Role of H1 and DNA methylation in selective regulation of transposable elements during heat stress

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Summary

- Heat-stressed Arabidopsis plants release heterochromatin-associated transposable element (TE) silencing, yet it is not accompanied by major reductions of epigenetic repressive modifications. In this study, we explored the functional role of histone H1 in repressing heterochromatic TEs in response to heat stress.
- We generated and analyzed RNA and bisulfite-sequencing data of wild-type and h1 mutant seedlings before and after heat stress.
- Loss of H1 caused activation of pericentromeric Gypsy elements upon heat treatment, despite these elements remaining highly methylated. By contrast, nonpericentromeric Copia elements became activated concomitantly with loss of DNA methylation. The same Copia elements became activated in heat-treated chromomethylase 2 (cmt2) mutants, indicating that H1 represses Copia elements through maintaining DNA methylation under heat.
- We discovered that H1 is required for TE repression in response to heat stress, but its functional role differs depending on TE location. Strikingly, H1-deficient plants treated with the DNA methyltransferase inhibitor zebularine were highly tolerant to heat stress, suggesting that both H1 and DNA methylation redundantly suppress the plant response to heat stress.

Introduction

Transposable elements (TEs) constitute a large proportion of many eukaryotic genomes, including plants (McClintock, 1984; SanMiguel et al., 1996; Kumar & Bennetzen, 1999; Havecker et al., 2004). TEs lead to genetic variability and are therefore considered a major driving force for genome evolution (Mirouze & Paszkowski, 2011; Lisch, 2013; Belyayev, 2014; Grandbastien, 2015). Nevertheless, their ability to transpose can induce mutations and overall genetic instability, which has enforced the evolution of a complex epigenetic regulatory network to repress TE activation and transposition (Slotkin & Martienssen, 2007; Lisch, 2009; Zemach et al., 2010; Gutzat & Mittelsten Scheid, 2012; Marí-Ordóñez et al., 2013). As a consequence, in plants, most TEs are quiescent and both transcriptionally and transpositionally inactive (Ito et al., 2011; Baubec et al., 2014). However, under stress conditions, such as seasonal and daily temperature changes, TEs can be activated and increase genetic instability (Pecinka et al., 2010; Tittel-Elmer et al., 2010; Ito et al., 2011; Bucher et al., 2012; Cavrak et al., 2014), yet only a small proportion of TEs become active under stress (Dubin et al., 2018), revealing that there are so far largely unexplored mechanisms maintaining TE repression under stress conditions.

DNA methylation in the regulatory region of genes and TEs is crucial for silencing (Zemach et al., 2010; Jones, 2012; Schübeler, 2015; Zhang et al., 2018). Plants have DNA methylation in all three cytosine contexts (CG, CHG and CHH; H = A, T, C) (Feng et al., 2010; Law & Jacobsen, 2010; Zemach et al., 2013; Bewick & Schmitz, 2017), which are established and maintained by different mechanisms. The establishment and maintenance of CHH methylation are mediated by the DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) that is targeted by 24-nt small interfering RNAs in the RNA-directed DNA methylation (RdDM) pathway, which is mainly active on euchromatic TEs (Law & Jacobsen, 2010; Matzke & Mosher, 2014). In heterochromatic histone H1-containing regions, CHH methylation is maintained by CHROMOMETHYLASE 2 (CMT2), which can be recruited by lysine 9 dimethylation on histone H3 (H3K9me2) (Stroud et al., 2014). Access of CMT2 to heterochromatic regions is mediated by the chromatin remodeler DECREASE IN DNA METHYLATION 1 (DDM1) (Zemach et al., 2013; Lyons & Zilberman, 2017). Thus, DDM1 together with CMT2 mediates CHH methylation in pericentromeric regions, whereas DRM2 targets mainly euchromatic regions (Zemach et al., 2013). CG and CHG methylation are established by the RdDM pathway, but the maintenance of CG methylation occurs by METHYLTRANSFERASE 1 (MET1) and CHG methylation by CMT3 (Law & Jacobsen, 2010; Du et al., 2015; Bewick & Schmitz, 2017; Zhang et al., 2018). CMT3 can be recruited by H3K9me2 (Law & Jacobsen, 2010; Du et al., 2012, 2015).
H3K9me2 and CHG methylation facilitate each other through regulatory feedback loops (Du et al., 2012, 2014). Heterochromatic regions are generally compact chromatin structures that are enriched for DNA methylation, H3K9me2 (Grewal & Jia, 2007; Vaillant & Paszkowski, 2007; Roudier et al., 2009) and histone H1 (Zemach et al., 2013; Rutowicz et al., 2019; Choi et al., 2020). H1 impedes the access of CMT2, causing increased DNA methylation on heterochromatic TEs upon H1 depletion (Zemach et al., 2013; Lyons & Zilberman, 2017). Loss of H1 causes only a few TEs to be upregulated, but the dual loss of H1 and DNA methylation causes strong TE activation, revealing that DNA methylation and H1 cooperatively maintain heterochromatic TEs in an inaccessible and silent state (Choi et al., 2020). In the vegetative cell of pollen, natural depletion of H1 allows access of the DNA glycosylase DEMETER to heterochromatic TEs, causing their activation by DNA demethylation (He et al., 2019).

