An Atypical Epigenetic Mechanism Affects Uniparental Expression of Pol IV-Dependent siRNAs

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Abstract

Background: Small RNAs generated by RNA polymerase IV (Pol IV) are the most abundant class of small RNAs in flowering plants. In Arabidopsis thaliana Pol IV-dependent short interfering (p4-si)RNAs are imprinted and accumulate specifically from maternal chromosomes in the developing seeds. Imprinted expression of protein-coding genes is controlled by differential DNA or histone methylation placed in gametes. To identify epigenetic factors required for maternal-specific expression of p4-siRNAs we analyzed the effect of a series of candidate mutations, including those required for genomic imprinting of protein-coding genes, on uniparental expression of a representative p4-siRNA locus.

Results: Paternal alleles of imprinted genes are marked by DNA or histone methylation placed by DNA METHYLTRANSFERASE 1 or the Polycomb Repressive Complex 2. Here we demonstrate that repression of paternal p4-siRNA expression at locus 08002 is not controlled by either of these mechanisms. Similarly, loss of several chromatin modification enzymes, including a histone acetyltransferase, a histone methyltransferase, and two nucleosome remodeling proteins, does not affect maternal expression of locus 08002. Maternal alleles of imprinted genes are hypomethylated by DEMETER DNA glycosylase, yet expression of p4-siRNAs occurs irrespective of demethylation by DEMETER or related glycosylases.

Conclusions: Differential DNA methylation and other chromatin modifications associated with epigenetic silencing are not required for maternal-specific expression of p4-siRNAs at locus 08002. These data indicate that there is an as yet unknown epigenetic mechanism causing maternal-specific p4-siRNA expression that is distinct from the well-characterized mechanisms associated with DNA methylation or the Polycomb Repressive Complex 2.

Introduction

Mendelian laws of inheritance state that a genetic element behaves identically when transmitted through maternal or paternal gametes. Genetic elements that break this law by exhibiting preferential or exclusive expression when inherited from one parent are genomically imprinted. Genomic imprinting is well described only in placental mammals and flowering plants, although a number of parent-of-origin-dependent effects are observed in other organisms [1,2,3,4,5].

Flowering plants are characterized by double fertilization, whereby two identical haploid sperm cells in the pollen grain fertilize two cells in the female gametophyte. Fertilization of the haploid egg cell generates the diploid embryo while fertilization of the diploid central cell generates the triploid endosperm. The endosperm is functionally analogous to mammalian placenta, acting as a conduit between maternal somatic tissues and the growing embryo but not contributing genetically to the next generation. Endosperm makes up the bulk of grains such as rice, wheat, and maize, making it a critical tissue for human nutrition. With a single exception in maize [6], all characterized imprinted genes in plants display uniparental expression specifically in the endosperm and some imprinted genes affect the growth and development of this tissue [7,8].

In plants, imprinted genes are associated with hypomethylated maternal DNA regardless of which allele is expressed. In Arabidopsis thaliana, differential DNA methylation is established by the opposing actions of DNA METHYLTRANSFERASE 1 (MET1) in the paternal gametophyte and the DNA glycosylase DEMETER (DME) in the central cell of the female gametophyte [9,10]. Loss of paternal DNA methylation through mutation of MET1 activates the normally silent paternal allele of FLOWERING WAGENINGEN (FW1), FERTILIZATION INDEPENDENT ENDOSPERM 2 (FIS2) and MATERNALLY EXPRESSED PAB C-
**Results**

Loss of DNA methylation does not alter uniparental p4-siRNA expression in endosperm

