ONSEN is a heat-activated LTR retrotransposon in *Arabidopsis thaliana*. Screens to identify transcriptional regulatory factors of *ONSEN* revealed a SWI/SNF-like chromatin remodeling protein, DRD1, which cooperates with plant-specific RNA polymerase and is involved in RNA-directed DNA methylation. *ONSEN* transcript level was increased in the *drd1* mutant relative to wild-type under heat stress, indicating that DRD1 plays a significant role in the silencing of activated *ONSEN* under the stress condition. The transcript level of *HsfA2*, which is directly involved in transcriptional activation of *ONSEN*, was not higher in the *drd1* mutant than in the wild-type. Interestingly, no transgenerational transposition of *ONSEN* was observed in the *drd1* mutant, even though DNA methylation levels were significantly reduced and expression levels were increased compared to the wild-type. These results suggest that other factors are involved in the regulation of *ONSEN* transposition in addition to the transcript level of *ONSEN*.

**Key words:** heat stress, DRD1, transposon, *ONSEN*, RNA-directed DNA methylation

**INTRODUCTION**

Transposable elements (TEs) of various classes, including both DNA transposons and retrotransposons, are abundant in plant genomes and impact genome evolution and gene expression (Wessler, 1996; Kumar and Bennetzen, 1999; Kazazian, 2004; Makarevitch et al., 2015). Despite their potential to either disadvantage or benefit the host genome, most TEs are silent and rarely transpose due to genetic aberrations such as point mutations, deletions or recombination that disrupt their activities. Even though full-length autonomous transposons are intact and have the ability to transpose, host plants have evolved various types of epigenetic regulation, such as DNA methylation or histone modification, to defend their genome against such transposition.

One of the well-studied mechanisms for TE regulation is RNA-directed DNA methylation (RdDM), in which small interfering RNAs (siRNAs) direct the cytosine methylation of DNA sequences that are complementary to the siRNAs (Wierzbicki et al., 2008; Gao et al., 2010). RdDM requires transcriptional machinery that involves two plant-specific RNA polymerases, RNA polymerase IV (Pol IV) and RNA polymerase V (Pol V) (Kanno et al., 2005b; Onodera et al., 2005; Pontier et al., 2005). Pol IV generates primary RNA transcripts and Pol V generates non-coding transcripts to introduce siRNA-mediated DNA methylation on the target site. *Arabidopsis thaliana*, RNA-dependent RNA polymerase 2 (RDR2) converts a transcript produced by Pol IV to double-stranded RNA, and DICER-LIKE 3 (DCL3) subsequently processes these precursor RNAs into 24-nt siRNAs (Zhang et al., 2007; Mosher et al., 2008). The siRNAs bind to an RNA-induced silencing complex, RISC, that contains ARGONAUTE 4 (AGO4). AGO4 interacts with Pol V and recruits the DNA methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), which
directs de novo DNA methylation of target TEs (Cao and Jacobsen, 2002; Matzke and Birchler, 2005).

Although most TEs have their own modes of regulation, some are activated under stress conditions (Chandler and Walbot, 1986; Bennetzen, 1987; Hirochika, 1993; Grandbastien et al., 1997, 2004; Scotti et al., 1997; Steward et al., 2000; Hashida et al., 2003; Henderson and Jacobsen, 2007; Hirayama et al., 2009; Lisch, 2009; Zeller et al., 2009). The stress might induce a change in the epigenetic state of the TE, generating stress-responsive elements in host genes in the proximity of TEs. Epigenomic diversity may allow phenotypic plasticity and the ability to cope with environmental variation.

Previously, we found heat stress-induced activation of a Ty1/copia-like retrotransposon named ONSEN in A. thaliana (Ito et al., 2011). The activation of ONSEN requires a heat stress transcription factor, HsfA2, which distinctively binds to a cis-regulatory sequence (heat response element, HRE) in the promoter of the ONSEN LTR (Cavrak et al., 2014). Furthermore, the activated ONSEN is transposed in stressed plants that are defective in the RdDM pathway (Ito et al., 2011; Matsunaga et al., 2012).

