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Fertilization-independent seed development in *Arabidopsis thaliana*

(ovule/apomixis)

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ABSTRACT **We report mutants in *Arabidopsis thaliana* (fertilization-independent seed: *fis*) in which certain processes of seed development are uncoupled from the double fertilization event that occurs after pollination. These mutants were isolated as ethyl methanesulfonate-induced pseudo-revertants of the *pistillata* phenotype. Although the *pistillata* (*pi*) mutant has short siliques devoid of seed, the *fis* mutants in the *pi* background have long siliques containing developing seeds, even though the flowers remain free of pollen. The three *fis* mutations map to loci on three different chromosomes. In *fis1* and *fis2* seeds, the autonomous endosperm nuclei are diploid and the endosperm develops to the point of cellularization; the partially developed seeds then atrophy. In these two mutants, proembryos are formed in a low proportion of seeds and do not develop beyond the globular stage. When *FIS/fis* plants are pollinated by pollen from *FIS/FIS* plants, ≈50% of the resulting seeds contain fully developed embryos; these seeds germinate and form viable seedlings (*FIS/FIS*). The other 50% of seeds shrivel and do not germinate; they contain embryos arrested at the torpedo stage (*FIS/fis*). In normal sexual reproduction, the products of the *FIS* genes are likely to play important regulatory roles in the development of seed after fertilization.**

Arabidopsis seed, like the seed of other angiosperms, is a product of double fertilization, in which one of the two sperm cells fertilizes the haploid egg cell, giving rise to a diploid embryo, and the other sperm cell fertilizes the polar nuclei in the central cell, giving rise to the triploid endosperm (1). As the embryo and the endosperm develop, the ovule enlarges into a seed; the maternal tissues of the inner and outer integuments surrounding the embryo sac form the seed coat.

We have proposed a strategy for identifying genes that uncouple components of seed development from the fertilization process (2). Inactivation of genes that normally repress seed development may lead to precocious seed development without fertilization. In many apomictic plants, seed development does occur without fertilization or with only partial fertilization (3). In autonomous apomixis, seed development occurs without pollination and thus without fertilization of either the egg cell or the polar cell. In pseudogamous apomixis, pollination is required; in some cases, the pollination event results in fertilization of the polar cell but not of the egg cell. Apomixis has been described in a close relative of *Arabidopsis*, *Arabis holboellii* (3), and some genetic data support a one or two gene control of apomixis (3), so we reasoned that a mutational approach in *Arabidopsis* might detect mutants displaying some components of apomixis.

For the isolation of these mutants, we used stamenless *pistillata* (*pi*) (4). If *pi* plants are not pollinated, the siliques remain short; they only elongate when seed is formed. We identified mutants in which the siliques elongated without pollination (5), and recently Ohad *et al* (6) described a mutant that forms endosperm without fertilization.

In the present paper, we describe *fis1* (*f*ertilization-*i*ndependent *s*eed), *fis2*, and *fis3*, whose developmental and genetic characterization indicates that these genes normally have a controlling role in seed development after pollination and double fertilization.

MATERIALS AND METHODS

Mutagenesis and Mutant Identification. Heterozygous *PI/pi* seeds were generated by pollinating a female *pi/pi* with pollen from a *PI/PI* plant. For each mutagenesis, 2 g of F1 seeds was mutagenized as described (7). Each pot of the M1 plants was harvested separately by collecting at least 10 mature siliques from each plant to ensure that sufficient seeds were obtained from each M1 plant. In the M2 population, one–fourth of the progeny plants were *pi/pi*. *PI/pi* and *PI/PI* plants were identified by the presence of petals and stamens and were removed. Putative mutants were detected, in the *pi/pi* population, on the basis of elongation of siliques without formation of stamens.

Mapping of the *fis* Genes by Their Linkage to Morphological Markers. *FIS/fis* heterozygous plants in a phenotypically self-fertile background were used as pollen donors in a cross to a multiply marked tester line, W100F (a male-fertile derivative of line W100) (8). In the segregating F2 population, recombination frequency was estimated as the proportion of *FIS/fis* plants in the total of *FIS/fis* plus *FIS/FIS* plants homozygous for each marker gene. Recombination frequency was converted to map distance (9).

Microscopy. Fertilized and unfertilized siliques were dissected under a dissecting microscope. For optical microscopy, tissue was prepared according to Craig and Miller (10). For scanning electron microscopy, dissected ovaries were attached with colloidal graphite to a copper stub, frozen under vacuum, and examined according to Craig and Beaton (11).

