Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 296–301, January 1999
Plant Biology

Genes controlling fertilization-independent seed development in *Arabidopsis thaliana*

MING LUO*, PIERRE BILODEAU*, ANNA KOLTUNOW†, ELIZABETH S. DENNIS*, W. JAMES PEACOCK*, AND ABDUL M. CHAUDHURY*‡

*Commonwealth Scientific and Industrial Research Organization, Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia; and †Commonwealth Scientific and Industrial Research Organization, Plant Industry, Horticulture Unit, GPO Box 350, Glen Osmond, SA 5001, Australia

Contributed by W. James Peacock, October 26, 1998

ABSTRACT **We have cloned two genes, *FIS1* and *FIS2*, that control both fertilization independent seed development and postpollination embryo development in *Arabidopsis*. These genes confer female gametophytic phenotypes. *FIS2* encodes a protein with a C_2H_2 zinc-finger motif and three putative nuclear localization signals, indicating that it is likely to be a transcription factor. *FIS1* encodes a protein with homology to the *Drosophila Polycomb* group gene *Enhancer-of-zeste* and is identical to the recently described *Arabidopsis* gene *MEDEA*. FIS1 is a protein with a number of putative functional domains, including the SET domain present in Enhancer-of-zeste-related proteins. Comparison of the position of the lesions in the *fis1* and *medea* mutant alleles indicates that *fis1* is a null allele producing a truncated polypeptide lacking all the protein domains whereas the deduced protein from *medea* lacks only the SET domain. We present a model of the role of *FIS1* and *FIS2* gene products in seed development.**

Plants have two alternating life phases, a gametophytic phase giving rise to male and female gametes and, after gamete fusion during fertilization, a sporophytic phase initiating with the zygote that develops into the mature plant (1). In most diploid, sexually reproducing plants, seed development begins when the haploid egg and the homodiploid central cells of the female gametophyte are fertilized by two sperm cells, producing a diploid zygote and triploid endosperm, respectively. In apomictic plants, unreduced egg cells or diploid nucellar cells develop parthenogenetically to produce the zygote, and in some cases endosperm development is autonomous. To isolate genes that might control components of apomixis, we have isolated mutants of *Arabidopsis* in which some stages of seed development are initiated without pollination (2, 3). In *fis1* and *fis2,* autonomous diploid endosperm development progresses to the cellularized stage, and occasionally zygote-like and early embryo-like structures form. In the *fis3* mutant, endosperm development stops at the free-nuclear stage and autonomous embryo-like structures have not been seen (3). Another mutant, *fie*, also showing endosperm development without fertilization and mapping to chromosome 3, is likely to be an allele of *fis3* (4). After pollination in *fis1*, *fis2,* and *fis3* mutants, most embryos are arrested at the globular to torpedo stages. We suggested that the *FIS* genes define a complex that suppresses the development of the seed, including the embryo and endosperm, in the absence of fertilization, and after fertilization, plays a role in promoting embryo development (3). To further define the roles of these gene products in seed development we have cloned the *FIS1* and *FIS2* genes.

In this paper we describe the *FIS1* and *FIS2* genes, their deduced products, and a model of their role in triggering fertilization-independent seed development and postpollination embryo development. Altered regulation of these genes and their homologs might play a role in the development of apomictic seeds.

MATERIALS AND METHODS

Genetic Mapping of *fis1* and *fis2*. Two separate mapping populations were obtained by crossing line E_{12} (CS8116, ABRC, Ohio State University (Columbus, OH) (Nossen background), and *short integument* 1–2 (*sin1–2*) (Ws background) obtained from Aminesh Ray (Rochester University, Rochester, NY) to *fis1/fis1* (L.*er* background). In E_{12} a Ds element (*DsG*) with the reporter gene *GUS* and *NPTII* conferring kanamycin resistance (Kan^R) was located south of *angustifolia* (*an*). In the *sin1–2* mutant, the *SIN1* gene is disrupted by a T-DNA insertion carrying Kan^R. The resulting F1 hybrids were back crossed to Landsberg *erecta* (L.*er*) and the F2 progeny were scored for recombinants between Kan^R contained in the T-DNA or the *DsG* and *fis1*. *fis1*/Kan^R recombinants were analyzed with simple sequence length polymorphism markers AthACS, nF21B7, and nT1G11 (http://genome.bio.upenn.edu/sslp_info/sslp_html), and two simple sequence length polymorphism markers found by our group from bacterial artificial chromosome (BAC) T7I23. Plant genomic DNA preparation and Southern blot analyses were performed as previously described (5). BAC IGF 11O10 was obtained from the Resource Center of the German Human Genome Project (Max-Planck-Institut, Berlin).