Loss of DNA METHYLTRANSFERASE 1 (MET1) does not alter maternal-specific expression of p4-siRNAs at locus 08002 in Arabidopsis endosperm [18]. MET1 is the primary methyltransferase in Arabidopsis and is responsible for maintenance of CG dinucleotide methylation [23]. Methylation at CHH sites (where H is A, T, or C) is performed by CHROMOMETHYLTRANSFERASE 3 (CMT3) and asymmetric methylation (at CHH sites) is placed by DOMAINS REARRANGED METHYLTRANSFERASES (DRM1 and DRM2) [23]. To determine whether non-CG DNA methylation represses paternal p4-siRNA alleles or induces expression of maternal alleles, we crossed non-CG DNA methylation represses paternal p4-siRNA alleles or FERASES (DRM1 and DRM2) [23]. To determine if other chromatin modifications might repress paternal p4-siRNAs at locus 08002, we tested several candidate genes as above. HISTONE DEACETYLASE 6 (HD6) is associated with silencing of transposable elements [25] and rDNA repeats [26,27]. KRYPTONITE (KYP) encodes a histone methyltransferase that catalyzes dimethylation at lysine 9 of histone H3 (H3K9me), the canonical mark of silent chromatin [26]. **DECREASE IN DNA METHYLATION 1 (DDM1) and MORPHEUS**

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*Terminal (MPC)*, and reduces expression of the paternal-specific imprinted gene *PHERES (PHE)* [11,12,13,14]. Similarly, loss of DME activity inhibits the maternal expression of at least *FIS2* and *MPC* [13,15], and ectopic expression of DME outside of the central cell is sufficient to induce expression of another maternal-specific gene, *MEDEA (MEA)* [16]. These observations demonstrate the importance of DNA methylation patterns in the expression of imprinted genes.

Over 125 genes in Arabidopsis are imprinted [17]. In contrast, thousands of intergenic regions producing RNA Polymerase IV-dependent small interfering (p4-si) RNAs are maternally expressed in the developing seed [18]. Many p4-siRNAs are produced from transposable elements, but others coincide with imprinted genes such as *FWA, MPC*, and *MEA* [19,20] indicating that there may be a connection between parent-of-origin specific expression of protein-coding genes and non-coding RNAs. Recent genome-wide analyses of DNA methylation in the endosperm further support this connection between p4-siRNA expression and imprinting of genes because maternal chromosomal undergo extensive DME-mediated DNA demethylation at regions of p4-siRNA production [21,22]. A plausible scenario was that p4-siRNAs and imprinted genes might be coordinately regulated by DME and MET1.

To examine the genetic requirements for maternal expression and paternal silencing of p4-siRNAs at a representative locus, we show that differential DNA methylation does not explain uniparental expression of p4-siRNAs at locus 08002 and that various histone modifications, including Histone H3 Lysine 27 methylation (H3K27me), do not establish maternal-specific expression at this site. Furthermore, demethylation of maternal chromosomes by DME is dispensable for p4-siRNA expression in the endosperm.
DME is part of a small family of glycosylases involved in maternal expression of p4-siRNAs, we first assayed p4-siRNAs. In DME heterozygotes, seeds inheriting a maternal dme allele abort early in development while seeds inheriting a maternal DME allele develop normally. To determine whether DME is necessary for accumulation of p4-siRNAs from maternal chromosomes, we dissected aborted and developed seeds from heterozygous dme-2 self-fertilized siliques during mid-embryo development (10–12 days post-fertilization). Unexpectedly, p4-siRNA accumulation in dme seeds was higher than in wild-type siblings (figure 5). To determine whether this was due to lack of demethylation by DME or due to the developmental arrest of mutant seeds during an earlier period of high p4-siRNA accumulation, we analyzed wild-type and dme seeds from the same developmental stage. When transmitted maternally the weaker dme-1 allele does not always trigger seed abortion, making homozygous mutant lines possible. Developing siliques from dme-1 and wild type were collected at 5 days post anthesis and p4-siRNA accumulation was assayed (figure 5). dme-1 siliques display wild-type expression of p4-siRNAs, indicating that demethylation by DME is not necessary for p4-siRNA production from maternal chromosomes in the endosperm.

Discussion

Differential methylation of maternal and paternal DNA is extensive in the endosperm of Arabidopsis, primarily due to DEMETER-mediated demethylation of transposable elements in the central cell [21,22]. Many transposable elements produce p4-siRNAs, leading to the hypothesis that demethylation of these elements in the endosperm causes maternal-specific production of p4-siRNAs [22]. However, loss of DNA methylation in developing seeds is insufficient for paternal p4-siRNA expression from the representative p4-siRNA locus 08002 (figure 1), and loss of maternal DNA methylation does not eliminate p4-siRNA expression (figure 5). We also demonstrate that several known histone modifications, including H3K27 and H3K9 methylation, are dispensable for paternal repression of locus 08002 (figure 2). These data indicate an additional, as yet uncharacterized chromatin signal affects maternal expression and paternal repression of 08002 and possibly other p4-siRNA loci in developing seeds.

