Active DNA Demethylation in Plant Companion Cells Reinforces Transposon Methylation in Gametes

Christian A. Ibarra *et al.*

Science **337**, 1360 (2012);
DOI: 10.1126/science.1224839

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

corpus callosum and optic nerve. Furthermore, in the mPFC, hypomyelination occurs even when oligodendrocyte ErbB3 is lost at later stages (after P19). Moreover, mPFC myelination in CNP-DN-ErbB4 mice is not different from wild type at P21 but is clearly altered by P35 (fig. S10). ErbB4 KO mice do not have alterations in CNS myelin even upon reaching adulthood (*24*), and loss of ErbB3 produces the same phenotype as expression of DN-ErbB4, which blocks both ErbB3 and ErbB4 function; this indicates that ErbB3 is the critical ErbB receptor for CNS myelination. Our data suggest that normal type III NRG1 expression is necessary for mature mPFC myelination, in agreement with the observation that reduced type III NRG1 expression leads to hypomyelination in several brain regions (*22*), whereas NRG1 overexpression results in thicker CNS myelin (*24*).

The effects of social experience on mPFC myelination depend, at least in part, on the social experience–dependent regulation of NRG1-ErbB3 signaling. We propose that lack of social interactions during the juvenile period leads to reduced type III NRG1 expression by mPFC neurons, resulting in reduced oligodendrocyte ErbB3 signaling and thus incomplete oligodendrocyte maturation and myelination. Previous studies showed that neuronal activity influences expression of type I but not type III NRG1 mRNA in the intact brain and cultures of cortical neurons (*33*), a finding we replicated in primary cultures of frontal cortex neurons (fig. S11A). Furthermore, expression of type III NRG1 was unaffected in the mPFC of mice with oligodendrocyte ErbB3 KO or those expressing DN-ErbB4 in oligodendrocytes (fig. S11B). These results suggest that social experience affects mPFC myelination by influencing the levels of type III NRG1 in mPFC neurons independent of neuronal depolarization and ErbB receptor signaling in oligodendrocytes. Nevertheless, the possibility that other molecular events also contribute to the effects of social experience on myelination should not be dismissed. For example, in vitro studies by Wake *et al*. (*34*) showed that release of glutamate along axons induces myelination in part by increasing synthesis of myelin proteins in the associated oligodendrocytes. Thus, electrical activity in mPFC might influence the translation of mRNA species that are regulated by type III NRG1 in an experience-dependent fashion and so contribute to normal myelination.

Our findings indicate that the effects of childhood isolation and neglect on adult mental health might be caused, at least in part, by alterations in oligodendrocytes and myelin development. Furthermore, we provide a cellular and/or molecular context and genetic models in which to begin to understand the effects of juvenile social experience on brain development in general and myelin maturation in particular. Our results also may be relevant to neuropsychiatric disorders such as schizophrenia and mood disorders, which usually manifest after the juvenile period and have been linked to alterations in white matter and myelination (*35*, *36*). In this respect, it is noteworthy that the NRG1-ErbB signaling pathway has been genetically linked to neuropsychiatric disorders (*36*, *37*), which suggests that genetic liabilities in this pathway and life experience may interact in the pathogenesis of these diseases.

