H3K27me3 demethylases alter HSP22 and HSP17.6C expression in response to recurring heat in Arabidopsis

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Acclimation to high temperature increases plants’ tolerance of subsequent lethal high temperatures. Although epigenetic regulation of plant gene expression is well studied, how plants maintain a memory of environmental changes over time remains unclear. Here, we show that JUMONJI (JMJ) proteins, demethylases involved in histone H3 lysine 27 trimethylation (H3K27me3), are necessary for Arabidopsis thaliana heat acclimation. Acclimation induces sustained H3K27me3 demethylation at HEAT SHOCK PROTEIN22 (HSP22) and HSP17.6C loci by JMJs, poising the HSP genes for subsequent activation. Upon sensing heat after a 3-day interval, JMJs directly reactivate these HSP genes. Finally, jmj mutants fail to maintain heat memory under fluctuating field temperature conditions. Our findings of an epigenetic memory mechanism involving histone demethylases may have implications for environmental adaptation of field plants.

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The ability to adapt to environmental changes is essential for the survival of plants, which are sessile organisms. Plants "remember" exposure to heat over several days, which affects their responsiveness to subsequent heat exposures. Exposure to moderately elevated temperatures enables plants to acquire thermotolerance to subsequent lethal high temperatures: this phenomenon is known as acquired thermotolerance or heat acclimation. Although epigenetic regulation of plant gene expression is well studied, how plants maintain heat memory over time remains unclear. In Arabidopsis thaliana, HEAT SHOCK TRANSCRIPTION FACTOR A2 (HSFA2) is necessary for the maintenance of acquired thermotolerance, while HSFA1s play important roles in basal thermotolerance that underlie the basis of heat memory. HEAT SHOCK PROTEIN (HSP) genes encode molecular chaperones that protect cellular proteins from denaturation. When high temperature is sensed, HSFA2 levels rise. The transient binding of HSFA2 to HSP genes causes a sustained increase in histone marks, such as H3K27me3 trimethylation (H3K27me3), and the expression of HSP genes.

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Heat acclimation defects in H3K27me3 demethylases prime HSP22 and HSP17.6C activation. Next, we conducted RNA-seq to identify JMJ-dependent targets whose expression changed in response to acclimation and heat shock (Fig. 2a and Supplementary Fig. 5). In general, plants acclimated to a stress often respond to the triggering stress faster, earlier, and/or more strongly than non-acclimatized plants. Thus, we screened for genes that showed no difference in expression immediately after HS in acclimatized wild-type and jmj mutants, but showed significant differences after either 4 or 24 h of HS (Fig. 2a). These analyses identified 142 genes that were differentially expressed in the jmj mutant compared to the wild type (FDR < 0.05) (Fig. 2b, c and Supplementary Data 1 and 2). As expected, stress-related Gene Ontology (GO) terms such as "response to stress" and "response to heat" were significantly enriched among these genes (Fig. 2b and Supplementary Data 3). Figure 2c shows a clustering heatmap of the expression changes observed for these 142 genes in all datasets. To determine which of the 142 candidate genes are up- or downregulated during heat acclimation, we used k-means clustering. An optimal number of six clusters were identified using Pearson correlation based on levels of gene expression observed at 0, 4, and 24 h (Fig. 2c and Supplementary Fig. 6). In all, 62 genes were upregulated and 80 were downregulated in jmj relative to the wild type. Two clusters (1 and 6; 62 genes) comprised genes upregulated in the jmj mutant. Four clusters (2, 3, 4, and 5; 80 genes) comprised genes downregulated in the jmj mutant (Supplementary Fig. 6). Cluster 2 (25 genes) showed a rapid increase in expression after HS in acclimatized wild-type seedlings (Fig. 2d and Supplementary Fig. 6), but this induction was delayed in acclimatized jmj mutants (Fig. 2d). This cluster included eight differentially expressed HSP genes whose products function as molecular chaperones (Fig. 2d, e and Supplementary Data 2). Some of these genes were differentially expressed in jmj mutants grown under HS conditions, but only at later time points (Fig. 2d, e).
H3K27me3 demethylase activity is required for acquired thermotolerance. **a** Schematic representation of the temperature conditions used in this study. The time of phenotyping is indicated with a green dot. Left, normal plant growth conditions. Center, basal thermotolerance conditions (+HS). Right, heat-stress memory conditions (+ACC +HS). **b**–**g** Wild type (**b**) and *jmj30 jmj32 ref6 elf6* quadruple mutants (*jmjq*) (**c**) grown under control conditions. **d**–**g** Wild type (**f**) and *jmjq* (**g**) grown under +HS conditions. Insets show scanning electron microscope images of representative shoot apical meristems. **h** Quantification of survival rate. In all, 10-day-old seedlings grown under the three different temperature conditions were categorized into three groups based on phenotypic severity: green, normal growth; light green, partially damaged; and white, perished. Different letters indicate significant differences. Significance was determined by the χ² test followed by a post-hoc test. **i** Quantification of chlorophyll contents. Sample minimum (lower bar); lower quartile (box); median (middle line); upper quartile (box); and sample maximum (upper bar). Light green dots and white circles represent the chlorophyll contents of individual samples and statistical outliers, respectively. One-way ANOVA test, *p < 1.0 × 10⁻³; **p < 1.0 × 10⁻⁴. Different letters indicate significant differences based on post-hoc Tukey’s HSD test. p-values based on post-hoc Tukey’s HSD test between wild type and *jmjq* mutants are shown. p < 0.05. NS nonsignificant. n = 6.
Among the cluster 2 HSP genes, the small HSP genes HSP22, HSP17.6C, and HSP21 are known for their importance in heat acclimation\textsuperscript{17,18}. To explore the expression dynamics of these small HSP genes during heat acclimation, we performed qRT-PCR. In the acclimatized wild-type plants, HSP22, HSP17.6C, and HSP21 were rapidly induced to high levels after exposure to 20-min ACC at 37 °C is complete (hereinafter referred to as "0 h after ACC"), and were moderately activated within 4 h of HS (Fig. 2f, g and Supplementary Figs. 7 and 8). This relatively moderate and delayed activation of HSP genes upon HS might reflect the time needed to recover from cell damage caused by the HS\textsuperscript{19} (Supplementary Fig. 9). The expression of HSP22, HSP17.6C, and HSP21 following ACC and after 4 h of HS was significantly lower in acclimatized \textit{jmjq} mutants than in acclimatized wild-type seedlings (Fig. 2f, g and Supplementary Figs. 7 and 8). Similar expression patterns of HSP22 gene were observed in heat acclimation-defective mutants, such as \textit{hsfa2}\textsuperscript{9} (Supplementary Fig. 10). In addition, basal thermotolerance-defective \textit{hsfa1a/b/d}\textsuperscript{11} mutants did not show upregulation of HSP genes upon either ACC or HS (Supplementary Fig. 10). The similar HSP expression patterns of the \textit{jmjq} and \textit{hsfa2} mutants support our idea that JMJ demethylases contribute more to heat acclimation than to basal
This is also consistent with our phenotypic observations (Fig. 1) and transcriptome analysis (Fig. 2a–e). Furthermore, the accumulation of HSP22, HSP17.6, and HSP21 proteins was lower in acclimatized jmjq mutants compared to that in the wild type 4h after HS.20,21 (Fig. 2h, i and Supplementary Figs. 11 and 12). These data indicate that JMJs play an important role in the rapid induction of small HSP genes.