Measurement of Ploidy of the Endosperm Nuclei. To measure the DNA content of endosperm nuclei of *fis1* and *fis2* mutants and pollinated homozygous *pi/pi* plants, 2-μm sections were stained with 0.5 μg/ml 4′, 6-diamidino-2-phenylindole (12) in water for 4 minutes and mounted in water. All observations were made with a Nikon Optiphot fluorescence microscope using a ×40 objective (Nippon Kogaku, Tokyo). At least 50 nuclei were measured for each mutant. Images were collected and analyzed using the Image 1 pro-

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Abbreviation: HAF, hour(s) after fertilization.
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cessing system (Universal Imaging, Media, PA). The Total Intensity Function was used to measure the fluorescence intensity of each endosperm nucleus.

RESULTS

Mutants in Which Siliques Elongate Without Pollination. In a screen of 50,000 *pi/pi* M2 plants, six putative mutants with elongated siliques but no reversion of the floral *pistillata* phenotype were identified. These mutants contained seed of size and external morphology comparable to the seeds developing after pollination of the *pi/pi* plants (Fig. 1). We have analyzed three of these fertilization-independent seed mutants (*fis1*, *fis2*, and *fis3*).

Genetic Transmission of the *fis* Mutations. Plants with the *fis* phenotype originally were isolated in a male-sterile *pi/pi* background so that the *fis* trait could not be transmitted via pollen and had to be transmitted via the female gamete. When *pi/pi FIS1/fis1*, *pi/pi FIS2/fis2*, and *pi/pi FIS3/fis3* plants were pollinated with pollen from a *PI/PI FIS/FIS* plant, the ratios of the *FIS/fis*-to-*FIS/FIS* plants were, respectively, 3:200, 1:151, and 0:140, rather than the expected ratio of 1:1. Heterozygous *FIS/fis* plants were identified by the 1:1 ratio of normal and embryo-arrested seeds. No egg transmission of the *fis3* locus was detected, so the *pi/pi FIS3/fis3* mutant plant was screened for a rare phenotypic reversion of the *pi* phenotype, which produced some pollen. One flower of ≈500 screened in the mutant plant was found to have some pollen; the pollen from this plant was used to transfer the mutant *fis3* trait to progeny. These results indicate that the *fis* traits have a low frequency of transmission via the female gamete.

In contrast, the pollen transmission of the *fis* trait was unaffected by the *fis* mutation. Pollen from *FIS/fis PI/pi* heterozygotes of each of the three *fis* mutants was used to pollinate a *FIS/FIS pi/pi* female line. Among the resulting F1 plants, *FIS1/fis1* and *FIS1/FIS1* genotypes segregated 63:66, *FIS2/fis2* and *FIS2/FIS2* segregated 59:59, and *FIS3/fis3* and *FIS3/FIS3* segregated 82:83, indicating normal pollen transmission of the *fis* trait in each of the three *fis* mutants. Together these results indicate that the *fis* mutations are female gametophytic in action.

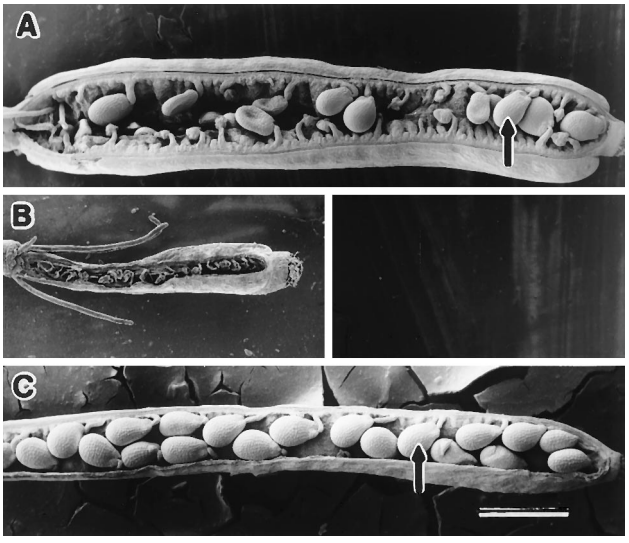

FIG. 1. Pollination-independent silique and seed development in a *FIS/fis* mutant plant. (*A*) Autonomous silique and seed development of a *pi/pi FIS3/fis3* plant; unpollinated (*B*) and pollinated (*C*) silique from a *pi/pi FIS/FIS* plant also are shown. Note the comparable development of fertilization-independent seed (*A*, arrow) and sexually developed seed (*C*, arrow). (Bar = 1 mm.)

To obtain *fis/fis* homozygous plants, progeny of *FIS/fis PI/PI* plants were screened for plants in which all seeds in a silique were embryo-arrested. One of the 450 plants for the *FIS1* locus and 2 of the 1621 plants for *FIS2* were homozygous *fis1/fis1* and *fis2/fis2*. No plants homozygous for *fis3* were found among 2000 plants tested.