In this study we performed a genetic screening to investigate the regulation mechanism of ONSEN and revealed that an epigenetic regulator, DRD1 (defective in RNA-directed DNA methylation), regulates ONSEN expression. DRD1 is a member of the plant-specific subfamily of SW12/SNF2-like proteins (Kanno et al., 2004, 2005b). It associates with many subunits of the Pol V complex and is required for the accumulation of Pol V-dependent transcripts to facilitate RdDM and gene silencing of homologous DNA sequences (Kanno et al., 2005a; Law et al., 2010). Although the activation of ONSEN has been studied in some mutants (Ito et al., 2011; Matsunaga et al., 2012), the precise mechanism of transcriptional regulation remains unknown. Here, we provide new insights into chromatin remodeling protein-mediated regulation of ONSEN.

MATERIALS AND METHODS

Plant material and stress treatments The A. thaliana plants used in the experiments included wild-type Columbia-0 (Col-0) and Landsberg erecta (Ler), mutants nrpd1-1-3 (Herr et al., 2005) and drd1-6 (Kanno et al., 2004), and transgenic lines that possessed a full-length LTR (genome position: 4212570–4213146) of ONSEN (AT5G13205) fused with a GFP gene (Matsunaga et al., 2015). The plants were grown on Murashige and Skoog medium under continuous light at 21 °C. For heat stress treatment to analyze ONSEN expression and DNA methylation, seven-day-old seedlings were subjected to a temperature shift from 21 °C to 37 °C for 24 h. The transcript level of HsfA2 was analyzed on seven-day-old seedlings that were subjected to a temperature shift from 21 °C to 37 °C for 1 h.

Real-time PCR Total RNA was extracted from seedlings using TRI Reagent (Sigma-Aldrich), according to the supplier’s recommendations. Five individual plants were pooled prior to RNA extraction. Around 3 to 5 µg of total RNA was treated with RQ1 RNase-free DNase (Promega) and reverse-transcribed using the ReverTraAce qPCR RT Kit (Toyobo) with random primers. Real-time PCR was performed using the Applied Biosystems 7300 Real-Time PCR System with the Power SYBR Green PCR Master Mix (Thermo Fisher Scientific). Three biological repetitions were performed and standard deviation was determined.

Southern blot analysis Plant genomic DNA was isolated using the Nucleon PhytoPure DNA Extraction Kit (GE Healthcare). Southern blots were performed as described previously (Miura et al., 2004). We detected hybridization signals in a highly concentrated sodium dodecyl sulfate hybridization buffer (Church and Gilbert, 1984) using a radiolabeled ONSEN-specific probe (Supplementary Table S1) that was generated with the Amer sham Megaprime DNA Labeling System (GE Healthcare).

Mapping of mutation by whole-genome sequencing To map the gene responsible for the boil mutant, the boil mutant (Col-0) was first crossed with wild-type (Ler), and then self-pollinated to produce a segregating population of boil lines. Seedlings of this population were heat-stressed at 37 °C for 24 h, and then grown for about two weeks in pots, one group with high expression of GFP signal (High population) as in the boil mutant, and the other with weak expression of GFP fluorescence (Low population) as in the GFP:wild-type (Col-0). The true leaves were then sampled together in three pieces. The number of individuals used in the analysis was 22 for the High sample and 23 for the Low sample in boil1, and 20 for the High sample and 21 for the Low sample in boil5. Genomic DNA was extracted using the Nucleon PhytoPure DNA Extraction Kit (GE Healthcare). The extracted DNA was fragmented by sonication (S220, Covaris) and then used to construct a library using TruSeq DNA Library Prep Kits (Illumina) according to the manufacturer’s protocol. The library was sequenced by NextSeq500 (Illumina). The output bc files were converted to fastq files by bc1fastq (Illumina). Based on the read sequences, a search for the gene responsible was performed in Mitsucal (Suzuki et al., 2018). For each mutant, the areas where Col-0-type bias was observed were selected. The search conditions were as follows: [number of substitutions: 1–200; substitution rate: 80–100; QV substitution rate: 80–100; number of control substitutions: blank; only mutation positions affecting the
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Amino acid sequence: checked; type of base substitution: blank.