References and Notes

1. B. Egeland, L. A. Sroufe, M. Erickson, *Child Abuse Negl.* **7**, 459 (1983).
2. C. S. Widom, K. DuMont, S. J. Czaja, *Arch. Gen. Psychiatry* **64**, 49 (2007).
3. K. Bos *et al*., *Harv. Rev. Psychiatry* **19**, 15 (2011).
4. S. D. Pollak *et al*., *Child Dev.* **81**, 224 (2010).
5. H. T. Chugani *et al*., *Neuroimage* **14**, 1290 (2001).
6. T. J. Eluvathingal *et al*., *Pediatrics* **117**, 2093 (2006).
7. M. M. Sánchez, E. F. Hearn, D. Do, J. K. Rilling, J. G. Herndon, *Brain Res.* **812**, 38 (1998).
8. R. J. Zatorre, R. D. Fields, H. Johansen-Berg, *Nat. Neurosci.* **15**, 528 (2012).
9. B. S. Mallon, H. E. Shick, G. J. Kidd, W. B. Macklin, *J. Neurosci.* **22**, 876 (2002).
10. J. C. Murtie, W. B. Macklin, G. Corfas, *J. Neurosci. Res.* **85**, 2080 (2007).
11. O. Yizhar *et al*., *Nature* **477**, 171 (2011).
12. R. Dias, J. P. Aggleton, *Eur. J. Neurosci.* **12**, 4457 (2000).
13. H. A. Van de Weerd *et al*., *J. Appl. Anim. Welf. Sci.* **5**, 87 (2002).
14. K. T. Winterfeld, G. Teuchert-Noodt, R. R. Dawirs, *J. Neurosci. Res.* **52**, 201 (1998).
15. T. Hol, C. L. Van den Berg, J. M. Van Ree, B. M. Spruijt, *Behav. Brain Res.* **100**, 91 (1999).
16. S. K. Park, R. Miller, I. Krane, T. Vartanian, *J. Cell Biol.* **154**, 1245 (2001).
17. J. Y. Kim, Q. Sun, M. Oglesbee, S. O. Yoon, *J. Neurosci.* **23**, 5561 (2003).
18. S. Chen *et al*., *J. Neurosci.* **26**, 3079 (2006).
19. G. V. Michailov *et al*., *Science* **304**, 700 (2004).
20. C. Taveggia *et al*., *Neuron* **47**, 681 (2005).
21. K. Roy *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **104**, 8131 (2007).
22. C. Taveggia *et al*., *Glia* **56**, 284 (2008).
23. J. Schmucker *et al*., *Glia* **44**, 67 (2003).
24. B. G. Brinkmann *et al*., *Neuron* **59**, 581 (2008).
25. S. Qu *et al*., *Genesis* **44**, 477 (2006).
26. N. H. Doerflinger, W. B. Macklin, B. Popko, *Genesis* **35**, 63 (2003).
27. V. Prevot *et al*., *J. Neurosci.* **23**, 230 (2003).
28. D. L. Falls, *Exp. Cell Res.* **284**, 14 (2003).
29. J. L. Gariépy, P. L. Gendreau, R. B. Mailman, M. Tancer, M. H. Lewis, *Pharmacol. Biochem. Behav.* **51**, 767 (1995).
30. S. Vijayraghavan, M. Wang, S. G. Birnbaum, G. V. Williams, A. F. Arnsten, *Nat. Neurosci.* **10**, 376 (2007).
31. C. R. Li, G. B. Huang, Z. Y. Sui, E. H. Han, Y. C. Chung, *Brain Res.* **1346**, 183 (2010).
32. B. Rico, O. Marín, *Curr. Opin. Genet. Dev.* **21**, 262 (2011).
33. X. Liu *et al*., *J. Neurosci.* **31**, 8491 (2011).
34. H. Wake, P. R. Lee, R. D. Fields, *Science* **333**, 1647 (2011).
35. R. D. Fields, *Trends Neurosci.* **31**, 361 (2008).
36. G. Corfas, K. Roy, J. D. Buxbaum, *Nat. Neurosci.* **7**, 575 (2004).
37. A. Buonanno, *Brain Res. Bull.* **83**, 122 (2010).

Acknowledgments: We thank T. Schwarz, C. J. Woolf, R. A. Corfas, M. Rao, F. Sher and M. Lozada for critical reading of the manuscript; C. Hart for help with the mouse colony; G. Gorski for help with electron microscopy; C. Arteaga for the generous gift of the ErbB3 floxed mouse line; J. C. Dodart for advice on behavioral tests; M. Fagiolini for allowing us to use behavioral equipment. This research was supported in part by the National Institute of Neurological Disorders and Stroke, NIH, grant R01 NS35884 (to G.C.), by Lori and Joel Freedman (to G.C.), an NIH Intellectual and Developmental Disabilities Research Center grant P30-HD 18655, and Nara Medical University (M.M.).

Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6100/1357/DC1
Materials and Methods
Figs. S1 to S11
Table S1
References (*38*)

21 February 2012; accepted 13 July 2012
10.1126/science.1220845

Active DNA Demethylation in Plant Companion Cells Reinforces Transposon Methylation in Gametes

Christian A. Ibarra,[1]* Xiaoqi Feng,[1]* Vera K. Schoft,[2]* Tzung-Fu Hsieh,[1]* Rie Uzawa,[1] Jessica A. Rodrigues,[1] Assaf Zemach,[1] Nina Chumak,[2] Adriana Machlicova,[2] Toshiro Nishimura,[1] Denisse Rojas,[1] Robert L. Fischer,[1]† Hisashi Tamaru,[2]† Daniel Zilberman[1]†

The *Arabidopsis thaliana* central cell, the companion cell of the egg, undergoes DNA demethylation before fertilization, but the targeting preferences, mechanism, and biological significance of this process remain unclear. Here, we show that active DNA demethylation mediated by the DEMETER DNA glycosylase accounts for all of the demethylation in the central cell and preferentially targets small, AT-rich, and nucleosome-depleted euchromatic transposable elements. The vegetative cell, the companion cell of sperm, also undergoes DEMETER-dependent demethylation of similar sequences, and lack of DEMETER in vegetative cells causes reduced small RNA–directed DNA methylation of transposons in sperm. Our results demonstrate that demethylation in companion cells reinforces transposon methylation in plant gametes and likely contributes to stable silencing of transposable elements across generations.