Plants used for *fis2* Restriction Fragment Length Polymorphism analyses were recovered from two F2 populations generated by crosses with morphological markers *erecta* (*er*) and *asymmetric leaves* (*as*). In cross #1, *fis2 er* (L.*er*) plants were crossed to *FIS2 ER* (Columbia). In cross #2, *fis2 AS* (L.*er*) plants were crossed to *FIS2 as* (Columbia) plants. Recombinant *FIS2/fis2 er/er* and *FIS2/fis2 as/as* were analyzed with restriction fragment length polymorphism markers mi277 and m323. YUP9D3 ends were cloned according to published procedures (6). Cosmid 18H1 was analyzed by restriction mapping by using enzymes *Eco*RI and *Bam*HI (Fig. 1*B*). The YUP library and the TAMU BAC library were obtained from Joe Ecker (University of Pennsylvania, Philadelphia) and from Rod Wing (Clemson University, Clemson, SC), respectively. The pOCA18 cosmid library was obtained from Neil Olszewski (University of Minnesota, St. Paul, MN).

Tagging of *FIS2*. *DsG1* and *Ac1* lines were obtained from V. Sundaresan (Institute of Molecular Agrobiology, Singapore) and have been described (7). F_1 plants of the *DsG/Ac1* cross

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Abbreviations: BAC, bacterial artificial chromosome; Kan^R, kanamycin resistance.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos.: AF096094–AF096096).

‡To whom reprint requests should be addressed. e-mail: abdul@pi.csiro.au.

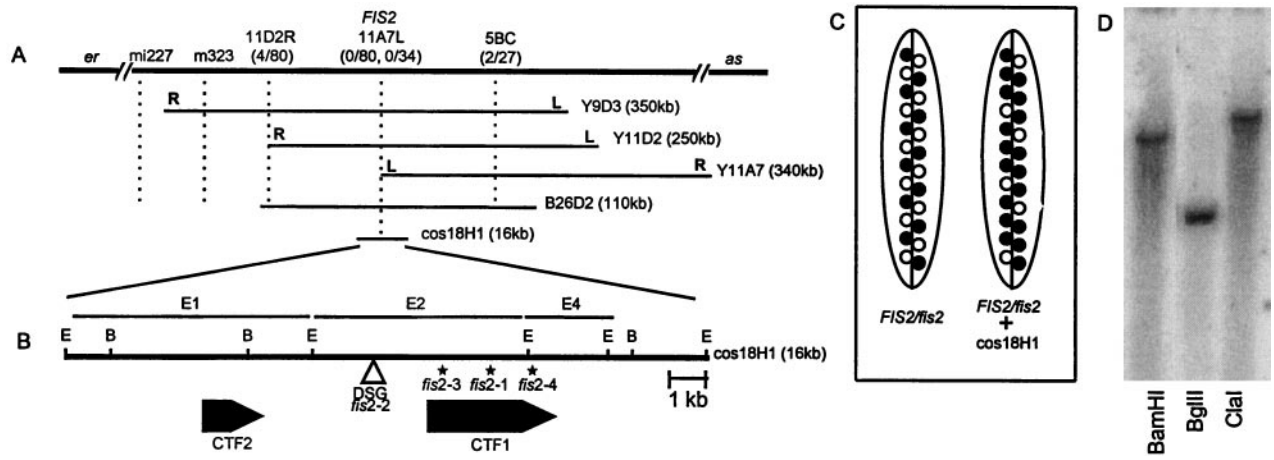

FIG. 1. (*A*) Position of the *FIS2* locus on chromosome II. The relative position of the *FIS2* locus and restriction fragment length polymorphism markers m323, mi227, YUP9D3, YUP11D2, YUP11A7 left (L) and right (R) ends, and the BAC 26D2 fragment 5BC are shown. (*B*) The position of the *DsG* insertion and the two cDNAs are shown. Asterisks (*) mark the locations of the mutations in the three ethyl methanesulfonate-induced *fis2* alleles. E, *Eco*RI; B, *Bam*HI. (*C*) Complementation of *fis2* with the cosmid 18H1. The silique on the left is from a *FIS2*/*fis2* plant showing a ratio of 50:50 of normal and *fis* seeds. On the right is a silique from a *FIS2*/*fis2* plant with an introduced 18H1 *FIS2* segment. The ratio of normal to *fis* seeds is now 75:25 as expected for the complementation of a gametophytic mutation (see data in Table 1). ○, *fis* seeds; ●, *FIS* seeds. (*D*) Southern blot of *Arabidopsis* L*er* genomic DNA digested with either *Bam*HI, *Bgl*II, or *Cla*I and hybridized with the 2.38 kb *FIS2* cDNA insert.

were screened for chimeric plants containing sectors that showed both the female gametophytic mutant phenotype and the fertilization-independent seed phenotype. One out of the 20 plants tested contained a presumptive *FIS2*/*fis*2–2 sector. A library was made from DNA of a *FIS2*/*fis2*–2 plant and screened with the DNA fragment E2 from cosmid 18H1 and the *DsG* 5′ end probe.