DNA methylation analysis For bisulfite sequencing analysis, genomic DNA was extracted from seedlings using the Nucleon PhytoPure DNA Extraction Kit. Bisulfite treatment was performed using the MethylCode Bisulfite Conversion Kit (Thermo Fisher Scientific). Bisulfite-treated DNA was amplified by PCR with the EpiTaq HS (Takara Bio). Primers for the analysis are listed in Supplementary Table S1. PCR products were cloned into the pANT vector using the TA-Enhancer Cloning Kit (Nippon Gene), and fifteen clones were sequenced for the 5’ LTR region in each line. Methylated sites were analyzed using CyMATE (http://www.cymate.org).

RESULTS

Upregulation of ONSEN in EMS mutants subjected to heat stress To understand the molecular mechanism of ONSEN regulation, we tried to find a new regulatory factor by mutant screening. We used a transgenic Arabidopsis that possessed an intact LTR of ONSEN fused with a gene for green fluorescent protein (GFP). The transgenic plants with a single-copy insert were mutagenized by ethyl methanesulfonate and the resulting M2 progeny were screened for mutations. The GFP signals of 5,000 heat-stressed mutants were compared with that of the parental line subjected to heat stress. Twenty-four individual seedlings with stronger GFP signals were self-pollinated and endogenous ONSEN expression in their progeny was analyzed by quantitative reverse transcription PCR. More than twice the expression level of ONSEN relative to wild-type was observed in 16 of the lines. We named the mutant lines boils (burst of ONSEN induction lines). We focused on one of the boils, boil5, which has strong GFP signals in the seedlings (Fig. 1A). We analyzed the expression level of endogenous ONSEN in the self-pollinated progeny of boil5. The result showed that the expression level of ONSEN in

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Fig. 1. Activation of ONSEN in boil5 subjected to heat stress. (A) Photographs of bright field and GFP fluorescence images. Wild-type transgenic plants (WT-GFP) and boil5 plants were observed immediately after heat treatment (HS) or under non-stress (NS) conditions as a control. (B) Relative transcription levels of endogenous ONSEN. HS, heat-stressed samples; NS, non-stress samples. Error bars represent the mean ± SEM, n = 3; values are relative to heat-stressed transgenic plants (WT-GFP). (C) Structure of the DRD1 gene and the predicted protein domains. Boxes represent exons, and black and gray colors indicate coding and non-coding regions, respectively, on the gene structure (upper). The positions of the point mutation in boil5 and drd1-6 are indicated, together with nucleotide numbers. The amino acid substitutions caused by boil5 and drd1-6 are shown in the protein structure (lower) with the predicted amino acid domains. #, the mutation generates a stop codon. (D) Relative transcript levels of endogenous ONSEN in heat-stressed seedlings including wild-type transgenic plants (Col-0), boil5, drd1-6, and F1 progeny of boil5 × drd1-6 (F1). Error bars represent the mean ± SEM, n = 3; values are relative to heat-stressed transgenic plants (Col-0). Asterisks mark significant differences from Col-0 (P ≤ 0.05, Student’s t-test).
heat-stressed boil5 was six times higher than that in the parental line (Fig. 1B).

Next, we analyzed the transgenerational transposition of ONSEN in boils subjected to heat stress. To detect new inserted copies of ONSEN in the progeny of heat-stressed boil5, Southern blot analysis was conducted on the next generation in boil5 plants subjected to heat stress. As a control, ONSEN transposition in boil1 progeny was also analyzed. boil1 has a mutation in the NRPD1 gene, which encodes the largest subunit of Pol IV. The mutation causes a non-synonymous substitution in the NRPD1 protein and strong GFP signals in the seedlings (Fig. 2A and 2C). The expression level of ONSEN in heat-stressed boil1 plants was six times higher than that in the parental line (Fig. 2B). Southern blot analysis detected transgenerational transposition of ONSEN in the next generation in boil1 but not in boil5 subjected to heat stress (Fig. 2D). This result indicated that transcriptional activation was necessary but not sufficient for ONSEN transposition.

