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Mutations in *FIE*, a WD Polycomb Group Gene, Allow Endosperm Development without Fertilization

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A fundamental problem in biology is to understand how fertilization initiates reproductive development. Higher plant reproduction is unique because two fertilization events are required for sexual reproduction. First, a sperm must fuse with the egg to form an embryo. A second sperm must then fuse with the adjacent central cell nucleus that replicates to form an endosperm, which is the support tissue required for embryo and/or seedling development. Here, we report cloning of the Arabidopsis *FERTILIZATION-INDEPENDENT ENDOSPERM* (*FIE*) gene. The FIE protein is a homolog of the WD motif–containing Polycomb proteins from Drosophila and mammals. These proteins function as repressors of homeotic genes. A female gametophyte with a loss-of-function allele of *fie* undergoes replication of the central cell nucleus and initiates endosperm development without fertilization. These results suggest that the FIE Polycomb protein functions to suppress a critical aspect of early plant reproduction, namely, endosperm development, until fertilization occurs.

INTRODUCTION

The higher plant life cycle alternates between multicellular haploid (i.e., gametophyte) and diploid (i.e., sporophyte) generations. In Arabidopsis, the ovule generates the female gametophyte, which is composed of egg, central, synergid, and antipodal cells (Figure 1A). Surrounding and protecting the female gametophyte are maternal cell layers, which are called the integuments (Figure 1A). Double fertilization within the female gametophyte activates four reproductive developmental programs. (1) The fertilized egg initiates embryogenesis (Figure 1D). (2) The fertilized central cell generates the primary triploid endosperm nucleus that replicates to form the endosperm (Figure 1D), which is the tissue that supports the development of the embryo. (3) The maternal integument cell layers of the ovule become the seed coat (Figures 1D and 1H). (4) The ovary differentiates and elongates to form the fruit, or silique (Figure 1F).

The two products of fertilization, embryo and endosperm, display distinct patterns of development. In Arabidopsis, the embryo proceeds through a series of stages that have been defined morphologically as preglobular, globular (Figure 1D), heart, cotyledon, and maturation (Figure 1H). During these stages, an axis of polarity is fixed, shoot and root meristems are formed, and storage organs are generated (Goldberg et al., 1994). In contrast, the fertilized central cell (i.e., the primary endosperm nucleus) divides mitotically to produce a syncytium of nuclei that fills an expanding central cell (Figure 1D). After cytokinesis occurs, endosperm cells produce storage proteins, starch, and lipids (Lopes and Larkins, 1993).

Ultimately, the endosperm is absorbed by the developing embryo (Figure 1H). It is thought that endosperm evolved from a supernumerary fertilization event that originally produced multiple embryos and may represent a highly modified embryo that enhances the fitness of its sister embryo (Friedman, 1992, 1995).

To understand the mechanisms controlling the onset of reproduction, we isolated gametophytic mutations in Arabidopsis, designated *fie* (for *fertilization-independent endosperm*), that initiate aspects of reproductive and maternal development in the absence of fertilization (Ohad et al., 1996). In this study, we extend our analysis of the mutant phenotype conferred by *fie* and report the cloning of the *FIE* gene. We show that the *FIE* gene encodes a polypeptide related to the family of WD Polycomb group proteins. Our results suggest that one function of the *FIE* gene is to suppress transcription of genes for central cell nuclear replication in the female gametophyte until fertilization occurs.

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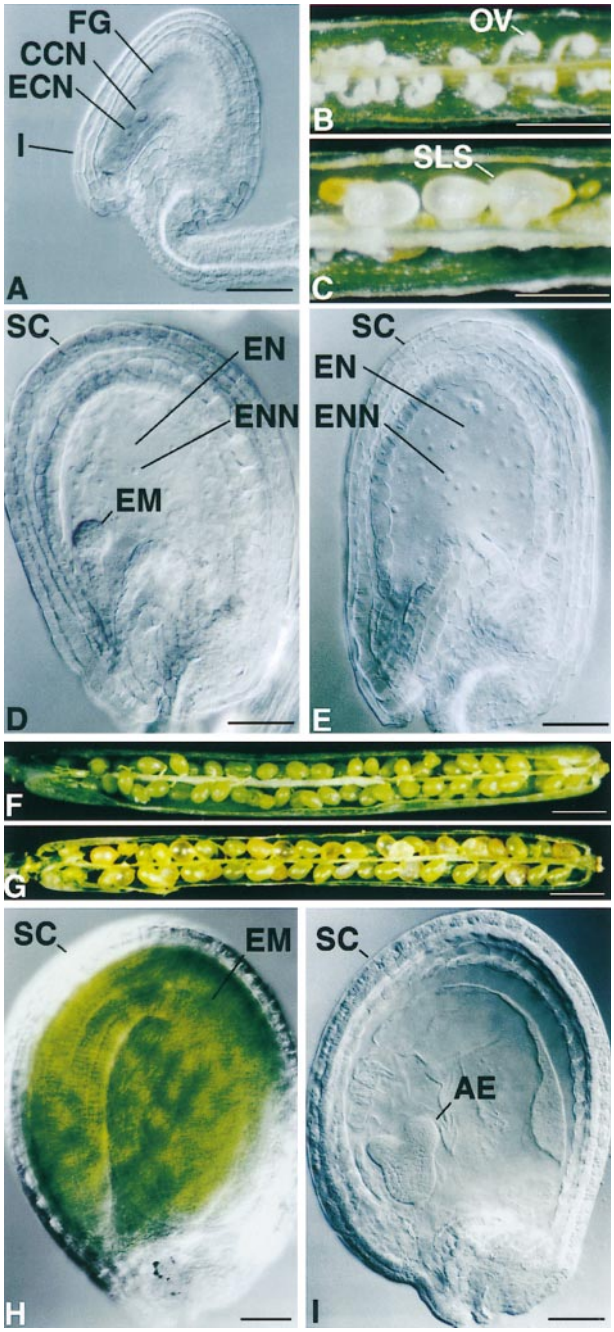