Complementation of *fis2*. Cosmid pOCA18H1 was mobilized in *Agrobacterium tumefaciens* AGL1, and the T-DNA introduced into *Arabidopsis* ecotype C24 using the root explant transformation protocol (8). Four independent T1 Kan^R plants were crossed as females to *fis2*/*fis2*. Siliques from Kan^R and kanamycin-sensitive (Kan^S) F_1 plants were scored for female gametophytic lethality.

cDNA Library Construction and Screening. Polysomal poly(A) mRNA was extracted from *Arabidopsis* L*er* siliques ranging from heart/torpedo to maturing seed stages using standard procedures (9). A cDNA library was made using the Choice cDNA Synthesis System (GIBCO/BRL, catalog no. 18090) according to the manufacturers protocol except that the size fractionated cDNA was cloned into Lambda Zap II vector (Stratagene). DNA probes derived from the *Eco*RI fragments E1 and E2 (Fig. 1*B*) were used to screen 200,000 clones from the cDNA library. Prehybridization and hybridization were performed in 10% PEG6000, 7% SDS, 0.25 M NaCl, 0.05 M $NaPO_4$ (pH 7.2), 1% BSA, 1 mM EDTA at 65°C for 2 hr and 16 hr, respectively. The filters were washed at room temperature (once in 2× standard saline citrate, 1% SDS, once in 1× standard saline citrate, 1% SDS for 30 min each). Lambda phagemid DNA containing the cDNA insert was excised from positive plaques according to the Stratagene protocol.

DNA and Protein Sequence Analysis. An Applied Biosystems Model 370A DNA Sequencer with fluorescent dye-labeled dideoxy terminators was used for sequencing. *FIS2* sequence (exons and introns) from the homozygous *fis*2 mutants was obtained by PCR amplification of a 3,754-bp region using seven different primer pairs. Sequence of the *MEDEA* gene from the homozygous *fis1* mutant was obtained by PCR amplification of a 4,155-bp segment using five different primer pairs designed from the *MEDEA* cDNA sequence (10). The sequence data were analyzed by using the GCG software (11). Sequence comparisons were performed using BLAST searches (12) and multiple sequence alignments were performed and analyzed with the computer programs CLUSTALW (13), GENEDOC (http://www.cris.com/~Ketchup/genedoc.shtml), and PHILIP (14).

Southern Hybridization Analysis. Genomic DNA from *Arabidopsis* seedlings was prepared by the hexadecyltrimethylammonium bromide protocol (15). Five micrograms of genomic DNA was digested with restriction enzymes before 1% agarose gel electrophoresis. The DNA was then transferred to a Hybond N membrane, prehybridized for 1 hr, hybridized, and the filters washed according to Church and Gilbert (16). The membrane containing the digested genomic DNA was probed with the radiolabeled 2.38-kb *FIS2* cDNA insert.

RESULTS

Localization of *FIS2*. Genetic mapping localized the *FIS2* gene between the markers *er* and *as* on chromosome 2 (Fig. 1*A*). A yeast artificial chromosome clone, YUP9D3, that mapped in this region was used to link the genetic location of *FIS2* to the molecular map. Analyses of recombinants at the *fis2-er* and *fis2-as* interval indicated that the left end of 9D3 mapped to the *as* side of the *FIS2* gene. By using YUP9D3 as a probe, two additional yeast artificial chromosomes, 11D2 and 11A7 were isolated from the YUP library. The right end of 11D2 mapped to the *er* side of *FIS2* and the left end of 11A7 showed no recombination with *FIS2* indicating close proximity of this marker to *FIS2* (Fig. 1*A*).

To locate *FIS2* on a DNA fragment smaller than a yeast artificial chromosome, the left end of 11A7 was used as a probe to isolate a BAC clone, 26D2, and the cosmid 18H1. A physical map of BAC 26D2 was constructed with the enzymes *Bam*HI and *Cla*I, and the fragments were tested on the *fis2-er* and *fis2-as* recombinant population to delineate the position of the *FIS2* gene. The results of these analyses suggested that the *FIS2* gene is contained in fragment E2 of BAC 26D2 (Fig. 1 *A* and *B*).