The *FIS* Genes Map to Different Chromosomes. To map the *FIS* loci, pollen from *FIS/fis PI/PI* plants was used to pollinate W100F, a male-fertile derivative of W100 that contains 10 morphological mutations distributed on the arms of the five *Arabidopsis* chromosomes (8). Among the F2 progeny of *FIS/fis* W100F/+, plants homozygous for the different recessive morphological mutations were scored for *FIS/FIS* (all seeds in the siliques were normal) and for *FIS/fis* (the siliques contained a mixture of fully developed and embryo-arrested seeds). With these data, *fis1* and *an* at the top end of chromosome 1 had a recombination frequency of 0.92 ± 0.91, translating to a map distance of 0.93 cM (n = 109). Similarly, the *fis2-py* recombination frequency of 9.28 ± 1.56 (map distance of 10.26; n = 345) and the *fis2-er* recombination frequency of 13.07 ± 2.73 (map distance of 15.14; n = 153) positioned *fis2* between *er* and *py* on chromosome 2. Recombination frequencies of 14.45 ± 2.67 (map distance of 17 cM; n = 173) between *fis3* and chromosome 3 marker *gl1* and of 19.47 ± 2.9 (map distance of 24.67 cM; n = 187) between *fis3* and *hy2* positioned *fis3* between *hy2* and *gl1* in chromosome 3.

Characterization of the *fis* Mutants. Fertilized ovules of *pi/pi* plants developed into seeds, but unfertilized ovules remained small and eventually shrivelled (Fig. 2*D*). In unpollinated heterozygotes of the *fis* mutants, one–third to one–half of the ovules in the elongated siliques were transformed into seed-like structures resembling normal, sexually produced seed in external morphology and size (Fig. 2; ref. 13). All sexually fertilized seeds turn green and mature after pollination whereas seeds from pollinated *FIS/fis* heterozygotes contained green (mature) and white (embryo-arrested seed) at a 1:1 ratio. The *fis* ovules were similar to *FIS* ovules in early stages of ovule development. Both inner and outer integuments and the nucellar tissues of the *fis* mutants were indistinguishable from those of *FIS* plants (Fig. 2).

Endosperm and Embryo Development in the *fis* Mutants. Embryo sac, embryo, and endosperm development in ovules from the *fis* mutants were compared with those of ovules of the congenic L*er FIS* plants (Fig. 3). In *pi/pi* ovules, no embryo or endosperm cells were seen (Fig. 3*A*). Three days after pollination of the *pi/pi* plant with pollen from a *PI/PI* plant, the ovules contained an embryo and free nuclear endosperm cells, and each ovule had expanded to the size of the mature seed (Fig. 3*B*).

Fig. 3 *C* and *D* shows the autonomous development of *fis1* mutant ovules from a *FIS1/fis1* heterozygous plant. As shown in Fig. 3*C*, cellularized endosperm cells have formed inside the ovule, and the ovule has developed to the same size as the pollinated ovule, 3 days after pollination (Fig. 3*B*). More rarely, the *fis1* ovule contained embryo-like structures at the micropylar end as well as free nuclear endosperm cells (Fig. 3*D*). In the mutant ovules from a *FIS2/fis2* heterozygous plant, the ovule development was equivalent to the development of *pi/pi* ovules 3 days after pollination, and cellularized endosperm cells occasionally were accompanied by an embryo-like structure at the micropylar end (Fig. 3*E*). However, in the mutant ovules of *fis3*, even though the ovule developed in size as much as the *fis1* and *fis2* mutant ovules, the endosperm developed only to the free nuclear stage and not to the cellularized stage (Fig. 3*F*). In the ovules of a *fis1* homozygous mutant plant (Fig. 3 *G* and *H*), giant multicellular structures often were seen at the micropylar end (Fig. 3*G*), and the endosperm development was either free nuclear (Fig. 3*H*) or cellularized (Fig. 3*G*).