Temperature shifts from cold to high temperature cause transcriptional activation of genes and heterochromatic TEs in Arabidopsis (Grandbastien, 1998; Lämke & Bäurle, 2017). H2A.Z eviction leads to gene expression by affecting DNA accessibility upon high temperature, whereas the loss of DNA methylation on TEs enhances TE activation in response to heat (Kumar & Wigge, 2010; Tittel-Elmer et al., 2010; Cavrak et al., 2014). Resetting of this stress-induced epigenetic state on TEs requires the redundant action of DDM1 and MORPHEUS’ MOLECULE 1 (MOM1) and CHROMATIN ASSEMBLY FACTOR 1 (CAF1) (Pecinka et al., 2010; Iwasaki & Paszkowski, 2014). Given the connection between DDM1 and H1 and the enrichment of H1 on heterochromatic TEs, we hypothesized that H1 has a functional role in repressing TEs during temperature stress.

We explored this question using the h1.1 h1.2 double mutants and found that H1 is required in particular for TE repression in response to heat stress. We discovered that the response to H1 loss differs depending on TE location; although pericentromeric GYPSY TEs became activated in heat-treated h1 mutants despite maintaining high levels of DNA methylation, nonpericentromeric COPIA elements became activated concomitantly with loss of DNA methylation. Many of those activated COPIA elements also became activated in heat-treated cmt2 mutants, suggesting that H1 repressed COPIA elements through maintaining DNA methylation. Heat-induced COPIA elements previously were shown to be strongly activated in DNA methylation impaired mutants upon heat treatment (Ito et al., 2011; Thieme et al., 2017). Most strikingly, H1-deficient plants treated with the DNA methyltransferase inhibitor zebularine were highly tolerant to heat stress, suggesting that both H1 and DNA methylation redundantly suppress the plant response to heat stress.

Materials and Methods

Plant material, growth conditions, heat stress and chemical treatments

All wild-type and mutant plants were in the Arabidopsis thaliana accession Columbia (Col-0) background. The h1.1-1 (SALK_128430C) and h1.2-2 (GK-116E08) plant lines were described previously (Rutowicz et al., 2015); h1.1-1 h1.2-2 double mutants were generated by crossing (H1, histone1). The cmt2-5 (SAIL_906_G03) and cmt3-11 (SALK_148381) mutants were described previously (Chan et al., 2006; Shen et al., 2014) (CMT, CHROMOMETHYLASE 2).

Plants were grown on plates with 1/2 Murashige & Skoog medium including vitamins (Duchefa, Haarlem, The Netherlands) and 1% sucrose. Zebularine-treated plants were grown on 1/2 MS medium supplemented with 40 µM zebularine (Abcam, Cambridge, UK). Plates were sealed with micropore tape and stratified for 2 d at 4°C in the dark. The plates were then transferred to a growth chamber with a 16 h : 8 h, light (110 µmol m⁻² s⁻¹, 22°C) : dark (20°C) photoperiod. Ten-day-old seedlings were used for RNA extraction. For heat stress treatments, we incubated 10-d-old seedlings at 4°C for 1 h followed by 37°C for 24 h in the dark, as published previously (Shen et al., 2014). The survival rate of heat-treated plants was determined by incubating 10-d-old seedlings in the dark at 4°C for 1 h followed by 37°C for 36 h and then removing to normal conditions for 2 d. Seedlings were defined as lethal if they showed bleaching of shoot apices and leaves.

RNA isolation and quantitative reverse transcription (qRT)-PCR

RNA extraction was performed using the MagMAX™ Plant RNA Isolation Kit (Thermo Fisher Scientific, Göteborg, Sweden) followed by cDNA synthesis using a RevertAid first-strand cDNA synthesis kit (Thermo Fisher Scientific). Experiments were performed in biological triplicates. Quantitative PCR with gene-specific primers (Supporting Information Table S1) was performed using a HOT FIREPol EvaGreen qPCR Supermix (Solis biodyne, Tartu, Estonia) according to the manufacturer’s instructions. The reactions were performed using the CFX connect Real-time PCR cycler detection system (Bio-Rad). For qPCR analysis of ONSEN copy numbers, ACTIN2 was used to normalize DNA levels.