Genetic Complementation of *FIS2*. The cosmid 18H1 containing the E2 fragment was introduced into the *Arabidopsis* ecotype C24 and the transgenic plant was pollinated by a *fis2*/*fis2* plant. Kan^R F1 plants containing the introduced cosmid with the *FIS2* gene were selected and their siliques scored for normal and arrested seeds (Table 1). As *fis2* is a female gametophytic mutant, the expected ratio of wild-type to arrested seed in a *FIS2*/*fis2* plant is 50:50. An introduced

Table 1. Ratio of normal:arrested seen in *FIS/fis2* plants segregating for the cosmid 18H1

Line	Kan^R progeny (+18H1)		Kan^S progeny (−18H1)	
	Ratio	X^2	Ratio	X^2
1	179:57	0.051	Not tested	–
6	153:55	0.23	Not tested	–
7	139:47	0.01	131:119	0.576
10	210:73	0.1	54:61	0.43
Control C24	NA	–	211:216	0.058

Kan^R plants contain the cosmic and Kan^S do not. Each line is derived from an independent transformation event. Expected ratios for Kan^R and Kan^S plants are 75:25 and 50:50, respectively, if the 18H1 cosmid complements the *fis2* mutation. Control cross with C24 shows the segregation of *fis2* without complementation. NA, not available.

single copy *FIS2* transgene will segregate 1:1 in the gametes so that 50% of the *fis2* seeds will contain the transgene and have a *FIS2* phenotype. This will shift the ratio to 75:25 in favor of normal seed. In F_1 plants derived from four independent transgenic plants containing the E2 fragment, the ratio of arrested to wild-type seeds fitted the 75:25 segregation ratio (Fig. 1*C* and Table 1). In contrast, a 50:50 ratio was observed in the Kan^S plants of the same cross. These data show that the cosmid 18H1 does complement the mutant phenotype of *fis2* (Table 1) and also suggest that the *fis2* mutation is a gametophytic loss-of-function allele.

Isolation of *FIS2* cDNA. Analyses of DNA from *FIS2/fis2–2* indicated that the *DsG* transposon was inserted in the E2 segment of the genomic DNA, further delineating the position of *FIS2*. The E2 segment, used to probe a late silique cDNA library, hybridized to two cDNA clones of 1.43 kb and 2.38 kb. The 1.43-kb insert was identical to the 3′ end of the 2.38-kb insert indicating that they came from the same gene, *CTF1* (*C*lose *T*o *FIS* #1). The 5′ end of the *CTF1* cDNA was 750-bp downstream of the *DsG* insertion (Fig. 1*B*). In a second screen, a 1-kb *Bam*HI–*Eco*RI DNA fragment from E1 identified another cDNA, *CTF2*, that mapped 2.5 kb upstream of the *DsG* insertion point (Fig. 1*B*). Thus, the tagged *fis2* allele did not define which of the two cDNAs encodes *FIS2*.

To ascertain which of the two cDNAs does encode *FIS2,* both *CTF1* and *CTF2* sequences of three ethyl methanesulfonate-induced mutant alleles of *fis2* were compared with the wild-type sequences. *CTF2* sequences in these mutant alleles of *fis2* were identical to the wild-type sequence, eliminating this gene as a contender for being the *FIS2* gene. Each mutant allele of *fis2* contained a different alteration in the *CTF1* sequence. In the *fis2–1* allele, there is a single nucleotide deletion at position 1,472 of the *CTF1* cDNA that induces a frameshift resulting in a stop codon four amino acids downstream of the deletion. A *G* to *A* base conversion in the *fis2–3* mutant occurs at the 3′ splice junction of intron four of *CTF1*, altering the consensus splice acceptor sequence A*G* to A*A* and probably resulting in missplicing of the *FIS2* gene. In *fis2–4*, a *G* to *A* base substitution occurs at position 2,017 of the *CTF1* cDNA creating a stop codon. The *DsG* tagged allele, *fis2–2*, did not show any nucleotide changes in either the *CTF1* or *CTF2* sequences. Therefore, *DsG* could have disrupted a critical regulatory sequence in the upstream region of the *FIS2* gene or, the Ds insert may have altered chromatin structure modifying the normal expression of *FIS2*. These results confirm that *CTF1* corresponds to the *FIS2* gene. Southern hybridization with the *FIS2* probe showed a single band at high and low stringency (Fig. 1*D*), indicating that *FIS2* is encoded by a single copy gene.

Characteristics of *FIS2*. Comparison of the *FIS2* cDNA and genomic sequences from the *Arabidopsis* ecotype L.*er* defined 12 exons and 11 introns. The largest exon (#7) is 1,365-bp long and contains a 66-bp sequence that is repeated 12 times (repeat A) and a 51-bp sequence that is repeated seven times (repeat B). The *FIS2* gene from the *Arabidopsis* ecotype Columbia (Col) contains a 180-bp deletion in exon #7 from nucleotide 1,434 to 1,613 of the L.*er FIS2* cDNA spanning one of the B repeats and two of the A repeats. Subsequent PCR analysis of this region in ecotypes L.*er*, Col, WS, and C24 showed that the deletion was present only in Col (data not shown). Col also contains a 26-bp deletion in intron #6. These deletions do not appear to have any effect on *FIS2* function because Col plants do not exhibit a *fis* phenotype and the cosmid 18H1 that complemented the *fis2* mutation in the L.*er* ecotype originated from the Col ecotype.

