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Transcriptional Regulation of the Ambient Temperature Response by H2A.Z Nucleosomes and HSF1 Transcription Factors in *Arabidopsis*

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ABSTRACT

Temperature influences the distribution, range, and phenology of plants. The key transcriptional activators of heat shock response in eukaryotes, the heat shock factors (HSFs), have undergone large-scale gene amplification in plants. While HSFs are central in heat stress responses, their role in the response to ambient temperature changes is less well understood. We show here that the warm ambient temperature transcriptome is dependent upon the HSFA1 clade of *Arabidopsis* HSFs, which cause a rapid and dynamic eviction of H2A.Z nucleosomes at target genes. A transcriptional cascade results in the activation of multiple downstream stress-responsive transcription factors, triggering large-scale changes to the transcriptome in response to elevated temperature. H2A.Z nucleosomes are enriched at temperature-responsive genes at non-inducible temperature, and thus likely confer inducibility of gene expression and higher responsive dynamics. We propose that the antagonistic effects of H2A.Z and HSF1 provide a mechanism to activate gene expression rapidly and precisely in response to temperature, while preventing leaky transcription in the absence of an activation signal.

Key words: gene expression regulation, plant temperature sensing and signaling, transcriptomics, nucleosome dynamics, histone variant H2A.Z, heat shock transcription factors

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INTRODUCTION

Warm temperature is an important cue for plants, which must be able to adapt to their environment (Wigge, 2013). In *Arabidopsis*, many of the growth and developmental responses to temperature are mediated by the basic helix-loop-helix transcription factor PHYTOCHROME INTERACTING FACTOR4 (Koini et al., 2009; Kumar et al., 2012), which is controlled by the thermosensor phyB (Jung et al., 2016; Legris et al., 2016). The phyB temperature perception mechanism relies on dark reversion, and consequently expression of the warm-temperature transcriptome controlling development occurs at night (Jung et al., 2016). Plants are most likely to encounter higher temperatures in sunlight, however, suggesting an additional mechanism to sense temperature during the day. Consistent with this, genes involved in response to heat stress are predominantly expressed in the light (Jung et al., 2016).

Thermal stress is a major threat to the cell, causing protein denaturation and compromising membrane integrity. Rising

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global temperature is estimated to reduce crop yields by 2.5%–16% for every additional 1°C of warming during hot summers (Battisti and Naylor, 2009). It is therefore important to understand the pathways and mechanisms by which warm temperature influences the cell. Work in yeast, *Drosophila*, plants, and mammalian cells has led to a widely established model that genes encoding heat shock proteins (HSPs) are transcriptionally induced by heat shock factor (HSF)-class transcription factors (TFs) upon activation by heat stress (Shivaswamy and Iyer, 2008; Zobeck et al., 2010; Jacob et al., 2017). A genetic screen in plants revealed that in addition to HSFs (Miozzo et al., 2015), the chromatin state also influences the expression of warm temperature-induced genes (Kumar and Wigge, 2010). Mutants deficient in the incorporation of H2A.Z nucleosomes show a higher *HSP70* expression and many phenotypes associated with warm temperature growth. Moreover, it was shown that H2A.Z-nucleosome occupancy decreases in response to temperature at *HSP70* and a few other genes (Kumar and Wigge, 2010), and heat stress results in a global increase in chromatin accessibility at responsive loci (Sullivan et al., 2014). It is unclear, however, whether these dynamics of H2A.Z nucleosomes reflect a passive process by which the stability of H2A.Z nucleosomes responds directly to temperature or results from increased transcriptional responsiveness of the loci, or a combination of these mechanisms.

The transcriptional activation of heat stress genes in plants is potentially complicated by the high degree of gene duplication. While yeast has a single HSF, *Arabidopsis* for example has 21 HSF family members (von Koskull-Döring et al., 2007). One clade in particular, the HSFA1 group, appears to be important for the early responses to heat stress (Yoshida et al., 2011). Activation of the HSF pathway in *Arabidopsis* is complex, with multiple downstream TFs being involved (Schramm et al., 2008). While the role of HSFA1 TFs in the response to heat stress is well established, it is still not clear whether these factors are involved in the transcriptional response to warm temperature in the ambient range. While H2A.Z nucleosomes have been implicated in regulating the warm-temperature transcriptome, how they interact with TFs and other *cis*-acting factors is not clear.

In this study, we use genome-wide datasets to investigate the dynamics of both nucleosome and TF behavior to determine their contributions to the temperature transcriptome. We found that the day-time warm ambient temperature transcriptome is dependent on HSFA1 TFs that are rapidly and robustly recruited to the promoters of responsive genes, activating their transcription. Moreover, we show that HSFA1a TFs are essential for H2A.Z eviction occurring in response to warm temperature at the responsive genes. Activation of downstream TFs by the HSFA1-class TFs results in a transcriptional cascade that can account for a large proportion of the day-time warm-temperature transcriptome. Genes responding rapidly to warmer temperature display distinctive promoter architecture of heat shock elements (HSEs) and nucleosome positions. We propose that both HSFA1-class TFs and H2A.Z nucleosomes enable a dynamic transcriptional response system to be activated upon passing a threshold temperature.

RESULTS

A Warm-Temperature Transcriptome Defined by the HSFA1 TFs

To analyze the daytime temperature response, we measured the temperature transcriptomes of plants shifted 1 h after dawn from 17°C to 27°C for 0.25, 1, and 4 h (Supplemental Figure 1A). A total of 1035 transcripts show significant changes in these conditions (see Methods), and we refer to these as the "temperature-responsive" transcripts (Supplemental Figure 1B). Hierarchical clustering of these temperature-responsive genes reveals six major patterns of transcriptional response to warmer temperature (Figure 1A, Supplemental Figure 2A, and Supplemental Dataset 1).

Cluster 1 (350 genes, gray sidebar in Figure 1A) contains the genes that are predominantly repressed over time, and is enriched in genes involved in metabolic processes (based on a gene ontology [GO] enrichment analysis) (Eden et al., 2009), which are likely to be transcribed at a lower rate under stresses (more details on GO term analyses are provided in Supplemental Table 1). **Cluster 2** (193 genes, blue) shows partial upregulation after 1 and 4 h at 27°C, but the patterns are similar to those of the 17°C control samples at these time points, suggesting the transcriptional dynamics may be endogenous to the experimental setup and/or related to the circadian rhythm. The transcriptional activation of cluster 2 genes appears to be more rapid at 27°C than 17°C after 1 h (Supplemental Figure 2B). **Cluster 3** (110 genes, cyan) demonstrates partial transcriptional repression but is not significantly enriched for any functional GO term. **Cluster 4** (130 genes, green) demonstrates rapid and transient transcriptional activation at both 17°C and 27°C, followed by repression after 4 h, and is enriched in genes that are involved in stress and defense responses. Both transcriptional activation and repression seems to be faster at 27°C for the genes in this cluster (Supplemental Figure 2B). **Cluster 5** (105 genes, pink) is the smallest cluster with a slight increase in transcriptional level at 27°C, but not enriched in a GO term. **Cluster 6** (147 genes, red) genes are termed "rapidly temperature responsive," as they show maximal expression within 15 min of 27°C treatment. The peak of expression at 15 min is transient, as the expression of these genes returns to near basal levels after 4 h. These genes are highly enriched in biological process GO terms of heat and light responses (Supplemental Table 1), and include genes involved in the response to heat stress such as *HSP70* and other *HSP*s, *HSFA7A* and *DREB2A* (Supplemental Dataset 1). The role of higher temperature in activating these genes is particularly clear when gene expression at 27°C is normalized to that at 17°C (Supplemental Figure 2B). To confirm that these results are a consequence of rapid transcriptional activation, we investigated RNA polymerase II (Pol II) occupancy by chromatin immunoprecipitation sequencing (ChIP-seq). We observe a corresponding increase in the relative amount of Pol II in the gene bodies of the cluster 6 genes in response to ambient temperature increase, which is absent in the control set of all genes (Figure 1B).

The *Arabidopsis* genome encodes 21 HSFs and members of the HSFA1 class are of particular importance in the early responses

