

# An invariant aspartic acid in the DNA glycosylase domain of *DEMETER* is necessary for transcriptional activation of the imprinted *MEDEA* gene

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Helix–hairpin–helix DNA glycosylases are typically small proteins that initiate repair of DNA by excising damaged or mispaired bases. An invariant aspartic acid in the active site is involved in catalyzing the excision reaction. Replacement of this critical residue with an asparagine severely reduces catalytic activity but preserves enzyme stability and structure. The *Arabidopsis DEMETER* (*DME*) gene encodes a large 1,729-aa polypeptide with a 200-aa DNA glycosylase domain. *DME* is expressed primarily in the central cell of the female gametophyte. *DME* activates maternal allele expression of the imprinted *MEDEA* (*MEA*) gene in the central cell and is required for seed viability. We mutated the invariant aspartic acid at position 1304 in *DME* to asparagine (*D1304N*) to determine whether the catalytic activity of the DNA glycosylase domain is required for *DME* function *in vivo*. Transgenes expressing wild-type *DME* in the central cell rescue seed abortion caused by a mutation in the endogenous *DME* gene and activate maternal *MEA:GFP* transcription. However, transgenes expressing the *D1304N* mutant *DME* do not rescue seed abortion or activate maternal *MEA:GFP* transcription. Whereas ectopic expression of the wild-type *DME* polypeptide in pollen is sufficient to activate ectopic paternal *MEA* and *MEA:GUS* expression, equivalent expression of the *D1304N* mutant *DME* in pollen failed to do so. These results show that the conserved aspartic acid residue is necessary for *DME* to function *in vivo* and suggest that an active DNA glycosylase domain, normally associated with DNA repair, promotes gene transcription that is essential for gene imprinting.

DNA glycosylases are typically low-molecular-weight (200–300 aa) enzymes responsible for recognizing base lesions in the genome and initiating base excision repair. These proteins excise mispaired or damaged (e.g., oxidized, alkylated, deaminated, or methylated) bases (1, 2). After base excision, the DNA is further processed by the concerted action of an endonuclease, a DNA polymerase, and a DNA ligase. DNA glycosylases have been conserved during evolution and four structural families have been identified based on similarity to a uracil DNA glycosylase (UDG) family, an alkyladenine DNA glycosylase (AAG) family, a bacterial 8-oxoguanine DNA glycosylase (MutM/Fpg) family, and a HhH family that share a HhH active-site motif (3). The mispaired or damaged bases that are repaired by DNA glycosylases arise from the inherent chemical instability of DNA, from errors arising from the activity of DNA polymerase during replication, and from exposure to DNA-damaging agents present in the environment (4). Such damage has the potential to cause mutations and cell death, and bacterial strains deficient in DNA glycosylases (Ung, MutY, and MutM) show a mutator phenotype (1, 5). However, whereas DNA glycosylase-deficient mice show marked sensitivity to the DNA-damaging agents at the cellular level, they do not display any developmental abnormalities (6, 7). Therefore, little is known about the function of DNA glycosylases during eukaryote development.

*DEMETER* (*DME*) encodes a large polypeptide (1,729 aa) with a nuclear localization signal and a 200-aa DNA glycosylase

domain. The *DME* DNA glycosylase domain is most closely related to the HhH family of DNA glycosylases (8). Within the *DME* DNA glycosylase domain are conserved amino acid residues essential for DNA glycosylase activity. *DME* is required for maternal allele expression of the imprinted genes *MEDEA* (*MEA*), a Polycomb group gene, and *FWA*, a transcription factor gene, in the central cell of the female gametophyte and in the endosperm of *Arabidopsis* (8, 9). The central cell gives rise to the endosperm, an embryo-nourishing tissue, on fertilization. *MEA* function is required for seed viability; a seed inheriting a mutant maternal *mea* allele aborts regardless of genotype of the silent paternal allele (10–13). Seed viability also depends solely on the maternal *DME* allele, and seeds that inherit a mutant maternal *dme* allele abort regardless of the paternal *DME* allele genotype. *DME* is primarily expressed in the central cell of the female gametophyte, where it activates maternal *MEA* allele expression. *DME* and *MEA* are not expressed in the stamens, which produce the male gametophyte or pollen. After fertilization of the central cell, *DME* expression is greatly reduced. Therefore, only the maternal *MEA* allele, not the paternal *MEA* allele, is exposed to *DME* activity. Thus, *DME* establishes *MEA* imprinting (maternal allele expressed, paternal allele not expressed) in the endosperm.