Cytosine methylation regulates gene expression and represses transposable elements (TEs) in plants and vertebrates (*1*). DNA methylation in plants is catalyzed by three families of DNA methyltransferases that can be roughly grouped by the preferred sequence context: CG, CHG, and CHH (H = A, C, or T). The small RNA (sRNA) pathway targets de novo methylation in all sequence contexts and is required for the maintenance of CHH methylation.

Flowering plant sexual reproduction involves two fertilization events (*2*). The pollen vegetative cell forms a tube that carries two sperm cells to the ovule, where one fuses with the diploid central cell to form the triploid placenta-like endosperm, and the other fertilizes the haploid egg to produce the embryo. Endosperm DNA of *Arabidopsis thaliana* is modestly, but globally, less methylated than embryo DNA in all contexts (*3*). The DEMETER (DME) DNA glycosylase that excises 5-methylcytosine is highly expressed in the central cell before fertilization and is at least partially required for the demethylation observed in endosperm (*2*, *3*), which has been inferred to occur on the maternal chromosomes inherited from the central cell. Passive mechanisms, such as down-regulation of the MET1 DNA methyltransferase, have also been proposed to contribute to demethylation of the maternal endosperm genome (*2*). The global differences between embryo and endosperm are consistent with passive demethylation and suggest that the process may have little sequence specificity (*3*, *4*). However, DNA methylation of the maternal and paternal endosperm genomes has not been compared except for a few loci, and therefore, it is difficult to make general inferences about the mechanism and specificity of central cell demethylation. Why the central cell should undergo extensive DNA demethylation is also unclear.

To understand the extent, mechanism, and biological significance of active demethylation in the central cell, we used reciprocal crosses between the Col and L*er* accessions of *Arabidopsis* that differ by >400,000 single-nucleotide polymorphisms (SNPs) (*4*) to identify DNA methylation that resides on either the maternal or paternal endosperm genome (*5*) by shotgun bisulfite sequencing (table S1). The wild-type maternal genome is substantially less methylated than the paternal genome in the CG context (Fig. 1A and figs. S1 and S2), with slight global hypomethylation accompanied by strong local demethylation (Fig. 1B and figs. S3 and S4). The local demethylation is nearly fully reversed in *dme* mutant endosperm (Fig. 1, A and B, and figs. S2, S4, and S5), which indicates that DME is either the only or by far the major enzyme required for excision of 5-methylcytosine in the central cell and demonstrating that active DNA demethylation of at least 9816 specific sequences spanning 4,443,250 bp (table S2) accounts for the methylation differences between the maternal and paternal endosperm

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA. [2]Gregor Mendel Institute, Austrian Academy of Sciences, 1030 Vienna, Austria.

*These authors contributed equally.
†To whom correspondence should be addressed. E-mail: rfischer@berkeley.edu (R.L.F.); hisashi.tamaru@gmi.oeaw.ac.at (H.T.); danielz@berkeley.edu (D.Z.)