Evidence indicates that this activating mark is established before fertilization because p4-siRNA expression in the maternal flowers is required for p4-siRNA expression in the developing seed [18]. Lack of p4-siRNA expression in mature pollen would restrict this activating mark to maternal chromosomes [33]. This unidentified mark could also be used by protein-coding genes because many maternal-specific transcripts in the Arabidopsis seed transcriptome identified are unaffected by loss of DNA or H3K27 methylation [17].

We had previously concluded, based on dissection and genetic analysis, that the activating mark would be carried on the maternal alleles of the fertilized endosperm. This remains a plausible explanation. However there is also the possibility that the maternal p4-siRNAs are expressed in the maternal seed coat and of a different ecotype and determined parental origin of locus 08002 p4-siRNAs at 5 days after fertilization. If demethylation is required for expression, crosses generated with the transgenic lines as pollen donors should result in biallelic expression of p4-siRNAs. Instead, strict maternal-specific expression was detected for all crosses (figure 4), indicating that ectopic demethylation of the paternal genome by overexpression of DME family glycosylases is insufficient to induce paternal expression at locus 08002.

To further assess the role of DME in accumulation of p4-siRNAs, we assayed p4-siRNA expression in dme mutant endosperm, which is not demethylated at p4-siRNA loci [21,22]. In dme-2 heterozygotes, seeds inheriting a maternal dme allele abort early in development while seeds inheriting a maternal DME allele develop normally. To determine whether DME is necessary for accumulation of p4-siRNAs from maternal chromosomes, we dissected aborted and developed seeds from heterozygous dme-2 self-fertilized siliques during mid-embryo development (10–12 days post-fertilization). Unexpectedly, p4-siRNA accumulation in dme seeds was higher than in wild-type siblings (figure 5). To determine whether this was due to lack of demethylation by DME or due to the developmental arrest of mutant seeds during an earlier period of high p4-siRNA accumulation, we analyzed wild-type and dme seeds from the same developmental stage. When transmitted maternally the weaker dme-1 allele does not always trigger seed abortion, making homozygous mutant lines possible. Developing siliques from dme-1 and wild type were collected at 5 days post anthesis and p4-siRNA accumulation was assayed (figure 5). dme-1 siliques display wild-type expression of p4-siRNAs, indicating that demethylation by DME is not necessary for p4-siRNA production from maternal chromosomes in the endosperm.

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Figure 3. **DEMETER** family glycosylases are insufficient to induce vegetative expression of p4-siRNAs. Ectopic expression of the **DEMETER** glycosylase behind the strong, nearly constitutive 35S promoter (35S::DME) does not cause ectopic accumulation of type I p4-siRNAs (00687, 02815, 08002, and siRNA 02) in leaves, nor does it alter expression of type II p4-siRNAs (AtRep2, Simplehat, and siRNA1003) in leaves. Similarly, overexpression of the related glycosylases **REPRESSOR OF SILENCING** (35S::ROS1), **DEMETER-LIKE 2** (35S::DML2), or **DEMETER-LIKE 3** (35S::DML3) has no affect on p4-siRNA expression. Two independent transgenic lines were assayed for each overexpression construct. 35S::ROS1 lines are in the C24 background [34]; all other lines are in the Col background [16].

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Figure 4. **DEMETER** family glycosylases do not trigger paternal expression of p4-siRNAs. Small RNAs were isolated from inter-ecotype crosses between wild type and transgenic lines and parental origin of small RNA was determined as described in figure 1. Expression of the **DEMETER** glycosylase in the male gametophyte from the strong, nearly constitutive 35S promoter (35S::DME) does not trigger paternal expression of p4-siRNAs in endosperm. Similarly, overexpression of the related glycosylases **REPRESSOR OF SILENCING** (35S::ROS1), **DEMETER-LIKE 2** (35S::DML2), or **DEMETER-LIKE 3** (35S::DML3) does not affect imprinted p4-siRNA expression in endosperm. Two independent transgenic lines were assayed for each overexpression construct. 35S::ROS1 lines are in the C24 background [34]; all other lines are in the Col background [16].