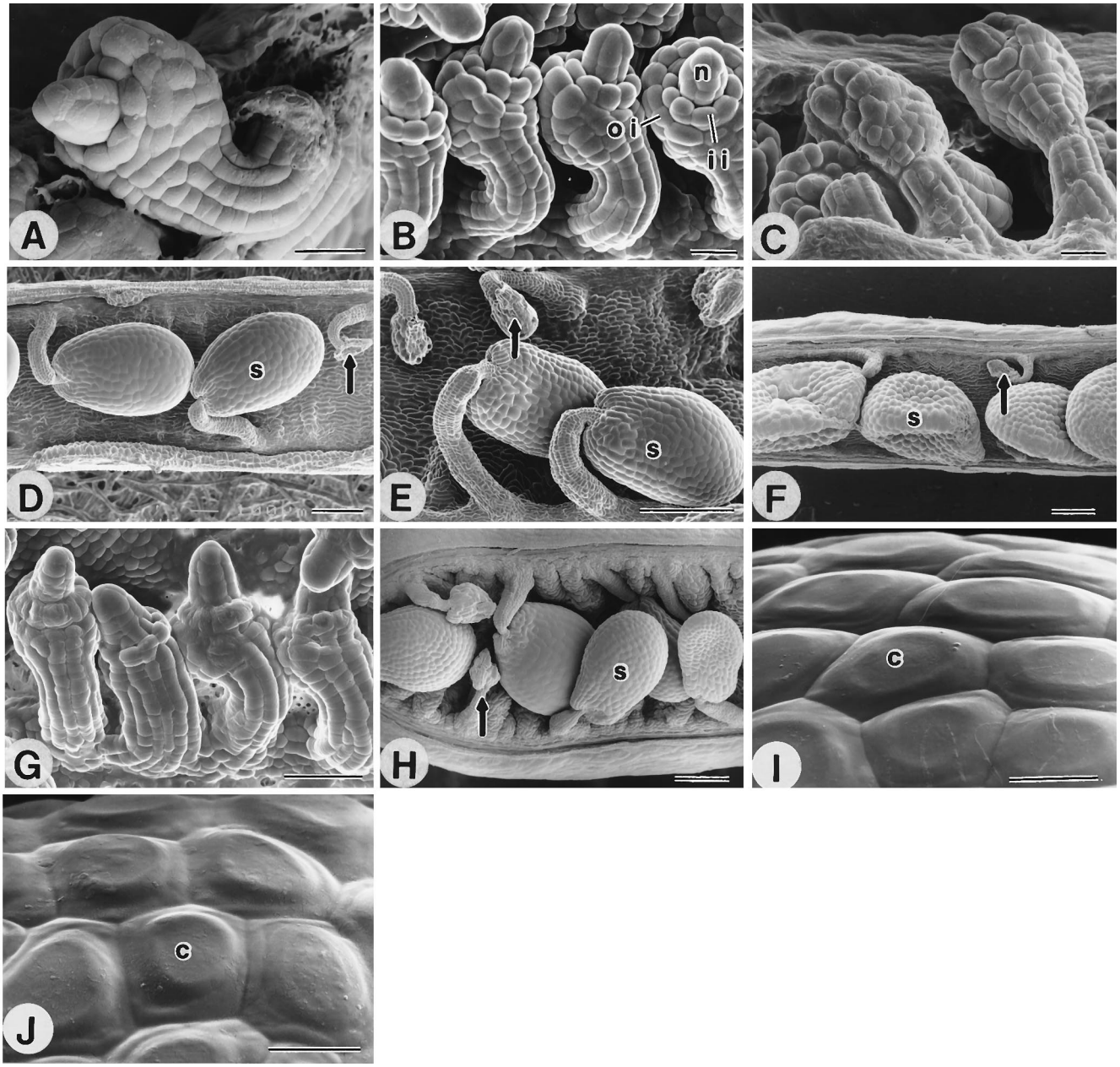

FIG. 2. Cryo-scanning electron microscopy micrographs of ovules and seeds of *fis* mutants and fertilized wild-type plants. Developing ovules [nucellar column (n) protruding from the inner integument (ii) and the outer integument (oi) as shown in *B*] of (*A*) wild-type, (*B*) *fis1/fis1* homozygote, (*C*) *fis2/fis2* homozygote, and (*G*) *FIS3/fis3* heterozygote. (*D*) Sexually fertilized seeds(s) of *pi/pi FIS/FIS* plants 7 days after fertilization. Unfertilized ovules shrivel (arrow). Seeds developing without fertilization(s) of (*E*) *fis1/fis1* homozygote, (*F*) *fis2/fis2* homozygote, and (*H*) *FIS3/fis3* heterozygote. Collumella (c) on the surface of (*I*) sexually fertilized seed of wild type and (*J*) autonomously developing *fis2* homozygous seeds. (Bar = 20 μm for *A–C*, G, I, and J, 100 μm for *D–F*, and 200 μm for *H*.)

Pollination Induces Changes in the Ovules of the *fis* Mutants. When the *fis1/fis1* or the *fis2/fis2* homozygous mutant plants were pollinated with pollen from a *FIS/FIS* plant, embryos developed further than they did in the unpollinated *fis1/fis1* and *fis2/fis2* plants (Figs. 3*I* and 4). Heterozygous *FIS3/fis3* plants, pollinated with pollen from a *FIS/FIS* plant, produced normal seed and torpedo-stage, embryo-arrested seed in a ratio of 1:1.