**Mapping of BOIL5** To identify the gene responsible for ONSEN regulation in boil5, the mutant was outcrossed with Ler. Twenty of 134 F2 seedlings showed a strong GFP signal, indicating that the mutation of the gene responsible is recessive. Approximately 20 F2 progeny each that showed high or low expression of GFP were collected, and DNA was extracted from each group in bulk. A DNA library was constructed for each, and sequenced using a high-throughput sequencer. To identify the mutation responsible for the phenotypes of boil5, we used the Mitsucal software (Suzuki et al., 2018), which aligned all reads for boil5 with reference genes by Bowtie with a parameter permitting multiple alignments. Within the 5–10-Mbp region of chromosome 2, 24 mutations with a > 90% ratio of mismatch were detected. Fourteen muta-

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**Fig. 2.** Activation of ONSEN in boil1 subjected to heat stress. (A) Photographs of bright field and GFP fluorescence images. Wild-type transgenic plants (WT-GFP) and boil1 plants were observed immediately after heat treatment (HS) or under non-stress (NS) conditions as a control. (B) Relative transcript levels of endogenous ONSEN. HS, heat-stressed samples; NS, non-stress samples. Error bars represent the mean ± SEM, n = 3; values are relative to heat-stressed transgenic plants (WT-GFP). (C) Structure of the NRPD1 gene and the predicted protein domains. Boxes represent exons, and black and gray colors indicate coding and non-coding regions, respectively, on the gene structure (upper). The position of the point mutation in boil1 is indicated, together with nucleotide numbers. The amino acid substitution caused by boil1 is shown in the protein structure (lower) with the predicted amino acid domains, together with amino acid numbers. #, the mutation generates a stop codon. DUF, domain of unknown function. (D) Southern blot analysis of ONSEN in a heat-stressed boil1 and boil5 plant and in self-pollinated siblings. A gel stained with ethidium bromide (EtBr) is shown at the bottom of each panel as a loading control.
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Fig. 3. Relative transcript levels of endogenous ONSEN (A) and HsfA2 (B) in heat-stressed seedlings of wild-type (Col-0) and drd1-6. Error bars represent the mean ± SEM, n = 3; values are relative to heat-stressed transgenic plants (Col-0). The asterisk marks a significant difference from heat-stressed Col-0 (P ≤ 0.05, Student’s t-test).

Fig. 4. Comparison of DNA methylation in wild-type (Col-0) and drd1-6 in the ONSEN 5′ LTR region. (A) DNA methylation was determined by bisulfite-sequencing of the LTR region of At1g11265. Black and white arrowheads represent individual cytosine methylation status within the analyzed region (black: methylated; white: unmethylated). The first half of the analyzed 779 bp full-length LTR is shown. Circles, squares and triangles indicate CG, CHG and CHH, respectively. (B) DNA methylation level of the LTR region in wild-type (Col-0), nrpd1-3 and drd1-6. Percentage of methylation was determined based on the number of methylated cytosines divided by the total number of cytosines within the analyzed sequences from each PCR clone. Asterisks mark significant differences from the methylation level of Col-0 (P ≤ 0.05, Generalized Linear Model with Poisson error distribution was used to deal with count data).

mutations had no effect on protein structure (mutation in intron, or synonymous substitution). Seven mutations of the remaining ten were a substitution from G to A (or C to T), the most typical mutation caused by EMS. Four mutations were observed only in the F2 progeny that showed high expression of GFP. One of the four was
in a gene encoding a WD-40 repeat family protein; two
others were in genes encoding proteins of unknown func-
tion. The remaining one of the four mutations was found
in the gene encoding DRD1, an SNF2 domain-containing
protein. This mutation was located at the 5′ end of the
fourth exon of the gene labeled AT2G16390 and causes
a non-synonymous substitution (Fig. 1C). To assess
whether this mutation corresponded to the phenotype of
boil5, boil5 was crossed with a disruptant for AT2G16390
(designated drd1-6) obtained from the Arabidopsis Bio-
logical Resource Center (numbered CS69758, http://abrc.
osu.edu/). The transcript level of ONSEN in F1 plants
was significantly higher than that in the wild-type (Fig.1D).
These results strongly suggest that the causative
factor of boil5 is a mutation in the DRD1 gene.