The crystal structures of four proteins in the HhH family of DNA glycosylases (EndoIII, AlkA, MutY, and hOGG1) revealed a conserved HhH motif followed at a fixed distance by a glycine–proline-rich loop and an invariant aspartic acid residue in the active site (3, 14–21). Mutation of the invariant aspartic acid residue to asparagine reduces the catalytic activity of DNA glycosylases *in vitro*; depending on the enzyme, *in vitro* activity is either abolished or reduced by ≈65-fold. Biochemical and crystallographic analyses indicate that this mutation preserves the DNA glycosylase structure and stability but reduces enzymatic activity (19, 20), establishing an essential role for the invariant aspartic acid in the catalysis of base excision.

The function of the predicted *DME* DNA glycosylase domain in the activation of gene transcription in the central cell is not known. One possibility is that the DNA glycosylase domain is required to activate transcription. Alternatively, the *DME* DNA glycosylase domain might not be needed and other *DME* domains are responsible for activation of transcription. To distinguish between these alternatives, we mutated the conserved aspartic acid at position 1304 in *DME* to asparagine (*D1304N*) and compared the *in vivo* function of wild-type *DME*

Abbreviations: *DME*, *DEMETER*; *MEA*, *MEDEA*; *GUS*,  $\beta$ -glucuronidase; *DME:DME*, *DME* promoter ligated to *DME* cDNA; *DME:DME(D1304N)*, *DME* promoter ligated to a mutagenized *DME* cDNA where aspartic acid at position 1304 is changed to asparagine; *CaMV:DME*, cauliflower mosaic virus promoter ligated to *DME* cDNA; *CaMV:DME(D1304N)*, cauliflower mosaic virus promoter ligated to mutant *DME(D1304N)* cDNA; *MEA:GFP*, *MEA* promoter ligated to *GFP* cDNA; *MEA:GUS*, *MEA* promoter ligated to *GUS* cDNA; HhH, helix–hairpin–helix.

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and mutant DME(D1304N) polypeptides in *Arabidopsis* plants. We show that the invariant aspartic acid residue D1304 is necessary for DME function in the central cell, suggesting that an active DME DNA glycosylase is essential to regulate *MEA* gene imprinting and seed viability. Thus, the DNA glycosylase domain that is usually associated with DNA repair can also function during plant reproduction as a regulator of gene transcription, imprinting, and endosperm development.

## Materials and Methods

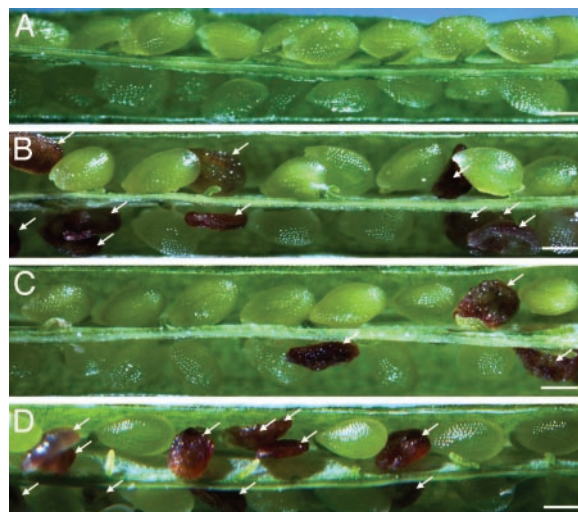
**Plant Materials and Microscopy.** Wild-type and mutant *dme* alleles (Landsberg *er* ecotype) are as described (8). Methods for growing plants, fixing tissues, photography,  $\beta$ -glucuronidase (GUS) activity localization, and fluorescence microscopy were as described (22).