FIS2 expression was not detected in shoots, leaves, bolting stem, flower buds or siliques after Northern blot analysis using 20 μg of total RNA for each tissue sample. Similarly, RNAse protection assays did not generate a protected fragment, suggesting that *FIS2* is expressed at low levels. The only confirmed expression of the gene is in late silique RNA where a *FIS2* cDNA was isolated from the library at a frequency of 1:100,000.

The predicted polypeptide sequence of *FIS2* shows a putative C_2H_2 zinc-finger motif within the first 35 residues of the protein and 3 putative bipartite nuclear localization signals distributed between residues 267–540 (Fig. 2). The presence of the zinc finger motif and nuclear localization signal suggest that *FIS2* might be a transcription factor. Like other zinc finger proteins (17), *FIS2* has a high serine content (12.9%). The A and B repeats produce a distinctive signature when the *FIS2* protein product is compared with itself by dot matrix analysis (Fig. 3). The consensus sequences for A and B repeats are H-V-N-D-D-N-V-S-S-P-P-[R/K]-A-H-S-S-K-K and L-T-T-T-Q-P-A-I-A-E-S-S-E-P-K-V. Following the consensus sequence described above, half of the A repeats have a T-S-D-I sequence and the other half have N-E-S-T. The central part of the A repeat has a consensus SPP[R/K] with similarity to the motif [T/S]PXX (X is usually a basic amino acid) that has been shown to bind in the minor groove of DNA (18).

***FIS1* Is Allelic to *MEDEA*.** We localized the *FIS1* gene to a short region on top of chromosome 1, linked to *an* (3). Using further genetic and physical mapping, we identified yeast artificial chromosome and BAC contigs that were likely to span the *FIS1* gene. While this work was in progress, the *medea* mutant was described exhibiting embryo arrest in 50% of seeds after fertilization (10). *MEDEA* maps to the same region as *fis1* (U. Grossniklaus, personal communication). *MEDEA* encodes a SET domain protein (*S*uppressor of variagation 3-9, *E*nhancer of zeste, *T*rithorax) similar to *Enhancer-of-zeste* (Ez) from *Drosophila*, a member of the *Polycomb* group of proteins. We investigated whether *medea* and *fis1* were allelic mutations by sequencing the *MEDEA* gene from wild-type and homozygous *fis1* plants (Fig. 4). In *medea,* the *DsG* element is inserted at position 1,755 of the cDNA (10). In *fis1* the same cDNA shows a *C* to *T* substitution at position 320 creating a stop codon (Fig. 4), indicating that *medea* is an allele of *FIS1*.

We sequenced the *FIS1* genomic region and compared it with the cDNA sequence (10). *FIS1* has 17 exons and 16 introns with the introns ranging from 75 bp to 566 bp in length (Fig. 4). There are conserved TATAAT and CCAAT boxes located at positions −123 and −143 relative to the initiating ATG codon. In addition to its similarity to Ez (37% similarity), the *FIS1* predicted polypeptide shares homology with two *Arabidopsis* proteins, *CURLY LEAF (CLF)* (39% similarity), a regulator of floral homeotic gene expression (19), and *EZA1* (49% similarity), a recently identified gene of unknown function (GenBank accession no. AF100163). The C-terminal regions of FIS1 and Ez that contain the conserved SET domain and a cysteine-rich domain (CXC) show 65% sequence similarity.