Figure 1. Phenotype of Wild-Type and *fie-1* Plants.

(A) Wild-type ovule 6 days after anther removal.
(B) Wild-type silique 6 days after anther removal.
(C) *fie-1* heterozygote silique 6 days after anther removal.
(D) Wild-type seed with globular embryo 2 days after pollination.
(E) *fie-1* seedlike structure 6 days after anther removal.
(F) Self-pollinated wild-type silique.
(G) Self-pollinated silique from *fie-1* heterozygote.
(H) Viable seed obtained from silique in **(G)**.
(I) Defective seed from silique in **(G)**.

RESULTS

Phenotype Conferred by *fie*

When fertilization was prevented in plants heterozygous for the *fie-1* mutant allele, siliques elongated (Ohad et al., 1996) and contained seedlike structures (Figure 1C) composed of an endosperm surrounded by a seed coat (Figure 1E). No embryo development was observed (Figure 1E). In wild-type plants, when fertilization was prevented by the removal of pollen-producing anthers, no reproductive development was detected (Figure 1B). Genetic analysis suggested that the *fie-1* mutation acts first in the female gametophyte to initiate endosperm development that in turn stimulates maternal seed coat and silique formation (Ohad et al., 1996).

The maternally derived *FIE* allele plays an essential role during embryo development (Ohad et al., 1996). Control wild-type siliques only rarely contained defective seeds (Figure 1F). However, inheritance of the *fie-1* allele by the female gametophyte caused embryo abortion after fertilization. That is, heterozygous *fie-1* plants, after self-pollination or when pollinated with wild-type pollen, displayed equal numbers (Figure 1G) of normal seeds with viable embryos (Figure 1H), and defective seeds with embryos aborted at the heart stage (Figure 1I). As shown in Figure 2, in wild-type embryos at 6 days after pollination, there was a dramatic increase in cell proliferation associated with growth and development of the embryo axis and cotyledons. In contrast, embryo cell proliferation was greatly reduced in embryos with a maternally derived mutant *fie-1* allele, even though they inherited a wild-type paternally derived allele (Figure 2). These results indicate that the maternally derived *fie* mutant allele, directly or indirectly, inhibits embryo cell proliferation and morphogenesis.

Cloning the *FIE* Gene

We used a map-based strategy to clone the *FIE* gene. As shown in Figure 3, the *FIE* gene was mapped between morphological markers *emb29* (Meinke, 1985) and *axr2* (Timpte et al., 1994) and between molecular markers NDR (Century et al., 1997) and the end of yeast artificial chromosome clone ClC3D12 (Creusot et al., 1995). Fine-structure mapping localized the *FIE* gene between molecular markers CH18S and KN1N (Figure 3). To further define the position of the *FIE* gene, we introduced overlapping wild-type

AE, aborted embryo; CCN, central cell nucleus; ECN, egg cell nucleus; EM, embryo; EN, endosperm; ENN, one of the endosperm nuclei in the endosperm; FG, female gametophyte; I, integument; OV, ovule; SC, seed coat; SLS, seedlike structure. Bars in **(B)**, **(C)**, **(F)**, and **(G)** = 1 mm; bars in **(A)**, **(D)**, **(E)**, **(H)**, and **(I)** = 0.1 mm.