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Parent-of-origin chromatin signals might be more prevalent than previously thought. Although imprinted expression of endogenous protein-coding genes has only been described in placental mammals and flowering plants, parent-of-origin phenomena exist throughout the animal kingdom. Some transgenes in the nematode Caenorhabditis elegans and the zebrafish Danio rerio are imprinted [1,5], and Drosophila melanogaster transgenes inserted near regions of heterochromatin or within the Y chromosome are also imprinted [2,37]. Parent-of-origin effects are not limited to uniparental gene expression. The first published case of parental “imprints” is Sciarid flies, where paternal chromosomes are eliminated from specific cell lineages [3,39]. In coccid insects the entire paternal genome is either heterochromatinized or eliminated from somatic tissues [4], while in C. elegans the X chromosome adopts specific histone modifications depending on the parent of origin [39]. It seems likely that parent-of-origin chromatin signatures are widespread throughout sexual eukaryotes, and it will be interesting to discover what role small RNA-directed chromatin modification might play in establishing or responding to these signals.

Materials and Methods

Plant growth conditions and genotypes

All plants were grown under standard conditions including 16 hours of light each day. Mutant alleles were as follows. Columbia ecotype: met1-1 [40], drm 1-2 (SALK_031705) [41], drm2-2 (SALK_150863) [41], cmr3-1 (SALK_148381) [41], hda6-9 (E. Havecker, C. Melnyk, and D. Baulcombe, unpublished allele), ddm1-2 [42], mon1-2 (SALK_141293) [30], and fis (GABI 362D08); Landsberg erecta ecotype: cmr3-7 [43], and kyp-2 [29]; Wassilewskija ecotype: drm1-1 [44] and drm2-1 [44]. The drm1 and drm2 mutations were isolated in Columbia and backcrossed to Landsberg erecta [16]. The drm1 drm2 double mutant contained drm1-1 and drm2-1; the ddc triple mutant contained drm1-2, drm2-2, and cmr3-11. Mutations were confirmed using molecular markers or visible phenotypes. Wassilewskija and C24 contain the Columbia-0 allele at locus 008002 (figure S2).

To eliminate possible self-fertilization, crosses were performed 24 hours after manual emasculation of immature flowers. For each cross, six to ten siliques were collected 5 days after fertilization. To determine the effect of the loss-of-function dme-2 allele, dme-2 heterozygotes were allowed to self-fertilize. The resulting seeds were dissected 10–12 days after fertilization and divided into DME and dme based on development of the embryo. For analysis of the weaker dme-1 allele, flowers were inspected daily and marked upon anthesis. Siliques were collected 5 days after anthesis.

Generation of transgenic lines

Total RNA from wild-type Columbia-0 leaf tissue was used to reverse transcribe and amplify full-length cDNAs of DML2 and DML3 with the following primers: DML2: 5’-CACCATGGAA-GTGGAAGTGAAGGTGGAAG-3’ and 5’-TCATATCTCGTTTGTCTAT-CTTTTCTTGGTCTG-3’; DML3: 5’-CACCATGTTGAACAGAGTTTGAACAC-3’ and 5’-CTATATCATCATCAGTCT- CATATAAATGCTTGGC-3’. PCR products were introduced into pENTR D-TOPO (Invitrogen) and the resulting entry vectors were recombined into pEARLEYGATE 202 [45]. 35S::DML2 and 35S::DML3 constructs were stably transformed into wild-type Columbia-0 using standard protocols. Generation of 35S::DME and 35S::ROS1 are described elsewhere [16,34].

Overexpression of DME-family glycosylases was verified with quantitative reverse transcription-PCR using Quantifast SYBR Green One-Step RT-PCR Kit (Qiagen) and the following primers:

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RNA extraction and northern hybridizations

RNA was extracted from leaves using TRIP Reagent (Sigma-Aldrich) according to the manufacturer’s protocol. RNA from crossed silicites or dissected seeds was extracted as follows: 5-6 silicites were frozen in liquid nitrogen and ground to a fine powder. 500 μL of room temperature extraction buffer (100 mM glycine pH 9.5, 10 mM EDTA, 100 mM NaCl, 2% SDS) was added and once thawed, samples were further homogenized and placed on ice. Lysates were extracted once with cold Tris-saturated phenol (pH 8.0), twice with cold 25:24:1 Tris-saturated phenol:m:isoamyl alcohol before precipitation with sodium acetate and ethanol.