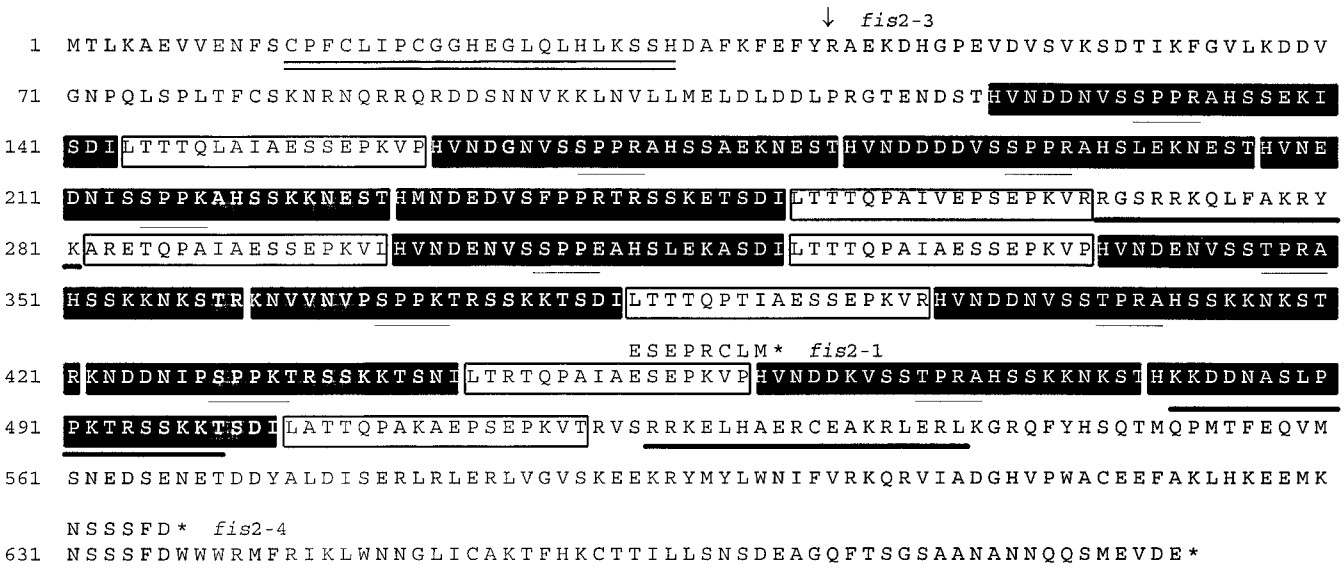

FIG. 2. Deduced protein sequence of *FIS2*. The amino acid sequence corresponding to the C_2H_2 zinc finger motif is double underlined. The three putative nuclear localization signals are underlined with thick lines. The 12 A repeats are shown in filled boxes and the 7 B repeats are shown in open boxes. The [T/S]PXX motifs within the A repeats are underlined with thin lines. The position of the *fis*2–1 (deletion of T) and the *fis*2–4 (G → A) mutations are shown with the resulting modifications of the encoded peptide. The location of the *fis*2–3 mutation (G → A) at the junction of intron 5 and exon 6 is indicated by an arrow (↓). Stop codons are indicated with asterisks (*).

DISCUSSION

***FIS2*, a C_2H_2 Zinc Finger Transcriptional Regulator.** We isolated the *FIS2* gene using positional cloning and tagging. The presence of a C_2H_2 zinc finger signature, and three putative nuclear localization signal motifs suggests that *FIS2* encodes a transcriptional regulator. Other than these motifs the *FIS2* sequence does not have extensive homology to any sequence in the database. Several members of the C_2H_2 zinc-finger protein family, also known as the TFIIIA-like zinc finger protein gene family, play important roles in growth and development in *Drosophila* (20, 21) and in plants (22). Unlike animal C_2H_2 zinc finger proteins that contain multiple zinc finger motifs, present as tandem arrays, the plant C_2H_2 zinc finger proteins contain only one to four fingers (22). The region in the center of the FIS2 protein containing A and B type repeats (Fig. 2), with most of the A-type repeats containing a [T/S]PXX motif, could form a 3-dimensional structure involved in protein-protein interactions. Alternatively, it could form fingers where the [T/S]PXX motifs positioned at the tip of each finger could directly bind A+T-rich DNA sequences. Histones H1, H2A, and H2B are essential for chromatin condensation and each of them contains several [T/S]PXX motifs (23, 24).

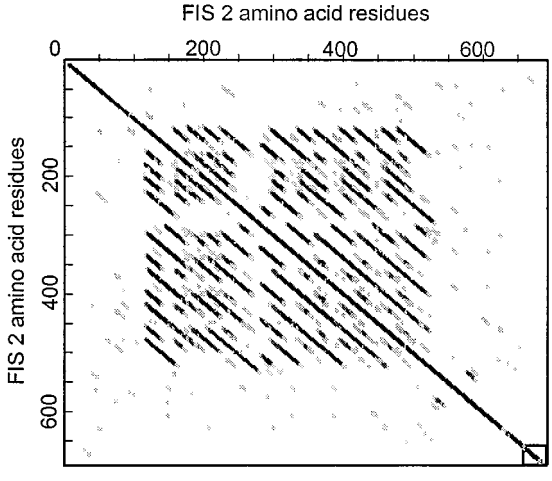

FIG. 3. Bi-dimensional plot of the FIS2 predicted protein sequence showing the tandem repeats between residues 120 and 520. Within the matrix, the dark dots indicate high homology and open dots indicate no significant homology. The dot matrix was obtained using the software ANTHERPROT 3.2 (35) with a window size = 19 and a similarity threshold = 10. The principle of the method is described in Staden (1982) *Nucleic Acid Res.* 10, 2951–2961.