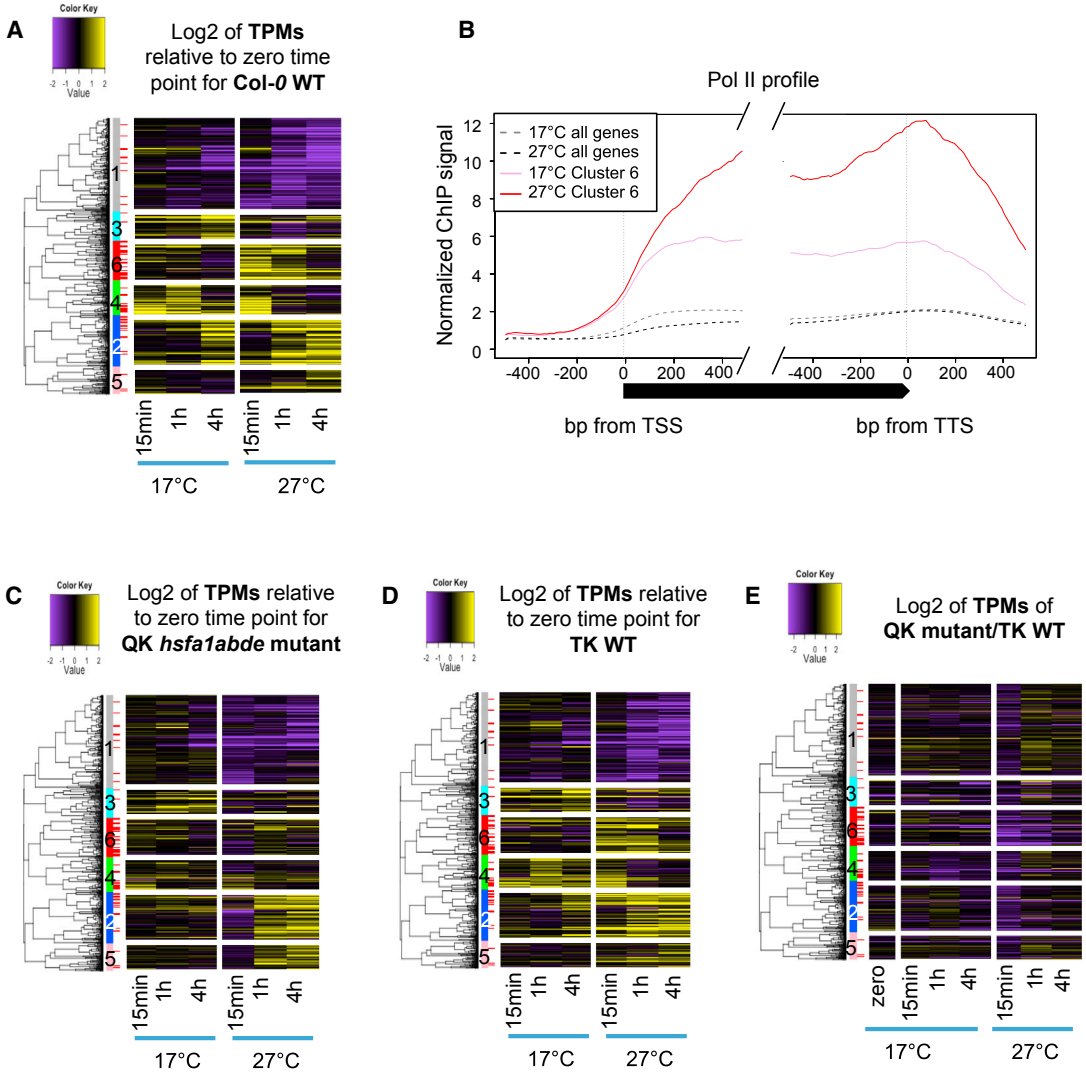

Figure 1. HSFA1 Transcription Factor Family Is Regulating the Transcriptional Response to Ambient Temperature.

(A) Transcriptional patterns and dynamics of temperature-responsive genes (1035 genes) in response to ambient temperature shift (17°C–27°C) in Col-0 WT. The temperature-responsive genes were hierarchically clustered into six groups, based on the $\log_2$ ratio to the zero time point of transcript per million (TPM) values. Upregulated genes are in yellow and downregulated genes are in purple. The first sidebar to the left of the heatmap indicates the six clusters of temperature-responsive genes. The second sidebar indicates target genes of HSFA1a at 27°C based on HSFA1a ChIP-seq performed on seedlings shifted for 15 min from 17°C to 27°C.

(B) Average RNA Pol II occupancy profiles at TSS and TTS of cluster 6 genes (solid) and genome average (dotted) at 17°C (pink for cluster 6, gray for all genes) and after 15 min of shift to 27°C (red for cluster 6, black for all genes).

(C) Transcriptional patterns in the QK *hsfa1abde* mutant. The genes and clusters are in the same order as in **(A)**.

(D) Transcriptional patterns in the TK WT control, keeping the same order of genes and clusters as for Col-0 WT in **(A)**. As the QK *hsfa1abde* mutant genome is a combination of the Ws and Col-0 backgrounds (Liu et al., 2011), we used the TK WT as a control as it was generated at the same time and thus serves as a suitable reference (Liu et al., 2011).

(E) Transcriptional changes between the QK *hsfa1abde* mutant and the TK WT control ($\log_2$(QK/TK) for each time point and temperature shift). The genes and clusters are in the same order as in **(A)**.

to heat, since the *hsfa1abde* quadruple (QK) mutant is more sensitive to mild heat stress (Liu et al., 2011; Yoshida et al., 2011). We therefore investigated whether the QK mutant alters the warm-temperature transcriptome. Consistent with a major role for this HSF clade, there is a global reduction in temperature responsiveness across all clusters in our experiment (Figure 1C and 1E; Supplemental Figure 2C), particularly clearly evident after 15 min of shifting to 27°C. Clusters 2, 4, and 6 are most strongly perturbed, with many transcripts showing little or no temperature responsiveness. Interestingly, more than half the genes in cluster 6 (83/147; 56%) lose temperature responsiveness (the same criteria used to extract temperature-responsive transcript, see Methods) after a 15-min temperature shift in the QK mutant, as compared with its corresponding TK wild-type (WT), which has the same background as QK but with *HSFA1ABDE* activity (Figure 1D). Lower proportions of genes in clusters 2 (59/193; 31%) and 4 (21/130; 16%) become unresponsive to temperature in QK.

HSFA1a targets	No. of genes	No. of HSFA1a target genes	Fisher's exact test P value (vs. all genes)
All genes	27 206	1325	n.a.
Cluster 1	350	39	9.95e−06
Cluster 2	193	44	7.30e−15
Cluster 3	110	7	0.5035
Cluster 4	130	36	1.32e−14
Cluster 5	105	5	1
Cluster 6	147	68	<2.2e−16

Table 1. Enrichment of HSFA1a Targets in the Six Clusters of Temperature-Responsive Genes.
Number of HSFA1a targets in each cluster and Fisher's exact test *P* values are provided.

HSFA1a Binds to Rapidly Responsive Genes and Initiates a Transcriptional Cascade in Response to Warm Temperature

To determine whether the effect of HSFA1a, a representative of the HSFA1 family TFs, on the warm ambient temperature transcriptome is direct, we performed ChIP-seq of HSFA1a on seedlings shifted from 17°C to 27°C for 15 min, and in controls kept at 17°C (Supplemental Figure 1A). We identified 1371 genes that are directly bound by HSFA1a within 15 min of 27°C (Supplemental Dataset 2). Specifically, ~46% of cluster 6 genes are directly bound by HSFA1a, and in clusters 1–5, 17% of genes are bound by HSFA1a, marked in red on the second sidebar of Figure 1A (Fisher's exact *P* value of the overlaps <2.2e−16 for both groups) (Supplemental Figure 3A). By comparison, HSFA1a binds to only ~5% of the genes in *Arabidopsis*. Consistent with HSFA1a binding the promoters of temperature-responsive genes, we observe strong enrichment for predicted HSEs in clusters 2 and 6 (Supplemental Table 2). For cluster 6 genes we found predicted HSEs within 44/84 (~52%) and 17/39 (~45%) of HSFA1a ChIP-seq peaks at 27°C and 17°C, respectively.

While HSFA1a targets are significantly enriched in clusters 1, 2, 4, and 6 (Table 1), HSFA1a signal increases specifically for cluster 6 genes after 15 min of shift from 17°C to 27°C (Figure 2A, Wilcoxon test *P* value <2.2e−16 when comparing cluster 6 genes with genes in clusters 1–5). For rapidly temperature-responsive genes (cluster 6), there is significant signal for HSFA1a at the non-inductive temperature of 17°C at HSEs and over the gene body, and this markedly increases at 27°C (Figure 2B). An increase in HSFA1a occupancy upon temperature increase can be observed in most of cluster 6 genes (Figure 2C, red dots represent predicted HSEs). This increase in HSFA1a occupancy with temperature is not seen in other clusters, including cluster 2, which is also enriched for predicted HSEs (Supplemental Figure 3B–3E).

Consistent with the central role of HSFA1a in shaping the rapid transcriptional responses to warm temperature, there is a positive correlation between changes in transcription and HSFA1a binding occupancy in response to shifting at 27°C only for cluster 6 genes (*P* value from linear model fitting = 1.5e−11, Figure 2D), in line with a previous study showing the major role of HSFA1a in regulating the response to heat stress (Liu et al., 2011). Within cluster 6, ~56% (83/147) of the genes become unresponsive to temperature in the QK mutant. Interestingly, we observe direct binding of HSFA1a to ~58% of these (48/83, Fisher's exact test *P* value <2.2e−16). HSFA1a binding also occurs at ~32% (35/111, *P* = 2.6e−15) of the other temperature-responsive genes that lose responsiveness in the *hsfa1abde* background in clusters 1–5 (Figure 2E). Despite HSEs being found in multiple clusters, the rapid increase in transcript abundance of the cluster 6 genes, accompanied by increased HSFA1a occupancy, is specific for this group of genes. Taken together, these results indicate that the presence of an HSE alone is insufficient to predict rapid gene induction by temperature. We note that the HSFA1a target genes identified here might be underestimated, as the ChIP was performed using an HSFA1a-tagged line in a WT background, and thus might be subjected to interference by the native HSFA1a that could potentially decrease the ChIP signal. We nonetheless observe a strong overlap of ~43% (83/194) between identified HSFA1a targets in all temperature-responsive genes (clusters 1–6) and the genes becoming unresponsive to temperature in the QK mutant.

While HSFA1a only binds directly to ~17% of the temperature-responsive genes outside of cluster 6, we observe that the expression of up to 32% of these genes is perturbed in *hsfa1abde* (Figure 2E). This could be because some targets require HSFA1b, d, or e and cannot be bound by HSFA1a. We think this is unlikely, however, since the HSFA1 clade has a highly conserved DNA binding domain and the HSFA1A knockout mutants are highly redundant (Liu et al., 2011; Yoshida et al., 2011). Furthermore, many of the temperature-responsive genes outside of cluster 6 do not have a clearly identifiable HSE in their promoters. As we show, several of the direct targets of HSFA1a are themselves TFs, so it is plausible that these are part of a transcriptional cascade that activates a broader range of transcriptional targets. We identified nine TFs in cluster 6 that are directly bound by HSFA1a and have previously been implicated in transcriptional responses, particularly to stresses. These HSFA1a targets are two members of the *HFSB2* family (*HSFB2A* and *HFSB2B*), two members of the *HSFA7* family (*HSFA7A* and *HSFA7B*), two closely related myb homeodomain TFs (*EARLY PHYTOCHROME RESPONSE1* [*EPR1*] and At3g10113), and genes encoding the stress-responsive TFs bZIP28, RAP2.4, and DREB2A. We were able to identify potential targets for six of these TFs in all temperature-responsive clusters (HSFB2A, HFSB2B, EPR1, At3g10113, bZIP28, and DREB2A), using data generated by DNA affinity purification coupled with sequencing (DAP-seq) (O'Malley et al., 2016). Our analysis reveals a cascade by which these intermediate TFs are able to transmit the warm-temperature signal to genes in other clusters. This cascade can therefore account for a large proportion of the temperature transcriptome, and likely contributes to the temporal variation in the responses we see (Figure 2F and Supplemental Table 3).