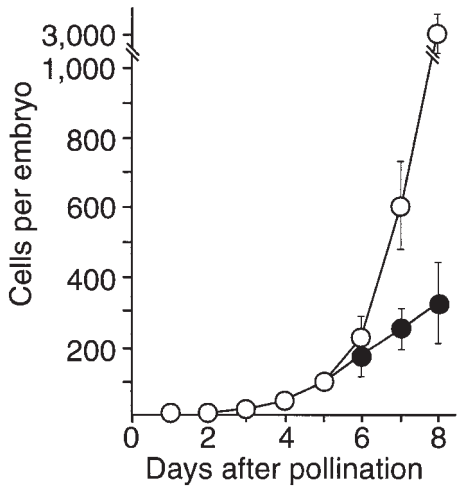

Figure 2. Cell Proliferation in Wild-Type and Mutant Embryos.

The average number of cells (±SD; $n > 10$) is shown for each time point. Open circles indicate wild-type embryos; closed circles indicate heart-shaped embryos with a maternally derived *fie-1* mutant allele and a paternally derived wild-type *FIE* allele. Stages of wild-type control embryos are as follows: 1 day after pollination (DAP), zygote; 2 DAP, two- to three-cell embryo proper; 3 DAP, four- to eight-cell embryo proper; 4 DAP, early- to mid-globular stage; 5 DAP, late-globular stage; 6 DAP, early-heart stage; 7 DAP, heart stage; 8 DAP, torpedo- to walking-stick stage.

cosmid clones into heterozygous *fie-1* plants by Agrobacterium-mediated transformation, and we observed genetic complementation for cosmids GM15, AD21, and pCLD36 (Figure 3). For lines homozygous for both the mutant *fie-1* allele and the transgenic wild-type *FIE* allele, self-pollinated siliques contained viable seed, and the aborted embryos associated with the maternal *fie-1* mutation (Figure 1I) were not observed (data not shown). When fertilization was prevented by the removal of anthers, *fie-1* seedlike structures with an endosperm and seed coat were not formed, and the transgenic ovule resembled a wild-type ovule (Figure 1A). These complementation experiments suggest that the entire *FIE* gene resides within a 12-kb region delineated by the end points of cosmids AD21 and pCLD36 (Figure 3). Because *fie* is a gametophytic mutation, it was not possible to determine whether the mutant allele is dominant or recessive by using classic genetic procedures. However, these complementation experiments indicate that the mutant *fie-1* allele is recessive to the wild-type *FIE* allele, suggesting that FIE represses endosperm development before fertilization.

As shown in Figure 4A, comparison of genomic and cDNA sequences revealed a gene with 13 exons in the region that complements the *fie-1* mutation. When compared with the wild type, nine mutant alleles, each causing a similar mutant phenotype, were found to contain DNA sequence alterations in this gene, conclusively demonstrating that the *FIE* gene had been identified. For the *fie-1*, *fie-2*, and *fie-3* alleles, a G residue was changed to an A residue at the 3′ border of the first, third, and eighth introns, respectively (Figure 4A). This 3′ intron border G residue is present in all wild-type Arabidopsis introns sequenced to date, and mutations that change the G to an A residue have been shown to disrupt the splicing reaction (Brown, 1996). The *fie-4*, *fie-5*, *fie-6*, and *fie-9* alleles have single-base changes that resulted in the formation of stop codons in the ninth, seventh, thirteenth, and fifth exons, respectively (Figure 4A). The *fie-7* allele has a 27-bp deletion in the tenth exon, whereas the single-base change plus a two-base deletion associated with the *fie-8* allele disrupted the reading frame in the seventh intron. All nine mutations are likely to represent loss-of-function alleles, which is consistent with *fie* being recessive to the wild type and further supports the idea that the wild-type *FIE* allele prevents the central cell from initiating endosperm development.

As shown in Figure 4B, a search of the databases revealed that the FIE protein is related (i.e., ~40% amino acid sequence identity) to the family of WD Polycomb group proteins, which are encoded by genes such as *Esc* (for *Extra sex combs*) from Drosophila (Gutjahr et al., 1995; Sathe and Harte, 1995) and *Eed* (for *Embryonic ectoderm development*) from mouse (Schumacher et al., 1996) and humans (Sewalt et al., 1998). The predicted FIE protein is a polypeptide with 369 amino acids and a molecular mass of 41 kD and displays sequence identity to Esc and Eed over its entire length. Like Esc and Eed, FIE contains seven predicted

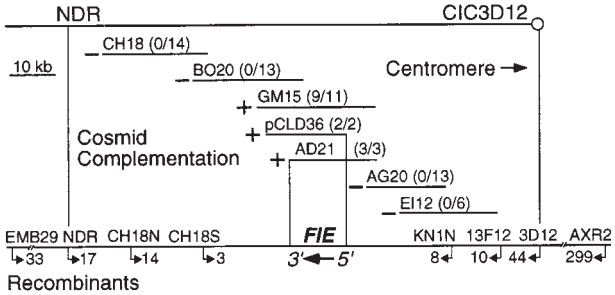

Figure 3. Positional Cloning of *FIE*.