**Generation of Transgenic Lines.** To mutate the conserved aspartic acid at position 1304 to asparagine, *JHDME1* primer (CCCT-GTTaACACGAATGTTGGAAGGATAGC; codon for asparagine is underlined, mutagenized base is lowercase) and *SKEN-5* (8) primer were used in a PCR to amplify a 1.3-kb *DME* (*D1304N*) cDNA. A 0.5-kb DNA fragment spanning the D1304 codon was excised from the wild-type *DME* cDNA clone by digestion with *HincII* and *XmaI* restriction endonucleases and was replaced with the 0.5-kb *HincII* and *XmaI* DNA fragment with the D1304N mutation, creating the full-length *DME*(*D1304N*) cDNA clone. The *DME* promoter ligated to *DME* cDNA [*DME:DME* (8)] and the *DME* promoter ligated to a mutagenized *DME* cDNA where aspartic acid at position 1304 is changed to asparagine [*DME:DME*(*D1304N*)] transgenes were generated by ligating 3.4 kb of *DME* 5' flanking sequences to the wild-type full-length *DME* cDNA and *DME*(*D1304N*) cDNA, respectively. Transgenes were inserted into the *Agrobacterium* vector, pBI-GFP(S65T), and transgenic wild-type (Landsberg *er* ecotype) *Arabidopsis* lines were generated (8). Four independent *DME:DME*(*D1304N*) T<sub>1</sub> transgenic lines were pollinated with *DME/dme-2* pollen to generate F<sub>1</sub> lines heterozygous *DME/dme-2* and hemizygous *DME:DME*(*D1304N*). Likewise, four independent *DME:DME* T<sub>1</sub> transgenic lines were pollinated with *DME/dme-2* pollen to generate F<sub>1</sub> plants, heterozygous *DME/dme-2* and hemizygous *DME:DME*. Identification of these plants was facilitated by the fact that the *dme-2* mutant allele is due to insertion of a pSKI015 T-DNA (23) with a *BAR* gene that confers resistance to glufosinate ammonium herbicide (Basta, Crescent Chemical, Islandia, NY). Plants bearing the *DME:DME* or *DME:DME*(*D1304N*) transgenes were identified by PCR amplification of a 460-bp DNA by using 3' *RACE1*(5'-GCCTCAAGCCAGTGGGATAG-3') and *SKB4* (5'-GGATG-GACTCGAGCACTGGG-3') primers.

Plants homozygous for a *MEA* promoter ligated to *GFP* cDNA (*MEA:GFP*) transgene (8), heterozygous *DME/dme-2*, *DME*(*D1304N*) transgenes were generated by standard genetic crosses. Plants bearing the *MEA:GFP* transgene were identified by PCR amplification of an 860-bp DNA by using *UCB3-F2* (5'-AGGAATTTAACCCGTATATATGTC-3') and 5' *sGF-Prev* (5'-GAACTTGTGGCCGTTACAGTCGCC-3') primers.

The cauliflower mosaic virus promoter (*CaMV*) was ligated to a full-length *DME* cDNA to create a *CaMV:DME* transgene as described (8). To generate a *CaMV* promoter ligated to mutant *DME*(*D1304N*) cDNA [*CaMV:DME*(*D1304N*)] transgene, a 2.3-kb *BsrGI* and *SmaI* DNA spanning the D1304N mutation was excised and used to replace the 2.3-kb *BsrGI* and *SmaI* DNA from a full-length *DME* cDNA, creating the *CaMV:DME*(*D1304N*) cDNA clone.

**Pollen Collection and RNA Analysis.** Pollen were isolated and processed by using procedures modified from Preuss *et al.* (24). About 30 open flowers were harvested in an Eppendorf tube on



**Fig. 1.** Effect of the D1304N mutation on seed viability. Siliques in A–D were dissected and photographed 14 days after self-pollination. (Scale bars = 0.5 mm.) Arrows indicate aborted seeds. (A) Wild-type silique. (B) Heterozygous *DME/dme-2* silique. (C) Silique is heterozygous *DME/dme-2* and hemizygous for a *DME:DME-4* transgene. (D) Silique is heterozygous *DME/dme-2* and hemizygous for a *DME:DME*(*D1304N*)-3 transgene.

ice with 750  $\mu$ l of tobacco pollen germination medium (20 mM Mes-KOH, pH 6.0/0.07%  $\text{Ca}(\text{NO}_3)_2$ /0.02%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ /0.01%  $\text{KNO}_3$ /0.01%  $\text{H}_3\text{BO}_3$ /2% sucrose/15% PEG 4000). Tubes were vortexed, and then the pollen was sedimented at 9,000 rpm for 30 s. After supernatant and flower debris were removed, pollen was resuspended by vortex mixing in 50  $\mu$ l of ice-cold pollen germination medium. Pollen from  $\approx$ 600 flowers was pooled into a single tube, sedimented at 9,000 rpm for 30 s, resuspended by vortex mixing in 800  $\mu$ l of ice-cold *Arabidopsis* pollen germination medium (17% sucrose/2 mM  $\text{CaCl}_2$ /1.625 mM boric acid, pH 7.5, with 4 M KOH), and incubated for 1 h at room temperature to induce germination. Pollen was then sedimented at 3,000 rpm for 5 min. RNA was isolated with 1 ml of TRIzol (Invitrogen), and RT-PCRs were carried out as described (25). Primers for amplifying *DME* were *cDNA-5* (CAGAAGTGTGGAGGGAAAGCGTCTGGC) and *SKEN-5* (8). Primers for *MEA* were as described (13).