Homozygous *fis1* and *fis2* plants, as well as heterozygous *fis3* plants, were pollinated with pollen from a *FIS/FIS* plant, homozygous for a ^{35}S-*Gus* reporter gene. The resulting torpedo-stage embryos were stained to detect the product of the *Gus* gene. As shown in Fig. 4, all of the embryos resulting from self-pollination of the *FIS/FIS* ^{35}S-*Gus*/^{35}S-*Gus* plant stained blue, as did the embryos resulting from a pollination of a *pi/pi FIS/FIS* plant with pollen from a ^{35}S-*Gus*/^{35}S-*Gus* plant. In contrast, when ^{35}S-*Gus* pollen was used to pollinate *fis1/fis1* (Fig. 4), *fis2/fis2* homozygotes, or *FIS3/fis3* heterozygotes (data not shown), the resulting torpedo stage embryos were either *Gus*-positive or *Gus*-negative, suggesting that both zygotic and maternal embryos were present. The presence of *Gus* sequences in the blue embryos and their absence in the white embryos has been confirmed by PCR using primers from the *Gus* genes.

Wild-Type and *fis* Mutant Seeds Are Similar in Seed Coat Development. After fertilization, the outer integuments of the *Arabidopsis* wild-type ovule develop polygonal structures with a central elevation called the columella (13). These structures were not seen in unfertilized ovules that did not develop any mature seed characters before they atrophied (Fig. 2*D*). Al-