RNA sequencing

Except for the h1 samples from where only duplicates were generated, RNA of biological triplicates was extracted (50 mg seedlings per replicate) using a RNeasy Plant Mini Kit (Qiagen). Libraries were generated using DNA-free RNA with the TruSeq RNA Library Prep Kit v2 (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. Sequencing was performed on an Illumina HiSeq2000 in 50-bp single-end mode. The correlation between replicates is shown Fig. S1 and details on the data in Table S2. A comparison between our RNA-seq data and previously published data (Choi et al., 2020) of h1 mutants under nonheat conditions is shown in Fig. S2.

DNA isolation and bisulfite sequencing

For a single replicate, four seedlings of wild-type or the h1 mutants were collected before and after heat stress. Around
Bioinformatic analysis

For RNA analysis, untrimmed reads were mapped to the TAIR10 Arabidopsis reference genome using STAR (v2.5.3.a, (Dobin et al., 2013)). Expression counts were generated using the R function summarizeOverlaps from the package HTSeq in union mode on exons from the reference transcriptome AtRTD (Zhang et al., 2017). Differential expression analysis was performed using the R/DESeq2 (v1.20.0, (Love et al., 2014)). Genes with a log2 fold change ≥1 or ≤−1 and Bonferroni-adjusted P-value (padj) ≤0.05 were considered to be differentially expressed. Likewise, expression counts along transposable elements (TEs) were generated using summarizeOverlaps from R/HTSeq in union mode along the coordinates of the 31 189 TAIR10 annotated TEs. Differential expression analysis was performed using DESeq2, using the same threshold values as for gene transcripts.

For DNA methylation analysis, the 150-bp-long pair-end reads were first quality trimmed by removing the first five bases from the 5′ end and the last 20 bases from the 3′ end. Reads were mapped to the reference genome TAIR10 in PE mode (–score_min L 0, –0.6) using Bismark v0.16.3. Mapped reads were deduplicated and cytosine methylation values calculated using the Bismark Methylation Extractor. Methylation reports were pooled for both replicates for further analyses.

Differentially methylated regions (DMRs) in each context were calculated only for demethylation in 50-bp bins considering as the fractional methylation threshold the bins with differences below the 1st decile. Those were parsed if they passed a fisher test (P<0.01).

Published datasets were processed as follows: H1 chromatin affinity purification (ChAP) reads from GSE122394 (Choi et al., 2020) control libraries were mapped to the TAIR10 genome using Bowtie2. Coverage was calculated using the coverage function from R/NUCLER (Flores & Orozco, 2011) and normalized by the total number of mapped reads. The normalized input signal was subtracted from the H1.1 and H1.2 ChAP signal. Small RNA reads from GSE116067 (Tan et al., 2018) were mapped to the TAIR10 genome using Bowtie2 (–v 0-best). Pooled mapped reads were separated into two categories (21/22- and 24-nucleotides-long) and remapped using SHORTSTACK (Johnson et al., 2016). The alignments were normalized by converting coverage values to reads per million values (Wang et al., 2020).

Pericentromeric heterochromatin was considered to span the regions between the following coordinates: Chr1: 11 500 020–17 696 331; Chr2: 1100 003–7192 918; Chr3: 10 298 763–17 289 015; Chr4: 1500 001–2300 002; Chr4: 2800 003–6300 004 and Chr5: 8999 997–5982 772 (Copenhaver et al., 1999).