## Results

**The Invariant Aspartic Acid Is Essential for Seed Viability.** We mutated the conserved aspartic acid at position 1304 to asparagine (D1304N) to determine whether DNA glycosylase activity is critical for DME function. The full-length cDNA clone with the mutation, *DME*(*D1304N*), as well as the control wild-type full-length *DME* cDNA clone, was ligated to 3.4-kb of *DME* 5' flanking sequences that activate transcription in the central cell of the female gametophyte (8). We transformed *Arabidopsis* plants with *DME:DME*(*D1304N*) and obtained four independently isolated transgenic lines designated *DME:DME*(*D1304N*)-1, -2, -3, and -4. We also transformed *Arabidopsis* plants with the control *DME:DME* transgene and obtained four independently isolated transgenic lines designated *DME:DME*-1, -2, -3, and -4. Transgenes were crossed into a *dme-2* heterozygous background so that we could compare the function of the *DME*(D1304N) and *DME* proteins during seed development.

Wild-type *Arabidopsis* seeds rarely abort (Fig. 1A). Self-pollinated heterozygous *DME/dme-2* siliques display 50% seed abortion (Fig. 1B and Table 1) because seed viability depends on the presence of a wild-type maternal *DME* allele (8). Seeds that

**Table 1. Effect of the D1304N mutation on ratios of viable and aborted seeds**

Genotype	n*	%†	P for 1:1‡	P for 3:1§
<i>DME/dme-2</i>	762	51	0.5	—
<i>DME/dme-2</i> , <i>DME:DME(D1304N)-1</i>	181	45	0.2	—
<i>DME/dme-2</i> , <i>DME:DME(D1304N)-2</i>	986	49	0.6	—
<i>DME/dme-2</i> , <i>DME:DME(D1304N)-3</i>	1,588	48	0.1	—
<i>DME/dme-2</i> , <i>DME:DME(D1304N)-4</i>	764	50	0.8	—
<i>DME/dme-2</i> , <i>DME:DME-1</i>	699	30	—	<0.005
<i>DME/dme-2</i> , <i>DME:DME-2</i>	525	33	—	<0.005
<i>DME/dme-2</i> , <i>DME:DME-3</i>	322	23	—	0.5
<i>DME/dme-2</i> , <i>DME:DME-4</i>	1,041	25	—	0.6

\*Number of seeds checked.

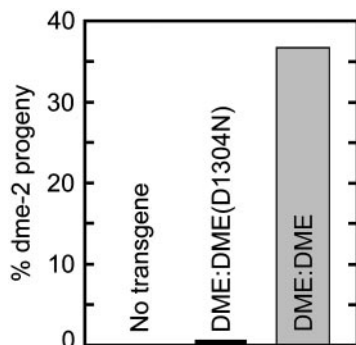
†Percentage of aborted seed.

‡Probability that deviation from a 1:1 segregation of viable aborted seeds is due to chance.

§Probability that deviation from a 3:1 segregation of viable aborted seeds is due to chance.

inherit a wild-type maternal *DME* allele are viable, and seeds that inherit the mutant maternal *dme-2* allele abort. Self-pollinated heterozygous *DME/dme-2* siliques that are also hemizygous for the *DME:DME* transgene show a reduced level of seed abortion. Two transgenic lines (*DME:DME-3* and *DME:DME-4*) displayed 25% seed abortion (Table 1 and Fig. 1C). In these two lines inheritance of a mutant *dme-2* allele and a transgene produced a viable seed, indicating that the *DME:DME* transgene had suppressed the *dme-2* allele and rescued seed viability. Two other transgenic lines (*DME:DME-1* and *DME:DME-2*) displayed ≈30% seed abortion and therefore partially suppressed the *dme-2* mutation (Table 1). By contrast, four independent lines heterozygous for *DME/dme-2* and hemizygous for a mutant *DME:DME(D1304N)* transgene had siliques with 50% seed abortion (Table 1 and Fig. 1D), indicating that seeds with a *dme-2* allele and the mutant transgene were not viable, indicating that the *DME:DME(D1304N)* transgene had not suppressed the *dme-2* mutant allele. These results show that the conserved aspartic acid is essential for DME function in developing seeds.