FIS1 Is Related to the *Polycomb* Group Protein Ez of *Drosophila*. We determined that the *FIS1* gene is the same as *MEDEA*, a recently described gene related to the *Polycomb*-group gene *Ez* from *Drosophila* (10). Mutants of *Ez* exhibit defects in oogenesis, maternal-effect lethality, and zygotic lethality (25). Several lines of evidence indicate that Ez-related proteins play a central role in chromosome architecture and the nucleation of repressive protein complexes in chromatin (26). Comparison of the sequences between *fis1* and *medea* indicates that whereas all of the characteristic domains of Ez-related proteins are eliminated by the N-terminal stop codon in the *fis1* allele, all domains except the SET domain remain intact in the C-terminal disruption of the *medea* mutant allele (Fig. 4). *fis1* exhibits an autonomous endosperm phenotype in 50% of seeds and an arrested embryo phenotype after pollination (3); *medea* has been reported to have the arrested embryo phenotype (10) but also shows a weak autonomous endosperm phenotype (27).

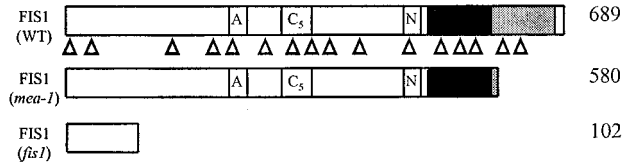

FIG. 4. Schematic representation of the FIS1 protein. The SET domain is shown as ▨. The cysteine-rich domain CXC is shown as ■. Boxes labeled A, N, and C5 represent an acidic domain, a putative nuclear localization signal, and a second conserved cysteine-rich domain. The truncated FIS1 peptides resulting from the *mea*-1 and *fis1* mutations are shown beneath the wild-type FIS1 protein. Numbers on the right indicate the number of amino acid residues in the corresponding FIS1 protein products. Triangles (△) under the schematic representation of FIS1 protein indicate the position of the 16 introns in the corresponding DNA sequence.

Do *Caenorhabditis* and *Arabidopsis* Use Similar Genes to Maintain their Germlines? In *Caenorhabditis elegans*, an Ez homolog (MES2) appears to play a central role in maintaining a repressed chromatin state in the germline (28). Active *MES* genes are essential for normal germline development (29). It has been suggested that *MES* proteins act in a complex that is required to maintain a germline specific organization of chromatin from one generation to the next and that this chromatin state is essential to initiate the correct pattern of gene expression in the germline. Tandem arrays of transgenes are efficiently expressed in somatic cells but are specifically silenced in the germline of wild-type worms (30). In *mes* mutant lines, these tandem transgenes are activated in the germline, supporting the concept that the *MES* proteins repress gene expression through an influence on chromatin state. The similarities between *FIS1* and *MES*2 in being Ez homologs and in repressing germline functions suggest that FIS1 may also act by maintaining a repressed chromatin state. Another *FIS* gene, *FIS3,* may be a homolog of another *MES* gene such as *MES*6 (a homolog of *Extra sex combs* in *Drosophila)* (28). Mutation in all three *fis* loci leads to autonomous partial seed development so perhaps they too, like the MES proteins, may act in a complex with cooperative roles in maintaining a repressed seed development program, normally relieved by pollination.

In *C. elegans*, the PIE-1 protein keeps the germline blastomeres transcriptionally quiescent whereas the surrounding somatic cells become transcriptionally active (31). When PIE-1 decays, the primordial germ cells become transcriptionally active and the *MES* genes are required to repress certain genes. PIE-1 is a zinc finger protein, as is FIS2. FIS2 may play a role analogous to that of PIE-1.

Model of Action of the *FIS* Genes. Formerly, we presented a model in which the *FIS* gene products act in a complex to repress genes required for seed development (3). We can now modify this model to incorporate our new data that suggest that FIS2 is a transcription factor and FIS1 a homolog of the *Polycomb* group protein Ez (Fig. 5*A*). In the revised model, FIS1 represses the activity of one or more seed development genes (*SDG*) and FIS2, together with FIS1 and FIS3, maintains the repressed state of the *SDG*. A mutation to a nonfunctional allele in any of *FIS1*, *FIS2*, or *FIS3* would result in derepression of the seed development genes. *SDG* expression may trigger heterochronic developmental steps, such as cell division in the central or egg cells that are normally quiescent in the absence of pollination. These steps may initiate a cascade of developmental events leading to partial seed development.

During normal seed development, fertilization may introduce derepressed *SDG* from pollen to initiate seed development. The derepression of *SDG* in pollen could be a result of epigenetic silencing of one or more of the *FIS* genes in the male gamete. These processes resemble genomic imprinting that has been shown to occur in plant endosperm (32, 33).

In a second model (Fig. 5*B*), the *FIS2* product positively regulates the *FIS1* gene that represses *SDG* as part of a silencing mechanism. In *fis2* mutants, *FIS1* would not be expressed and thus *FIS1* mediated silencing of *SDG* does not occur. *FIS3* may also be under *FIS2* control and/or may be in a functional complex with FIS1. This sequential model is based on the indication that FIS2 is a transcription factor and that FIS1 may play a role in gene repression. This model predicts that *FIS1* gene expression would be down-regulated in a *fis2* mutant.