A portion of yeast artificial chromosome CIC3D12 and an overlapping series of cosmid clones derived from this clone are shown. (−), no complementation; (+), complementation. After the name of each cosmid, the ratio of complementing *fie-1* heterozygous T_1 transgenic lines to total *fie-1* heterozygous T_1 transgenic lines is shown within parentheses. A genetic map indicating the position of *FIE* relative to morphological and molecular markers is shown at the bottom of the panel. (→), number of upstream recombinant chromosomes between indicated marker and *fie-1*; (←), number of downstream recombinant chromosomes between indicated marker and *fie-1*.

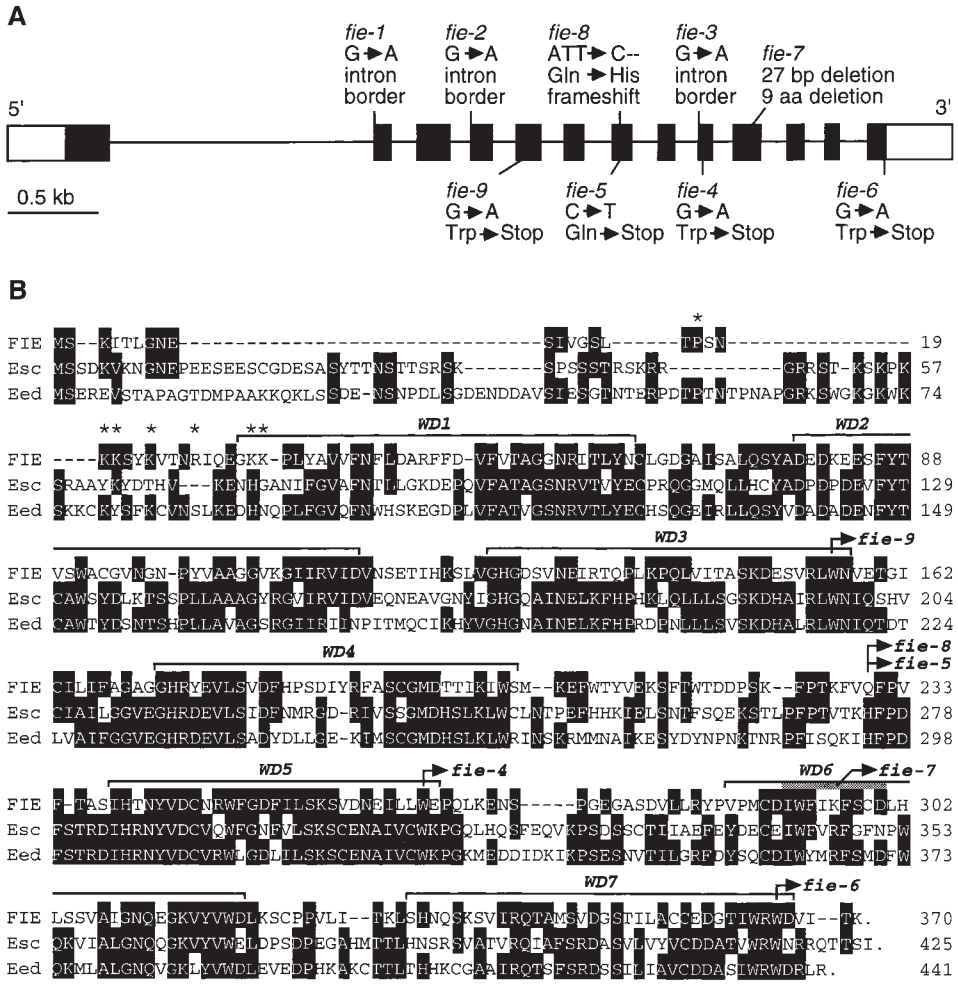

Figure 4. *FIE* Gene Structure and Sequence.

(A) Position of mutant *fie* alleles. Black and open boxes represent translated and untranslated sequences, respectively. The nucleotide change for mutations is shown. aa, amino acid; Stop, mutation created a stop codon.