H2A.Z-Nucleosome Signal Transiently Decreases at Rapidly Responsive Genes at 27°C

As H2A.Z nucleosomes at the +1 nucleosome of *HSP70* can be evicted in response to warm temperature (Kumar and Wigge, 2010) we used ChIP-seq of FLAG-tagged HTA11, a broadly expressed H2A.Z gene, to determine whether this is a global

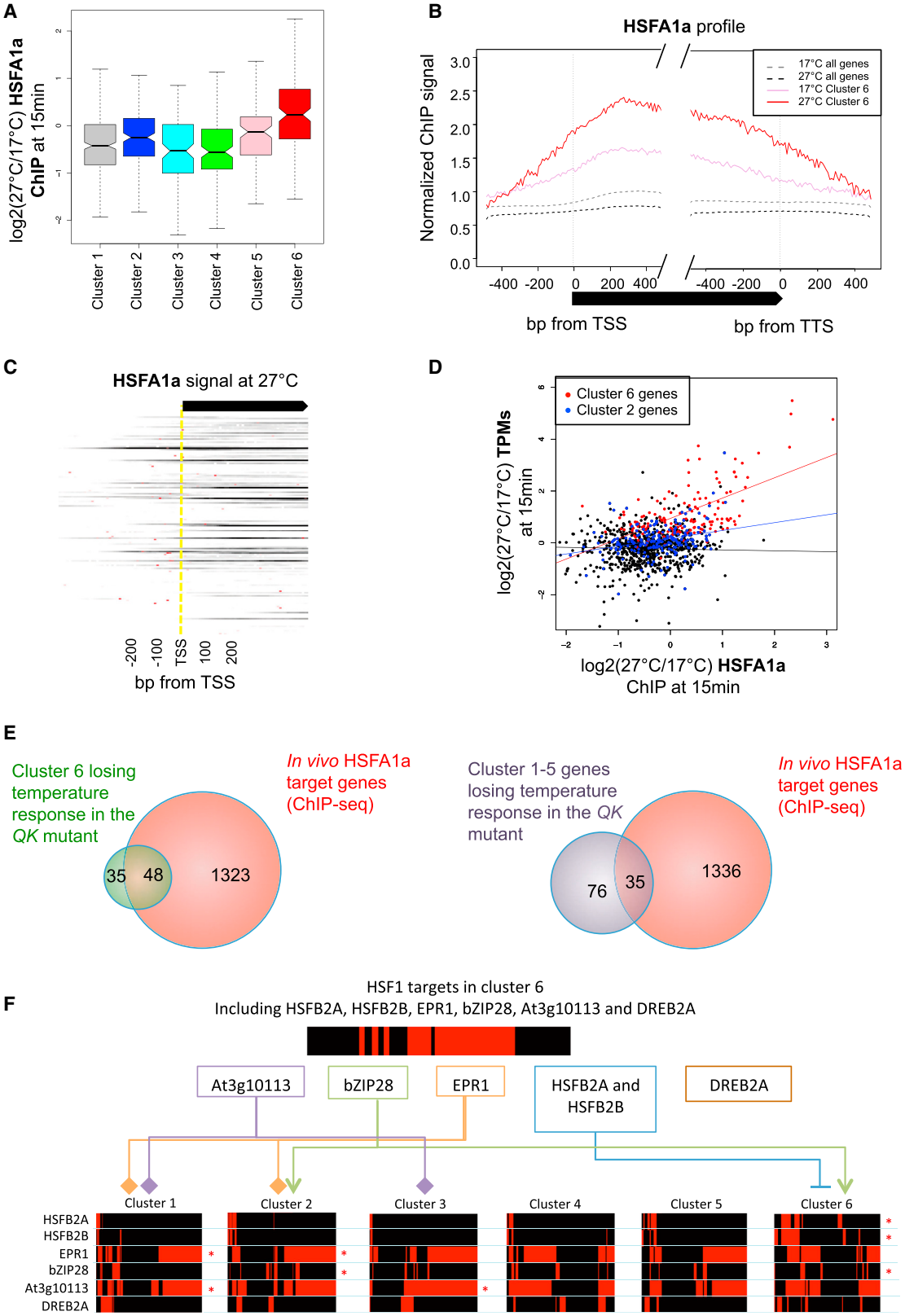

Figure 2. HSFA1a Transcription Factor Binding Is Increasing at 27°C at the Cluster 6 Genes.

(A) Boxplot of changes for HSFA1a signal in the gene body between 17°C and 27°C after 15 min for genes in each temperature-responsive cluster. HSFA1a ChIP signal increases after temperature shift to 27°C specifically for cluster 6 genes (Wilcoxon test *P* value <2.2e−16 when comparing cluster 6 genes with genes in clusters 1–5). Non-overlapping notches indicate significant differences between populations' medians.

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response of temperature-responsive genes. We observe that cluster 6 genes show a rapid loss of HTA11 ChIP signal at the transcriptional start site (TSS) in response to 27°C (Figure 3A). We were able to confirm this dynamic behavior for the occupancy of HTA9, a separate H2A.Z protein, using anti-HTA9 antibodies (Yelagandula et al., 2014), at *HSP70* (Figure 6B and Supplemental Figure 8B), indicating that HTA11 ChIP signal is representative of H2A.Z-nucleosome occupancy for temperature-responsive genes. The decrease of HTA11 ChIP signal on temperature-responsive promoters is dynamic, since we observe signal returning after 1 h, and by 4 h the HTA11 ChIP signal is comparable with the starting levels at 17°C. This depletion of H2A.Z-nucleosome occupancy is not confined to the +1 nucleosome, but also occurs in the gene body and the region surrounding the transcriptional termination site (TTS) (Figure 3B and Supplemental Figure 4A). We also sought to determine whether temperature responsiveness is a general property of H2A.Z nucleosomes or whether this occurs only at specific loci. Interestingly, we see that H2A.Z-nucleosome eviction is only clearly apparent at cluster 6 and not at the other clusters (Figure 3C, Wilcoxon test P value <2.2e−16 when comparing cluster 6 genes with genes in clusters 1–5; Supplemental Figure 4D). We also observed a strong negative correlation between transcription and HTA11 occupancy in response to shifting to 27°C for 15 min (Figure 3D and Supplemental Figure 4B). This negative correlation is specific to cluster 6, as it is not observed elsewhere, even in clusters 2 and 4 (Supplemental Figure 5) where partial increases of transcription levels are observed, together with occurrence of predicted HSEs and HSFA1a binding.

H2A.Z Loss at Temperature-Responsive Genes Is Associated with Increased Chromatin Accessibility

A key question is the extent to which H2A.Z-nucleosome eviction is required for transcriptional activation or is a consequence of it. The occupancy of HTA11 at cluster 6 genes (solid lines, Figure 3E and Supplemental Figure 4C) is markedly higher than that of the genome-wide average at 17°C (dotted lines), but within 15 min at 27°C this drops below the average. Again, this high baseline HTA11 ChIP signal at 17°C for the +1 nucleosome is not observed in clusters 2 and 4 (Supplemental Figure 5C–5F). This trend is also apparent within cluster 6, as we can identify two subgroups based on HTA11 dynamics: 6A, which shows robust decreases in HTA11 ChIP signal, and 6B, where the change in HTA11 is less clear (Figure 3F). Interestingly, genes in 6A are also characterized by higher baseline HTA11 ChIP signal at 17°C compared with both the genome average and subgroup 6B (Figure 3G). While transcripts in both 6A and 6B are activated within 15 min at 27°C, 6A genes show a greater dynamic range with little or no expression at 17°C compared with 6B genes, which show moderate expression at this temperature (Figure 3H). These observations suggest that H2A.Z nucleosomes may act to enhance the transcriptional dynamic range by keeping genes transcriptionally repressed under non-inductive conditions via restriction of promoter and gene accessibility.

We also assessed the influence of H2A.Z nucleosomes on the temperature transcriptome by comparing transcriptional responses of WT plants shifted to 27°C for 15 min with *arp6-1*, which is deficient in H2A.Z incorporation. Interestingly, transcriptional changes due to temperature shift and in *arp6-1* appear to be more correlated in cluster 6, as compared with genome-wide (Pearson correlations, 0.24 for cluster 6 versus 0.12 genome-wide, Supplemental Figure 5G) and with any other cluster (Pearson correlations of 0.004, −0.01, −0.04, 0.05, and −0.06 for clusters 1–5, respectively). This further supports a model whereby H2A.Z nucleosomes create a local chromatin environment refractory to transcription at 17°C for genes in cluster 6, which is diminished when shifted to 27°C or in *arp6-1*.