**Results**

Absence of H1 affects gene and TE activation after heat stress

Histone H1 is abundant throughout the whole genome and mediates global nucleosome positioning in plants and animals, but only few genes and TEs become deregulated when H1 is deficient (Geeven et al., 2015; Rutowicz et al., 2019; Choi et al., 2020). In plants, heat stress causes dispersal of heterochromatin, similar to H1-deficient mutants (Pecinka et al., 2010; Rutowicz et al., 2019). We therefore addressed the question of whether H1 has a role in antagonizing heat stress. Because H1.1 and H1.2 are constitutively expressed and highly redundant (Rutowicz et al., 2019; Choi et al., 2020), we used h1.1 h1.2 double mutants, henceforth referred to as h1 mutants. Expression levels of H1.3 are very low under normal conditions (Ascenzi & Gantt, 1997; Rutowicz et al., 2015) and H1.3 was not induced by heat stress (Fig. S3), justifying the use of the h1 mutants to explore the role of H1 in the heat response. We generated RNA-seq profiling data of wild-type and h1 mutants before and after heat stress (4°C for 1 h followed by 37°C for 24 h in dark conditions). It was previously shown that loss of H1 activates gene expression but only weakly derepresses TEs (Rutowicz et al., 2019; Choi et al., 2020), so we therefore focused initially on upregulated genes in h1 mutants. We found that 301 genes (log2 fold change ≥1 and Bonferroni-adjusted P-value (padj) ≤0.05) were upregulated in h1 mutants compared to wild-type (Fig. 1a; Table S3). Surprisingly, only 116 genes (log2 fold change ≥1 and Bonferroni-adjusted P-value (padj) ≤0.05) were activated in h1 after heat stress compared to heat-treated wild-type (Fig. 1a; Table S3). Many genes (265), including 68 heat-responsive genes in wild-type that were activated in h1, were not changed or became downregulated in h1 upon heat (Figs 1a,c, S4a,b), suggesting that heat attenuates the effect of loss of H1 on genes. To test whether H1 functions as a repressor after heat stress, we analyzed previously published H1 enrichment data (Choi et al., 2020) on upregulated genes and downregulated genes in h1 mutants upon heat stress. We found that upregulated genes in heat-treated h1 mutants were significantly more enriched for H1 than downregulated genes (Fig. S5), indicating that H1 has a mainly repressive role after heat stress. We identified three over-represented gene ontology (GO) functional categories among the upregulated genes in h1 mutants upon heat stress, corresponding to carbohydrate binding, hydrolase activity and nucleoside-triphosphatase (Fig. S6). Consistent with previous findings (Choi et al., 2020), we found only few (13) TEs upregulated in h1 mutants, but this number substantially increased (86 upregulated TEs) in h1 upon heat compared to heat-treated wild-type (Fig. 1b,d; Table S4). Most of these TEs also were heat responsive in wild-type (Figs 1d, S4c). This reveals a repressive role of H1 on TEs in response to heat stress. Previous work revealed that loss of H1 together with loss of DNA methylation causes TE de-repression (Choi et al., 2020). To understand the H1 repressive mechanism in response to heat, we generated bisulfite-sequencing data of wild-type and h1 mutants with and without heat treatment. We found that CG
methylation levels in the 2-kb upstream region of the 265 upregulated genes in \( h1 \) mutants without heat treatment (Fig. 1a) did not significantly change compared to wild-type, irrespective of heat treatment (Fig. 1c). By contrast, CHG and CHH methylation decreased in \( h1 \) mutants without heat treatment, and also decreased to similar levels in wild-type and \( h1 \) mutants upon heat treatment (Fig. 1f,g). Loss of CHH methylation in \( h1 \) was partly attenuated by heat stress, providing a possible explanation why many genes became activated in \( h1 \), but not in heat-treated \( h1 \) mutants (Fig. 1a,c). We identified CHG and CHH differentially methylated regions (DMRs) in \( h1 \) mutants with and without heat treatment. We focused on the 2-kb upstream region of those 265 genes that were upregulated in untreated \( h1 \) mutants but did not change expression upon heat treatment (Fig. 1a,h). Upon heat treatment, we found that 49 genes gained hypermethylated CHG or CHH regions (Hyper DMRs), whereas 81 genes lost hypomethylated regions (Hypo DMRs; Fig. 1b) in \( h1 \) mutants, which restored CHG or CHH methylation to wild-type levels upon heat (Fig. 1f,g), consistent with fewer genes being activated in \( h1 \) mutants after heat stress. Together, our data reveal that heat treatment and loss of H1 both caused a reduction of CHG and CHH methylation (Fig. 1f,g), likely a consequence of opened chromatin structure (Zemach et al., 2013; Rutowicz et al., 2019). However, heat treatment of \( h1 \) mutants partly restored CHH methylation (Fig. 1g,h), indicating that heat ameliorates the effect of loss of H1 on genes.

Gain of DNA methylation is not sufficient to repress \textit{GYPSY} TEs in \( h1 \) mutants after heat stress