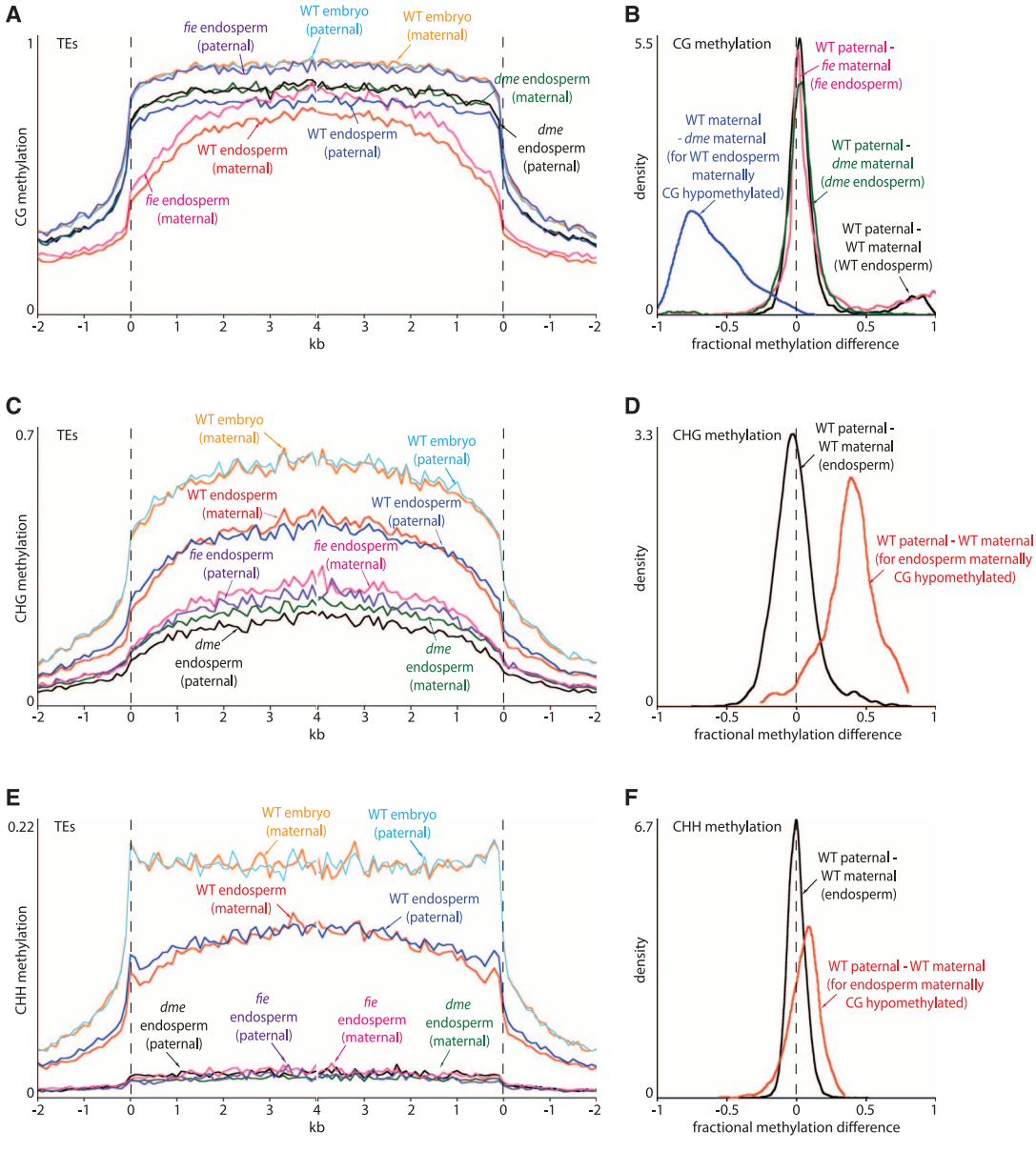

Fig. 1. Local DME-dependent demethylation of maternal endosperm chromosomes. (**A**, **C**, and **E**) Transposons were aligned at the 5′ and 3′ ends (dashed lines) and average methylation levels for each 100-bp interval are plotted. (**B**, **D**, and **F**) Kernel density plots trace the frequency distribution of endosperm methylation differences for all 50-bp windows with an informative SNP. A shift of the peak with respect to zero represents a global difference; shoulders represent local differences.

genomes. Global CG methylation of both maternal and paternal genomes is slightly elevated by lack of DME compared with wild type (Fig. 1A and fig. S5), consistent with overexpression of genes that mediate CG methylation in *dme* endosperm (*4*).

Global CHG methylation of the wild-type endosperm maternal genome is similar to that of the paternal genome (Fig. 1, C and D), but loci that are maternally demethylated in the CG context show strong maternal CHG demethylation (Fig. 1D), consistent with the reported in vitro activity of DME on methylation in all sequence contexts (*6*). A similar but weaker correspondence exists for CHH methylation (Fig. 1, E and F), presumably because sRNA-directed DNA methylation (RdDM) patterns are more variable, and may be partially restored after fertilization. We did not observe major methylation differences between parental genomes in embryo (Fig. 1, A, C, and E; and figs. S1, S2, S4, and S6).

As we showed previously, *dme* endosperm has greatly reduced CHG methylation and almost no CHH methylation (*3*), and our present data show that this applies similarly to both parental genomes (Fig. 1, C and E, and fig. S2). As this is the opposite of the outcome expected from a mutation in a demethylating enzyme, we hypothesized an indirect mechanism. DME activity is required for functionality of the Polycomb repressive complex 2 (PRC2) in endosperm, prompting us to examine methylation in endosperm lacking maternal activity of the core PRC2 protein FIE (*2*). Lack of FIE had an effect similar to that of lack of DME on non-CG methylation (Fig. 1, C and E, and table S1), which indicates that PRC2 promotes non-CG DNA methylation in endosperm. In contrast, local maternal CG demethylation was mostly unaffected by lack of FIE (Fig. 1B and figs. S4 and S5), as would be expected with direct activity of DME. Global CG methylation levels were increased even more than in *dme* endosperm (Fig. 1A and fig. S5), consistent with strong overexpression of genes that mediate CG methylation in *fie* endosperm (*4*).