To measure chromatin accessibility, we investigated the accessibility of chromatin to micrococcal nuclease (MNase) genome-wide using sequencing (MNase-seq) on seedlings subjected to the same temperature-shift regime as described above. Since nucleosomes protect DNA from MNase cleavage, this provides a robust assay of nucleosome accessibility. Genes within cluster 6 are enriched for MNase-protected sequences at 17°C, but these become accessible after shifting to 27°C (Supplemental Figure 6A). This higher protection of the +1 nucleosome at 17°C is less distinct for genes in clusters 2 or 4 (Supplemental Figure 6B). Loss of HTA11 ChIP signal correlates with loss of MNase-sequencing (MNase-seq) signals at the +1 nucleosome position for cluster 6 genes (Supplemental Figure 6C, red

(B) Genome-wide average HSFA1a binding profiles of HSFA1a show a higher HSFA1a occupancy at genes in cluster 6 (solid, pink) compared with at 17°C (dotted, gray). The HSFA1a occupancy markedly increases after 15-min shift to 27°C (solid, red), compared with 17°C (solid, pink).
(C) Gene-by-gene HSFA1a ChIP signal of cluster 6 genes at 27°C. Red dots indicate predicted HSEs from a known consensus motif.
(D) Correlation between changes in transcripts level (TPMs) and HSFA1a signal after 15 min of shift from 17°C to 27°C. A strong positive correlation is observed between increase in transcript level and HSFA1a signal for cluster 6 genes (red), compared with an equal number of random genes from other temperature-responsive gene clusters (black) and cluster 2 genes (blue).
(E) Comparison of *in vivo* HSFA1a targets genes with genes losing temperature response in the *QK hsfa1abde* mutant that are either in cluster 6 (left) or in clusters 1–5 (right). Of all the 147 temperature-rapid-response genes (cluster 6), 83 (~56%) become temperature irresponsive in the *QK* mutant as compared with the TK WT. Temperature responsiveness is defined by $\log_2$(TPM 27°C 15 min/TPM 17°C 15 min) ≥ 0.5, or the TPM 27°C at 15 min is less than 2 (undetectable), while TPM 17°C at 15 min is not. Of these 83 genes, 48 (~58%) are shown to be direct targets of HSFA1a (Fisher's exact test P value <2.2e−16), signifying the crucial role of HSFA1a and other TFs in the HSFA families in transcriptional regulation temperature rapid responsive genes. For other temperature-responsive genes (clusters 1–5), 35 out of 111 (~32%) genes losing temperature responsiveness are direct targets of HSFA1a. Note that there are 1371 genes (~5%) predicted as targets of HSFA1a in the *Arabidopsis* genome.
(F) Representation of the transcriptional cascade in the genes in clusters 1–6 (bottom) by the TFs that are in the cluster 6 and are HSFA1a targets (top). The direct targets of these TFs were determined based on the available DAP-seq dataset (O'Malley et al., 2016) and are represented in red stripes for each cluster (bottom). The linkers and asterisks indicate the temperature-responsive clusters whose members are significantly enriched in target genes of these temperature-responsive TFs. Known positive and negative regulatory relationships are indicated by arrows and blunt arrows, respectively. HSFB2A/B, EPR1, bZIP28, DREB2A, and At3g10113 TFs in cluster 6 are transcriptionally activated by HSFA1a, and they themselves regulate downstream temperature-responsive targets.

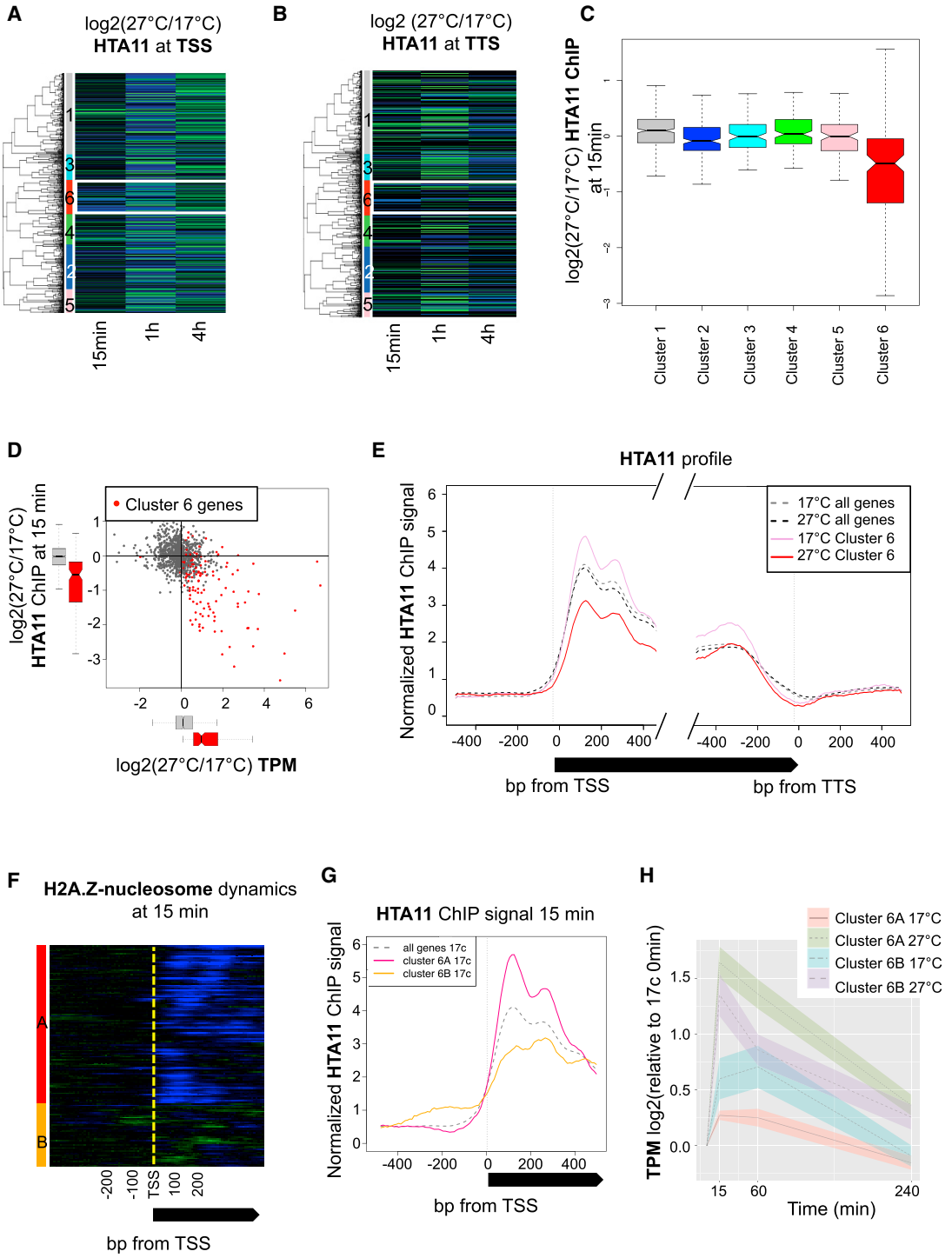

Figure 3. HTA11 ChIP Signal Is Transiently Reduced at Cluster 6 Genes in Response to the Shift to 27°C.

(A) Dynamic profiles of +1 HTA11 ChIP (used to represent H2A.Z nucleosomes) of temperature-responsive genes around TSS (same orders and clusters as in Figure 1A) reveal rapid loss of H2A.Z-nucleosome occupancy among the rapid temperature-responsive genes at 15 min (cluster 6, highlighted by white box). Green represents relative gain and blue represents relative loss of HTA11 ChIP signal between 17°C and 27°C.

(B) Dynamic profiles of HTA11 ChIP signal around the TTS of temperature-responsive genes (same orders as in Figure 1A). A rapid loss of H2A.Z-nucleosome occupancy is observed among the rapid temperature-responsive genes at 15 min (cluster 6, highlighted by white box).

(C) Changes for HTA11 signal at the TSS between 17°C and 27°C after 15 min for all genes in each temperature-responsive gene cluster. A strong reduction of HTA11 signal at 27°C is specifically observed in cluster 6 genes (Wilcoxon test *P* value < .2e−16 when comparing cluster 6 genes with genes in clusters 1–5). Non-overlapping notches indicate significant differences between populations' medians.

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dots) when shifted to 27°C. This suggests that the genomic DNA at these genes becomes more accessible when H2A.Z is evicted compared with the other temperature-responsive genes (Supplemental Figure 6C, black dots). To investigate whether the changes in DNA accessibility are specific to H2A.Z-containing nucleosomes or whether they reflect broader changes in nucleosome dynamics, we investigated how other histones changed under the same temperature shift. H3 occupancy is indistinguishable between cluster 6 genes and the rest of the genome at 17°C and 27°C (Supplemental Figure 6D and 6E) or genes in clusters 2 and 4 (Supplemental Figure 6F). This suggests that the decrease in H2A.Z signal in response to heat does not reflect a general depletion of nucleosomes in response to greater transcription of these loci, but rather the specific exchange of H2A.Z nucleosomes for H2A.

Promoter Architecture of Genes Responding Rapidly to Temperature May Facilitate Transcriptional Activation

We have seen that HSFA1a binds to and activates transcription of a large proportion of the cluster 6 genes, and consistent with this we observe 130 instances of predicted HSEs among these genes, statistically greater than expected by chance (Figure 4A, Fisher's exact test *P* value <2.2e−16). We do not observe this degree of enrichment for the other clusters, highlighting the importance of the HSF class of TF in mediating this response. Furthermore, in the promoters of cluster 6 genes, HSEs appear to be strongly positioned within 200 bp upstream of the TSS, adjacent to the +1 nucleosome that exhibits loss of HTA11 signal after the temperature shift (Figure 4B). This is also true for predicted HSEs in cluster 6 genes with bound HSFA1a (ChIP-filtered HSEs), based on our ChIP-seq experiments. Examining the target genes of HSFA1a, we also observed significant enrichment of HSE occurrences close to the TSS compared with other genes (Figure 4C). In contrast, the distribution of HSEs elsewhere in the genome, or even in cluster 2 where HSEs are also statistically enriched, do not show such a pattern in their distribution (Figure 4D). Consistent with the importance of HSF TFs in this response, we observe specific H2A.Z-nucleosome depletion upon shifting to 27°C for cluster 6 genes with HSEs (red box) when compared with other genes (black) (Wilcoxon test *P* values <2.2e−16) (Figure 4E). This reduction of HTA11 signal is still greater than when compared with other temperature-responsive gene clusters 1–5 (gray) or even cluster 6 genes without HSEs (pink). In addition, 59 of 65 of cluster 6 genes with HSEs show a reduced H2A.Z signal when shifted to 27°C, and in 31 of these genes the H2A.Z signal is reduced by more than half, providing further evidence that the HSEs at close proximity of H2A.Z nucleosomes in cluster 6 genes are strongly associated with H2A.Z reduction (Figure 4F).