Sustained H3K27me3 demethylation and H3K4me3 methylation alter HSP22 and HSP17.6C induction. We hypothesized that acclimation-dependent rapid HSP activation mediated by JMJs was associated with sustained demethylation of H3K27me3. Based on our observation that the acclimatized wild-type plants remembered heat exposure for at least 3 days, we harvested samples from wild-type and jmjq mutant plants 3 days after ACC and from equivalent non-acclimatized control plants and analyzed them by chromatin immunoprecipitation followed by sequencing (ChIP-seq) (Fig. 3a–c). In agreement with a role for JMJs in H3K27me3 removal, >2500 genes were H3K27me3 hyper-methylated in the gene body in jmjq compared to the wild type, irrespective of whether the plants were non-acclimatized or acclimated (Fig. 3b and Supplementary Figs. 13–15). For each condition, 55% of genes identified by RNA-seq as being down-regulated in jmjq also showed H3K27me3 hypermethylation (Fig. 3b and Supplementary Data 4). An overlap that was significantly greater than that expected by chance (p = 6.2 × 10$^{-26}$).

Among eight HSP genes that were downregulated in jmjq mutants, the HSP22 and HSP17.6C loci showed acclimation-dependent removal of H3K27me3 by JMJs (Fig. 3b, c and Supplementary Data 4 and 5). Thus, we mainly focused on these two genes for further analysis.

To understand the histone modification dynamics of these small HSP genes during heat acclimation, ChIP followed by qPCR (ChIP-qPCR) was conducted. In the wild type, H3K27me3 levels at the HSP22 and HSP17.6C loci in the gene body (HSP22-a and HSP17.6C-a, respectively) reduced gradually and kept lower following ACC and HS (Fig. 3c, d (#)). The gradual reduction of H3K27me3 levels at the HSP22-a and HSP17.6C-a between ACC and HS was not observed without ACC (Supplementary Fig. 16).

In the jmjq mutant, however, the H3K27me3 levels after acclimation were significantly higher than those in the wild type (Fig. 3d (*) and Supplementary Fig. 16). This could be due to failure to reestablish H3K27me3 deposition at the TA3 locus (transposable element) negative control loci in the jmjq mutant background. This rescued line had jmj30 expression levels similar to those of the wild type (Supplementary Fig. 20). Consistent with the levels of H3K27me3 and gene expression of HSP22 and HSP17.6C, jmj30 directly bound to these genes in response to ACC and HS (Fig. 3f), whereas jmj30 binding was not observed at the TA3 locus (Supplementary Fig. 20). The highest jmj30 binding peaks were observed in the gene bodies of HSP22-a and HSP17.6C-a (Supplementary Fig. 20), suggesting that the distribution of jmj30-HA overlaps with that of the changes in histone modification over the length of these regions. Ref6 binding peaks at these two loci were also examined. Two independent public Ref6 ChIP-seq data sets2,23 showed no Ref6 binding at HSP22 or HSP17.6C (Supplementary Fig. 21). Furthermore, Ref6 did not bind to these two genes in response to ACC and HS based on ChIP-qPCR. Taken together, our results suggest that jmj30 directly removes H3K27me3 at HSP22 and HSP17.6C in response to heat.

Furthermore, the jmjq mutant had higher H3K27me3 levels at the HSP22-a and HSP17.6C-a than jmj30-2 elf6-3 ref6-1 and jmj32-1 elf6-3 ref6-1 (Supplementary Fig. 18), suggesting that H3K27me3 levels are also regulated by functional redundancy. There was no difference between wild type and jmjq mutants in histone H3 signals at these two loci following ACC and HS (Supplementary Fig. 19), suggesting that it is histone modifications, rather than the locations of histones (or nucleosomes) along the DNA that are affected by acclimation. Hence, JMJs such as JMJ30 and JMJ32 mediate H3K27me3 removal at HSP22 and HSP17.6C in response to ACC at least in part.

The delicate balance between H3K27me3 and H3K4me3 results in a poised transcriptional state.15,22 The levels of the permissive H3K4me3 histone mark at HSP22-a and HSP17.6C-a increased after ACC and HS in wild type, but each gene shows different histone modification patterns (Fig. 3e and Supplementary Data 5 and 6). In the case of H3K4me3 at HSP22-a, the first significant differences between wild type and jmjq were seen just after acclimation (0h after ACC). The HSP22 locus is decorated with H3K4me3 during the phase between ACC and HS, as reported previously.14 Consistent with the high levels of H3K27me3 at HSP22-a in jmjq, H3K4me3 deposition was greatly attenuated in the mutant (see Discussion). On the other hand, H3K4me3 deposition at the HSP17.6C-a locus in wild type was observed just after ACC and after HS and this deposition was not observed in jmjq, suggesting that other permissive histone modification(s) might antagonize H3K27me3 at HSP17.6C to switch on the gene. Furthermore, H3K4me3 levels in the TA3 negative control loci were overall lower than those in the HSP22-a locus regardless of genotype (Supplementary Fig. 17). Thus, JMJs mediate the balance between H3K27me3 and H3K4me3 at HSP22.

We also performed the ChIP assay using the previously described biologically functional JMJ30-HA tagged line in the jmj30 mutant background. This rescued line had jmj30 expression levels similar to those of the wild type (Supplementary Fig. 20). Consistent with the levels of H3K27me3 and gene expression of HSP22 and HSP17.6C, jmj30 directly bound to these genes in response to ACC and HS (Fig. 3f), whereas jmj30 binding was not observed at the TA3 locus (Supplementary Fig. 20). The highest jmj30 binding peaks were observed in the gene bodies of HSP22-a and HSP17.6C-a (Supplementary Fig. 20), suggesting that the distribution of jmj30-HA overlaps with that of the changes in histone modification over the length of these regions. Ref6 binding peaks at these two loci were also examined. Two independent public Ref6 ChIP-seq data sets2,23 showed no Ref6 binding at HSP22 or HSP17.6C (Supplementary Fig. 21). Furthermore, Ref6 did not bind to these two genes in response to ACC and HS based on ChIP-qPCR. Taken together, our results suggest that jmj30 directly removes H3K27me3 at HSP22 and HSP17.6C in response to heat.