DME-mediated DNA demethylation in the central cell is required to establish monoallelic (imprinted) expression of a number of genes in the endosperm (*2*, *4*). We examined the location of loci that are significantly less methylated in wild-type endosperm than in *dme* endosperm (table S2) in relation to imprinted endosperm genes (fig. S4). Maternally and paternally expressed genes are preferentially associated with such differentially methylated regions (DMRs), particularly just upstream of the gene (fig. S7). Maternally expressed genes also exhibit DMRs that span the transcriptional start site (fig. S7), consistent with the strong correlation between methylation of this region and gene silencing (*1*). Paternally expressed genes are enriched in DMRs within the gene body (fig. S7), which suggests that gene body demethylation can disrupt gene expression, for example, by revealing repressor binding sites, as has been proposed for the DMR downstream of the *PHERES1* gene (fig. S4) (*2*).

Several TEs were reported to be less methylated in the pollen vegetative cell of *Arabidopsis* compared with sperm (*7*), and we recently showed that DME is required for demethylation of two genes in the vegetative cell that are demethylated by DME in the central cell (*8*). These data suggest that DME-mediated DNA demethylation may proceed similarly in the central and vegetative cells. To examine this issue, we compared DNA methylation patterns in sperm and vegetative cell nuclei that were purified by fluorescence-activated cell sorting (*8*, *9*). Most CG sites are heavily and similarly methylated in both cell types, but a subset is specifically demethylated in the vegetative cell (Fig. 2, A and B, and figs. S3, S4, and S8), corresponding to at least 9932 loci spanning 4,068,000 bp (table S2). The demethylated vegetative cell CG sites overlap 45.5% of those demethylated in the maternal endosperm genome and, by extension, in the central cell (Fig. 2B, fig. S4, and table S2).

We examined methylation in pollen from heterozygous *dme*/+ plants (strong *dme* alleles cannot be made homozygous), in which half of the pollen lacks DME (*8*). CG sites that are demethylated in wild-type vegetative cells showed much greater methylation in vegetative cell nuclei isolated from *dme*/+ pollen (Fig. 2, A and B, and fig. S4), which indicates that DME is required for demethylation in the vegetative cell. CHG methylation is generally higher in the vegetative cell than in the sperm cell (Fig. 2, C and D), but loci demethylated at CG sites in the vegetative cell are also demethylated at CHG sites (Fig. 2D), as they are in endosperm (Fig. 1D). CHH methylation is very high in the vegetative cell and low in sperm (Fig. 2E), consistent with the strong RdDM activity reported in the vegetative cell (*9*). Nonetheless, vegetative cell CG-demethylated loci tend to show lower CHH methylation in vegetative cells than in sperm (Fig. 2F). Overall, our data strongly support the hypothesis that DNA demethylation