Transcriptional Response of the Warm-Temperature Genes Is Triggered by Absolute Temperature

The transcriptional changes we observe could reflect a response to a relative change in temperature or be triggered by crossing an absolute temperature threshold. To distinguish between these possibilities, we generated transcriptomes, in conjunction with HTA11 and HSFA1a ChIP-seq datasets, for seedlings shifted from 17°C to 22°C and from 22°C to 27°C, as well as from 17°C to 37°C. While shifting seedlings from 22°C to 27°C elicits a robust induction of the warm-temperature response (Figure 5A), comparable with the previous shift from 17°C to 27°C, only a very mild change in gene expression is seen for the 17-to-22°C shift (Supplemental Figure 7A). This indicates that absolute temperature plays a pivotal role in determining the transcriptional response, since both these shifts were of the same magnitude (5°C change) but had very different transcriptional outcomes. These results are supported by the HTA11 occupancy behavior, whereby shifting to 27°C had a strong effect but 22°C did not (Supplemental Figure 7A and 7B). Furthermore, increasing the temperature to 37°C causes a strong induction of the cluster 6 genes (Figure 5B and Supplemental Figure 7C), concomitant with a strong increase in HSFA1a signal at the responsive genes (Figure 5C), and a greater loss of HTA11 and MNase signals that are maintained over 4 h (Figure 5D; Supplemental Figure 7D and 7E). Interestingly, the transcriptional induction of *HSP70* in plants shifted from 17°C to 27°C was observed only during the day but not in the dark, whereas *HSP70* induction occurs in both light and darkness when shifted to 37°C (Supplemental Figure 7F). Taken together, these results indicate that there is a key threshold temperature between 22°C and 27°C that must be passed for the induction of cluster 6 genes during the day, and, once this is activated, a further increase in temperature results in a quantitative increase in gene activation.

HSFA1a Binding at Warm Temperature Is Necessary to Promote H2A.Z Loss and Transcriptional Activation

To understand the relationship between HTA11, gene expression, and HSFA1a more clearly, we asked whether HTA11 and/or HSFA1a occupancy are good predictors of transcriptional level among the cluster 6 genes. We see a strong positive correlation between transcription and HSFA1a occupancy ($P = 1.5e{-}11$ for linear model fitting) and a negative relationship between

(D) Negative correlation between HTA11 occupancy at TSS and transcriptional changes (as TPMs) in response to 15-min shift from 17°C to 27°C for all temperature-responsive genes (black), and most prominently in cluster 6 genes (red).

(E) Average HTA11 occupancy profiles among the temperature-responsive genes in cluster 6 (solid), and genome-wide average (dotted). The loss of +1 H2A.Z-containing nucleosomes after 15 min shift to 27°C is observed only in cluster 6 genes (red compared with pink).

(F) Two subgroups of genes can be observed in the cluster 6 based on HTA11 dynamics in the plants shifted from 17°C to 27°C for 15 min: 6A (red), which exhibit a predominant loss of H2A.Z occupancy at the +1 nucleosomes, as well as into the gene bodies, and 6B (orange) without any clear H2A.Z pattern. The yellow line marks transcriptional start site.

(G) Average profiles of H2A.Z-containing nucleosomes of the genes in subclass 6A (pink), is higher than for genes in subclass 6B (orange), and the genome-wide average (dotted black).

(H) Average transcriptional changes of temperature-responsive genes ($\log_2$ ratio of TPMs normalized to the zero time point) for the subclasses 6A (orange for 17°C and green for 27°C) and 6B (blue for 17°C and purple for 27°C) in Col-0 WT, which were previously identified by the reclustering of cluster 6 genes based on HTA11 dynamics. The dynamic of transcriptional activation is stronger in genes of the subclass 6A, notably because of a lower baseline transcriptional level compared with the subclass 6B.

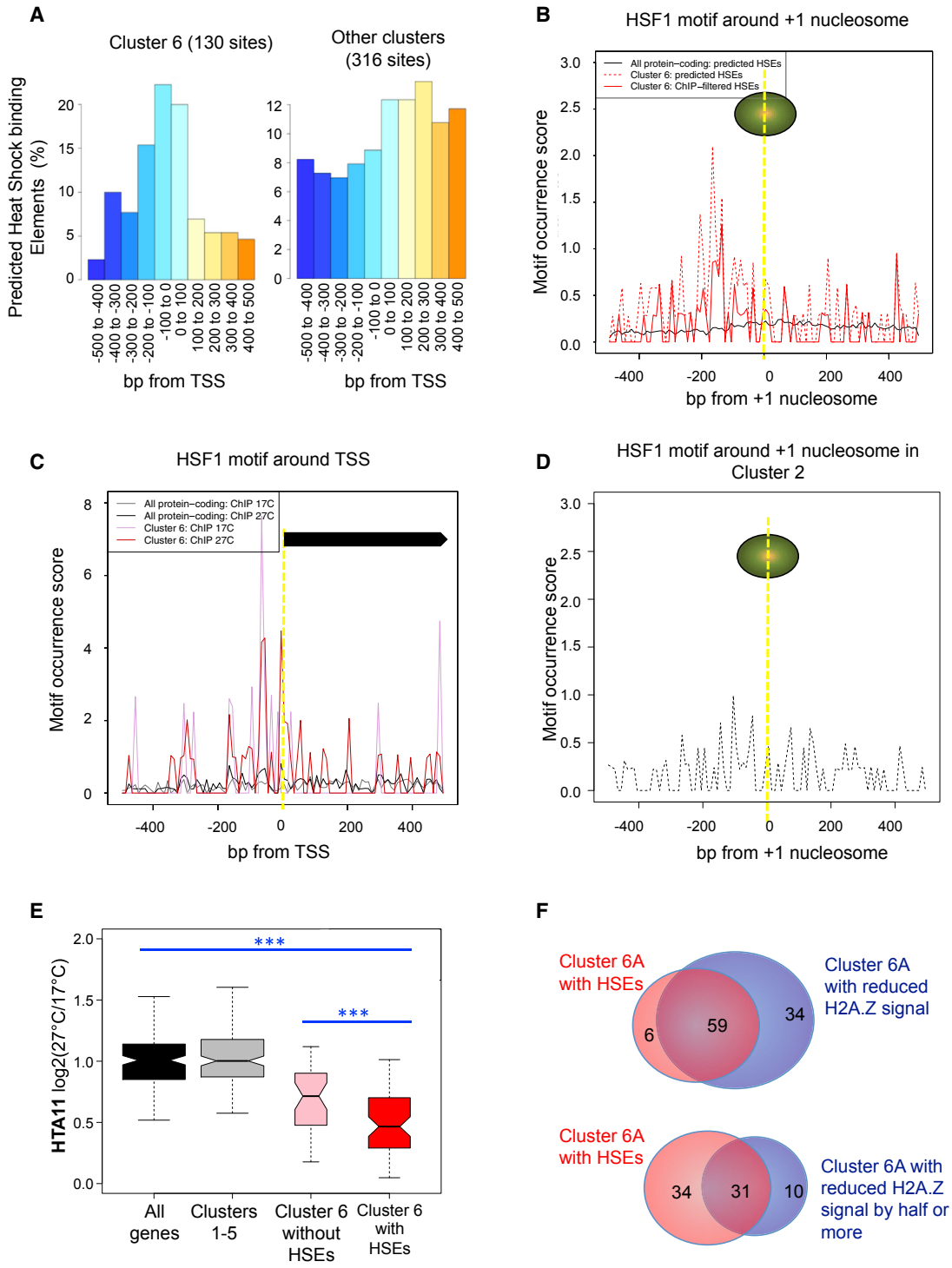

Figure 4. Promoter Architecture of Temperature Rapid-Response Genes May Facilitate Transcriptional Activation.

(A) Predicted heat shock binding elements (HSEs) are specifically enriched in temperature-responsive gene cluster 6, particularly within ±100 bp upstream of TSS (Fisher's exact test *P* value <2.2e−16). The same is not observed in other temperature-responsive genes.

(B) Predicted HSEs (dashed), and those confirmed and filtered by ChIP-seq evidence of HSFA1a from this study (solid), are specifically enriched in rapid temperature-responsive gene cluster 6 (red), just upstream of the +1 nucleosome. The same is not observed in other genes in the genome (black). The yellow line marks the center of +1 nucleosome.

(C) ChIP-filtered HSEs are enriched in temperature-responsive gene cluster 6 around TSS. For the target genes of HSFA1a with ChIP-seq peaks (17°C ChIP in pink and 27°C ChIP in red) within 500 bp from TSS, predicted HSEs are specifically enriched within ±100 bp upstream of TSS, compared with HSEs found in other genes (17°C ChIP in gray and 27°C ChIP in black). The yellow line marks transcriptional start site.

(legend continued on next page)

transcription and HTA11 signal (Supplemental Table 4) (P = 1.1e−9), indicating that both HTA11 and HSFA1a can predict the transcriptional response to 27°C to a certain extent. Interestingly, combining HSFA1a and HTA11 occupancies provides a prediction of gene expression upon shifting from 17°C to 27°C with higher confidence, based on a linear model fitted using transcriptional rate as a response (P = 8.5e−15, Figure 6A and Supplementary Table 4). Within cluster 6, the subclass 6A genes (red dots) demonstrate a greater reduction of H2A.Z signal, gain of HSFA1a, and transcriptional induction as compared with the subclass 6B (orange). Such a correlation is not observed in randomly selected non-temperature-responsive genes (P = 0.38, Supplemental Figure 8A and Supplemental Table 4). These results show that both HSFA1 class TFs and H2A.Z nucleosomes are necessary for rapid transcriptional responses to warmer temperatures, but how they influence each other is not clear.

To investigate whether H2A.Z-nucleosome eviction is mediated by temperature directly or via other factors, we performed *in vitro* ChIP on purified nucleosomes from *Arabidopsis* seedlings grown at 17°C (see Supplemental Figure 8C and Methods). We observe that the H2A.Z nucleosomes at the +1 position near *HSP70* are now not responsive to temperature *in vitro* (Supplemental Figure 8D). In addition, we also looked into the temperature responsiveness of reconstituted H2A.Z nucleosomes *in vitro* using a fluorescence resonance energy transfer (FRET) assay. This allows us to directly assay the behavior of nucleosomes in response to temperature in the absence of other cellular components such as remodelers or TFs. We observe no significant change in DNA dynamics on the nucleosome at a wide range of ambient temperatures (Supplemental Figure 8E–8F).