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To formulate the dynamics of H3K27me3 and H3K4me3 histone modification by JMJs, we established a mathematical model using the HSP22 data24,25 (Fig. 3g). Recovery from HS was considered to explain the gradual upregulation of HSP22 after HS in acclimatized wild type and jmjq mutants (Fig. 2f and Supplementary Fig. 9). On the basis of our calculations from the model, HSP22 expression is immediately induced upon ACC and gradually induced after HS (Fig. 3h). Furthermore, the model predicted that HSP22 expression after HS would be lower in acclimatized jmjq mutants than in acclimatized wild type plants (Fig. 3h). Consistent with the biochemical function of JMJs, a comparison of the best-fit parameters of the wild type and the
Fig. 3 JUMONJI demethylases remove H3K27me3 histone modifications from key heat-memory genes in response to heat. a Schematic representation of the temperature conditions used. Colored dots indicate the times of different assays: ChiP-seq, red; ChIP-qPCR, gray. Left, basal tolerotolerance condition (+H5); right, heat-stress memory condition (+ACC +H5). b Venn diagram showing the overlaps between genes downregulated in jmjq mutants and genes with elevated H3K27me3 in the mutant, with and without acclimation (p = 6.2 × 10^-26 for elevated H3K27me3). c H3K27me3 levels by ChiP-seq along HSP gene regions in the wild type and jmjq, without and with acclimation. HSP22-a and HSP17.6C-a indicate the ChiP amplicons used in the ChiP assays. d–f H3K27me3 (d) and H3K4me3 (e) levels in the wild type and jmjq, and JMJ30-HA (f) levels in the wild type and jmjq, as determined by ChIP-qPCR. Gray dots represent the levels of individual samples. A two-tailed Student’s t test compared to the wild type before ACC, *p < 0.05. A two-tailed Student’s t test comparing wild type and jmjq mutants at the same time point, **p < 0.01. NS not significant. g Diagram of the mathematical model describing the histone modification process. h The profile of HSP22 expression in the wild type and jmjq predicted by the model under the conditions shown in Fig. 3a. Experimental data from the wild type (crosses) and jmjq (diamonds) are also plotted. i, j Wild type (i) and hsp22 hsp17.6C mutants (j) grown under +ACC +H5 conditions. k Quantification of chlorophyll contents. Sample minimum (lower bar); lower quartile (box); median (middle line); upper quartile (box); and sample maximum (upper bar). One-way ANOVA test; *p < 0.05. Different letters indicate significant differences based on a post-hoc Tukey’s HSD test (p < 0.05). NS nonsignificant. n > 7.

jmjq mutant indicated that the jmjq mutant transitioned from the repressively silenced state to a less severe state (V_{0–L–U}) at a lower rate (Fig. 3h and Supplementary Note 1). The model highlighted the importance of the presence of multiple modified nucleosomes for lengthening the duration of the memory effect (Supplementary Fig. 22).

To address the functions of HSP22 and HSP17.6C during heat acclimation, double mutants were generated. Genetic analysis revealed that hsp2218 hsp17.6C double mutants exhibited reduced heat-acclimation ability in terms of chlorophyll content and fresh weight, although hsp17.6C could be a weak allele (Fig. 3i–k and Supplementary Fig. 23). Unlike the jmjq mutants, acclimated hsp22 hsp17.6C double mutants had the same survival rate as acclimatized wild-type plants (Supplementary Fig. 24). The heat-acclimation defects in hsp22 hsp17.6C double mutants were less severe than those in jmjq mutants, the conferral of gene memory by these two genes accounts for only a part of the phenotypic effect of jmjq mutants. Ectopic expression of HSP17.6C in the jmjq mutant led to increased heat tolerance (Supplementary Fig. 24), similar to the effect of HSP22 overexpression.18 These results indicated that HSP22 and HSP17.6C function to confer heat tolerance.

JMJ30 induction prior to acclimation results in sustained H3K27me3 demethylation. To investigate whether the timing of demethylation activity is important for heat acclimation, we introduced a JMJ30 transgene with an estradiol-inducible promoter into the jmjq mutant background26 (Fig. 4a). Upon application of estradiol, JMJ30 expression was immediately induced to high levels. A 100-fold increase in JMJ30 transcript abundance was detected at 0 h after ACC, suggesting that JMJ30 is present immediately after induction (Supplementary Fig. 25).

Induction of JMJ30 before ACC (①) rescued the jmjq mutant phenotype, whereas induction just before HS (②) did not (Fig. 4b, c and Supplementary Fig. 26). jmjq mutants with JMJ30 induced prior to ACC contained significantly more chlorophyll than jmjq mutants with JMJ30 induced prior to HS (Fig. 4c). These results imply that heat memory requires JMJ30 activity prior to acclimation.

We next used RNA-seq to compare the changes in gene expression in jmjq mutants with JMJ30 induced prior to ACC versus HS. The identification of differentially expressed genes revealed that similar gene pathways were affected in JMJ30-induced plants prior to ACC and in the jmjq mutant (Supplementary Fig. 27 and Supplementary Data 7 and 8). The overlap between these two datasets was significantly larger than expected by chance (p = 3.0 × 10^-18) (Supplementary Fig. 27). As expected, heat acclimation-related genes, including seven out of the eight HSP genes downregulated in jmjq mutants, were differentially expressed after 4 h of HS when JMJ30 was significantly induced before ACC, based on RNA-seq (Fig. 4d, Supplementary Fig. 27, and Supplementary Data 7 and 8). Time-course qRT-PCR revealed that induction of JMJ30 expression prior to ACC enables jmjq mutants to sustain HSP22, HSP17.6C, and HSP21 expression until at least from 4 h after ACC (Fig. 4e and Supplementary Fig. 28). Furthermore, expression of the three HSP genes was activated within 4 h after HS in jmjq mutants with JMJ30 induced prior to ACC (Fig. 4e and Supplementary Fig. 28). This activation was significantly delayed in jmjq mutants with JMJ30 induced just before HS (Fig. 4e and Supplementary Fig. 28). In addition to an increase in chlorophyll content, higher HSP expression was also observed in jmjq plants with JMJ30 induced prior to ACC than in the wild type, suggesting that JMJ30 activity in induced plants is higher than that of the endogenous JMJ30 in the wild type (Supplementary Fig. 29). Nevertheless, JMJ30 induction before ACC was responsible for the JMJ-regulated small HSP gene expression in response to HS.