In contrast with genes that were less affected in heat-treated \( h1 \) mutants compared to heat-treated wild-type plants, we found increased numbers of TEs upregulated in heat-stressed \( h1 \) mutants compared to heat-stressed wild-type plants (Fig. 1b,d). In order to understand the role of H1 on TE repression in response to heat, we analyzed H1 enrichment data (Choi et al., 2020) to test whether upregulated TEs differ in their H1 enrichment compared to nonupregulated TEs. Indeed, we found that in wild-type, upregulated TEs in heat-treated \( h1 \) mutants were more strongly enriched for H1 compared to TEs not affected by heat (Fig. 2a). Those upregulated TEs also had higher wild-type methylation levels in all three sequence contexts compared to nonupregulated TEs (Fig. 2b), consistent with H1 being associated with heavily methylated heterochromatic TEs (Rutowicz et al., 2015; Choi et al., 2020). Upregulated TEs belonged to different TE families, among which LTR/\textit{GYPSY} (\( P = 2.81e-24 \)), LTR/\textit{COPIA} (\( P = 9.88e-05 \)) and DNA/\textit{En-Spm} (\( P = 0.4045 \)) families were significantly enriched (Fig. 2c) (LTR, long terminal repeat). Among the most enriched retrotransposons, LTR/\textit{COPIA} TEs were mildly increased in \( h1 \) mutants after heat stress, whereas LTR/\textit{GYPSY} TEs were more strongly upregulated (Fig. 2d). We analyzed DNA methylation levels of all \textit{GYPSY} TEs and found that they gained DNA methylation in all sequence contexts in \( h1 \) compared to wild-type, independent of being subjected to heat stress or not (Fig. 2e–g). This is consistent with previous findings revealing that absence of H1 causes gain of methylation in heterochromatic regions, but loss of methylation in euchromatic regions (Zemach et al., 2013; Lyons & Zilberman, 2017). Upregulated \textit{GYPSY} TEs in \( h1 \) upon heat followed this trend and likewise gained DNA methylation of all three sequence contexts in \( h1 \) mutants vs WT both before and after heat stress (Fig. 2h–j). This suggests that in the absence of H1, high levels of DNA methylation are not sufficient to repress a subset of TEs in response to heat stress. Thus, H1 has an indispensable and DNA methylation-independent repressive function in response to heat stress.

H1 and DNA methylation cooperatively control a subset of \textit{COPIA} elements in response to heat

Upregulated \textit{COPIA} elements in \( h1 \) mutants upon heat were mainly \textit{COPIA78/ONSEN} elements, including the eight full-length heat responsive \textit{ONSEN} TEs (Ito et al., 2013; Cavrak et al., 2014; Pietzenuk et al., 2016). In contrast with \textit{GYPSY} TEs that strongly gained DNA methylation in \( h1 \) mutants after heat stress, there were only mild changes in DNA methylation on \textit{COPIA} TEs (Fig. 3a–c). However, those heat-responsive \textit{COPIA78/ONSEN} elements that became upregulated upon heat stress, had increased DNA methylation levels in \( h1 \) mutants without heat treatment, but experienced a strong loss of CG and CHG methylation upon heat treatment, independent of the presence or absence of H1 (Fig. 3d–f). Stronger activation of \textit{COPIA78/ONSEN} elements in \( h1 \) mutants upon heat compared to heat-treated wild-type suggests that under heat stress, DNA methylation and H1 cooperatively repress a subset of \textit{COPIA} TEs.

Among those upregulated \textit{COPIA} TEs were three \textit{COPIA78/ONSEN} elements (\textit{ONSEN} 1 (\textit{AT1}TE12229), \textit{ONSEN} 2 (\textit{AT3}TE92522) and \textit{ONSEN} 3 (\textit{AT5}TE15240) that had more strongly reduced DNA methylation in all three sequence contexts in heat-treated \( h1 \) mutants compared to heat-treated wild-type (Fig. 3g, S7). Heat treatment in combination with impaired epigenetic repression previously was shown to cause \textit{ONSEN} transposition (Ito et al., 2011; Thieme et al., 2017), however, the copy numbers of \textit{ONSEN} remained the same in the second generation of heat-treated \( h1 \) mutants as in wild-type, revealing that H1 does not control \textit{ONSEN} transposition (Fig. S8).

\textit{CMT2} controls \textit{COPIA78/ONSEN} element silencing during heat stress

Natural \textit{CMT2} variation was shown to correlate with CHH methylation variation and temperature seasonality in \textit{Arabidopsis}, and \textit{cmt2} mutants are more heat tolerant (Shen et al., 2014; Dubin et al., 2015). Because \textit{CMT2} acts on \textit{H1} containing loci (Zemach et al., 2013), we explored the connection between \textit{H1} and \textit{CMT2} under heat stress. We generated transcriptome data of \textit{cmt2} mutants before and after heat stress. Only 26 TEs became activated in \textit{cmt2} mutants upon heating (Fig. 4a; Table S4). Among those, six TEs belonged to the \textit{GYPSY} family, whereas 13 TEs belonged to the \textit{COPIA78/ONSEN} subfamily. Among the upregulated TEs, 15 overlapped with TEs upregulated in heat-treated \( h1 \) mutants (Fig. 4a), including 12 \textit{COPIA78/ONSEN} TEs. The \textit{COPIA78/ONSEN} elements were mildly upregulated.
in heat-treated h1 mutants but became strongly upregulated in heat-treated cm2 mutants (Fig. 4b), revealing that CMT2, like H1, plays a major repressive role on TEs of the COPIA78/ONSEN subfamily in response to heat stress. Heat stress-induced expression of COPIA78/ONSEN also was observed in RNA-directed DNA methylation (RdDM) mutants (Ito et al., 2011), suggesting that COPIA78/ONSEN elements are common targets of the CMT2 and RdDM pathways. Consistently, upregulated COPIA elements strongly accumulated 24-nt siRNAs, differing from nonupregulated COPIA elements and Gypsy elements.