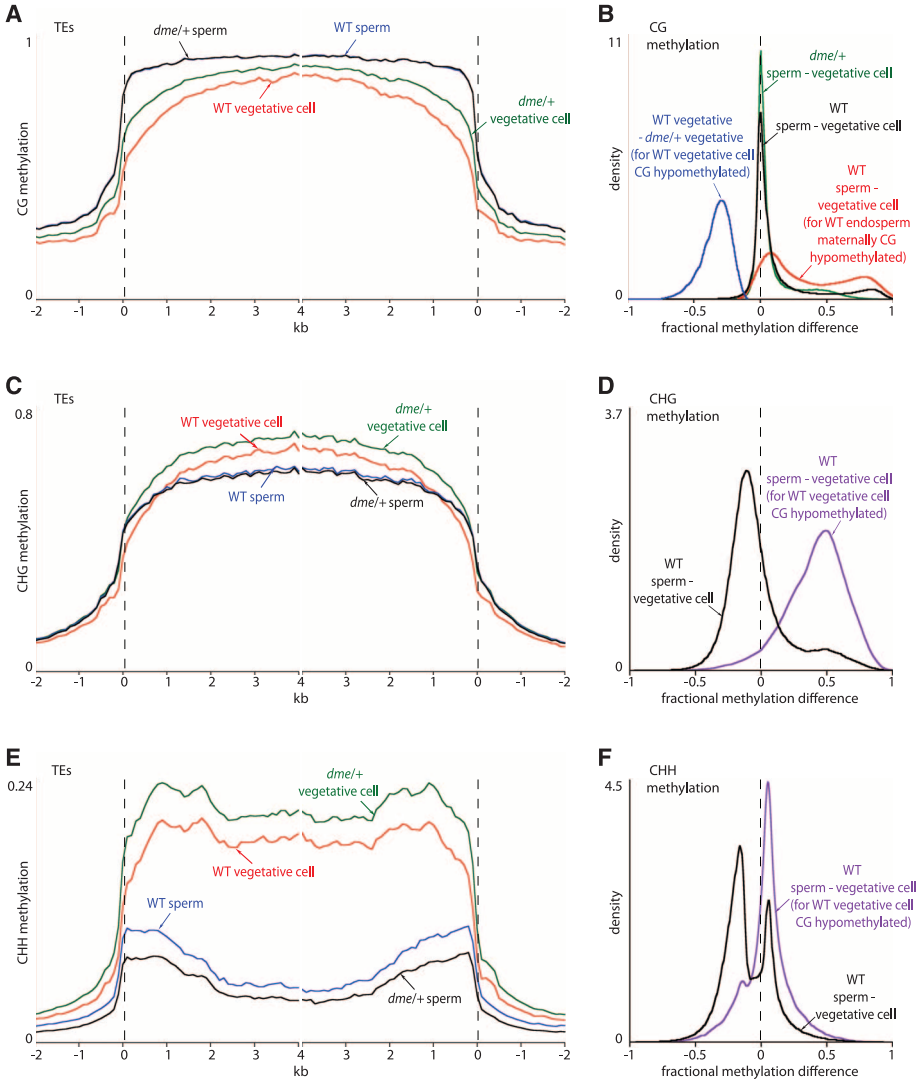

Fig. 2. Local DME-dependent demethylation in the pollen vegetative cell. (**A**, **C**, and **E**) Average methylation in transposons was plotted as in Fig. 1. (**B**, **D**, and **F**) Kernel density plots, as in Fig. 1, of pollen methylation differences in 50-bp windows.

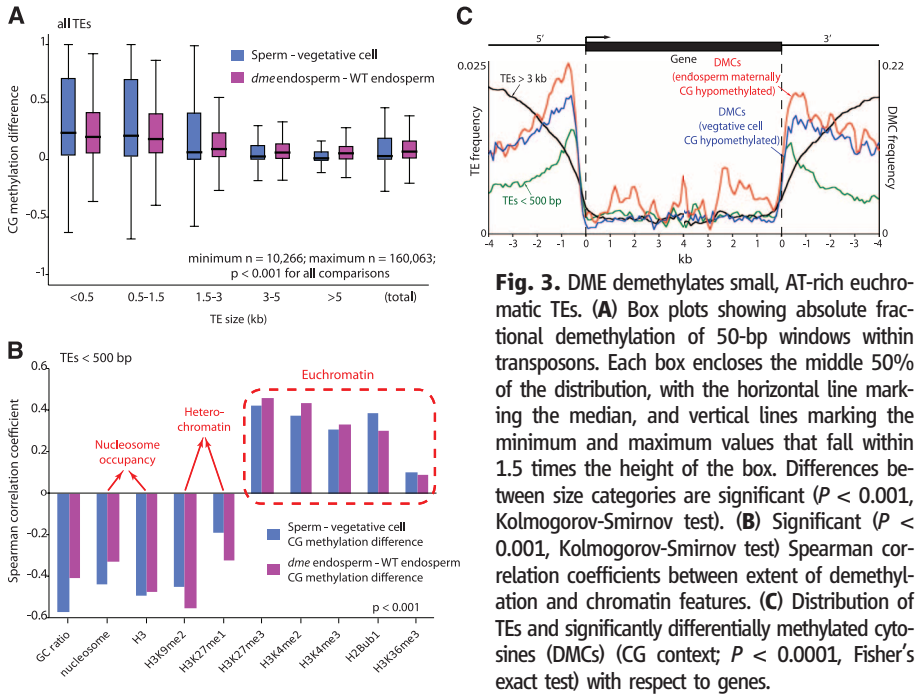

Fig. 3. DME demethylates small, AT-rich euchromatic TEs. (**A**) Box plots showing absolute fractional demethylation of 50-bp windows within transposons. Each box encloses the middle 50% of the distribution, with the horizontal line marking the median, and vertical lines marking the minimum and maximum values that fall within 1.5 times the height of the box. Differences between size categories are significant ($P < 0.001$, Kolmogorov-Smirnov test). (**B**) Significant ($P < 0.001$, Kolmogorov-Smirnov test) Spearman correlation coefficients between extent of demethylation and chromatin features. (**C**) Distribution of TEs and significantly differentially methylated cytosines (DMCs) (CG context; $P < 0.0001$, Fisher's exact test) with respect to genes.

in the male and female companion cells proceeds by an active, DME-dependent mechanism.

Loci demethylated in companion cells tend to be within smaller AT-rich TEs that are enriched for euchromatin-associated histone modifications (*10*) and depleted of nucleosomes with heterochromatin-associated modifications (Fig. 3, A and B, and figs. S9 and S10), which suggests that chromatin structure plays an important role in regulating active DNA demethylation (*11*), consistent with the decondensation of heterochromatin observed in central and vegetative cells (*9*, *12*). TEs longer than 3 kb are rarely demethylated (Fig. 3A and fig. S3), except at the edges (fig. S9), consistent with our published observations in rice (*13*). The preference for smaller TE demethylation holds for TEs of various types (fig. S10). Demethylation of small TEs accounts for the shapes of the wild-type maternal endosperm methylation traces (Fig. 1, A, C, and E) and the wild-type vegetative cell methylation traces (Fig. 2, A and C), which are closer to the paternal and sperm traces, respectively, in the middle (long TEs) than at the points of alignment (mostly small TEs). Small *Arabidopsis* TEs, like