The *FIS* genes also play a positive role in embryo development after pollination. In *fis* mutants, after pollination, embryo development is generally incomplete, with embryos arrested at the globular to torpedo stages; occasional embryos develop to maturity (3). In *fis* mutants, the auto-activation of genes in the female gametophyte might initiate an abnormal development pattern leading to embryo arrest even in fertilized ovules. The occasional escapees that develop to mature seeds presumably result from the leakiness of the mutation. The *Polycomb* group protein Ez of *Drosophila* has been associated with a gene activating function during particular developmental stages and acting through a specific promoter

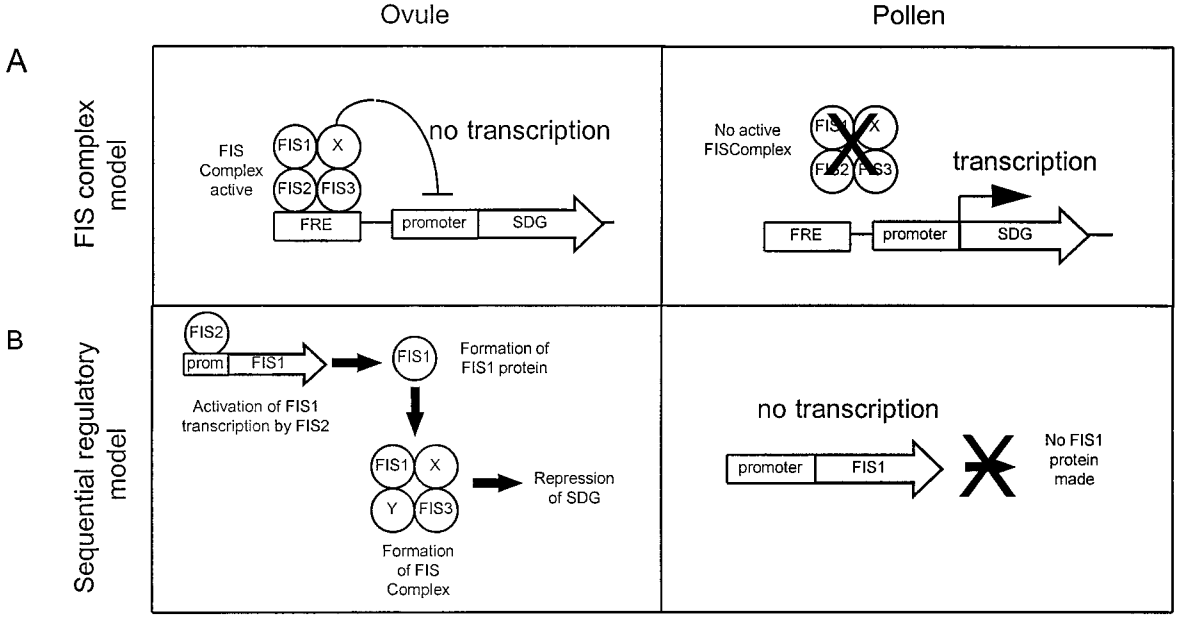

FIG. 5. Model for the role of FIS protein products in seed development. The cartoon represent possible roles of FIS protein in maintaining *S*eed *D*evelopment *G*ene (*SDG*) repression. Molecular events taking place in ovule and pollen are described. X and Y represent additional proteins that could be part of the FIS complex. (*A*) The "FIS complex" model. FIS1, 2, and 3 bind as a complex (FIS complex) to specific sites in *SDG* regulatory regions termed FRE for *F*IS *R*esponse *E*lement. The FIS complex represses by interfering with the basal transcription machinery. (*B*) The "Sequential Regulatory" model. This model proposes that FIS2 transcriptionally regulates *FIS1* and/or other *FIS* gene(s) and subsequently forms an active FIS complex, repressing *SDG*. In both models, *FIS* genes are not active in pollen so that *SDG* can be active at the appropriate stage of development.

complex (34). FIS1 may have an activating role, again interacting in some way with FIS2 (and FIS3).

Apomixis requires many components of the reproductive processes to be integrated to produce autonomous viable seeds. Among these components are autonomous endosperm development and the initiation of zygotic development in the absence of fertilization. Both of these are seen to varying degrees in the *fis* mutants suggesting that the *FIS* genes may play a role in apomictic seed development and that apomixis may involve altered regulation of these genes.

We thank Janice Norman and Bjorg Sherman for technical support, Chris Helliwell for helping with the cloning the *FIS2* gene, and Ueli Grossniklaus for disclosing the map location of *MEDEA*. This research has been supported by the Rockefeller Foundation and by the Australian Center for International Agricultural Research.

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