(B) Comparison of the predicted FIE polypeptide to proteins with WD motifs encoded by the *Extra sex combs* (Esc) and *Embryonic ectoderm development* (Eed) genes. Optimal alignment of amino acid sequences was determined by using the Clustal V method (Higgins and Sharp, 1989). WD repeats (Ng et al., 1997) are bracketed. Asterisks denote amino acids that comprise a potential bipartite nuclear localization signal (Jans and Hubner, 1996). The gray bar shows the amino acids deleted in the *fie-7* mutant allele. Positions of the *fie-4*, *fie-5*, *fie-6*, *fie-7*, *fie-8*, and *fie-9* alleles are shown. Dashes were introduced to optimize alignment. Amino acids in black boxes are identical in at least two of the three Polycomb group proteins.

repeats of the WD motif (Ng et al., 1997). The WD repeats of the related heterotrimeric G protein and of insect Esc proteins are thought to form a propeller-like structure that functions in promoting protein–protein interactions (Wall et al., 1995; Sondek et al., 1996; Ng et al., 1997). The sequence identity displayed by the FIE Polycomb protein suggests that it also may form a similar tertiary structure that functions in protein–protein interactions. Gel blot hybridization experiments suggest that *FIE* is a single-copy gene in the Arabidopsis genome (data not shown).

Because *fie*-conferred phenotypes are detected both without fertilization (Figure 1E) and after fertilization (Figure 1I), we analyzed the pattern of *FIE* gene expression in immature floral buds that contain developing female gametophytes, open flowers that are undergoing fertilization, and young siliques after fertilization that have seeds with preglobular- to heart-stage embryos. RNA gel blot experiments detected a low-abundance RNA of the expected length of 1.5 kb at all of the above-mentioned stages (data not shown). As shown in Figure 5, more-sensitive reverse transcription–

polymerase chain reaction (RT-PCR) experiments also detected the presence of the *FIE* RNA. These results indicate that the *FIE* gene is expressed both before and after fertilization. Moreover, we also detected *FIE* RNA in cauline leaves, stems, and roots (Figure 5). Thus, the *FIE* gene is expressed in both reproductive and vegetative tissues.

DISCUSSION

We have found that mutations in the maternally derived *fie* allele cause multiple phenotypes. In the absence of fertilization, the central cell replicates and initiates endosperm development (Figure 1E). After fertilization, embryonic cell proliferation and morphogenesis are inhibited (Figure 1I). We have cloned the *FIE* gene and showed that it is related to WD domain Polycomb group proteins that control pattern formation during embryogenesis in insects and mammals (Figure 4B). These results reveal new biological functions for Polycomb proteins in plant reproduction.

FIE Suppresses Endosperm Development in the Female Gametophyte

The higher plant life cycle alternates between morphologically distinct gametophyte and sporophyte generations. Regulatory proteins must exist that control gametophyte-specific and sporophyte-specific developmental pathways. We have found that mutations in the *FIE* gene allow for replication of the central cell nucleus without fertilization. Thus, the *FIE* gene prevents a sporophytic pattern of development, namely, endosperm formation, from occurring in the female gametophyte before fertilization.

In Drosophila and mammals, long-term repression of homeotic genes requires the activity of Polycomb group proteins (Pirrotta, 1998). WD Polycomb proteins play an important role in the suppression of gene transcription during insect and mammal embryo development. As diagrammed in Figure 6A, by virtue of its multiple WD domains, this class of Polycomb group proteins has the capacity to bind simultaneously to various polypeptides and thereby contribute to the assembly of multiprotein complexes (Wall et al., 1995; Sondek et al., 1996; Ng et al., 1997). In this way, the Drosophila WD Polycomb group protein Esc is thought to inhibit transcription of homeotic genes by two mechanisms (Gutjahr et al., 1995). Initially recruited by interacting, either directly or indirectly, with a zinc finger transcription factor (i.e., Hunchback), Esc may interfere with the basal transcription machinery, TFIID. In addition, Esc recruits other Polycomb group proteins that form stable complexes at specific sites within the genome and most likely package chromatin into a condensed form that prevents gene transcription.

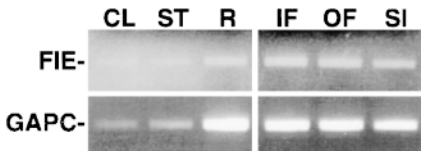

Figure 5. RT-PCR Analysis of *FIE* RNA Accumulation during Vegetative and Reproductive Development.

CL, cauline leaf; ST, stem; R, root; IF, immature floral buds; OF, open flowers; SI, young siliques. As a control, glyceraldehyde-3-phosphate dehydrogenase C (GAPC) RNA was amplified.