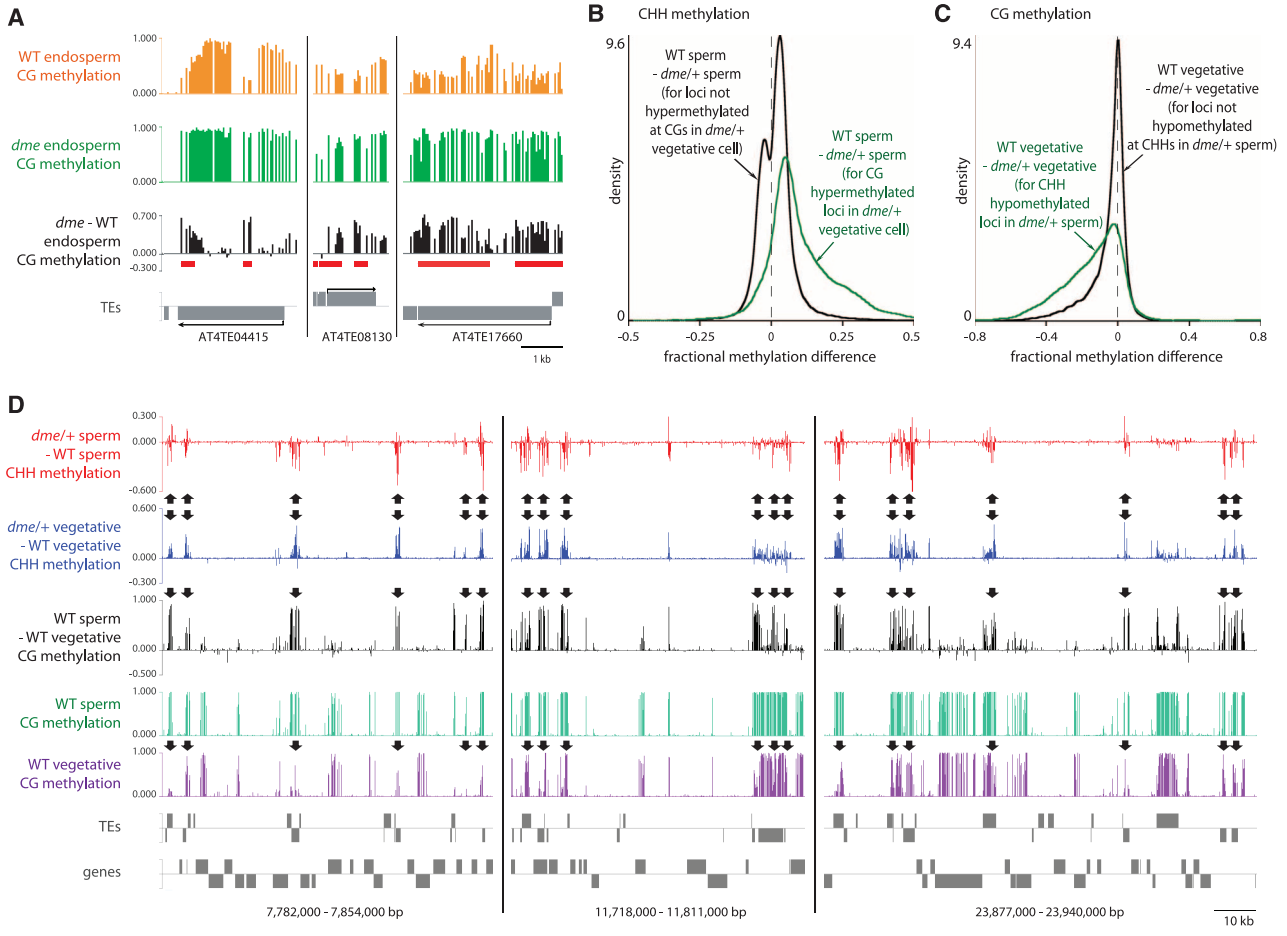

Fig. 4. Demethylation activates TEs in companion cells and reinforces methylation in sperm. (**A**) DME-dependent demethylation of TEs that are maternally expressed in wild-type, but not *dme*, endosperm (table S4). DMRs (table S2) are underlined. (**B**) Kernel density plots of the CHH methylation differences between sperm cells from wild-type and *dme*/+ plants. (**C**) Kernel density plots of the CG methylation differences between vegetative cells from wild-type and *dme*/+ plants. (**D**) Snapshots of CG and CHH methylation in pollen on chromosome 1. Arrows point out loci with decreased CHH methylation in sperm and increased CG methylation in vegetative cells from *dme*/+ pollen.

their rice counterparts (*13*), tend to occur near genes (Fig. 3C), explaining the observation that DME and related glycosylases preferentially demethylate gene-adjacent sequences (Fig. 3C) (*2*, *14*, *15*). This phenomenon explains why CHG and CHH methylation is lower in wild-type vegetative cells compared with sperm near genes (fig. S8), even though overall non-CG methylation is higher in vegetative cells than in sperm (Fig. 2, C to F).