Small RNA was enriched from 30–50 μg total RNA with mirVana miRNA isolation columns (Ambion) according to the manufacturer’s protocol. Small RNAs were resolved on a 7M urea/1X TBE/15% acrylamide gel (19:1 acrylamide:bisacrylamide mixture) and transferred to Hybond N+ membranes. Membranes were UV-crosslinked before pre-hybridization in 1X SSC/0.1% SDS. Hybridization and washing was at 35°C. Membranes were exposed to phosphor-storage screens for detection of siRNAs.

Probe sequences are as follows (underlined bases are LNA): siRNA002 5'-TCGAAACTCTCGACCTCAGGAT-3'; siRNA002.Col 5'-GCGGGACGGGTTTGGCAGGACGT-3'; siRNA002.Ler 5'-CATGTCGTTACCTTATGCGCCAGCC-3'; siRNA010 5'-GCAGGACGGGTTTGGCAGGACGT-3'; siRNA02 5'-GTTGACCAGTCCGCCAGCC-3'; siRNA03 5'-GGTTCCTCTTGACTCATGCTT-3'; siRNA06 5'-ATGCCAAGTTTGGCCTCACGG-3'; siRNA07 5'-ATGCCAAGTTTGGCCTCACGG-3'. All experiments were replicated with independent biological samples.

Supporting Information

Figure S1 Characterization of DEMETER family over-expression lines. Transline expressions giving the four members of the DEMETER family behind the nearly constitutive 35S promoter were assayed for transcript accumulation in leaves by quantitative reverse transcription-PCR. Overexpression of PRESSOR OF SILENCING (ROS) is in the C24 ecotype [34]; all other constructs are in Columbia (Col-0) [16]. All graphs are mean values for 3 biological replicates and were normalized to GAPDH expression. 35S::DME and 35S::ROS lines are homozygous; 35S::DML2 and 35S::DML3 are pooled samples of homozygous and hemizygous T2 individuals. Overexpression of DEMETER (DME) is weak, but sufficient to induce expression of MEDEA (MEA) in leaves (pink bars). (EPS)

Figure S2 The 08002 polymorphism in various Arabidopsis ecotypes. The p4-siRNA locus 08002 contains a six nucleotide indel between Arabidopsis ecotypes Columbia (Col) and Landsberg erecta (Ler). This polymorphism is the basis of the allele-specific probes 08002.Col and 08002.Ler (hybridizing to the region in bold type). To determine if these probes would also bind siRNAs from other ecotypes, the 08002 region from Wassilewskija (WS) and C24 was sequenced. These ecotypes are (Col)-like for the indel, but they also differ from Col at a single nucleotide (in red). However, this SNP does not appear to affect hybridization of the Col probe to C24 and WS siRNAs. (TIF)

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Author Contributions

Conceived and designed the experiments: RAM RLF CSP DCB. Performed the experiments: RAM. Analyzed the data: RAM. Contributed reagents/materials/analysis tools: EHT CSP. Wrote the paper: RAM.