Transmission of a maternal mutant *dme-2* allele is a more sensitive assay to compare DME and DME(D1304N) function during seed development. None of the viable F<sub>1</sub> progeny inherit the maternal mutant *dme-2* allele when a heterozygous *DME/dme-2* plant is pollinated with wild-type pollen (Fig. 2), confirming the importance of the wild-type maternal *DME* allele



**Fig. 2.** Effect of the D1304N mutation on transmission of the maternal mutant *dme-2* allele. No transgene, heterozygous *DME/dme-2* plant was pollinated with wild-type pollen and no F<sub>1</sub> progeny with the *dme-2* allele were detected (790 checked); *DME:DME(D1304N)*, plant heterozygous *DME/dme-2* and hemizygous for a *DME:DME(D1304N)-3* transgene was pollinated with wild-type pollen, and three F<sub>1</sub> progeny with the *dme-2* allele and *DME:DME(D1304N)-3* transgene were detected (379 checked); *DME:DME*, plant heterozygous *DME/dme-2* and hemizygous for a *DME:DME-4* transgene was pollinated with wild-type pollen and 36 F<sub>1</sub> progeny with the *dme-2* allele and the *DME:DME-4* transgene were detected (97 checked).

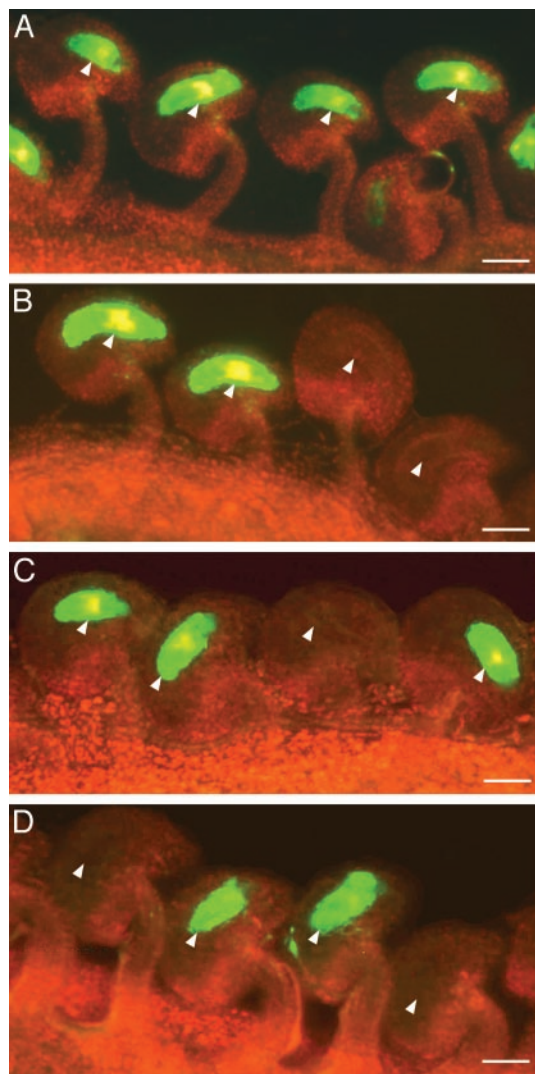
during seed development. When a plant that is heterozygous *DME/dme-2* and hemizygous for a *DME:DME-4* transgene is pollinated with wild-type pollen, viable F<sub>1</sub> progeny are detected that inherit both the maternal mutant *dme-2* allele and the transgene (Fig. 2), indicating that the *DME:DME-4* transgene suppressed the *dme-2* mutant allele. However, when a heterozygous *DME/dme-2*, hemizygous *DME:DME(D1304N)-3* plant is pollinated with wild-type pollen, the frequency of F<sub>1</sub> progeny that inherit the maternal mutant *dme-2* allele and the transgene is reduced ≈50-fold (Fig. 2). These results suggest that the D1304N mutation severely reduces but does not completely abolish DME activity during seed development. This is consistent with the finding that some glycosylases retain slight activity after mutation of the aspartic acid to asparagine (19, 21).