If H2A.Z nucleosomes are not intrinsically temperature responsive, could HSFA1a be one of the factors that influence DNA binding of H2A.Z nucleosomes in response to temperature shift? To assess the role of HSFA1a on H2A.Z eviction *in vivo*, we performed ChIP–qPCR for HTA9 in the QK *hsfa1abde* mutant and TK WT, in response to temperature shift, at the *HSP70* gene, at *CBF,* which is a positive control gene where H2A.Z signal remains after shift to 27°C, and at AT3G12590, which is a negative control gene where no H2A.Z signal is observed. Interestingly, +1 H2A.Z nucleosome at *HSP70*, as measured by ChIP, is no longer thermoresponsive in *hsfa1abde* (Figure 6B and Supplemental Figure 8B). This indicates that temperature-dependent H2A.Z-nucleosome depletion at induced genes requires HSFA1a.

DISCUSSION

A Dynamic Transcriptional Cascade Protects the Cell from Warm Temperatures

We have constructed a comprehensive dataset of temperature transcriptomes of WT and QK *hsfa1abde* mutant plants subjected to ambient temperature shift at three time points, 0.25, 1, and 4 h, with corresponding genome-wide binding profiles of the TF HSFA1a, Pol II, and histones H2A.Z and H3, in parallel with MNase nucleosome stability assays in consistent conditions (Supplemental Figure 1). We first observe a rapid induction of a core set of transcripts highly enriched for genes encoding cellular protective proteins such as chaperones within 15 min of shift from 17°C to 27°C. The activation of these genes is transient as they show a reduction of transcriptional levels after 1 h and return to the baseline by 4 h. The warm-temperature cellular protection pathway is therefore under tight transcriptional control and is rapidly attenuated when seedlings are maintained at warm temperatures, consistent with previous observations for heat stress (Ohama et al., 2016). Since chaperones function as ATPases, it may be important to control their expression levels for energy homeostasis within the cell. In *Drosophila*, maintaining flies at higher ambient temperature causes a depletion of body fat stores and a decline in fitness (Klepsatel et al., 2016). HSFA1a directly activates multiple activating TFs in cluster 6 genes, as well as the transcriptional repressors *HSFB2A* and *HSFB2*. These TFs may act in a negative feedback loop to fine-tune the response to warm temperature, and genes upregulated in *hsfb1-1/hsfb2b* (Ikeda et al., 2011) are highly enriched in cluster 6 (Fisher's exact test P value <2.2e−16, Supplemental Table 3). This indicates that HSFA1a and HSFB2A/B may act in an incoherent feedforward loop, a common regulatory motif in biology (Milo et al., 2002).

In addition to HSFB2A and HSFB2B, we show that HSFA1a also directly regulates multiple key TFs involved in stress response signaling in response to warm temperature: HSFA7A, DREB2A, RAP2.4, EPR1, and BZIP28 (Sakuma et al., 2006; Gao et al., 2008; Larkindale and Vierling, 2008; Lin et al., 2008; Yoshida et al., 2011; Ohama et al., 2017) (Figure 2F). This is well in line with earlier studies showing that the HSFA1 family members are key regulators of genes involved in response to heat as well as other abiotic stresses such as oxidative, osmotic, and salt stresses (Liu et al., 2011). Strikingly, these TFs that are HSFA1 targets bind to the promoters of multiple genes that are differentially expressed upon the shift to 27°C. It therefore appears that there is a transcriptional cascade from HSFA1a via these additional TFs, and this can partly account for differentially transcribed genes after shifting to 27°C, as previously described for heat stress (Ohama et al., 2016). Consistent with this model, *hsfa1abde* shows a greatly attenuated transcriptional response to 27°C, even for genes that are responsive to temperature but not bound by HSFA1a directly. This transcriptional cascade can help account for the diverse transcriptional responses to daytime warm temperature of genes in cluster 1–5 (Figure 1A) with both activation as well as repression of genes occurring over different timescales. To this end, the direct control of a key set

(D) Predicted HSEs (dashed) around +1 nucleosome for cluster two genes. HSEs are also enriched in this cluster, but without a clear positioning pattern in relation to the +1 nucleosome as in cluster 6 **(B)**. The yellow line marks the center of +1 nucleosome.
(E) Changes for HTA11 signal at the TSS between 17°C and 27°C after 15 min for all genes, genes in clusters 1–5, and genes in cluster 6 with our without HSEs. A strong reduction of HTA11 signal at 27°C is specifically observed in cluster 6 genes with HSEs compared with genes in all other clusters (Wilcoxon test P values <2.2e−16), or even to cluster 6 genes without HSEs (P = 8.0e−4), as highlighted by asterisks.
(F) In the subcluster 6A, comparison of genes with HSEs with genes showing a reduction of H2A.Z signal (top) or a reduction of H2A.Z signal by half or more (bottom). A strong enrichment in genes with HSEs is observed among genes with reduced H2A.Z signal in the subclass 6A.

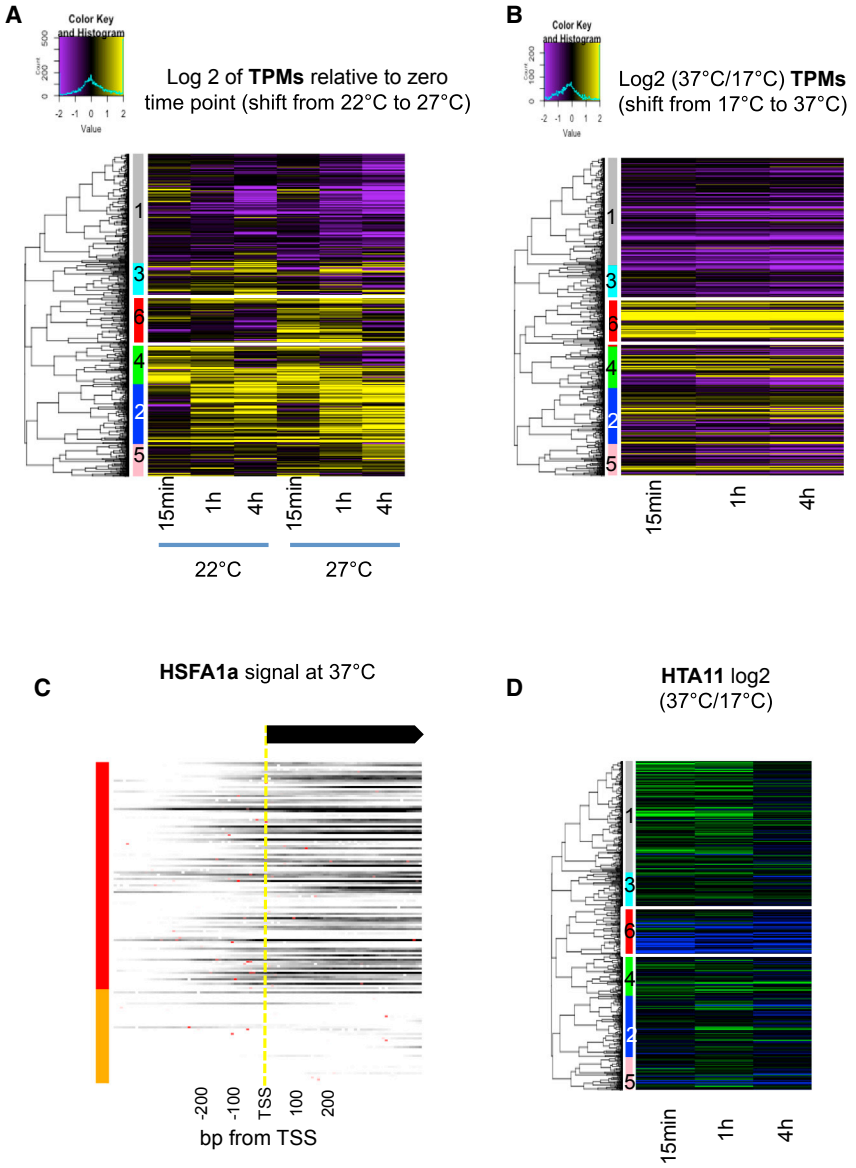

Figure 5. Transcriptional Response to Ambient Warm Temperature and Heat Stress.

(A) Transcriptional patterns in Col-0 WT seedlings shifted from 22°C to 27°C. The clusters are in the same order as in Figure 1A. Transcriptional activation is also observed for genes in cluster 6 (highlighted with white box). Upregulated genes are in yellow and downregulated genes are in purple.

(B) Transcriptional patterns in Col-0 WT seedlings shifted from 12°C to 37°C. Transcriptional activation of the cluster 6 genes is stronger and persists longer when shifted to 37°C as compared with 27°C (highlighted with white box).

(C) Gene-by-gene HSFA1a ChIP signal of cluster 6 genes at 37°C. Further recruitment of HSF1 to the promoters and gene bodies of the cluster 6A genes resulted in elevated HSF1 occupancy at 37°C. Red dots indicate predicted HSEs from a known consensus motif.

(D) Dynamic profiles of HTA11 ChIP signal around the TTS of temperature-responsive genes Col-0 WT shifted from 12°C to 37°C. In plants shifted to 37°C, the H2A.Z eviction for cluster 6 genes (highlighted with white box) is stronger than shifting to 27°C and takes longer to be incorporated back into the nucleosomes (after 4 h). Green represents relative gain and blue represents relative loss of HTA11 ChIP signal between 17°C and 27°C.

of TFs, including HSFB2A, HSFB2A, EPR1, At3g10113, and bZIP28, enables a complex transcriptional outcome in response to a single rapid stimulus. This role of HSFA1 TFs as master regulators of key TFs coordinating responses to heat stress and drought resembles the sequential waves of transcriptional activation observed in responding to the environment (Murray et al., 2004) and during cell differentiation (Boyd et al., 2014).