Fig. 1  Histone H1 is required for transposable element (TE) repression after heat stress in Arabidopsis. Venn diagrams show upregulated genes (log2-fold change ≥ 1, adjusted P-value (padj) ≤ 0.05, (a)) and TEs (log2-fold change ≥ 1, padj ≤ 0.05, (b)) in h1 mutants vs wild-type (h1/WT) and h1 plus heat vs WT plus heat (h1 + heat/WT + heat). The red dot in (a) represents the same group of genes marked in panel (h). Heat maps show upregulated genes (c) and TEs (d) in h1 mutants vs WT and h1 plus heat vs WT plus heat. Averaged CG (e), CHG (f) and CHH (g) methylation in the −2 kb to transcription start site (TSS) region of 265 upregulated genes in h1 mutants vs WT but not in h1 mutants WT plus heat or h1 plus heat WT plus heat (h1 + heat + WT + heat). * P < 0.05; ** P < 0.01; ns, not significant (Wilcoxon test). The lower and upper hinges of the boxplots correspond to the first and third quartiles of the data, the black lines within the boxes mark the median. CHG and CHH DMRs (h) in the −2 kb to TSS region of 265 upregulated genes in h1 vs WT. Hyper and hypo refer to hyper- or hypomethylated regions, respectively, present in the upstream region of genes in h1 vs WT or h1 plus heat vs WT plus heat.
The RdDM pathway targets the edges of long TEs, whereas CMT2 is required for TE body methylation on long heterochromatic TEs (Zemach et al., 2013). In agreement with the dual regulation of COPIA78 elements by both pathways, upregulated COPIA elements were significantly larger than upregulated GYPSY elements (Fig. 4d). Upregulated COPIA and GYPSY elements also differed in their chromosomal location: whereas upregulated GYPSY TEs were concentrated in the peri-centromeric region, upregulated COPIA TEs were mainly dispersed along the chromosome arms (Fig. 4e), which may explain their different mode of regulation.

H1-deficient plants treated with zebularine have improved heat-stress tolerance

In order to further explore the interaction between H1-mediated TE repression and DNA methylation, we grew wild-type and h1 plants on media supplemented with zebularine, a widely used inhibitor of DNA methyltransferases (Griffin et al., 2016). Consistent with the transcriptome data, COPIA78/ONSEN expression increased upon heat treatment and was further increased in h1 mutants upon heat as tested by qRT-PCR (Fig. 5a,b; note different axis scale). Zebularine treatment increased COPIA78/ONSEN expression upon heat treatment, but the effect was similar in wild-type and h1 mutants (Fig. 5b), consistent with the strong effect of cmt2 mutants on COPIA78/ONSEN expression upon heat treatment (Fig. 4b). By contrast, one GYPSY TE expression was increased in zebularine-treated wild-type and h1 mutants, and strongly increased in heat-treated h1 mutants compared to wild-type (Fig. 5c,d), indicating a synergistic repressive effect of H1 and DNA methylation on GYPSY repression upon heat treatment.

In order to functionally explore the impact of H1 on the temperature-stress response, we tested the sensitivity of wild-type and h1 plants on media supplemented with zebularine, a widely used inhibitor of DNA methyltransferases (Griffin et al., 2016). Consistent with the transcriptome data, COPIA78/ONSEN expression increased upon heat treatment and was further increased in h1 mutants upon heat as tested by qRT-PCR (Fig. 5a,b; note different axis scale). Zebularine treatment increased COPIA78/ONSEN expression upon heat treatment, but the effect was similar in wild-type and h1 mutants (Fig. 5b), consistent with the strong effect of cmt2 mutants on COPIA78/ONSEN expression upon heat treatment (Fig. 4b). By contrast, one GYPSY TE expression was increased in zebularine-treated wild-type and h1 mutants, and strongly increased in heat-treated h1 mutants compared to wild-type (Fig. 5c,d), indicating a synergistic repressive effect of H1 and DNA methylation on GYPSY repression upon heat treatment.