#### The Invariant Aspartic Acid Is Required for Activation of Maternal *MEA:GFP* Transcription in the Central Cell.

Essentially all prefertilization ovules (153 of 155 checked) from *MEA:GFP* homozygous plants showed strong GFP fluorescence in the central cell nucleus and cytoplasm (Fig. 3A). DME is necessary for the transcription of a *MEA:GFP* transgene in the central cell of the female gametophyte (8, 26), and plants homozygous for *MEA:GFP* and heterozygous for *DME/dme-2* display a 1:1 segregation ratio of fluorescent to nonfluorescent ovules (113:108,  $\chi^2 = 0.1$ ,  $P > 0.8$ ) (Fig. 3B). To determine whether DNA glycosylase activity is critical for *MEA* gene activation, we analyzed the effect of the D1304N mutation on transcription of the *MEA:GFP* transgene. In plants homozygous for a *MEA:GFP* transgene, heterozygous for *DME/dme-2*, and hemizygous for a *DME:DME-4* transgene, we detected a 3:1 segregation ratio of fluorescent to nonfluorescent ovules (557:169,  $\chi^2 = 1.1$ ,  $P > 0.4$ ) (Fig. 3C). These results suggest that female gametophytes inheriting *dme-2* and the *DME:DME* transgene transcribed *MEA:GFP* and that the *DME:DME* transgene suppressed the *dme-2* mutant allele. By contrast, in plants homozygous for a *MEA:GFP* transgene, heterozygous *DME/dme-2*, and hemizygous for a *DME:DME(D1304N)-3* transgene, we detected a 1:1 segregation ratio of fluorescent to nonfluorescent ovules (378:369,  $\chi^2 = 0.1$ ,  $P > 0.8$ ) (Fig. 3D). This result suggests that the female gametophytes inheriting *dme-2* and the *DME:DME(D1304N)* transgene did not transcribe the *MEA:GFP* transgene, and *DME:DME(D1304N)* did not suppress the *dme-2* mutant allele. Thus, the conserved aspartic acid D1304 is essential for activation of *MEA:GFP* transcription by DME.

#### The Invariant Aspartic Acid Is Required for Ectopic Paternal *MEA* Allele Expression.

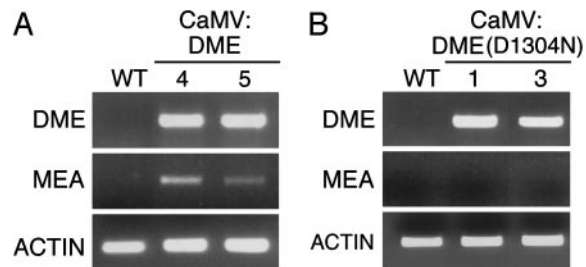
Ectopic *DME* expression in the leaf and endosperm is sufficient to induce *MEA* and paternal *MEA* allele expression, respectively (8). These results suggest that restriction of



**Fig. 3.** Effect of the D1304N mutation on maternal *MEA:GFP* transcription in the central cell. Fluorescence micrographs of ovules harvested from stage 12 flowers (46) are shown. GFP and chlorophyll fluorescence were converted to green and red, respectively. Arrows point to central cells. (Scale bars = 0.04 mm.) (A) Ovules from a wild-type flower that is homozygous for a *MEA:GFP* transgene. (B) Ovules from a flower that is heterozygous *DME/dme-2* and homozygous for a *MEA:GFP* transgene. (C) Ovules from a flower that is heterozygous *DME/dme-2*, hemizygous for a *DME:DME-4* transgene, and homozygous for a *MEA:GFP* transgene. (D) Ovules from a flower that is heterozygous *DME/dme-2*, hemizygous for a *DME:DME(D1304N)-3* transgene, and homozygous for a *MEA:GFP* transgene.

*DME* expression to the central cell of the female gametophyte is responsible, at least in part, for *MEA* imprinting in the endosperm. Consistent with this model, *DME* and *MEA* paternal allele RNA is not detected in the male gametophyte, pollen (Fig. 4), and GUS staining is not detected in *DME:GUS* (8) or *MEA* promoter ligated to *GUS* cDNA (*MEA:GUS*) (Fig. 5A) transgenic pollen. Thus, the pollen provides an opportunity to compare the ectopic activation of *MEA* and *MEA:GUS* expression by *DME* and *DME(D1304N)* in a cellular environment that is free from endogenous *DME* and *MEA* expression.

We generated *CaMV:DME* transgenic lines (8) where transcription of the wild-type *DME* cDNA is under the control of the *CaMV* promoter (27). Here, we show that both *DME* and *MEA* RNAs were present in pollen from independently isolated *CaMV:DME* transgenic lines (Fig. 4A). This result indi-