The Heat Protection Response Has an Activation Threshold above which It Increases Quantitatively

Temperature has diverse influences upon plant growth, development, and survival. An open question is the extent to which these response pathways share common or separate initial temperature-sensing events and downstream transcriptional cascades. In this study we have focused on the response to 27°C during the day, typically not regarded as a heat stress (Balasubramanian et al., 2006). Interestingly, however, much of the transcriptomic response appears to be conserved with higher temperature heat stress responses, suggesting that even 27°C is sufficient to activate these pathways. The key regulators we observe (HSFA1, DREB2A, HSFA7, RAP2.4, BZIP28, MBF1C, and HSFB2) have also previously been shown to be central in high-temperature stress responses (Gao et al., 2008; Larkindale and Vierling, 2008; Lin et al., 2008; Schramm et al., 2008; Ikeda et al., 2011; Liu et al., 2013). The HSFA1 class TFs appear to activate both heat stress and warm ambient temperature pathways during the day, in agreement with the previous observation that *hsfa1abde* is hypersensitive to prolonged exposure to 27°C (Liu and Charng, 2013). In contrast to the heat stress pathway, growth and developmental responses to warm temperature, mediated via the evening complex (Mizuno et al., 2014; Box et al., 2015; Raschke et al., 2015) or thermosensory phytochromes (Jung et al., 2016; Legris et al., 2016), occur predominantly at night and control different target genes (Jung et al., 2016; Ezer et al., 2017).

Our results indicate that an absolute temperature, rather than relative temperature, is important for cluster 6 gene activation and suggest the presence of a threshold between 22°C and 27°C for transcriptional induction of HSFA1a targets. Further experiments would be required to define the exact threshold temperature for cluster 6 gene activation, and this may vary from gene to gene depending on the promoter architecture. Above this activation threshold temperature there is a quantitative

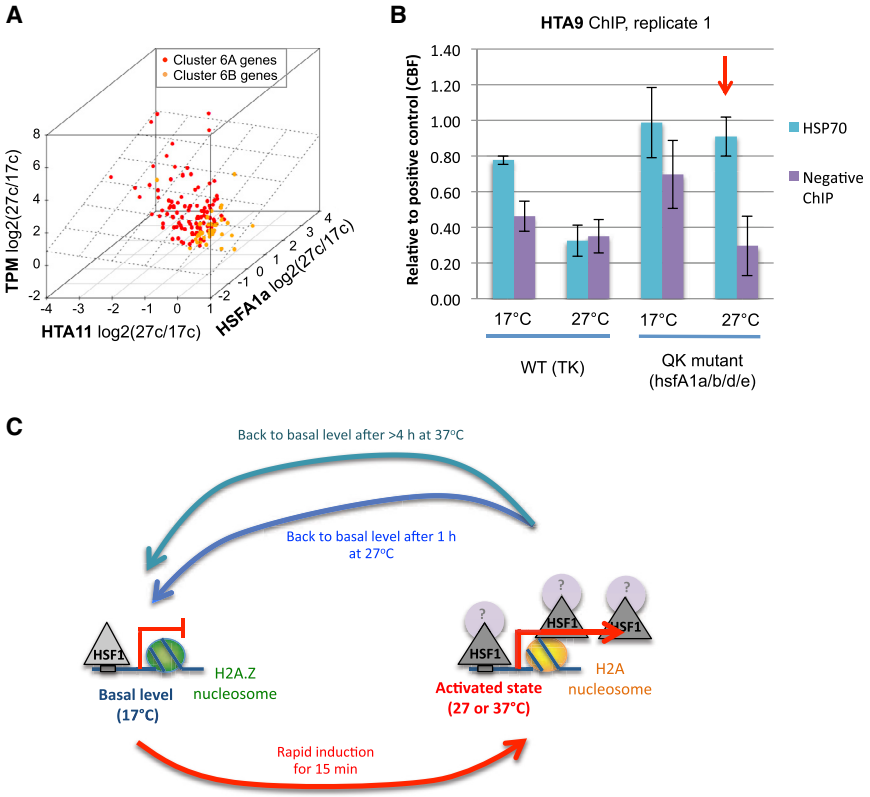

Figure 6. Interplay between HSFA1a- and H2A.Z-Containing Nucleosome in the Transcriptional Response to Temperature at Cluster 6 Genes.

(A) Changes of transcriptional levels (TPMs) among the cluster 6 genes are highly correlated with changes in HSF1 and HTA11 occupancies in response to 15 min of shift from 17°C to 27°C. This is particularly clear in the subclass 6A (red), and less so for 6B (orange).

(B) ChIP–qPCR for HTA9 in the QK *hsfA1a/b/d/e* mutant and TK WT at *HSP70* and AT3G12590, which is a negative control for H2A.Z. The ChIP results are first normalized by the INPUT and then by the positive control (CBF), so that they could be directly compared between samples. H2A.Z eviction at *HSP70* in response to temperature shift is no longer observed in the QK *hsfA1a/b/d/e* mutant at 27°C (red arrow). Error bars represent the SE of three technical replicates. The second biological replicate can be found in Supplemental Figure S8B.

(C) A model of transcriptional regulation of rapidly temperature-responsive genes (cluster 6). At a low ambient growth temperature (17°C), HSFA1a is detectable at the promoters of the rapidly temperature-responsive genes at a low level and at a small fraction of its target genes, insufficient to trigger transcriptional activation in Col-0 WT. Tightly bound H2A.Z nucleosome, especially at the +1 position of these genes, may play a role to help restrict transcriptional activation at lower temperatures. At elevated ambient temperature (27°C), HSF1 is transiently further recruited to HSEs around the TSS as well as to the gene bodies of the targets, an event concomitant with depletion of H2A.Z from these regions, and this is accompanied by robust transcriptional activation. The strong correlation between transcriptional activation, H2A.Z eviction, and HSF1 binding is also observed to a greater extent and for a longer period of time, when plants are subjected to heat stress (37°C).

increase in transcriptional induction with temperature, as seen in further activation of temperature-responsive genes in plants shifted to 37°C. Since HSFA1a is expressed constitutively in the plant, the temperature perception event in this pathway may occur at the level of the HSFA1 protein, consistent with the extensive post-translational modifications of HSFs in response to heat stress in plants (Ohama et al., 2016). HSFA1a binding signal is detectable by ChIP at a number of temperature rapid-response genes at 17°C (Figure 2B and Supplemental Figure 3D), suggesting that the TF may maintain genes in a poised state, ready for activation in response to warm temperature via the recruitment of additional HSFs and/or other components of transcriptional machinery complexes, a scenario which has been previously observed in yeast (Zanton and Pugh, 2006; Shivaswamy and Iyer, 2008).

A Combinatorial Role for H2A.Z and HSF1 to Mediate Temperature-Responsive Gene Expression

The daytime warm-temperature transcriptome is important for cellular survival and is able to be rapidly activated and shut down. While HSFA1 TFs are essential for this response, many genes with HSEs in their promoters are not rapidly activated by temperature, indicating that additional factors might be required to confer rapid and robust temperature-responsive gene expression. Nucleosome architecture at promoters can contribute to the transcriptional responsiveness of gene expression (Lam et al., 2008; Weber et al., 2014), and in this study we observe a strong tendency for temperature-responsive promoters to have well-positioned H2A.Z nucleosomes just downstream of HSEs (Figure 4B). Furthermore, we observe a specific temperature-dependent depletion of H2A.Z-nucleosome signal at these loci at higher temperature. This drop is also associated with an increase in nucleosome accessibility as shown by a reduction of signal in MNase-seq. However, this may not simply reflect a removal of nucleosomes by the transcriptional machinery upon the activation of transcription, because a concomitant drop in H3 is not observed. This is in agreement with a previous observation of an increase in nucleosome accessibility without reduction in nucleosome occupancy during rapid transcriptional activation (Mueller et al., 2017). This specific depletion of H2A.Z nucleosomes in response to temperature requires HSFA1a activity, and does not occur on chromatin *in vitro* or on reconstituted nucleosomes. Further experiments on H2A.Z occupancy changes in response to temperature using drugs blocking transcription may reveal whether H2A.Z eviction is indeed mediated by HSFA1a or is a consequence of transcriptional activation. Here, we also observe strong H2A.Z eviction at genes transcriptionally activated in response to warm temperature, whereas a previous study suggested that H2A.Z eviction might be independent of expression changes (Kumar and Wigge, 2010). This discrepancy could potentially have arisen from differences in temperature-shift regimes (shifting from 12°C to 27°C for 2 or 24 h, as opposed to 17°C–27°C for 15 min in daytime), and a more restricted set of genes being analyzed by ChIP–qPCR (six genes that showed a decreased

or flat transcriptional response to temperature showed some level of H2A.Z decline by ChIP–qPCR).

Being refractory to transcription *in vitro* (Park et al., 2004; Thakar et al., 2010), H2A.Z nucleosomes are well suited for creating inducible transcriptional switches (Figure 6C). At non-permissive conditions, loci can be maintained in a fully repressed state but, upon H2A.Z-nucleosome depletion, loci are fully accessible for rapid expression. In this way, the specific replacement of the H2A.Z-H2B dimer from nucleosomes may provide a particularly rapid and reversible transcriptional activation switch, and may also account for the paradoxical observation that H2A.Z nucleosomes anti-correlate with H3–H4 turnover (Weber et al., 2014). This depletion of H2A.Z at transcriptionally activated genes in response to elevated temperature is in agreement with a recent study demonstrating depletion of H2A.Z at genes transcriptionally activated in response to drought stress and an overrepresentation of genes affected by drought stress among the genes mis-regulated in an H2A.Z-depleted mutant (Sura et al., 2017). This, together with our results, suggests a repressive role of H2A.Z at genes responding to different environmental stresses, possibly by creating a closed chromatin environment refractory to transcription.