To gain more insight into the epigenetic basis of JMJ30 induction, we examined histone modifications by ChIP-qPCR. When the jmjq mutant carrying pER8:JM30 was treated with β-estradiol prior to ACC, we detected significant downregulation of H3K27me3 levels at HSP22-a and HSP17.6C-a initially after 72 h of acclimation (Fig. 4f). No significant differences in H3K27me3 levels at the TA3 locus were observed in jmjq mutants with JMJ30 induced prior to ACC or HS (Supplementary Fig. 30). Taken together, these results demonstrate that JMJ30 induction before ACC mainly functions to maintain proper H3K27me3 levels between ACC and HS for subsequent induction of HSP transcription upon heat stress.

H3K27me3 demethylases control heat acclimation under fluctuating temperature field conditions. To address the key function of JMJs in heat acclimation under natural conditions, we grew the wild type and the jmjq mutant under fluctuating temperature conditions based on actual meteorological data from five Japanese cities at different latitudes27 (Fig. 5a, b and Supplementary Data 9). Four-day-old seedlings grown in the laboratory at 22 °C were transferred to fluctuating temperature conditions, which included at least two temperature spikes to >30 °C separated by a 2-day gap. Under these fluctuating conditions, we referred to the first spike >30 °C as ACC and to the second spike as HS (Fig. 5b). Under all five of the fluctuating temperature conditions, the jmjq mutant contained significantly less chlorophyll than the wild type (Fig. 5c).

To examine the molecular basis of JMJ function in the response to naturally fluctuating temperatures, RNA-seq was performed 4 h after the second heat spike >30 °C in the treatment that mimicked temperatures in the city of Nara (Fig. 5b circle). The transcript levels of JMJ-regulated HSP genes, including HSP17.6C, HSP17.6II, HSP21, HSP22, and HSP23.6-MITO, were
all reduced in jmjq mutants grown under the Nara temperature conditions, similar to mutants grown under the standard laboratory conditions. By contrast, all five genes were significantly induced when JMJ30 expression was induced in the mutant before ACC (Fig. 5d, e, Supplementary Fig. 31, and Supplementary Data 10–12).

To further understand epigenetic control of gene expression under natural temperature conditions, we examined the effect of JMJs on gene expression and histone modification. qRT-PCR detected high temperature-dependent HSP22 and HSP17.6C transcript fluctuations in the wild type grown under the Nara conditions (6, 7, 9, and 10 Aug 2017 at 13:00) (Fig. 5f).
upregulation was significantly attenuated in the jmjq mutant (Fig. 5f). The stochastic model predicted that HSP22 gene expression would be reduced after heat stress in acclimated jmjq mutants under the Nara temperature conditions (Fig. 5g). Furthermore, JM30 directly bound to HSP22 and HSP17.6C genes in response to heat (6, 7, 9, and 10 Aug 2017 at 13:00) (Fig. 5h), whereas JM30 binding was not observed at the TA3 locus (Supplementary Fig. 32). This fluctuation of JM30-HA expression could be partially due to stabilization of JM30 mRNA (Supplementary Fig. 33).

To address the contribution of HSP22 and HSP17.6C to JM downstream effects under fluctuating field conditions, we examined the phenotype of hsp22 hsp17.6c double mutant plants. The double mutants exhibited reduced chlorophyll contents relative to the wild type under the Nara conditions, but not under the control conditions (Fig. 5i). However, this reduction was less significant than the reduction observed for hsp22 hsp17.6c double mutants grown under laboratory conditions (Figs. 3k and 5i). This difference in the severity of the phenotype could be due to differences in the activity of other HSP or heat stress-induced genes. Indeed, many other HSP or heat stress-induced genes were differentially expressed in jmjq mutants. In addition to the five small HSPs studied here, many other small HSPs were also differentially expressed in acclimated jmjq mutants under the Nara conditions, based on RNA-seq (Fig. 5e). qRT-PCR also revealed that a handful of heat response genes showed expression patterns similar to those of HSP22 and HSP17.6C in wild-type plants grown under the Nara conditions (Supplementary Fig. 34).

Furthermore, the expression levels of HSP18.2 and HSP2A genes were significantly decreased in jmjq mutants grown under the Nara conditions (Supplementary Fig. 34). Among those genes, JM30 directly bound to the gene body of the HSP18.2 gene after heat stress (Supplementary Figs. 32). These results suggest that other condition-dependent gene(s) in addition to HSP22 and HSP17.6C may promote heat acclimation under fluctuating field temperature conditions.

To examine further the role of JMJs in heat memory, wild-type and jmjq mutant plants were grown with or without ACC under fluctuating temperature conditions prior to gene expression and JM30 binding assays (Fig. 6a). qRT-PCR confirmed that acclimatized jmjq mutants had lower levels of HSP22, HSP17.6C, and HSP21 expression 4 h after exposure to HS under the Nara conditions compared to the wild type (9 Aug 2017 at 13:00). By contrast, there was no difference between the wild type and jmjq mutants in the expression of the same genes immediately after HS under the same conditions (9 Aug 2017 at 18:00) (Fig. 6a, b and Supplementary Fig. 35). Furthermore, upregulation of HSP22, HSP17.6, and HSP21 was significantly compromised in the absence of ACC between 5 Aug and 9 Aug (Fig. 6a, b). These results suggest that JM-mediated transcriptional changes are required at least in part to remember recurring heat, even under natural conditions.

To further test the hypothesis that histone modifications underlie the heat memory-related transcriptional changes, H3K27me3 levels at HSP22 and HSP17.6C were examined by ChIP-qPCR. Without ACC between 5 Aug and 9 Aug, we observed no differences in the H3K27me3 levels at HSP22 and HSP17.6C between 8-day-old wild-type and jmjq mutant plants grown under control conditions. Before HS under the Nara temperature conditions, the jmjq mutant had higher levels of H3K27me3 at HSP17.6C and HSP22 than the wild type (Fig. 6a, c). Because these two datasets were obtained from tissues at the same developmental stage, the changes in histone modifications were largely caused by the stress memory rather than developmental effects. H3K4me3 levels at HSP17.6C were not affected by ACC (Supplementary Fig. 36). Unlike under laboratory conditions, the wild type had higher levels of H3K4me3 at HSP22 than the jmjq mutant before HS under the Nara conditions (Supplementary Fig. 36). Under this condition, the accumulation of H3K4me3 at HSP22 in the wild type could be long lasting and this could be partially due to higher HSP2A expression, as reported previously. H3 levels were unaffected (Supplementary Fig. 37). Thus, the epigenetic memory implemented by H3K27me3 demethylases might facilitate environmental adaptation under natural conditions, although additional pathways probably also exist.