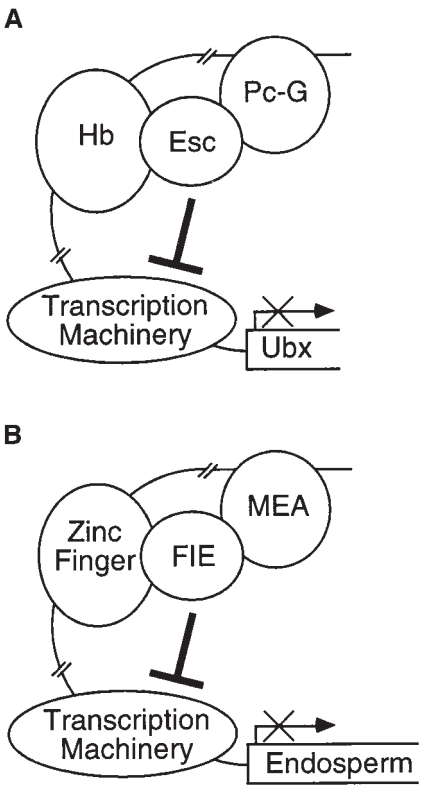

Figure 6. Models for the Silencing of Gene Transcription.

The T bars represent the inhibition of transcription by mechanisms described in the text.

(A) Proteins that may interact to silence homeotic gene transcription in Drosophila. Hb, hunchback transcription factor; Pc-G, Polycomb group protein complex; Ubx, ultrabithorax homeobox gene.

(B) Proteins that may interact to silence the transcription of genes needed to initiate endosperm development in Arabidopsis. Zinc Finger, zinc finger transcription factor; Endosperm, gene(s) required for endosperm formation.

Our results suggest that in the female gametophyte, the FIE protein functions to repress the transcription of genes required for replication of the central cell nucleus and subsequent endosperm development. Most likely, FIE, like insect and animal WD Polycomb proteins (Pirrotta, 1998), suppresses gene transcription by forming complexes with other Polycomb group proteins (Figure 6B). We propose that when fertilization occurs, alteration of complexes containing the FIE Polycomb protein may allow initiation of endosperm gene transcription. According to this model, loss-of-function mutations in the *FIE* gene prevent the formation of silencing complexes in the female gametophyte, resulting in fertilization-independent central cell nuclear replication and endosperm development. Finally, it is the developing endosperm that signals the maternal integuments and ovary to form the seed coat and fruit, respectively (Ohad et al., 1996).

The FIE WD Polycomb protein may participate in a complex similar to that found in other organisms. The Drosophila WD Polycomb group protein physically interacts with a SET (Jenuwein et al., 1998) domain Polycomb group protein, Enhancer of Zeste (E(z)) (Jones et al., 1998; Figure 6A). Similarly, the mouse (van Lohuizen et al., 1998) and human (Sewalt et al., 1998) WD homologs have been shown to interact with their respective SET domain orthologs. In plants, the maternally derived allele of an Arabidopsis SET domain homolog, *MEDEA* (*MEA*), like *FIE*, has been shown to be necessary for embryo development (Grossniklaus et al., 1998). Moreover, we have found that central cells that inherit loss-of-function *mea* alleles display endosperm development without fertilization, although the phenotype is weaker than that observed with *fie* mutants (Kiyosue et al., 1999). Thus, the mechanism for establishing and maintaining Polycomb group complexes that silence gene transcription may be conserved during the evolution of insects, mammals, and plants (Figure 6B). Specifically, the molecular partnership of WD (i.e., FIE) and SET domain (i.e., MEA) Polycomb proteins may be utilized in plants to suppress precocious endosperm development in the female gametophyte. Other mutations that allow for fertilization-independent endosperm development have been isolated (Chaudhury et al., 1997; N. Ohad and R.L. Fischer, unpublished results) and may represent genes that encode additional proteins that participate in the formation of the silencing complex.

FIE Is Required for Proper Embryo Development

Loss-of-function mutations in the maternally derived *FIE* allele caused two phenotypes: fertilization-independent endosperm development (Figure 1E) and embryo abortion (Figure 1I). The relationship between these two phenotypes is not known. It is possible that the maternally derived *FIE* allele performs distinct functions essential for endosperm and embryo development. Alternatively, perturbations in endosperm development may be responsible for subsequent embryo abortion (Scott et al., 1998). Moreover, it is not understood why the paternally derived *FIE* allele has no effect on seed development after fertilization. Perhaps essential *FIE* gene expression for embryo development occurs before fertilization in the female gametophyte. Although this model cannot be ruled out, it is important to note that the *FIE* gene is expressed in siliques after fertilization and in vegetative tissues (Figure 5). Alternatively, the paternally derived allele may not produce active FIE protein, perhaps due to transcriptional silencing (Martienssen, 1998).

Relation between Polycomb Proteins and Apomixis

Certain plant species display aspects of fertilization-independent reproductive development, including apomictic development of the embryo, endosperm, maternal seed coat, and fruit (Koltunow, 1993). The fertilization-independent phenotype associated with mutations in genes, such as *FIE*, *MEA*, and *FIS* (for *FERTILIZATION-INDEPENDENT SEED*), reveals that Arabidopsis, a sexually reproducing plant, has the genetic potential for aspects of fertilization-independent reproductive development (Ohad et al., 1996; Chaudhury et al., 1997; Kiyosue et al., 1999). It is possible that alterations in the activity of Polycomb proteins are responsible, at least in part, for the fertilization-independent reproductive development observed in naturally occurring apomictic plant species.