**DRD1 regulates the expression of a heat-activated ONSEN**
The heat shock transcription factor HsfA2 is an
important factor among a subset of stress response genes
and is required for ONSEN activation in plants subjected
to heat stress (Cavrak et al., 2014). To reveal whether
the amount of HsfA2 affected the expression level of ONSEN
in the drd1-6 mutant, we compared the transcript level of
HsfA2 between wild-type and drd1-6 mutant. There
was a significant increase of ONSEN transcript in drd1-6 under heat stress conditions, although the transcript
level of HsfA2 was not significantly different (Fig. 3A and
3B). This observation revealed that the increase in tran-
script level of ONSEN in drd1-6 was not correlated with
the transcript level of HsfA2.

**DRD1 regulates ONSEN expression by DNA methyla-
tion**
RdDM leads to methylation of cytosines in all
sequence contexts: CG, CHG and CHH (where H corre-
sponds to A, T or C). To understand the role of DRD1 in
the epigenetic regulation of ONSEN, DNA methylation
level was analyzed in the LTR promoter of the ONSEN
copy (At1g11265) that shows the highest expression level
upon heat stress (Cavrak et al., 2014). DNA methyla-
tion levels of this ONSEN LTR sequence showed a sig-
ificant difference for non-CG methylation between the
wild-type and the drd1-6 mutant: 76% of CHG was meth-
ylated in the wild-type, compared to 35% in the drd1-6
mutant. The methylation level of CHH was 7.1% in
drd1-6 compared to 60% in the wild-type. The drd1-6
mutant showed the same hypomethylation state of non-
CG as the nrpd1-3 mutant (Fig. 4A and 4B).

**Transgenerational transposition of ONSEN is not
detected in heat-stressed drd1-6**
To detect transgen-
erational transposition of ONSEN in drd1-6, Southern
blot analysis was performed in the offspring of drd1-6 plants subjected to heat stress. No individuals having
ONSEN transposition were found. In several individu-
als of that generation of nrpd1-3, which was used as a
control, new ONSEN insertions were detected, suggest-
ing that there are other important factors for ONSEN
transposition besides ONSEN expression levels and DNA
methylation levels on the ONSEN sequences (Fig. 5).

**DISCUSSION**
Here we report that a plant-specific SWI/SNF-like chro-
matin remodeling protein, DRD1, regulates the expres-
sion of a heat-activated retrotransposon. DRD1 was
first identified in a screen for mutants impaired in RdDM
(Kanno et al., 2004), and a subsequent study dem-

![Fig. 5. Southern blot analysis of ONSEN in progeny of heat-stressed drd1-6 and nrpd1-3. Each arrowhead indicates a new insertion of ONSEN. A gel stained with ethidium bromide (EtBr) is shown at the bottom of each panel as a loading control.]
posons or sequences that encode short RNAs (Huettel et al., 2006). Compared with the well-studied SNF2-like protein DDM1, which regulates global DNA methylation, DRD1 acts locally to regulate levels of non-CG methylation (Jeddeloh et al., 1999; Kanno et al., 2004). Several heterochromatic repeats lose CG methylation in ddm1 mutants but non-CG methylation in drd1 mutants, suggesting that DRD1 is important for non-CG methylation of target sequences (Huettel et al., 2006). The sequence of the ONSEN LTR contains 78 cytosines in non-CG contexts that could be targets for DRD1-mediated RdDM. As expected, the levels of CHG and CHH methylation on the ONSEN sequence were significantly reduced in the drd1 mutant compared to the wild-type.

Most transposons are silenced under non-stress conditions and are not activated in the drd1 mutant. We found that the expression of heat-activated ONSEN was upregulated in the drd1 mutant compared with the wild-type. The transcript level of ONSEN may be affected by chromatin state, which can be changed by heat stress. Although SWI2/SNF2 chromatin remodeling proteins play roles in stress responses (Shaked et al., 2006; Han et al., 2012; Gentry and Hennig, 2014), further research will be needed to determine the chromatin modification of ONSEN in the heat-stressed drd1 mutant.

One possible mechanism to explain the upregulation of ONSEN in the drd1 mutant subjected to heat stress is that the increase of ONSEN transcript is affected by the transcript level of HsfA2 in the drd1 mutant. Another possibility is that the physical association of HsfA2 with its targets is enhanced in the drd1 mutant under heat stress. The latter mechanism is supported by the fact that the level of HsfA2 transcription was not significantly increased in the drd1 mutant compared with the wild-type under heat stress conditions. However, we cannot exclude the possibility that a factor(s) other than the redundant heat-related transcription factor family is required for ONSEN activation under heat stress. The exact mechanisms underlying heat shock factor-mediated upregulation remain to be further elucidated.