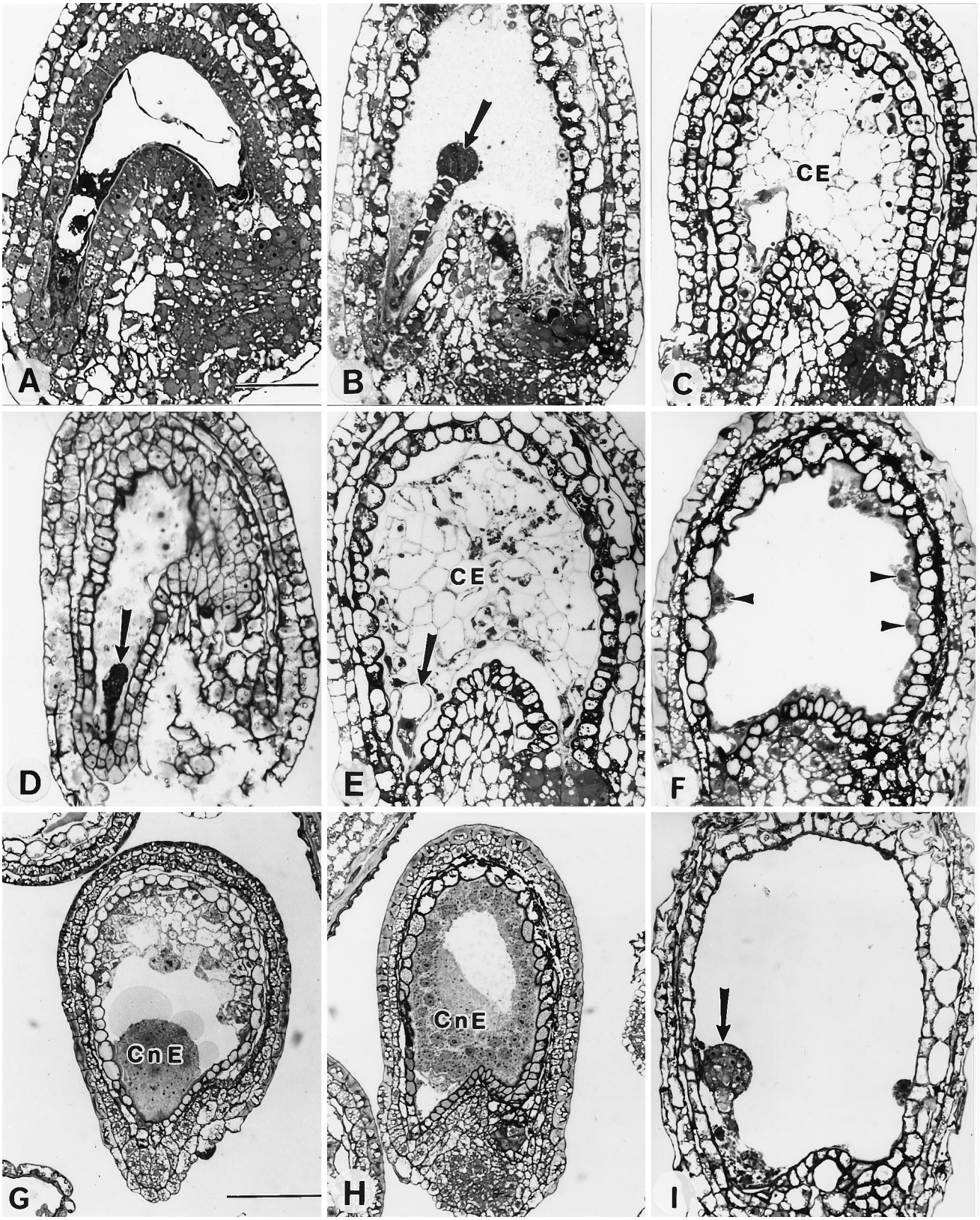

FIG. 3. Endosperm and embryo development of wild-type and fis seeds. (*A*) Longitudinal section through an ovule of an unfertilized *pistillata* (*pi*/*pi*) mutant. No endosperm or embryo development is visible. (*B*) Longitudinal section through an ovule of a 0.6-mm fruit from a *pi*/*pi* homozygote 3 days after fertilization with *FIS*/*FIS* pollen showing embryo (arrow). (*C* and *D*) Sections through an autonomously developing *fis1* seed from a *FIS1*/*fis1* heterozygous plant (from a 0.8-mm fruit). Notice cellularized endosperm (CE) and the embryo-like structure (*D*, arrow). (*E*) Section through an autonomously developing *fis2* seed from a *FIS2*/*fis2* heterozygote showing cellularized endosperm (CE) and a zygote-like structure (arrow) (from a 0.8-mm fruit). (*F*) Section through an autonomously developing *fis3* seed from a 0.9-mm fruit from a *FIS3*/*fis3* heterozygote showing free nuclear endosperm (arrowheads). (*G* and *H*) Autonomous endosperm development in a *fis1*/*fis1* homozygote showing free nuclear coenocytic endosperm (CnE) (from a 1-mm fruit). (*I*) Embryo arrested at the globular stage 5 days after pollination of a *fis1*/*fis1* homozygous plant with *FIS*/*FIS* pollen (arrow) (from a 1.2-mm fruit). (Bar = 50 μm for *A*–*F* and *I* and 100 μm for *G* and *H*.)

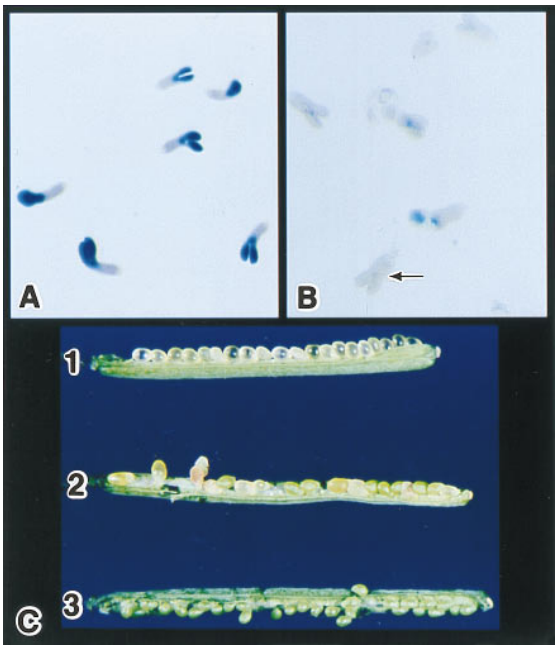

FIG. 4. Pollination-induced silique and embryo development in a *FIS1/fis1* heterozygote and a *fis1/fis1* homozygote. (*A*) Torpedo stage *Gus*-positive (blue) embryos resulting from the pollination of a *pi/pi FIS/FIS* homozygous plant with a plant homozygous for a ^{35}S-*Gus* construct. (*B*) Torpedo stage *Gus*-positive (blue) and *Gus*-negative (white, arrow) embryos from the pollination of a *fis1/fis1* homozygote with pollen from plants carrying a ^{35}S-*Gus* construct. (*C*) Silique development after self-pollination of (1) *fis1/fis1*, (2) *FIS1/fis1*, and (3) *FIS1/FIS1* plants.

though the *fis* seeds were not fertilized, they did form the columella in the outer integument cells (Fig. 2*J*), and they were indistinguishable from normal zygotic seeds before they shrivelled.