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mutants to severe heat stress (1 h at 4°C followed by 36 h at 37°C in dark conditions) by measuring the survival rate of heat-treated plants after returning to normal conditions for 2 d. The leaves of most plants gradually became yellow and bleached and the plants finally died during these two days, resulting in ≤20% survival rate of wild-type plants. The h1 mutants had a significantly higher survival rate (1.6-fold greater; P = 0.016, Wilcoxon signed-rank test) than wild-type (Fig. 5e,f). Strikingly, however, when the zebularine was present, the survival rate of wild-type and h1 mutants strongly increased after heat stress, reaching c. 50% in wild-type and c. 86% in h1 mutants (Fig. 5e,f), revealing a so far unknown potential of hypomethylation in combination with H1 deficiency to antagonize heat stress. We assessed whether the increased heat tolerance was a consequence of increased transposition rates of COPIA78/ONSEN elements in h1 mutants treated with zebularine and heat; however, we did not observe increased ONSEN copy numbers in the second generation of heat- and zebularine-treated h1 mutants compared to wild-type (Fig. S9).

Fig. 3 Histone H1 represses COPIA78/ONSEN transposable elements (TEs) upon heat through maintaining DNA methylation in Arabidopsis. Averaged CG (a), CHG (b) and CHH (c) methylation on all COPIA TEs in wild-type (WT), h1, wt plus heat (WT + heat) and h1 plus heat (h1 + heat). Averaged CG (d), CHG (e) and CHH (f) methylation on upregulated COPIA78/ONSEN elements in h1 plus heat vs WT plus heat in four different conditions of WT, h1, WT plus heat, and h1 plus heat. *, P < 0.05; **, P < 0.01; ns, not significant (Wilcoxon test). The lower and upper hinges of the boxplots correspond to the first and third quartiles of the data, the black lines within the boxes mark the median. Example of CG, CHG and CHH methylation levels (g) on ONSEN 1 (AT1TE12295). Triangle points to the decreased DNA methylation region.
In this work, we explored the function of the linker histone H1 in response to heat stress in plants and discovered a novel role of H1 in modulating the plant response to heat. Although loss of H1 causes only a mild effect under normal conditions (Rutowicz et al., 2015, 2019), we found that under heat stress, loss of H1 strongly activated transposable elements (TEs), mainly Gypsy and COPIA elements. Stress treatment of the h1.3 mutant previously was reported to cause increase of CHH methylation at defined loci and reduced gene expression (Rutowicz et al., 2015). Likewise, we observed that decreased CHH methylation in the 2-kb upstream region of upregulated genes in h1 mutants was partly restored in heat-treated h1, suggesting that increased CHH methylation accounts for the dampened gene expressed in stressed h1 mutants. Histone H1 together with DNA methylation were shown to jointly suppress aberrant intragenic transcription (Choi et al., 2020). Thus, this is also possible that the formation of aberrant transcripts in h1 at least in part account for reduced transcript levels.

In heterochromatic regions of Arabidopsis, H1 impedes DNA methyltransferase accessibility to chromatin, leading to increased DNA methylation upon H1 depletion (Zemach et al., 2013; Lyons & Zilberman, 2017). Although we found this effect very pronounced on Gypsy TEs, this effect was much weaker on COPIA TEs, indicating distinct regulation of both types of TEs by H1 (Fig. 6). In heat-treated h1 mutants, gain of DNA methylation was not sufficient to repress Gypsy TEs, demonstrating a key repressive role for H1 on Gypsy TEs. Depletion of DNA methylation by zebularine treatment caused strongly increased expression of Gypsy TEs in heat-treated h1 mutants, exceeding the upregulation in heat-treated h1 without zebularine, revealing that H1 and DNA methylation synergistically repress Gypsy TEs under heat, consistent with previous work (Choi et al., 2020).

Like Gypsy TEs that gained DNA methylation upon loss of H1, heat-induced COPIA TEs also gained DNA methylation in h1 mutants. However, in contrast with Gypsy TEs, which maintained high levels of DNA methylation in h1 mutants upon heat treatment, COPIA elements lost DNA methylation upon heat treatment in wild-type and h1 mutants. Because COPIA elements became more strongly activated in heat-treated h1 mutants than heat-treated wild-type, we conclude that the combination of reduced DNA methylation and loss of H1 caused transcriptional activation of COPIA elements upon heat. Nevertheless, most of the heat induced COPIA elements also were activated in heat-treated chromomethylase 2 (cmt2) mutants, indicating that a complete loss of CHH methylation has the activating effect on the same COPIA elements as loss of H1 under heat.