METHODS

Plant Material

Siliques, seedlike structures, and seeds were isolated, prepared, and photographed as described previously, except that whole-mount preparations were not stained with hematoxylin (Ohad et al., 1996). For counting embryonic cell number, wild-type or *fertilization-independent endosperm* (*fie-1*) heterozygous flowers were emasculated and pollinated with wild-type pollen. At the indicated time interval, seeds were harvested, cleared, and visualized with a Zeiss Axioskop microscope (Carl Zeiss, Inc., Thornwood, NY) by using Nomarski optics (Ohad et al., 1996). Each embryo was partitioned into optical sections, the number of cells per optical section was counted, and the total number of cells was determined by summing the values for the optical sections. Results for wild-type embryos were in good agreement with those obtained by others (Lindsey and Topping, 1993).

Genetic Mapping

A heterozygous *fie-1* mutant (*Arabidopsis thaliana* ecotype Landsberg *erecta*) was crossed as a male with a wild-type female plant (Columbia ecotype). Heterozygous progeny were crossed as males a second time to female wild-type (Columbia ecotype) plants, and 56 progeny were scored for the segregation of wild-type *FIE* and mutant *fie-1* alleles and for molecular markers distinguishing Landsberg and Columbia chromosomes (Konieczny and Ausubel, 1993; Bell and Ecker, 1994). The *FIE* gene initially was mapped to the region be-

tween the left ends of yeast artificial chromosome clones ClC6F6 and ClC7B4. For fine structure mapping, we visually selected plants bearing recombinant chromosomes with break points between *fie-1* and the downstream dominant morphological marker, *axr2*, that confers a dwarf phenotype (Timpte et al., 1994).

A *fie-1* heterozygote (Landsberg *erecta* ecotype) was crossed as a male with female (Columbia ecotype) dwarf *axr2* homozygotes. Dwarf F_1 progeny that displayed 50% seed abortion in self-pollinated siliques (i.e., heterozygous for *fie-1* and *axr2*) were self-pollinated. Three hundred F_2 recombinant plants (i.e., normal-sized plants with 50% aborted seed in self-pollinated siliques) were identified among 15,600 nonrecombinant dwarf F_2 progeny and used to map the *fie-1* mutation relative to downstream molecular markers (Figure 3). We also visually selected plants bearing recombinant chromosomes with break points between *fie-1* and the upstream morphological marker *emb29.* Self-pollinated siliques from heterozygous *emb29* plants display 25% embryo abortion (Meinke, 1985). A *fie-1* heterozygote (Landsberg *erecta* ecotype) was crossed as a male with female (Columbia ecotype) *emb29* heterozygotes. F_1 progeny that displayed 75% seed abortion in self-pollinated siliques (i.e., heterozygous for *fie-1* and *emb29*) were self-pollinated. Thirty-three recombinant F_2 plants (i.e., self-pollinated siliques displayed 25 or 50% aborted seed) were identified among 3586 nonrecombinant (i.e., self-pollinated siliques displayed 75% aborted seed) F_2 progeny, and they were used to map the *fie-1* mutation relative to upstream molecular markers (Figure 3).

Genetic Complementation

A plant transformation-competent cosmid library of yeast artificial chromosome ClC3D12 (Columbia ecotype) was made in vector pCLD04541, a cosmid contig was constructed, and *Agrobacterium tumefaciens*–mediated transformation of *fie-1* heterozygous plants (Landsberg *erecta* ecotype) was performed as described previously (Century et al., 1997). Siliques from kanamycin-resistant transgenic plants were harvested 10 days after self-pollination and were dissected, and the number of normal and aborted seeds was counted. Heterozygous *fie* T_1 lines displaying 50% seed abortion (e.g., 58 normal/74 aborted 1:1; $\psi^2 = 1.94$; $P > 0.24$) in self-pollinated siliques indicated lack of genetic complementation. T_1 lines displaying 25% seed abortion (e.g., 97 normal/34 aborted 3:1; $\psi^2 = 0.064$; $P > 0.86$) in self-pollinated siliques suggested that an unlinked transgenic *FIE* allele had complemented the mutant *fie-1* allele.