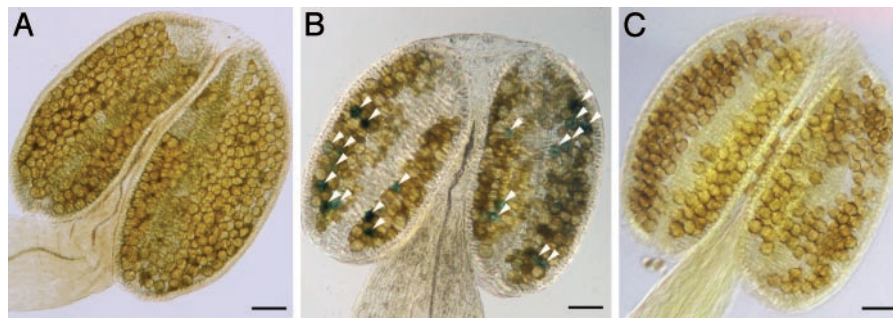
**Fig. 4.** Effect of the D1304N mutation on ectopic paternal *MEA* allele expression. *CaMV:DME-4* and *CaMV:DME-5* represent two independently isolated transgenic lines that ectopically express the wild-type *DME* cDNA (8). *CaMV:DME(D1304N)-1* and *CaMV:DME(D1304N)-3* represent two independently isolated transgenic lines that ectopically express the mutant D1304N form of *DME*. Total RNA was isolated from pollen harvested from open flowers, and the approximate level of *MEA* and *DME* RNA was determined by semiquantitative RT-PCR. (A) *CaMV:DME* transgenes activate paternal *MEA* allele gene expression in pollen. (B) *CaMV:DME(D1304N)* transgenes do not activate paternal *MEA* allele gene expression in pollen.

cates that expression of wild-type *DME* in pollen is sufficient to activate paternal *MEA* expression and supports the model that preventing *DME* expression in the male gametophyte is necessary for *MEA* gene imprinting. By contrast, *DME(D1304N)* RNA, but not *MEA* RNA, was detected in pollen harvested from multiple independently isolated *CaMV:DME(D1304N)* lines that express the mutant *DME(D1304N)* protein (Fig. 4B). The level of mutant *DME(D1304N)* and wild-type *DME* RNAs were similar in pollen harvested from the *DME:DME(D1304N)* and *DME:DME* lines, respectively, suggesting that the failure to induce *MEA* gene expression in the *DME:DME(D1304N)* lines is due to the replacement of aspartic acid by asparagine in the *DME(D1304N)* DNA glycosylase active site. We also crossed the *CaMV:DME-4* and *CaMV:DME(D1304N)-1* transgenes into a *MEA:GUS* genetic background. We detected GUS staining in pollen grains from plants hemizygous for a *CaMV:DME-4* transgene and hemizygous for a *MEA:GUS* transgene (Fig. 5B). By contrast, no GUS staining was detected in plants hemizygous for *CaMV:DME(D1304N)-1* and *MEA:GUS* transgenes (Fig. 5C). These results suggest that active *DME* DNA glycosylase is essential for activation of *MEA* gene transcription in pollen.

## Discussion

Most DNA glycosylases are low-molecular-weight enzymes that catalyze the first step in the base-excision DNA repair pathway by excising damaged or mispaired bases. These lesions are mutagenic and believed to play a role in cancer and aging, so the mechanism of base-excision DNA repair has been studied in great detail, and the mechanism of base excision is well understood at the atomic level (1, 3). Elucidation of DNA glycosylase 3D structures has led to a detailed understanding of their lesion recognition and catalysis mechanisms. However, the role of these DNA glycosylases in controlling development is largely unknown.

Compared with the well studied low-molecular-weight DNA glycosylases, the *Arabidopsis* *DME* protein has a distinct structure and function (8, 9). Embedded within the 1,729-aa *DME* polypeptide is a 200-aa domain related to the HhH family of DNA glycosylase. *DME* was discovered by a mutation that results in seed abortion. *DME* activates transcription of maternal alleles in the central cell of the female gametophyte, resulting in endosperm gene imprinting. We compared the *in vivo* function of wild-type *DME* and mutant *DME(D1304N)* polypeptides in *Arabidopsis* plants to understand the role of the



**Fig. 5.** Effect of the D1304N mutation on ectopic paternal *MEA:GUS* gene transcription. Light micrographs were taken 12 h after staining for GUS activity. (A) Stamen is hemizygous for a *MEA:GUS* transgene. (B) Stamen is hemizygous for a *CaMV:DME-4* transgene and hemizygous for a *MEA:GUS* transgene. (C) Stamen is hemizygous for a *CaMV:DME(D1304N)-1* transgene and hemizygous for a *MEA:GUS* transgene. (Scale bars = 0.005 mm.)