Discussion
In this study, we have uncovered a molecular mechanism for heat memory in *A. thaliana* based on the modulation of H3K27me3 histone modification at small *HSP* genes by JMJs. Our results suggest that H3K27me3 demethylases specifically control the level of histone modification at HSP22 and HSP17.6C genes by altering their reinduction to maintain heat memory. In response to ACC, JMJs contribute to induce faster HSP22 and HSP17.6C expression after HS. Five pieces of evidence support this. First, the jmjq mutant showed a reduced ability to remember heat. Second, the expression of HSP22 and HSP17.6C was significantly delayed in jmjq mutants than in the wild type in response to heat. Third, the level of H3K27me3 was significantly higher in the jmjq mutant than in the wild type, due to the failure to remove H3K27me3 in the mutant. Fourth, JM30 directly bound to the HSP22 and HSP17.6C loci in response to heat. Fifth, induction of JM30 in the jmjq mutant partially rescued not only its acclimation defects, but also its changes in gene expression and histone modification. Therefore, the maintenance of H3K27me3 at HSP22 and HSP17.6C by JMJs is likely to render these genes more sensitive to induction by subsequent heat (Fig. 6d). The proper removal of H3K27me3 at HSP22 and HSP17.6C through JMJs is
required for their repression during the memory phase (i.e., between ACC and HS). While ectopic expression of JMJD30 were able to remove H3K27me3, triple mutant did not have excess H3K27me3 and corresponding phenotypes (Figs. 1, 4 and Supplementary Fig. 18). Since, hyper trimethylation of H3K27me3 was observed in jmjq mutant (Fig.3 and Supplementary Fig. 18), this could be due to functional redundancy. To understand specific contribution of any of individual JMJs, genome-wide identification of JMJD30, JMJD32, REF6, and ELF6 targets during heat acclimation is required. Some genes are known to exhibit elevated expression levels between ACC and HS for heat acclimation2. But, our selection criteria is not designed for screening out such genes. Although a large set of stress-inducible genes are hypertrimethylated in jmjq compared to the wild type without heat stress (Fig. 3b), the role of those genes has not been characterized yet. To fully understand the role of JMJs on heat acclimation, further analysis is needed.

A previous report16 suggested that expression of the REF6 is regulated by heat under prolonged high temperatures, but we detected no REF6 binding at HSP genes during heat acclimation under our heat acclimation conditions. Different H3K27me3 demethylases may accumulate depending on the temperature and duration of the heat stress. Our findings that HSP genes were still induced in jmjq mutants in response to recurring heat and that
acclimation improved the ability of the mutant to survive heat stress suggested that additional, redundant H3K27me3 demethylase(s), such as JM113, and/or parallel pathway(s) contribute to heat acclimation. Furthermore, H3K27me3 levels are determined by the balance between the activities of histone demethylases and methyltransferases acting on H3K27. Although the Polycomb Group-mediated H3K27me3 methylation plays a critical role in plant growth and development\(^2\), nothing is known about the function of these methyltransferases during heat acclimation. In jmjq mutants, H3K27me3 levels at HSP22 and HSP17.6C were significantly increased within 24 h after ACC (Fig. 3d). The active removal of H3K27me3 from the gene body by JMJs is important for the activation of Polycomb-silenced genes during the developmental transition or in response to the internal or external stimuli\(^8\). Also, the plant H3K27me3 demethylases function not only delimiting Polycomb-silenced chromatin regions but also preventing the uncontrolled spreading of the epigenetic silencing\(^8\). During the memory phase, such mechanisms could be important for the proper H3K27me3 maintenance. A better understanding of the interactions between methyltransferases and demethylases is an important goal for future studies to uncover the molecular mechanisms underlying heat memory-mediated modulation of H3K27me3.

As expected, based on numerous previous studies\(^15\),\(^22\), there was a negative correlation between H3K27me3 and H3K4me3 levels at HSP22 throughout the memory phase, which is also consistent with a requirement of the mathematical model established for HSP22. In jmjq mutants, H3K4me3 at HSP22 were not accumulated. Our result suggests that the H3K27me3 removal and H3K4me3 deposition may be interconnected. A previous report showed that HSFA2 was essential for H3K4me3 accumulation at the HSP22 locus after ACC\(^14\). During the plant development, transcription factors act as a docking point for the different histone modification enzymes\(^30\). Such a mechanism might lead to the incorporation of the H3K27me3 removal and the H3K4me3 deposition to render the HSP22 gene more sensitive to induction by subsequent heat.

In the case of HSP17.6C, however, H3K4me3 levels increased in concert with gene expression only after heat exposure. This result suggests that deposition of permissive histone modification (s) other than H3K4me3 at HSP17.6C counteracts the inhibitory effects of H3K27me3. Other research has found no H3K4me3 accumulation at HSP70 after heat stress regardless of the transcriptional activity of this gene\(^14\),\(^31\). Therefore, it is likely that H3K4me3 enrichment does not always accompany transcriptional activation of HSP genes. The timing of deposition varies with different permissive histone modifications\(^32\), and their combinatorial effects on epigenetic modifications and the associated factors that bind neighboring chromatin sequences could be different for different genes. Thus, time-courses and comprehensive genome-wide analyses of histone modifications will be needed to better understand the interactions between histone modifications and their effects on gene expression.

In a parallel approach, genetic experiments using higher-order hsp mutants indicate that HSP22 and HSP17.6C play important roles during heat acclimation in A. thaliana. Based on differences in the severity of the phenotypes of the jmjq mutant and the hsp22 hsp17.6C double mutant, we conclude that HSP22 and HSP17.6C, along with additional genes or mechanisms, contribute to increased survival after heat acclimation. The additional genes might include the other differentially expressed HSP genes we identified in this study. Additional analyses of different histone modifications (other than H3K27me3) and changes in chromatin structure at multiple time points will reveal whether these candidate genes play regulatory epigenetic roles in enhancing plant survival. Furthermore, plants have evolved many acclimation strategies, including memory-based strategies involving proteins and metabolites\(^33\),\(^34\). To completely understand the heat acclimation phenotype of the jmjq mutant will require additional, less chromatin-biased analyses at multiple levels.

Interestingly, orthologs of key heat-memory genes, such as Ahg474482 (HSP17.6C), Ahg489869 (HSP22), and Ahg945273 (HSP21), were highly expressed in field-grown Arabidopsis halleri during the summer\(^35\). These results suggest that these demethylases play a key role in heat acclimation and HSP regulation in the field. Notably, more genes, such as HSP18.2 and HSFA2, were differentially expressed in jmjq mutants under fluctuating field temperature conditions than under laboratory conditions. Furthermore, the differences in gene expression and histone modification between the wild type and the jmjq mutant were larger under fluctuating field temperature conditions than they were under laboratory conditions. One explanation of this difference could be the previously reported stabilization of JM130 mRNA and JM130 proteins by heat\(^4\). However, differentially expressed genes between jmjq mutants under fluctuating field temperature and JM130-induced lines also are different, suggesting that a threshold mechanism of all-or-none transcriptional target activation by JM130 proteins is not the only explanation. In other words, additional regulatory mechanisms or pathways other than JM130 stabilization probably exist. Fluctuating field temperature-regulated associated co-activator(s) might allow JM complexes to bind different DNA binding sites and regulate additional target genes.