The mechanism by which H2A.Z nucleosomes are depleted in response to high temperature may involve additional chromatin remodeling complexes. For example, in *Drosophila*, HSF1 cooperates with the FACT complex to maintain the chromatin in an open state for transcription (Saunders et al., 2003). We observe an increase of HSFA1a signal at the HSEs at warm temperature, just upstream of the +1 nucleosome (Figures 2C and 4B), and the signal appears to extend into the gene body. The presence of HSFA1a around the +1 H2A.Z-containing nucleosome suggests that HSFA1a may recruit RNA Pol II machinery and/or chromatin remodelers to facilitate transcription and H2A.Z eviction, although this cannot yet be confirmed in this study. Indeed, the recruitment of chromatin remodelers and modifiers to the gene promoters by specific TFs appears to be a common theme enabling the intricate regulation of transcriptional rate (Stockinger et al., 2001; Lam et al., 2008; Charoensawan et al., 2012; Teichmann et al., 2012; Wu et al., 2012; Vercruyssen et al., 2014; Zhang et al., 2014; Zhao et al., 2015). We observe a rapid eviction and reinsertion of H2A.Z upon temperature increase, and it would be of interest to further investigate whether the chromatin regulators that have been shown previously to deposit H2A.Z into chromatin are involved in this process. H2A.Z is incorporated into chromatin by the SWR1 chromatin remodeling complex, composed of PIE1, ARP6, and SWC6 in *Arabidopsis* (Noh and Amasino, 2003; Deal et al., 2005; Choi et al., 2007; Lazaro et al., 2008; Zilberman et al., 2008; March-Diaz and Reyes, 2009). The INO80 complex has also been shown to be involved in H2A.Z deposition in yeast (Papamichos-Chronakis et al., 2011). On the contrary, aside from the human chaperone protein ANP32E that was shown to remove H2A.Z from chromatin (Obri et al., 2014), less is known about the H2A.Z eviction mechanism.

The broad pattern of activated HSFA1a we observe spreading across the gene body, beyond the predicted HSEs, is consistent with a role for HSFA1a in activating otherwise quiescent chromatin in a pioneer TF style of action (Fujimoto et al., 2012). It is also worth noting that HSF signal in gene bodies has already been observed for a few genes in mammals and *Drosophila* (Gonsalves et al., 2011; Mahat et al., 2016) and that DAP-seq peaks for HSF TFs have been seen to be enriched in the 5′ UTR and coding sequence of genes (O'Malley et al., 2016). In *Chlamydomonas*, HSF1 plays a key role in preventing gene silencing and maintains nearby loci in a transcriptional responsive state (Strenkert et al., 2013). It will be of interest to determine the temperature-dependent event activating HSFA1a that triggers this large-scale and rapid reprogramming of the transcriptome, as well as the mechanism by which H2A.Z-nucleosome can be rapidly removed and reinserted into chromatin at temperature-responsive loci.

METHODS

Plant Materials and Growth Conditions

We constructed FLAG-tagged HTA11 (AT3G54560) and HSFA1a (AT4G17750) lines under the native promoters, which were used for H2A.Z and HSF1 ChIP experiments. The *HTA11* and *HSFA1a* genomic clones were isolated from Columbia-0 (Col-0), tagged with 3×FLAG epitopes directly upstream to the stop codons. The vectors were transformed into Col-0 WT plants. The QK *hsfA1a/b/d/e* mutant and its corresponding TK WT, as well as an *arp6-1* mutant, were described previously (Deal et al., 2005; Liu et al., 2011). Seedlings were grown at constant 17°C in solid 1× Murashige and Skoog (MS) media in long days for H2A.Z, H3, H2B, and H2A ChIP-seq as well as all the RNA-sequencing (RNA-seq) experiments, and in short days for the HSF1 ChIP-seq. The seedlings were grown through a nylon mesh and for temperature shift, transferred to liquid MS media pre-incubated at 17°C (control), 22°C, 27°C, and 37°C at 1 h after dawn. The plant samples were collected at the shift (0 min) as well as 15 min, 1 h, and 4 h after the shift (Supplemental Figure 1A). For *arp6-1* mutant, seedlings were harvested after 15 min "mock" shift from 17°C in solid media to 17°C in liquid media.

RNA-Seq Library Preparation

Total RNA was isolated from 30 mg of ground seedlings. RNA quality and integrity were assessed on the Agilent 2200 TapeStation. Library preparation was performed using 1 μg of high-integrity total RNA (RIN>8) using the TruSeq Stranded mRNA library preparation kit and TruSeq RNA Library Preparation Kit v2 (Illumina, RS-122-2101 and RS-122-2001). The libraries were sequenced on a HiSeq2000 using paired-end sequencing of 100 bp in length and NextSeq500 using paired-end sequencing of 75 bp in length.

RNA-Seq Mapping and Differential Expression Analysis

The raw reads obtained from the sequencing facilities were analyzed using a combination of publicly available software and in-house scripts described in Supplemental Methods. No mis-match was allowed when mapping using TopHat, except for the QK *hfsa1abde* mutant and its corresponding TK WT for which data were analyzed for reads mapped with no mis-match as well with four mis-matches allowed, to account for potential discrepancies between the reference Col-0 genome, which was also used to map TK WT and QK mutant, which contain parts of Ws genome. The genes whose transcription affected by temperature ("temperature-responsive" genes) were identified using DESeq (Anders and Huber, 2010) through R Bioconductor. Further analyses on these genes were performed using their TPM values (Wagner et al., 2012), whereby different number of reads in libraries and transcript lengths were taken into account and normalized. The TPM values of different samples were normalized by those from the zero time point. Raw reads and process files were deposited in the Gene Expression Omnibus (GEO: GSE79355). Hierarchical clustering of transcriptomic data was performed using the statistical program R (R Development Core Team, 2011). Over-represented biological functions of temperature mis-regulated genes were assessed using GOrilla (Eden et al., 2009).

ChIP and ChIP-Seq Library Preparation

The ChIP experiment was performed as described by Gendrel et al. (2002) with minor modifications described in Supplemental Methods. In-house library preparation was done using the TruSeq ChIP sample preparation kit (Illumina, IP-202-1012) following the manufacturer's instructions. The libraries were sequenced on HiSeq2000 and NextSeq500.

Analyses of ChIP-Seq and Nucleosome Profiles

Sequenced ChIP-seq data were analyzed in house, following the same quality control and pre-processing as in RNA-seq. The read counts mapped to each base pair in each sample were normalized by the sample's genome-wide mappable reads coverage per base pair, and used in the subsequent statistical analyses. The nucleosome and ChIP profiles were binned to generate "pile-up" ChIP profiles for different groups of genes/promoters using in-house R and Perl scripts. Nucleosome positioning and occupancy was determined using DANPOS (Chen et al., 2013). Plus one (+1) nucleosomes were defined as the first nucleosome found downstream from TSS, but not more than 250 bp from TSS. Peaks of HSFA1a ChIP-seq were called using MACS (Zhang et al., 2008).

In Vitro Chromatin Immunoprecipitation

Seedlings expressing gHTA11::HTA11-3×FLAG were grown in liquid 0.5× MS medium supplemented with 1% sucrose and vitamin B for 7 days at 22°C in long days and shifted to 17°C 3 days prior to the material collection. The plant material was collected 1 h after dawn and immediately flash-frozen in liquid nitrogen. Nuclei were purified as previously described (Folta and Kaufman, 2006). The chromatin was digested with MNase to obtain mononucleosomes and an aliquot was taken as "Input before IP." HTA11-3×FLAG containing nucleosomes were purified by immunoprecipitation and divided into five tubes: "Input after IP," "17°C 15 min," "17°C 1 h," "27°C 15 min," and "27°C 1 h" (Supplemental Figure 8C). Input after IP sample was eluted with 100 ng/μl of 3×FLAG in TE buffer. Other samples were incubated at 17°C or 27°C for 15 min or 1 h before washes. The nucleosomes were released with 100 ng/μl 3×FLAG in TE buffer.

In Vitro Nucleosome Stability Analysis by Single-Molecule FRET

A 155-bp DNA template containing a single 601-nucleosome positioning sequence with two fluorophores was generated by PCR. In all DNA constructs the donors and acceptors were separated by 76–81 bp (~24 nm), and positioned so that the FRET pair are close together in space in reconstituted nucleosomes. This way, the signal of the acceptor fluorophore is strong when the nucleosome is closed, and this signal is reduced when the nucleosome is destabilized (Supplemental Figure 8E). Two DNA constructs were generated: one with an FRET pair at the nucleosome extreme to measure DNA breathing (position Z), and the other at 27 bp from one nucleosome end to measure the nucleosome stability (position Y). WT *Arabidopsis thaliana* H2A, H2A.Z, H2B, H3, and H4 histones were expressed in *Escherichia coli* and purified as described previously (Robinson et al., 2008), and reconstituted by refolding an equimolar mixture of each of the four denatured histones by dialysis against a buffer containing 2 M NaCl. The intact histone octamers were fractionated from histone tetramers and hexamers by size-exclusion chromatography as described by Robinson et al. (2008). Two samples containing H2A and H2A.Z nucleosomes were mounted simultaneously on a 2-well culture insert (Ibidi) of two confined chambers. Samples and slides were equilibrated to reach the desired temperature before mounting or after changing the temperature of the setup. Bursts of fluorescence were detected using the method described previously by Buning et al. (2015).

Prediction of Heat Shock Elements, Regulatory Motifs, and Target Genes

Potential regulatory sequences of HSEs were predicted based on the consensus binding motifs using FIMO (Grant et al., 2011), as part of the MEME suite (Bailey et al., 2009). The HSE motifs were analyzed using Protein Binding Microarray by Franco-Zorrilla et al. (2014). Target genes were identified using PeakAnalyzer where their TSS are within 1 kb from the predicted TF binding sites or the peaks of ChIP-seq (Salmon-Divon et al., 2010).

ACCESSION NUMBERS

Sequence data from this article can be found in the Gene Expression Omnibus under accession number GEO: GSE79355.

SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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AUTHOR CONTRIBUTIONS

S.C., V.C., and P.A.W. conceived and designed the research. S.C., V.C., A.B., R.B., C.R., and K.E.J. performed the experiments, under the supervisions of J.v.N., D.R., and P.A.W. S.C. and V.C. analyzed the data. S.C., V.C., and P.A.W. wrote the manuscript, which was proofread and approved by all the authors.

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