The endogenous targets of DRD1 silencing machinery are short-RNA-encoding elements that are located in the 5′ flanking region of the target sequences (Huettel et al., 2006). In general, the target sequences that were upregulated in the drd1 mutant had a euchromatic character and reside in gene-rich regions (Huettel et al., 2006). In the Col-0 accession, seven full-length ONSEN copies exist in euchromatic regions and one copy is located in centromeric heterochromatin. Further analysis may reveal whether the expression of each copy can be equally regulated by DRD1.

It is necessary to separate transpositional activity from transposable activity when discussing the activation of transposons. An increase in the level of transcription is not necessarily associated with an increase in the frequency of transposition. As shown in our study, ONSEN is upregulated by heat stress in the drd1 mutant to the same level as in the nrpd1 mutant, but transgenerational transposition of ONSEN was not observed in the drd1 mutant, whereas it was observed in the nrpd1 mutant. To explain the difference, the properties of the ONSEN insertion sites need to be investigated, but at present, no common motifs have been found in the primary sequences of ONSEN insertion sites, and it is not clear what targets are used to determine the insertion sites. Previous studies, however, have shown that ONSEN tends to be inserted into euchromatin genes (Ito et al., 2016). Therefore, it is possible that DNA demethylation is induced in the drd1 mutant, but chromatin condensation is maintained. This distinction may account for the lack of transgenerational transposition of ONSEN even though the element has similar methylation levels and increased expression in both the drd1 and nrpd1 mutants.

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REFERENCES

Bennetzen, J. L. (1987) Covalent DNA modification and the regulation of Mutator element transposition in maize. Mol. Gen. Genet. 208, 45–51.

Cao, X., and Jacobsen, S. E. (2002) Role of the Arabidopsis DRM methyltransferases in de novo DNA methylation and gene silencing. Curr. Biol. 12, 1138–1144.

Cavrak, V. V., Lettner, N., Jame, S., Kosarewicz, A., Bayer, L. M., and Scheid, O. M. (2014) How a retrotransposon exploits the plant's heat stress response for its activation. PLoS Genet. 10, e1004115.

Chandler, V. L., and Walbot, V. (1986) DNA modification of a maize transposable element correlates with loss of activity. Proc. Natl. Acad. Sci. USA 83, 1767–1771.

Church, G. M., and Gilbert, W. (1984) Genomic sequencing. Proc. Natl. Acad. Sci. USA 81, 1991–1995.

Gao, Z., Liu, H.-L., Daxinger, L., Pontes, O., He, X., Qian, W., Lin, H., Xie, M., Lorkovic, Z. J., Zhang, S., et al. (2010) An RNA polymerase II- and AGO4-associated protein acts in RNA-directed DNA methylation. Nature 465, 106–109.

Gentry, M., and Hennig, L. (2014) Remodelling chromatin to shape development of plants. Exp. Cell Res. 321, 40–46.

Grandbastien, M.-A. (2004) Stress activation and genomic impact of plant retrotransposons. J. Soc. Biol. 198, 425–432. (in French)

Grandbastien, M.-A., Lucas, H., Morel, J.-B., Mhiri, C., Vernhettes, S., and Casacuberta, J. M. (1997) The expression of the tobacco Tnt1 retrotransposon is linked to plant defense responses. Genetica 100, 241–252.

Han, S.-K., Sang, Y., Rodrigues, A., BIOL425 F2010, Wu, M.-F., Rodriguez, P. L., and Wagner, D. (2012) The SWI2/SNF2 chromatin remodeling ATPase BRAHMA represses abscisic
Kumar, A., and Bennetzen, J. L. (1999) Plant retrotransposons. Plant Cell 24, 4892–4906.

Hashida, S., Kitamura, K., Mikami, T., and Kishima, Y. (2003) Temperature shift coordinates the activity and the methylation state of transposon Tam3 in Antirrhinum majus. Plant Physiol. 132, 1207–1216.

Henderson, I. R., and Jacobsen, S. E. (2007) Epigenetic inheritance in plants. Nature 447, 418–424.