The abundance of DME targets in gene-poor heterochromatin (fig. S11) and the overlap among DME targets in the central and vegetative cells (table S2), despite their different functions and developmental fates, suggest that establishment of genomic imprinting is not the basal function of DME. DNA demethylation and activation of TEs in the vegetative cell was proposed to generate sRNAs that would reinforce silencing of complementary TEs in sperm (*7*). If such TEs are demethylated in the central cell, their maternal copies should remain active in endosperm. Indeed, we identified 11 TEs demethylated by DME that are specifically maternally expressed in wild-type, but not *dme*, endosperm (Fig. 4A and table S4) (*4*). We also tested whether sRNAs can travel from the central cell to the egg by expressing a microRNA in the central cell that targets cleavage of green fluorescent protein (GFP) RNA expressed from a transgene in the egg, analogous to an experiment performed earlier in pollen (*7*). The central cell-expressed microRNA substantially reduced GFP fluorescence in the egg (fig. S12 and table S5), which suggests that central cell sRNAs can travel into and function in the egg.

If silencing induced by companion cell sRNAs occurs at the transcriptional level, lack of DME in the companion cell would be expected to reduce RdDM of DME target sequences in gametes. Indeed, overall CHH methylation of TEs is decreased in *dme*/+ sperm compared with wild-type sperm (Fig. 2E), and CG sites demethylated by DME in vegetative cells show preferential CHH hypomethylation in *dme*/+ sperm (Fig. 4, B and D). Conversely, loci that exhibit decreased CHH methylation in *dme*/+ sperm show increased CG methylation in *dme*/+ vegetative cells (Fig. 4, C and D). Thus, DME activity in the vegetative cell is required for full methylation of a subset of sperm TEs, which indicates that demethylation in companion cells generates a mobile signal—probably sRNA—that immunizes the gametes against TE activation. This conclusion is supported by the specific CHH hypermethylation of small, endosperm-demethylated TEs observed in the rice embryo (*13*).

References and Notes

1. J. A. Law, S. E. Jacobsen, *Nat. Rev. Genet.* **11**, 204 (2010).
2. M. J. Bauer, R. L. Fischer, *Curr. Opin. Plant Biol.* **14**, 162 (2011).
3. T.-F. Hsieh *et al.*, *Science* **324**, 1451 (2009).
4. T.-F. Hsieh *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 1755 (2011).
5. Materials and methods are available as supplementary materials on *Science Online*.
6. M. Gehring *et al.*, *Cell* **124**, 495 (2006).
7. R. K. Slotkin *et al.*, *Cell* **136**, 461 (2009).
8. V. K. Schoft *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 8042 (2011).
9. V. K. Schoft *et al.*, *EMBO Rep.* **10**, 1015 (2009).
10. F. Roudier *et al.*, *EMBO J.* **30**, 1928 (2011).
11. W. Qian *et al.*, *Science* **336**, 1445 (2012).
12. M. Pillot *et al.*, *Plant Cell* **22**, 307 (2010).
13. A. Zemach *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 18729 (2010).
14. R. Lister *et al.*, *Cell* **133**, 523 (2008).
15. J. Penterman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 6752 (2007).

Acknowledgments: We thank S. Harmer for assistance with the analysis of histone modifications, the BioOptics team at the Vienna Biocenter Campus for sorting sperm and vegetative cell nuclei, K. Slotkin for the *LAT52p-amiRNA=GFP* plasmid, and G. Drews for the *DD45p-GFP* transgenic line. This work was partially funded by an NIH grant (GM69415) to R.L.F., NSF grants (MCB-0918821 and IOS-1025890) to R.L.F. and D.Z., a Young Investigator Grant from the Arnold and Mabel Beckman Foundation to D.Z., an Austrian Science Fund (FWF) grant P21389-B03 to H.T., a Ruth L. Kirschstein NIH Predoctoral Fellowship (GM093633) to C.A.I., a Fulbright Scholarship to J.A.R., a fellowship from the Jane Coffin Childs Memorial Fund to A.Z., and a Robert and Colleen Haas Scholarship to D.R. Sequencing data are deposited in GEO (GSE38935).

Supplementary Materials

www.sciencemag.org/cgi/content/full/337/6100/1360/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S12
Tables S1 to S5
References (*16*–*28*)

17 May 2012; accepted 11 July 2012
10.1126/science.1224839