***fis* Endosperm Cells Are Diploid.** The ploidy of the endosperm cells from *fis1* and *fis2* mutants was determined by measuring the fluorescence intensity of nuclei in 4′, 6-diamidino-2-phenylindole-stained sections. The average brightness of endosperm nuclei was 92.9 ± 20.8 (n = 57) for *fis1* and was 135.0 ± 27.2 (n = 51) for the pollinated L*er* control. The background values were 31.5 ± 6.9 (n = 50) and 34.3 ± 6.5 (n = 50), respectively. In a separate experiment, the brightness of autonomous *fis2* endosperm nuclei was found to be 79.4 ± 14.4 (n = 40), and that of wild-type control nuclei was 108 ± 23.1 (n = 42). The background values were 37.3 ± 7.8 and 35.5 ± 6.2, respectively. In both cases, the results were consistent with the autonomous endosperm being diploid in contrast to the triploid condition of the sexual endosperm nuclei.

DISCUSSION

Relationship of the *FIS* Alleles to the Recently Described *fie* Mutant. The *fis3* mutant described here and earlier by us (5) resembles *fie*, recently described by Ohad *et al.* (6). The phenotype of *fis3*, i.e., endosperm development to the free nuclear stage, lack of an embryo, and absence of female transmission, parallels that of *fie*. Like *fis3*, *fie* maps in chromosome 3 between *hy2* and *gl1*, which suggests that *fis3* and *fie* might be allelic. The absence of egg transmission and the gametophytic expression of the mutations preclude direct complementation testing between *fis3* and *fie*, and it remains possible that they are different loci.

In contrast, *fis1* and *fis2* map to chromosome locations different to either *fis3* or *fie*. Unlike *fis3* and *fie*, *fis1* and *fis2* have a low level transmission via the female gamete; this property enabled us to construct *fis1/fis1* and *fis2/fis2* homozygous plants. It was not possible to construct a *fis3/fis3* homozygote; the *fie/fie* homozygote has not been described. Unlike *fis3* and *fie*, the autonomous endosperm development in *fis1* and *fis2* goes beyond the free nuclear stage to the cellularized stage. *fis1* and *fis2* show a low frequency development of embryo-like structures that progress to the globular stage. These data indicate that the *fis1* and *fis2* mutations induce processes that are not under the control of *fis3* or *fie*. These processes include the transition of the endosperm from the free nuclear to the cellularized stage and autonomous formation of embryos. We conclude that *Arabidopsis* has at least three genes, *FIS1, FIS2*, and *FIS3(FIE)*, that prevent seed development without fertilization. We have identified seven other independent *fis* mutants, so it is possible that there are more than three loci.

In the *fis* Mutants, Partial Development of Seed Can Occur Without Pollination. In the *fis* mutants, a number of steps in seed development occur without pollination. These events include the autonomous development of diploid endosperm, a low frequency development of globular, embryo-like structures, and the partial development of ovules into seeds, indistinguishable from developing sexual seeds in size and external morphology. Most *fis* seeds do not develop beyond the endosperm cellularization stage before atrophying. These results indicate that a substantial program of activation of genes involved in seed development is induced in plants carrying the mutagen-generated alleles at the *FIS* loci.

Seed Development in the *fis* Mutants After Pollination. When homozygous *fis1/fis1* and *fis2/fis2* plants or heterozygous *FIS3/fis3* plants were pollinated, each of the *fis* seeds had an embryo arrested at the torpedo stage. When these plants were pollinated with pollen from a *FIS/FIS* plant homozygous for the *Gus* reporter gene driven by the ^{35}S promoter, the resulting torpedo stages were a mixture of *Gus*-positive (zygotic) and *Gus*-negative (maternal) embryos. In the *fie* mutant, all postpollination embryos were zygotic (6). If *fie* and *fis3* are allelic, this difference might indicate that *fis3* is a stronger allele than *fie*.

The postpollination arrest of embryos in the *fis* mutants indicates that factor(s) required for further embryo development are lacking. One explanation might be the ploidy of the endosperm; the autonomous diploid endosperm of the mutant might not nourish the embryo adequately so that neither the zygotic nor the apparently maternal embryos develop.

A Model of Action of the *FIS* Genes. Although a detailed model of the action of the *FIS* gene products must await further characterization of the mutants and their interactions, the action of the *FIS* genes can be considered in a formal model (Fig. 5). In our model, we have grouped stages of seed development (14) into four phases. In phase 1, at 0 h after fertilization (HAF), the ovule contains an 8-celled embryo sac with an egg, two synergids, a diploid central cell, and three antipodal cells; the silique is short. We propose that *FIS1*, *FIS2*, and *FIS3* specify polypeptides that act as negative regulatory elements repressing the conversion of a phase 1 ovule into a phase 2 developing seed (36 HAF). The *FIS1–FIS2* complex blocks both embryo and endosperm development. However, its effect on embryo development is only partial; other genes (X) also must block embryo development. The *FIS1–FIS2–FIS3* complex blocks endosperm development. We assume that seed development is initiated in wild type by a pollination-induced inactivation of the *FIS1–FIS2–FIS3* complex. The phase 2 partially developed seed normally proceeds through phase 3 (72 HAF) when the embryo is at the heart/torpedo stage and the endosperm is cellularized to the mature phase 4 seed (120 HAF, upturned U stage embryo). Our data suggest that there must be other control genes (Y) that are inactivated by pollination to facilitate this transition. For the sake of