Further support for our findings comes from a recent study of the acquisition of freezing tolerance in response to rapid versus
gradual decreases in temperature\textsuperscript{36}. Plants might have evolved more than one system to respond to and remember different rates of temperature change. Together with H3K27me3, other histone modifications or epigenetic changes, such as H3K36me3, DNA methylation, and chromatin structure alterations, might also contribute to heat-stress memory\textsuperscript{21,23,37}. Stress-induced nucleosome remodeling mediated by the FORGETTER (FGT) complex might be involved in this regulation, since direct targets of FGT, such as HSP18.2 and HSA32, were identified as differentially expressed genes in \textit{jmjq} mutants only under fluctuating field temperature (Fig. 5e). In addition to co-activators, epigenetic modifications and associated factors that bind neighboring chromatin sequences might contribute to additional target regulation. Whether histone modification and nucleosome abundance at memory genes act together or independently remains to be established. Alternatively, H3K27me3 demethylases might remove repressive histone marks repeatedly in response to each and every recurring heat stress under fluctuating field temperature conditions. Moreover, these two possibilities are not mutually exclusive. Our findings, coupled with potential future discoveries, will further elucidate the mechanisms of plant adaptation and memory.

**Methods**

**Plant materials and growth conditions.** \textit{Arabidopsis thaliana} plants were grown at 22 °C under continuous light unless otherwise specified. Culture medium consisted of half-strength Murashige and Skoog (MS) salts (Nacalai Tesque) and 0.8% agar (Nacalai Tesque) at pH 5.6 was used for all experiments except for crossing and transformation. For mature plant materials, seeds were sown in pots containing vermiculite and Metro-Mix. The following plant lines were previously described: \textit{jmj30-2}, \textit{jmj32-1}, and \textit{jmj30-2 jmj32-1} mutants and \textit{pJMJ30::JMJ30-HA jmj30-2; elf6-3, ref6-1}, and \textit{jmj13G} (Supplementary Data 13); and \textit{hsp22}, \textit{hsfa1a/b/d}, and \textit{hsfa2} (Fig. 6a). The \textit{hsp17.6c-1} (SALK_56782) mutant line was obtained from the Arabidopsis Biological Resource Center. All plants were in the Columbia background. Genotyping primers are listed in Supplementary Data 14.
Plasmid construction and plant transformation. For the GUS constructs, the genomic regions of the HSP22, HSP17.6C, and HSP21 loci, including sequence upstream of the translational start site, and coding regions, and excluding the translation termination codon, were amplified by PCR using PrimeSTAR GXL DNA polymerase (TaKaRa) and gene-specific primer sets. The resulting PCR products were subcloned into the pENTR/d-TOPO vector (Thermo Fisher Scientific). After sequences were confirmed with an ABI3130 sequencer (ABI), the insert fragments were transferred into the pB2GW7.0 destination vector (Thermo Fisher Scientific). All the GUS constructs were transformed into wild-type plants by floral dip using Agrobacterium tumefaciens (GV3101)40. T1 plants grown on soil were treated with the nonselective herbicide Basta. T2 seeds were harvested from Basta-resistant plants. Expression levels of HSPs, GUS expression, and the MUG assay were conducted using transgenses located in the same genomic position. Cloning primers are listed in Supplementary Data 4.

For the overexpression construct, the HSP17.6C cDNA was amplified by PCR using PrimeSTAR GXL DNA polymerase (TaKaRa) and gene-specific primer sets. cDNA prepared from Arabidopsis Columbia was used as a template. The resulting PCR products were subcloned into pENTR/d-TOPO vector (Thermo Fisher Scientific). After sequences were confirmed with an ABI3130 sequencer (ABI), the insert fragments were transferred into the pB2GW7.0 destination vector48 by LR clonase (Thermo Fisher Scientific). The construct was transformed into intoんplants by floral dip using Agrobacterium (GV3101)40. T1 plants grown on soil were treated with Basta. T2 seeds were harvested from Basta-resistant plants. Expression levels of HSPs, GUS expression, and the MUG assay were conducted using transgenses located in the same genomic position. Cloning primers are listed in Supplementary Data 4.

For the overexpression construct, the HSP17.6C cDNA was amplified by PCR using gene-specific primer sets with restriction enzyme sequences. cDNA prepared from Arabidopsis Columbia was used as a template. The amplified DNA fragment was Gateway-cloned into pENTR/d-TOPO vector (Thermo Fisher Scientific). After sequences were confirmed with gene-specific primer sets, the fragment was introduced into the pEPRvector29. The construct was transformed into intoんplants by floral dip using Agrobacterium (GV3101)40. T1 plants grown on MS medium supplemented with hygromycin for transgenic selection were shown on the independent lines. Representative lines were used for further analysis. Cloning primers are listed in Supplementary Data 14.

Phenotypic analyses. For all phenotypic analyses, plants to be directly compared were grown side by side at the same density per plate to minimize potential microenvironmental differences between chambers. To determine growth rate, phenotypic strength in the wild type and each mutant were categorized into three different classes42. If plants were vigorous and looked entirely green, those plants were categorized as "normal." If plants were largely pale, those were counted as "partially damaged." Dead plants were categorized as "perished." n > 138. At least three independent experiments were performed, and similar results were obtained. Representative plate images were photographed with a Nikon D750 camera. A chi-squared (χ²) test, followed by post-hoc test, was performed through IBM SPSS Statistics 26 (IBM).

For chlorophyll measurement, total chlorophyll levels were measured using \( \Delta N\) dimethylformamide (DMF) extraction and spectrophotometric quantification43.

Five seedlings were placed into a 2-ml tube containing 1 ml of DMF. The experiments were repeated more than five times. n > 5. Tubes were incubated overnight at 4 °C. The absorbance at 646.8 and 663.8 nm was measured in 1.00-cm cuvettes on spectrophotometer (IMPLEN NanoPhotometer P-Class). Total chlorophyll was calculated as reported previously48 (Chl a + b (\( \mu \)g) = 19.43 A663 + 8.05 A645).