Herr, A. J., Jensen, M. B., Dalmay, T., and Baulecombe, D. C. (2005) DNA polymerase IV directs silencing of endogenous DNA. Science 308, 118–120.

Hirayama, K., Wang, D., Matsuda, M., Lin, X., Namihiara, T., Takano, H., Takio, S., and Akiyama, H. (2009) Activation of retrotransposon in red algae by underwater pulsed discharge. Acta Phys. Pol. A 115, 1110–1111.

Hirochika, H. (1993) Activation of tobacco retrotransposons during tissue culture. EMBO J. 12, 2521–2528.

Huettel, B., Kanno, T., Daxinger, L., Aufsatz, W., Matzke, A. J. M., and Matzke, M. (2006) Endogenous targets of RNA-directed DNA methylation and Pol IV in Arabidopsis. EMBO J. 25, 2828–2836.

Ito, H., Gaubert, H., Bucher, E., Mirouzé, M., Vaillant, I., and Paszkowski, J. (2011) An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. Nature 472, 115–119.

Ito, H., Kim, J.-M., Matsunaga, W., Sahe, H., Matsui, A., Endo, T. A., Harukawa, Y., Takagi, H., Yasagishi, H., Masuta, Y., et al. (2016) A stress-activated transposon in Arabidopsis induces transgenerational abscisic acid insensitivity. Sci. Rep. 6, 23181.

Jeddeloh, J. A., Stokes, T. L., and Richards, E. J. (1999) Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat. Genet. 22, 94–97.

Kanno, T., Aufsatz, W., Jaligot, E., Mette, M. F., Matzke, M., and Matzke, A. J. M. (2005a) A SNF2-like protein facilitates dynamic control of DNA methylation. EMBO Rep. 6, 649–655.

Kanno, T., Huettel, B., Mette, M. F., Aufsatz, W., Jaligot, E., Daxinger, L., Kreil, D. P., Matzke, M., and Matzke, A. J. M. (2005b) Atypical RNA polymerase subunits required for RNA-directed DNA methylation. Nat. Genet. 37, 761–765.

Kanno, T., Mette, M. F., Kreil, D. P., Aufsatz, W., Matzke, M., and Matzke, A. J. M. (2004) Involvement of putative SNF2 chromatin remodeling protein DRD1 in RNA-directed DNA methylation. Curr. Biol. 14, 801–805.

Kazazian, H. H., Jr. (2004) Mobile elements: drivers of genome evolution. Science 303, 1626–1632.

Kumar, A., and Bennetzen, J. L. (1999) Plant retrotransposons. Annu. Rev. Genet. 33, 479–532.

Law, J. A., Ausin, I., Johnson, L. M., Vashisht, A. A., Zhu, J.-K., Wohlschlegel, J. A., and Jacobsen, S. E. (2010) A protein complex required for polymerase V transcripts and RNA-directed DNA methylation in Arabidopsis. Curr. Biol. 20, 951–956.

Lisch, D. (2009) Epigenetic regulation of transposable elements in plants. Annu. Rev. Plant Biol. 60, 43–66.

Makarevich, I., Waters, A. J., West, P. T., Stitzer, M., Hirsch, C. N., Ross-Ibarra, J., and Springer, N. M. (2015) Transposable elements contribute to activation of maize genes in response to abiotic stress. PLoS Genet. 11, e1004915.

Matsunaga, W., Kobayashi, A., Kato, A., and Ito, H. (2012) The effects of heat induction and the siRNA biogenesis pathway on the transgenerational transposition of OSEN, a copia-like retrotransposon in Arabidopsis thaliana. Plant Cell Physiol. 53, 824–833.

Matsunaga, W., Ohama, N., Tanabe, N., Masuda, Y., Masuda, S., Mitani, N., Yamaguchi-Shinozaki, K., Ma, J. F., Kato, A., and Ito, H. (2015) A small RNA mediated regulation of a stress-activated retrotransposon and the tissue specific transposition during the reproductive period in Arabidopsis. Front. Plant Sci. 6, 48.

Matzke, M. A., and Bircher, J. A. (2005) RNAi-mediated pathways in the nucleus. Nat. Rev. Genet. 6, 24–35.

