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both classes of genes after HS is not compromised in *hsfa2* mutants; however, the sustained expression of memory genes is strongly decreased.[8,14] This indicates that HSFA2 is required specifically for the memory phase and that initial transcriptional activation is separable from sustained expression later on.

The most parsimonious explanation for sustained induction of HS memory genes is the persistent binding of a transcription factor to these loci. Chromatin immunoprecipitation analysis of HSFA2 binding to its target genes after HS, however, indicates that it binds only transiently after HS and has mostly dissociated during the memory phase.[14] This is remarkable as only during the memory phase the mutant shows the most pronounced defects both at the organismal and the molecular level.[8,14] To investigate possible additional regulators, the authors focused their analysis on histone modifications, more specifically on histone H3K4 methylation. H3K4 trimethylation (H3K4me3) was strongly induced at memory-associated loci, where it was maintained at high levels for at least 2 days after HS. High H3K4me3 levels depended on functional HSFA2. Memory-associated loci were also enriched for H3K4 dimethylation (H3K4me2) after HS, and this enrichment became apparent only 1 day after HS. Thus, H3K4me2 levels did not correlate with transcriptional activity *per se*. As for H3K4me3, HSFA2 was required for peak levels of this mark.

The sustained induction of gene expression after a stress exposure of limited duration reflects a type of transcriptional memory. In the literature, transcriptional memory also describes a modified transcriptional response following a second stress that depends on the first stress exposure after an intervening period of inactivity.[15] In plants, such transcriptional memory had been described in response to recurring drought stress.[15,16] Lämke et al. now reported a similar phenomenon in HS memory; a subset of memory genes showed a stronger re-activation upon a recurring HS 2 days after the primary HS.[14] Interestingly, genes that showed this behavior were highly enriched in H3K4me2 and H3K4me3. Both, the transcriptional memory and the H3K4 me enrichment depended on functional HSFA2. Taken together, the data suggest that H3K4me2 and/or H3K4me3 act as a chromatin modifications that mark a locus for recent transcriptional activation and that persist after active transcription has subsided. The transiently binding HSFA2 transcription factor orchestrates this memory.

Although evidence so far remains correlative, it is tempting to speculate that H3K4me2 and H3K4me3 are involved in relaying transcriptional memory during HS memory. A prerequisite for efficient transcription is the transition of RNA Polymerase II from the initiating into the elongating phase of transcription. It was suggested that H3K4me3 is specifically required to enable this process.[17] Furthermore, H3K4me3 is thought to anchor the basic transcription machinery to specific loci.[18] This was suggested to be mediated through the interaction with the general transcription factor TFIID, a transcriptional activator that can specifically recognize this modification in mammalian systems. Of note, this interaction was enhanced in the presence of H3K9ac,[18] potentially explaining the modulation of transcript levels of the memory-associated genes over time. Another potential role of H3K4me3 could be its effect on transcript stability and efficient splicing. The maturation of mRNA molecules involves the addition of a 5′-cap (7-methylguanosine).[19] This cap is recognized and protected by the cap-binding-complex (CBC), which promotes splicing.[20] Recently, it was shown that there is an intricate connection between H3K4me3 and the recruitment of the CBC as the COMPASS-like complex (which is required for trimethlyation of H3K4) and subunits of the CBC interact physically. Loss of either H3K4me3 or the CBC lead to a higher proportion of un-capped and aberrantly spliced transcripts.[21] H3K4me3 also offers a potential explanation for the altered re-induction of some memory-associated genes after recurring HS. *In vitro* competition assays with a histone acetyl transferase showed a preference of H3 acetylation for histone tails already carrying H3K4me3.[22] Mechanistically, this suggests that H3K4me3 predisposes tails carrying this modification for acetylation of H3. This would be an attractive explanation for the observed super-induction of selected memory-associated genes as H3 acetylation has been suggested to increase the transcriptional rate.[23] Taken together, it is tempting to speculate that H3K4me3 is used to store information of past stress exposure and to modulate the responses to recurring stress.

The role of H3K4me2 is less well understood. H3K4me2 was suggested to play a role in differentiated cells in mammalian systems, as expressed cell type-specific genes of $CD4^+$ cells were enriched in

H3K4me2.[24] Similar observations were made for neuronal tissue.[25]

The findings reported in Lämke et al. provide new insights to better understand information storage and retrieval in plants. It will be interesting to determine if other histone modifications play a role in information storage after HS. Although H3K4me3 was suggested to have a similar role in drought stress memory,[15] we do not know to what extend the findings discussed can be generalized to other stress systems in plants and other organisms. A major open question is what enzymes set and regulate H3K4me3 and H3K4me2 in response to HS. In particular, it is unknown whether H3K4me2 is generated by degeneration of H3K4me3 or set independently, as suggested by others.[26] In summary, the recent findings shed light on the fundamental question of how plants can store information on recent stress exposure and how this might modify the response to further stress. At the same time, it opens up exciting avenues for future research.

Disclosure of potential conflicts of interest

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