References

1. Martin CC, McGowan R (1995) Parent-of-Origin Specific Effects on the Methylation of a Transgene in the Zebrafish, Danio-Rerio. Dev Genet 17: 233–239.
2. Lloyd VK, Sinclair DA, Grigliati TA (1999) Genomic imprinting and position-effect variegation in Drosophila melanogaster. Genetics 151: 1503–1516.
3. Goday C, Esteban MR (2001) Chromosome elimination in sciarid flies. Bioessays 23: 242–250.
4. Goday C, Esteban MR (2003) Chromosome elimination in sciarid flies. Bioessays 23: 242–250.
5. Sha K, Fire A (2005) Imprinting capacity of gamete lineages in Caenorhabditis elegans. Genetics 170: 1633–1632.
6. Sha K, Fire A (2005) Imprinting capacity of gamete lineages in Caenorhabditis elegans. Genetics 170: 1633–1632.
7. Sha K, Fire A (2005) Imprinting capacity of gamete lineages in Caenorhabditis elegans. Genetics 170: 1633–1632.
8. Sha K, Fire A (2005) Imprinting capacity of gamete lineages in Caenorhabditis elegans. Genetics 170: 1633–1632.
9. Sha K, Fire A (2005) Imprinting capacity of gamete lineages in Caenorhabditis elegans. Genetics 170: 1633–1632.
10. Gehring M, Hub JH, Hazl TF, Penterman J, Choi Y, et al. (2006) DEMETER DNA glycosylase establishes MEDEA polycistron gene self-imprinting by allele-specific demethylation. Cell 124: 495–506.
11. Kinoshita T, Miura A, Choi Y, Kinoshita Y, Cao X, et al. (2004) One-way control of PWA imprinting in Arabidopsis endosperm by DNA methylation. Science 303: 521–523.
12. Jullien PE, Katz A, Oliva M, Ohad N, Berger F (2006) Polycomb group complexes self-regulate imprinting of the Polycomb group gene MEDEA in Arabidopsis. Curr Biol 16: 486–492.
13. Tiwari S, Schulte R, Bood D, Dychau L, Bravo J, et al. (2008) MATERNALLY EXPRESSED PAB C-TERMINAL, a novel imprinted gene in Arabidopsis. Curr Biol 16: 486–492.
14. Makarevich V, Villar CB, Erolua A, Kohler C (2008) Mechanism of PHORES1 imprinting in Arabidopsis. Journal of Cell Science 121: 906–912.
15. Jullien PE, Kinoshita T, Ohad N, Berger F (2006) Maintenance of DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation. Cell 124: 495–506.
16. Gehring M, Hub JH, Hazl TF, Penterman J, Choi Y, et al. (2006) DEMETER DNA glycosylase establishes MEDEA polycistron gene self-imprinting by allele-specific demethylation. Cell 124: 495–506.
17. Kinoshita T, Miura A, Choi Y, Kinoshita Y, Cao X, et al. (2004) One-way control of PWA imprinting in Arabidopsis endosperm by DNA methylation. Science 303: 521–523.
18. Jullien PE, Katz A, Oliva M, Ohad N, Berger F (2006) Polycomb group complexes self-regulate imprinting of the Polycomb group gene MEDEA in Arabidopsis. Curr Biol 16: 486–492.
19. Tiwari S, Schulte R, Bood D, Dychau L, Bravo J, et al. (2008) MATERNALLY EXPRESSED PAB C-TERMINAL, a novel imprinted gene in Arabidopsis. Curr Biol 16: 486–492.
20. Makarevich V, Villar CB, Erolua A, Kohler C (2008) Mechanism of PHORES1 imprinting in Arabidopsis. Journal of Cell Science 121: 906–912.
21. Jullien PE, Kinoshita T, Ohad N, Berger F (2006) Maintenance of DNA methylation during the Arabidopsis life cycle is essential for parental imprinting. Plant Cell 18: 1369–1372.
22. Choi Y, Gehring M, Johnson L, Hannon M, Harada J, et al. (2002) DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in Arabidopsis. Cell 110: 33–42.
17. Hsieh T-F, Shin J, Uzawa R, Silva P, Cohen S, et al. (2011) Regulation of imprinted gene expression in Arabidopsis endosperm. Proc Natl Acad Sci U S A.

18. Mosher RA, Melnyk CW, Kelly KA, Dunn RM, Stadholme DJ, et al. (2009) Uniparental expression of PolIV-dependent siRNAs in developing endosperm of Arabidopsis. Nature 460: 283–286.

19. Chan SW-L, Zhang X, Bernatavichute YV, Jacobsen SE (2006) Two-step recruitment of RNA-directed DNA methylation to tandem repeats. PLoS Biol 4: 1923–1933.

20. Mosher RA, Schwach F, Stadholme D, Baulcombe DC (2008) PolIVb influences RNA-directed DNA-methylation independently of its role in siRNA biogenesis. Proc Natl Acad Sci U S A 105: 3145–3150.