Maximum, 75th percentile, 50th percentile, 25th percentile, and minimum values are shown in the graphs. At least three independent experiments were performed, and similar results were obtained. Normal distribution was verified by the Kolmogorov-Smirnov test for all analyses. Statistical significance was computed using a one-way ANOVA test followed by post-hoc Tukey’s HSD test (https://astatsta.com/OneWay_Anova_with_TukeyHSD/).

For fresh weight assay, 10-day-old seedlings were used. Ten Arabidopsis seedlings were grown in a 2-ml tube and measured with an analytical balance44 (Metler Toledo X104). n > 11. Maximum, 75th percentile, 50th percentile, 25th percentile, and minimum values are shown in the graphs. At least three independent experiments were performed, and similar results were obtained. Normal distribution was verified by the Kolmogorov-Smirnov test for all analyses. Statistical significance was computed using one-way ANOVA test followed by post-hoc Tukey’s HSD test (https://astatsta.com/OneWay_Anova_with_TukeyHSD/).

Scanning electron microscopy. For scanning electron microscopy (SEM), the aboverground parts of 10-day-old seedlings were placed in FAA (45% ETOH, 25% formalin, and 2.5% acetic acid) and placed under vacuum. The tissues were critical-point-dried with liquid CO2 in an EM CPD300 critical-point dryer (Leica Microsystems) and gold-coated with E-1010 (Hitachi) prior to SEM imaging. The tissues were imaged under a S-4700 SEM (Hitachi) with an accelerating voltage of 15 kV. At least five shoot apical meristems for each genotype and treatment were observed, and representative images are shown.

RNA analyses. For RNA extraction, total RNA was extracted from whole seedlings using the RNeasy Plant Mini Kit (Qiagen). About 100 mg of plant tissues was used to generate high-quality total RNA. Before use, the tissues were ground to a fine powder with ice-cold mortar and pestle. RNA extraction was performed following the manufacturer’s instructions. An RNase-Free DNase Set (Qiagen) was used to remove genomic DNA. Concentration was measured by spectrophotometer (IMPLEN NanoPhotometer P-Class). For qRT-PCR, cDNA was synthesized from 100 ng of RNA using a PrimeScript 1st strand cDNA Synthesis Kit (TaKaRa). The resulting cDNA was quantified with a LightCycler 480 (Roche) using FastStart Essential DNA Green Master mix (Roche). The signals were normalized against the internal control gene UBIQUITIN CONJUGATING ENZYMES 21β (UBC21, AT5G25760). Values in graphs are mean ± SEM. At least three independent experiments were performed, and similar results were obtained. Statistical significance was computed using a two-tailed t test. Primers for qRT-PCR are listed in Supplementary Data 14.

For RNA-seq, libraries were prepared as reported previously49 and sequenced by HighSeq 2500 using 50-base-pair single-end mode (Illumina). Mapping was performed using the Arabidopsis reference genome. Microarray reference count for each gene was calculated by RSEM48. After normalization, FDR and FC were calculated using the edgeR package49 following likelihood ratio test (version 3.48.0).
For GUS staining, tissues were kept in 90% acetone for 90 min to inactivate expression analysis. 5-bromo-4-chloro-3-indolyl-
D-glucuronic acid (X-Gluc) three times and put into 50 mM sodium phosphate (pH 7.0) for 30 min, and then left overnight or for two nights, depending on the expression levels. After GUS staining, chlorophyll was removed by allowing tissues to sit in 70% EtOH for at least 1 week. Representative images were taken under an AXIO Zoom. Its Supplementary Information and the list of results are available within the paper and its Supplementary Information files. The datasets, plant materials, and mathematical programming language-written source code file (nb format file) are available from corresponding authors upon request. The RNA-seq (DRA008818, DRA009425) and ChIP-seq (DRA011879) data have been deposited in the DDBJ database (DRA008818, DRA009425, DRA011879). Source data are available in the online version of the paper. Source data are provided with this paper.

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References
1. Lobell, D. B., Schlenker, W. & Costa-Roberts, J. Climate trends and global crop failure since 1980. Science 333, 616–620 (2011).
2. Lämke, J. & Baurle, I. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biol. 18, 124 (2017).
3. Ohama, N., Sato, H., Shinozaki, K. & Yamaguchi-Shinozaki, K. Arabidopsis genome, 2.27.0). To extend the 5′s of reads toward the 3′ direction, the slop function in the BEDTools was used. Mapping were performed on the NIG supercomputer at the ROIS National Institute of Genetics. The resulting reads were counted using the genome coverage function in the BEDTools. Hypermethylated regions of H3K27me3 and hypomethylated regions of H3K4me3 in jinjo compared to WT were identified using a fold enrichment threshold of 1.5 by R (Version 3.5.2). To visualize binding peaks, Integrative Genome Viewer (http://software.broadinstitute.org/software/igv/) was used. ngs.plot was used to generate metabolite and heatmap for ChIP-seq data (http://biotools.nature.com/). The sequence data were deposited into the DNA Data Bank of Japan (DRA011879).

Immunoblotting. For GUS staining, protein extracts were prepared from 10 mg of seedlings in 10 µl of SDS sample buffer. All of the eluate was loaded onto a 4–12% SDS-PAGE gel (Invitrogen) and the gel was run in a Bolt Mini Gel tank (Thermo Fisher Scientific). The resulting gel was subjected to immunoblot analysis using the iBlot2 dry blotting system (Thermo Fisher Scientific). Rabbit polyclonal anti-HSP22 (Eurofins Genomics) (1:1000 diluted), anti-HSP17.6 (ab80183; Abcam) (1:1000 diluted), anti-HSP17.11 (ab80175; Abcam) (1:1000 diluted) and anti-HRP conjugate (1:5000 diluted), Thermo Fisher Scientific) were used as primary and secondary antibodies, respectively. Signals were detected using chemiluminescence HRP substrates (Millipore) and an image analyser (LAS4000, GE healthcare).

Chromatin immunoprecipitation. For ChIP-qPCR, ChIP was carried out as described below (28). For each sample, 100–300 mg of seedlings were fixed with 1% formaldehyde for 15 min. After quenching of formaldehyde with glycine for 5 min, tissues were frozen in liquid nitrogen and kept at −80 °C until use. Tissues were ground to a fine powder with an ice-cold mortar and pestle. Using nuclear extraction buffer, ChIP DNA was isolated from a nuclear extract. Fragmentation was confirmed by using an Ultrasonic Disruptors UD-201 sonicator (TOMY). After pre-cleaning, antibodies were added and the mixtures were rotated overnight at 4 °C.

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5. Crevillén, P. et al. Epigenetic reprogramming that prevents transgenerational inheritance of the vernalized state. *Nature* 515, 587–590 (2014).