To rule out the possibility that the observed 25% seed abortion in T_1 plants was due to T-DNA disruption of a gene required for embryo viability (Meinke, 1985), a representative T_1 plant, heterozygous for *fie-1* and hemizygous for cosmid GM15 (Figure 3), was self-pollinated, and the extent of T_3 seed abortion in self-pollinated siliques from 171 T_2 plants was determined. We observed a 7:3:2 segregation ratio (105:45:21; $\psi^2 = 2.4$; $P > 0.3$) of T_2 plants displaying 0:25:50% T_3 seed abortion. These results are consistent with complementation of the mutant allele with the transgenic wild-type allele in the female gametophyte.

For lines homozygous for both the mutant *fie-1* allele and the transgenic wild-type *FIE* allele on cosmid GM15 (Figure 3), 14 self-pollinated siliques containing 814 seeds displayed 0% seed abortion. After fertilization was prevented, all ovules ($n = 48$) displayed a central cell with a single nucleus. After fertilization was prevented in control *fie-1* heterozygous plants, 44% ($n = 110$) of the ovules had central cells with two or more nuclei.

DNA Sequencing

Wild-type genomic DNA sequences were determined by Lark Technologies, Inc. (Houston, TX), using subcloned DNA fragments from cosmid clones that genetically complemented the *fie-1* mutation (see above). Because it was not possible to obtain homozygous mutant *fie* plants, sequence alterations associated with mutant alleles were obtained as follows. To sequence the *fie-1* mutant allele, we constructed a cosmid library using DNA from heterozygous Landsberg *erecta* ecotype *fie-1*/Columbia ecotype *FIE*+ plants. A cosmid clone was selected that contained a Landsberg *erecta*–specific polymorphism and the mutant *fie-1* Polycomb gene. The *fie-1* mutation was identified by comparing this sequence with that of the wild type (Landsberg *erecta* ecotype). The mutated sequence for the *fie-1* allele was confirmed, and the *fie-2*, *fie-3*, *fie-4*, *fie-5*, *fie-6*, *fie-7*, *fie-8*, and *fie-9* alleles were identified using a similar procedure in which heterozygous plant DNAs were polymerase chain reaction (PCR)-amplified, subcloned, and sequenced. Mutated sequences were confirmed within the genomes of each allele by converting them into a codominant cleaved amplified polymorphic sequence (Konieczny and Ausubel, 1993).

FIE cDNA clones were isolated from a floral cDNA library (Weigel et al., 1992). The labeled probe, extending from the fourth exon to the 12th exon of *FIE*, was amplified from genomic DNA by PCR. Primers for the amplification reactions were 5′-TCCATCGACAGACATGGCTG-3′ and 5′-CTCGCAAATGTGCAGAGTCTTGTG-3′. Seven *FIE* cDNA clones were identified among 200,000 plaques screened and were sequenced at the University of California, Berkeley, DNA Sequencing Facility. To obtain 5′ cDNA sequences, we performed 5′ rapid amplification of cDNA ends (RACE) by using the 5′ RACE system, as described by the manufacturer (Life Technologies, Inc., Gaithersburg, MD). The *FIE* cDNA sequence is available as GenBank accession number AF129516.

RNA Analysis

Cauline leaf, stem, root, and flower bud tissue containing stages 0 to 12 (Smyth et al., 1990) flowers and open flowers (stage 13) was harvested, and total RNA was isolated using the TRIZOL reagent as described by the manufacturer (Life Technologies, Inc.) and further purified by using the RNeasy minikit (Qiagen, Inc., Chatsworth, CA). Young silique (from preglobular- to late-heart-stage embryos) RNA was isolated using a modified hot borate extraction method (Wilkins and Smart, 1996). Reverse transcription–PCRs (RT-PCRs) were performed using a RETROscript kit (Ambion, Inc., Austin, TX) and contained 2 μg of total RNA as starting material. Primers used to amplify *FIE* RNA were 5′-CGAGTCATTGACGTCAACAGTG-3′ and 5′-TCCATCGACAGACATGGCTG-3′. Primers used to amplify control *GAPC* (for glyceraldehyde-3-phosphate dehydrogenase C) RNA (Shih et al., 1991) were 5′-CACTTGAAGGGTGGTGCCAAG-3′ and 5′-CCTGTTGTCGCCAACGAAGTC-3′.

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NOTE ADDED IN PROOF

While this article was under review, Luo et al. (Luo, M., Bilodeau, P., Koltunow, A., Dennis, E.S., Peacock, W.J., and Chaudhury, A.M. [1999]. Genes controlling fertilization-independent seed development in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **96,** 296–301) showed that the *FERTILIZATION-INDEPENDENT SEED2* (*FIS2*) gene encodes a C_2H_2 zinc-finger transcription factor and that the *fertilization-independent seed1* (*fis1*) mutation is a *medea* allele.

Mutations in *FIE*, a WD Polycomb Group Gene, Allow Endosperm Development without Fertilization

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