21. Gehring M, Bubb KL, Henikoff S (2009) Extensive demethylation of repetitive elements during seed development underlies gene imprinting. Science 324: 1447–1451.

22. Hsieh TF, Ibarra CA, Silva P, Zemach A, Eshed-Williams L, et al. (2009) Genome-Wide demethylation of Arabidopsis endosperm. Science 324: 1451–1454.

23. Law JA, Jacobsen SE (2010) Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 11: 204–220.

24. Henderson IR, Jacobsen SE (2008) Tandem repeats upstream of the Arabidopsis endogene SDC recruit non-CG DNA methylation and initiate siRNA spreading. Gene Dev 22: 1597–1606.

25. Lippman Z, May B, Yordan C, Singer T, Martienssen R (2003) Distinct mechanisms determine transposon inheritance and methylation via small interfering RNA and histone modification. PLoS Biol 1: E7.

26. Earley KW, Pontvianne F, Wierzbicki AT, Blevins T, Tucker S, et al. (2010) Mechanisms of HDAC6-mediated RNA gene silencing: suppression of intergenic Pol II transcription and differential effects on maintenance versus siRNA-directed cytosine methylation. Gene Dev 24: 1119–1132.

27. Jackson JP, Lindroth AM, Cao X, Jacobsen SE (2002) Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. Nature 416: 536–540.

28. Hirochika H, Okamoto H, Kakutani T (2000) Silencing of retrotransposons in arabidopsis and reactivation by the ddm1 mutation. Plant Cell 12: 357–369.

29. Vaillant I, Schubert I, Tourmente S, Mathieu O (2006) MOM1 mediates DNA-methylation-independent silencing of repetitive sequences in Arabidopsis. EMBO Rep in press.

30. Gong Z, Morales-Ruiz T, Ariza RR, Roldan-Arjona T, David L, et al. (2002) ROS1, a repressor of transcriptional gene silencing in Arabidopsis, encodes a DNA glycosylase/lyase. Cell 111: 803–814.

31. Ortega-Galisteo AP, Morales-Ruiz T, Ariza RR, Roldan-Arjona T (2008) Arabidopsis DEMETER-LIKE proteins DML2 and DML3 are required for appropriate distribution of DNA methylation marks. Plant Mol Biol 67: 671–681.

32. Agius F, Kapoor A, Zhu JK (2006) Role of the Arabidopsis DNA glycosylase/lyase ROS1 in active DNA demethylation. Proc Natl Acad Sci U S A 103: 11796–11801.

33. Slotkin RK, Vaughn M, Borges F, Tanurdzic M, Becker JD, et al. (2009) Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. Cell 136: 461–472.

34. Fujimoto R, Kinoshita Y, Kawai A, Kinoshita T, Takashima K, et al. (2008) Evolution and control of imprinted FWA genes in the genus Arabidopsis. PLoS Genet 4.

35. Maggert KA, Golic KG (2002) The Y chromosome of Drosophila melanogaster exhibits chromosone-wide imprinting. Genetics 162: 1245–1258.

36. Crouse HV (1960) The Controlling Element in Sex Chromosome Behavior in Sciara. Genetics 45: 1429–1443.

37. Bean CJ, Schaner CE, Kelly WG (2004) Meiotic pairing and imprinted X chromatin assembly in Caenorhabditis elegans. Nat Genet 36: 100–105.

38. Kankel MW, Ramsey DE, Stokes TL, Flowers SK, Haag JR, et al. (2003) Arabidopsis MET1 cytosine methyltransferase mutants. Genetics 163: 1109–1122.

39. Chan SW-L, Henderson IR, Zhang X, Shah G, Chien JS-C, et al. (2006) RNAi, DRD1, and histone methylation actively target developmentally important non-CG DNA methylation in Arabidopsis. PLoS Genet 2: 0791–0797.

40. Lindroth AM, Cao X, Jacobsen SE (2002) Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. Nature 416: 536–540.

41. Earley KW, Pontes O, Reuther R, Opper K, Juehne T, et al. (2006) Gateway-compatible vectors for plant functional genomics and proteomics. Plant J 45: 616–629.