6. Liu, F., Cui, X., Zhou, S., Juenemann, M. & Cao, X. Arabidopsis REF6 is a histone H3 lysine 27 demethylase. *Nature Genet.* 43, 715–719 (2011).

7. Cui, X. et al. REF6 recognizes a specific DNA sequence to demethylate H3K27me3 and regulate organ boundary formation in Arabidopsis. *Nature Genet.* 48, 694–699 (2016).

8. Yan, W. et al. Developmental and spatial restriction of Polycrom activity by plant histone demethylases. *Nat. Plants* 4, 681–689 (2018).

9. Charrng, Y. Y. et al. A heat-inducible transcription factor, HsfA2, is required for extension of acquired thermotolerance in Arabidopsis. *Plant Physiol.* 143, 251–262 (2006).

10. Nishizawa, A. et al. Arabidopsis heat shock transcription factor A2 as a key regulator in response to several types of environmental stress. *Plant J.* 48, 535–547 (2006).

11. Yoshida, T. et al. Arabidopsis HsfA1 transcription factors function as the main positive regulators in heat shock-responsive gene expression. *Mol. Genet. Genomics* 286, 321–332 (2011).

12. Liu, H.-C., Liao, H.-T. & Charrng, Y.-Y. The role of class A1 heat shock factors (HSF1A) in response to heat and other stresses in Arabidopsis. *Plant Cell Environ.* 34, 731–751 (2011).

13. Al-Whaihi, M. H. Plant heat-shock proteins: a mini review. *J. King Saud. Univ. Sci.* 23, 139–150 (2011).

14. Lämke, J., Brzezinka, K., Altman, S. & Baurle, I. A hit-and-run heat shock factor governs subtle histone methylation and transcriptional stress memory. *EMBO J.* 35, 162–175 (2016).

15. Xiao, J., Lee, U. S. & Wagner, D. Tug of war: adding and removing histone lysine methylation in Arabidopsis. *Curr. Opin. Plant Biol.* 34, 41–53 (2016).

16. Liu, J. et al. An H3K27me3 demethylase-HSFA2 regulatory loop orchestrates transgenerational thermomemory in Arabidopsis. *Cell Rep.* 39, 379–390 (2019).

17. Sedaghatmehri, M., Mueller-Roeber, B. & Balazadeh, S. The putative metalloprotease FtsH6 and small heat shock protein HSP21 jointly regulate thermomemory in Arabidopsis. *Nat. Commun.* 7, 12439 (2016).

18. Li, Y., Li, Y., Liu, Y., Wu, Y. & Xie, Q. The SHSP22 heat shock protein requires the A8I1 protein phosphatase to modulate polar auxin transport and downstream responses. *Plant Physiol.* 176, 2406–2425 (2018).

19. Izumi, M., Ishida, H., Nakamura, S. & Hidema, J. Entire photodamaged Arabidopsis plasma membrane during cold- and deacclimation in Arabidopsis thaliana. *Biochim. Biophys. Acta* 1877, 3770–3781 (2018).

20. Charng, Y. Y., Liu, H. C., Liu, N. Y., Hsu, F. C. & Ko, S. S. Arabidopsis HSA32, a positive regulator in heat shock-responsive gene expression. *Plant Cell Environ.* 42, 2196–2214 (2019).

21. Tsuge, T., Tsukaya, H. & Uchiyama, H. Two independent and polarized processes of cell elongation regulate leaf blade expansion in Arabidopsis thaliana (L.) Heynh. *Development* 122, 1589–1600 (1996).

22. Koch, E. & Slusarenko, A. Arabidopsis is susceptible to infection by a downy mildew fungus. *Plant Cell* 2, 437–445 (1999).

23. Orocuto-Cardenas, M. & Ryan, C. A. Hydrogen peroxide is generated systemically in plant leaves and systemically in the octadecanoyl phytol pathway. *Nat. Acad. Sci. USA* 105, 6553–6557 (1999).

24. Blázquez, M. Quantitative GUS Activity Assay Of Plant Extracts (Cold Spring Harbor Protocols, 2017).

25. Yamaguchi, N. et al. PROTOCOLS: chromatin immunoprecipitation from Arabidopsis. *Arabidopsis* 12, e0170 (2014).

26. Nagano, A. J. et al. Integrative genomic viewer for the agricultural genome. *Genome Biol.* 10, R25 (2009).

27. Li, H. et al. 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and SAM tools. *Bioinformatics* 25, 2078–2079 (2009).

28. Goodrich, J. et al. A Polycrom-group gene regulates homeotic gene expression in Arabidopsis. *Nature* 386, 44–51 (1997).

29. Xiaoj, I. & Wagner, D. Polycrom repression in the regulation of growth and development in Arabidopsis. *Curr. Opin. Plant Biol.* 23, 15–24 (2015).

30. Baile, F., Merini, W., Hidalgo, I. & Colanino, M. Dissection of PRC1 and PRC2 recruitment in Arabidopsis connects EAR repressor to PRC2 anchoring. *bioRxiv* https://doi.org/10.1101/2019.02.20.920030 (2020).

31. Friedrich, T., Faire, L., Baurle, I. & Schubert, D. Chromatin-based mechanisms of temperature memory in plants. *Plant Cell Environ.* 42, 762–770 (2019).

32. Rymen, B. et al. Histone acetylation enzymes wound-induced transcriptional activation and cellular reprogramming in Arabidopsis. *Commun. Biol.* 2, 1–10 (2019).

33. Miki, M., Takahashi, D., Kawamura, Y. & Uemura, M. Temporal proteomics of Arabidopsis plasma membrane during cold- and deacclimation. *J. Proteom.* 197, 71–81 (2019).

34. Hoermiller, I. I. et al. Subcellular reprogramming of metabolism during cold and photoinhibition in Arabidopsis. *Plant Cell Environ.* 40, 602–610 (2017).

35. Nagano, A., et al. Annual transcriptome dynamics in natural environments reveals plant seasonal adaptation. *Nat. Plants* 5, 74–83 (2019).
Author contributions

Conceptualization: N.Y. (lead) and all other authors (supporting); data curation: N.Y.; formal analysis: N.Y., S.M., and K.Y.; funding acquisition: N.Y., S.I., T.S., T.K., A.J.N., A.S., and T.I.; investigation: N.Y. (lead) and S.M., K.Y., M.S., K.H., M.K., Y.K., Y.N., K.N., S.I., T.S., E.-S.G., and T.T. (supporting); project administration: N.Y.; software: N.Y. (lead) and M.S., K.H., S.I., T.S., T.T., A.J.N., and A.S.; supervision: N.Y. and T.I.; validation: N.Y.; visualization: N.Y.; illustration: N.Y.; writing: N.Y. (original draft) and all authors (review and editing).

Competing interests

The authors declare no competing interests.

Additional information

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