Miura, A., Kato, M., Watanabe, K., Kawabe, A., Kotani, H., and Kakutani, T. (2004) Genomic localization of endogenous mobile CACTA family transposons in natural variants of Arabidopsis thaliana. Mol. Genet. Genomics 270, 524–532.

Mosher, R. A., Schwach, P., Studholme, D., and Baulecombe, D. C. (2008) Pol IVb influences RNA-directed DNA methylation independently of its role in siRNA biogenesis. Proc. Natl. Acad. Sci. USA 105, 3145–3150.

Onodera, Y., Haag, J. R., Ream, T., Costa Nunes, P., Pontes, O., and Pikaard, C. S. (2005) Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. Cell 120, 613–622.

Pontier, D., Yahubyan, G., Vega, D., Bulski, A., Saez-Vasquez, J., Hakimi, M.-A., Lerbs-Mache, S., Colot, V., and Lagrange, T. (2005) Reinforcement of silencing at transposons and highly repeated sequences requires the concerted action of two distinct RNA polymerases IV in Arabidopsis. Genes Dev. 19, 2030–2040.

Scortecchi, K. C., Dessaux, Y., Petit, A., and Van Sluys, M.-A. (1997) Somatic excision of the Ac transposable element in transgenic Arabidopsis thaliana after 5-azacytidine treatment. Plant Cell Physiol. 38, 336–343.

Shaked, H., Avivi-Ragolsky, N., and Levy, A. A. (2006) Involvement of the Arabidopsis SWI2/SNF2 chromatin remodeling gene family in DNA damage response and recombination. Genetics 173, 895–894.

Steward, N., Kusano, T., and Sano, H. (2000) Expression of ZmMET1, a gene encoding a DNA methyltransferase from maize, is associated not only with DNA replication in actively proliferating cells, but also with altered DNA methylation status in cold-stressed quiescent cells. Nucleic Acids Res. 28, 3250–3259.

Suzuki, T., Kawai, T., Takemura, S., Nishiwaki, M., Suzuki, T., Nakamura, K., Ishiguro, S., and Higashiyama, T. (2016) Development of the Mitsucal computer system to identify causal mutation with a high-throughput sequence. Plant Reprod. 31, 117–128.

Wessler, S. R. (1996) Turned on by stress. Plant retrotransposons. Curr. Biol. 6, 959–961.

Wierzbicki, A. T., Haag, J. R., and Pikaard, C. S. (2008) Noncoding transcription by RNA polymerase Pol IVb/Pol V mediates transcriptional silencing of overlapping and adjacent genes. Cell 135, 635–648.

Zeller, G., Henz, S. R., Widmer, C. K., Sachsenberg, T., Ratsch, G., Weigel, D., and Laubinger, S. (2009) Stress-induced changes in the Arabidopsis thaliana transcriptome analyzed using whole-genome tiling arrays. Plant J. 58, 1068–1082.

Zhang, X., Henderson, J. R., Lu, C., Green, P. J., and Jacobsen, S. E. (2007) Role of RNA polymerase IV in plant small RNA metabolism. Proc. Natl. Acad. Sci. USA 104, 4536–4541.
## Supplementary Table S1. Primer sequences

| Experiment          | Primer           | Sequence (5'-3')               |
|---------------------|------------------|--------------------------------|
| Southern probe      | ONSEN-F          | TAATGTTCCCTTCCAAGTCCC          |
|                     | ONSEN-R          | GCTTGTAATGACCCAAGAAGT          |
| Real-time PCR       | COPIA78-4129F_RT | CCACAAGAGGAACCAACGAA           |
|                     | COPIA78-4300R_RT | TTCGATCATGGAAGACCGG            |
|                     | 18Sr-FW          | CGTCCCTGCCCTTTGTACAC           |
|                     | 18Sr-RV          | CGAACACTTACCGGATCATT           |
|                     | HsfA2_qPCR_F     | TGGGATTTCATAAGTTCTCAACA        |
|                     | HsfA2_qPCR_R     | TGGATCAATCTTTCTGAATCCAT        |
| Bisulfite seq       | AT1G11265 BS F1  | GGTTGAAGGGTYAAAGGTAAYATAAG     |
|                     | COPIA78 BS R2    | ATTCTCACATCATCTARCTTCTCCRTT    |