The difference in chromosomal location may explain the different regulatory impact of DNA methylation on Gypsy and COPIA element expression in response to heat. Gypsy elements are located in compacted heterochromatic regions and possibly rely on Microrchidia (MORC) proteins for repression. MORC proteins act in heterochromatic regions and enforce silencing of TEs independently of DNA methylation, likely by affecting...
chromatin structure (Moissiard et al., 2012). Heat causes loss of chromocenter organization, similar to the effect caused by loss of H1 (Pecinka et al., 2010; Rutowicz et al., 2019; Choi et al., 2020). Thus, the combination of H1 loss together with heat may cause a synergistic effect, opening heterochromatic pericentric regions and activating GYPSY TE expression. By
contrast, nonpericentromeric COPIA elements affected by heat, likely rely on the combination of CMT2 and H1 for stable repression (Choi et al., 2020).

Most strikingly, we found that depletion of DNA methylation by zebularine enhanced heat tolerance and that this effect was strongly enhanced in h1 mutants. Previous work revealed that loci controlling adaptive responses to the environment are frequent targets of TE insertions (Ito et al., 2016; Quadrana et al., 2016). COPIA TEs contain heat responsive factor binding elements (HREs) that confer heat response to the nearby genes (Ito et al., 2011; Cavrak et al., 2014; Pietzenuk et al., 2016; Thieme et al., 2017), and transcriptional activation of COPIA78/ONSEN elements correlates with environmental heat stress in most species of the Brassicaceae (Ito et al., 2013). CHH methylation-deficient cmt2 mutants are more heat-tolerant than wild-type (Shen et al., 2014), consistent with the upregulated COPIA type TEs in both

![Figure 6: Model depicting the role of histone H1 and DNA methylation in selective repression of GYPSY and COPIA78/ONSEN retrotransposons in Arabidopsis. Upregulated GYPSY transposable elements (TEs) in heat-treated h1 mutants are located in pericentromeric regions, whereas upregulated COPIA78/ONSEN elements are dispersed in the chromosome arms. In h1 mutants, chromatin structure is opened and the two groups of TEs gain DNA methylation and remain silenced. After heat stress of wild-type plants (WT + heat), DNA methylation level remains unchanged on upregulated GYPSY TEs, but decrease on upregulated COPIA78/ONSEN elements, thus likely contributing to their activation. Opened chromatin structure is likely sufficient to induce expression of GYPSY TEs. In heat-treated h1 mutants (h1 + heat), GYPSY TEs gain DNA methylation, which, however, is not sufficient for repression and GYPSY TEs become more strongly expressed compared to heat-treated WT. By contrast, COPIA78/ONSEN TEs lose DNA methylation in heat-treated h1 mutants, which contributes to their upregulation, because COPIA78/ONSEN TEs, but not GYPSY TEs, are strongly activated in heat-treated chromomethylase 2 (cmt2) mutants (cmt2 + heat).](image-url)
cmt2 and h1 mutants upon heat stress. We speculate that reduced DNA methylation and loss of H1 exposes heat-responsive elements that are epigenetically silenced under normal conditions. The combination of loss of H1 and reduced DNA methylation acts cooperatively on COPIA and GYPSYTE activation, possibly explaining the strongly enhanced heat tolerance of zebularine-treated h1 seedlings. Whether or not this effect is inheritable remains to be explored.

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Author contributions
LH conceived the study; SL and JdJ designed and performed the experiments; MST-A and JS-G analyzed high-throughput sequencing data; and SL and CK interpreted the data and wrote the manuscript. All authors approved the final version of the manuscript.

Data availability
The RNA and bisulfite-sequencing data in this study have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE152402.

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**Supporting Information**

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Correlation analysis of RNA-seq samples.

**Fig. S2** Comparison of data in this study with previously published data (Choi et al., 2020).

**Fig. S3** *H1.3* expression does not change in heat-treated *h1* mutants compared to heat-treated WT.

**Fig. S4** A substantial proportion of upregulated genes in *h1* mutants and TE5s in *h1* upon heat are heat-responsive in WT.

**Fig. S5** H1 represses gene expression after heat stress.

**Fig. S6** Gene ontology terms of upregulated genes in *h1* plus heat vs WT plus heat.

**Fig. S7** Decreased CG, CHG and CHH methylation on upregulated *Copia*/*ONSEN* elements.

**Fig. S8** H1 does not affect *ONSEN* remobilization upon heat.
**Fig. S9** **ONSEN** does not amplify in the progeny of heat and zebularine-treated WT and *h1* mutants.

**Table S1** Primers used in the manuscript.

**Table S2** Mapping statistics of RNA sequencing data.

**Table S3** Lists of deregulated genes in *h1* mutants before and after heat stress.

**Table S4** Lists of upregulated TEs in *h1* mutants and *cmt2* mutants before and after heat stress.

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