DME DNA glycosylase domain in the activation of gene transcription in the central cell. We replaced the conserved aspartic acid residue with an asparagine residue because this mutation decreases DNA glycosylase activity without altering enzyme stability or structure *in vitro* (19, 20). We found that only the wild-type DME protein, not the mutant DME(D1304N) protein, rescued *dme*-mediated seed abortion (Figs. 1 and 2 and Table 1) and activated *MEA* transcription in the central cell (Fig. 3) and pollen (Figs. 4 and 5). These results are consistent with our finding that *in vivo* DME expression results in nicks in the *MEA* promoter that may be due to DME-mediated base excision (8). These experiments show that DME DNA glycosylase activity is essential for activation of imprinted gene transcription, a process that plays a critical role in reproductive development in plants.

**Multiple Mechanisms for Regulation of Gene Transcription by DNA Glycosylases.** Physical and functional linkages between DNA glycosylases and proteins that regulate gene transcription (e.g., transcription factors, receptors, and chromatin-remodeling proteins) have recently been discovered (28). Thymine DNA glycosylase has been reported to both activate and repress gene transcription by a variety of mechanisms, including binding to hormone receptors (29, 30), interacting with transcription factors (31), and associating with CBP/p300 acetylase, which remodels chromatin and activates transcription through histone acetyltransferase activity (32). Methylpurine DNA glycosylase has a synergistic effect on gene silencing by interacting with the methyl CpG-binding domain protein 1 (MBD1) transcriptional repressor (33) and 3-methyladenine DNA glycosylase interacts with estrogen receptor  $\alpha$  to inhibit gene transcription (34). Thus, DNA glycosylases are linked to the process of gene regulation by physical interactions that modulate the activities of transcription factors, receptors, and chromatin-remodeling proteins. However, it is not known whether base-excision activity is a requirement for regulation of gene transcription by DNA glycosylases in addition to direct physical association. Indeed, in one case it was shown that inactive and wild-type thymine DNA glycosylases bound estrogen receptors and activated them with equal efficiency (30), suggesting that receptor binding, rather than DNA glycosylase activity, was responsible for modulating the rate of gene transcription.

We have shown that an active DNA glycosylase domain is necessary to induce *MEA* gene transcription in the central cell. We recently found that DNA methylation plays an important role in *MEA* imprinting and seed viability. These processes are controlled by an antagonism between the MET1 methyltransferase and the DME DNA glycosylase in the central cell of the female gametophyte (26). MET1 is the *Arabidopsis* ortholog of mammalian Dnmt1 methyltransferase, which maintains DNA

methylation at CpG sites (35). One possibility is that DME initiates the replacement of 5-methylcytosine with cytosine, resulting in hypomethylation and activation of maternal *MEA* allele expression. In support of this model, ROS1 DNA glycosylase, an *Arabidopsis* protein related to DME, can excise 5-methylcytosine *in vitro* and represses DNA methylation-mediated transgene silencing (36). Also, an animal thymine DNA glycosylase can excise 5-methylcytosine *in vitro*, and its inhibition suppresses genome-wide hypomethylation, whereas overexpression causes promoter hypomethylation and activates transcription (37, 38). Alternatively, the antagonistic relationship between DME and MET1 may be indirect, whereas *MEA* gene transcription is promoted by DNA nicking associated with DME DNA glycosylase activity (8). This process may facilitate nucleosome sliding and alteration of chromatin structure (39).

DNA glycosylases may modulate gene transcription by two distinct mechanisms. One mechanism involves modulation by the association of DNA glycosylases with transcription factors and/or chromatin-remodeling proteins. A second mechanism invokes modification of the DNA by base-excision DNA glycosylase activity.

**Possible Function for Other DME Protein Domains.** *Arabidopsis* has numerous low-molecular-weight DNA glycosylases that are responsible for repairing DNA damage throughout the genome (40–45). By contrast, DME appears to act at very distinct sites in the *Arabidopsis* genome to activate transcription of specific genes such as *MEA* and *FWA* in the central cell (8, 9, 26). Thus, DME DNA glycosylase activity plays a precise and critical role in plant development. What accounts for the highly restricted genome target specificity of DME? One possibility is that the DME DNA glycosylase domain binds to a factor that directs it to its target in the genome. Alternatively, other domains within the high-molecular-weight DME polypeptide may play a role. Comparing the *Arabidopsis* DME to a related rice protein has revealed multiple regions of significant amino acid sequence homologies that are outside the shared DNA glycosylase domains (8). Although the function of these domains is unknown, they might direct DME, or interact with other molecules that direct DME, to specific sites within the genome where the DNA glycosylase activity of DME is needed to promote maternal allele transcription in the central cell, a process required for endosperm imprinting and seed viability.

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