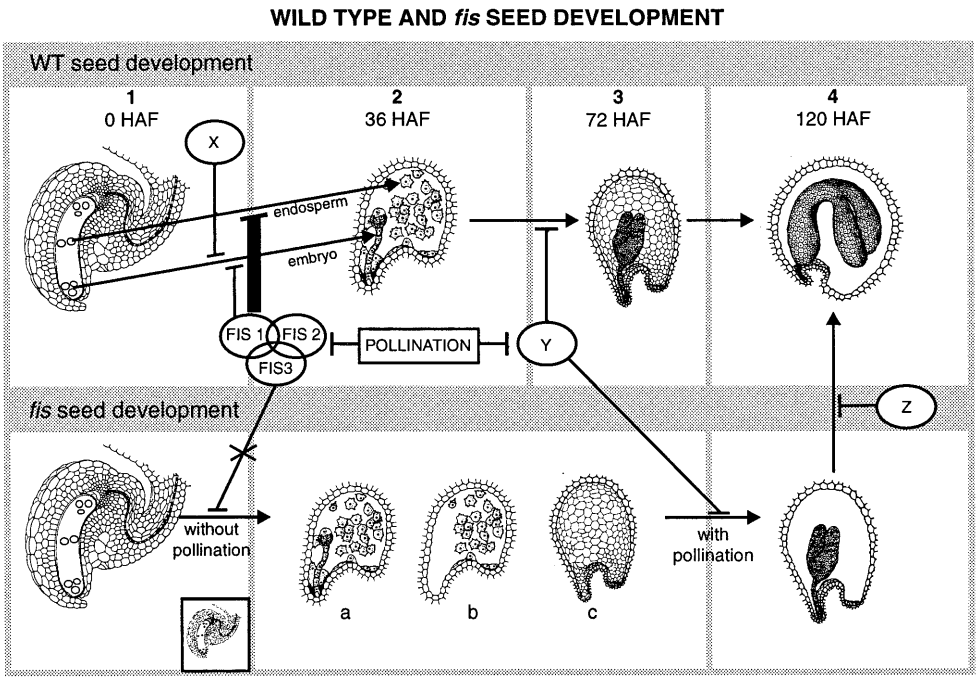

FIG. 5. Wild-type and *fis* seed development. Seed development of wild-type *Arabidopsis* and *fis* mutants are compared at developmental phases (14). Phase 1 shows ovules connected to the ovary wall by the funiculus; in the subsequent phases, only the developing seed is shown. The relative size of the ovule compared with the developing seed is shown by the *Inset*. The lengths of siliques at the different phases are: phase 1: 0.29 ± 0.04 mm (0 HAF); phase 2: 0.60 ± 0.08 mm (36 HAF); phase 3: 1.00 ± 0.07 mm (72 HAF); and phase 4 1.26 ± 0.07 mm (120 HAF). *a*, *b*, and *c* represent different developmental types seen in the *fis* mutants. *X*, *Y*, and *Z* represent postulated genes other than *FIS1*, *FIS2*, and *FIS3*. A detailed description of the model is given in *Discussion*.

simplicity, we are proposing a negative regulatory control; but these genes also could act as positive regulators.

In the *fis1*, *fis2*, and *fis3* mutants, the phase 1 ovule proceeds without pollination to phases analogous to phases 2 and 3 of wild type. The seed-like structures have free nuclear endosperm and an embryo (Fig. 5*a*, *fis1* and *fis2*), free nuclear endosperm without an embryo (Fig. 5*b*, *fis1*, *fis2*, and *fis3*), or seed-like structures with cellularized endosperm (Fig. 5*c*, *fis1* and *fis2*). Even with pollination, the *fis* seed develops only to an embryo-arrested heart/torpedo stage and does not become a mature seed with a U-shaped embryo. The conversion of the embryo-arrested seed to mature seed may be blocked by a gene *Z* that is not inactivated by pollination. Mutations in the hypothetical *Y* gene would convert the *fis* seeds into embryo-arrested seeds without pollination, and additional mutation of the *Z* gene would give rise to full normal seeds.

The *FIS* Genes Might Be Components of Apomictic Seed Development Seen in Many Angiosperms. The gametophytic nature of the *fis* mutations indicates that their time of action is downstream from the point at which apomictic processes operate in plants that have either aposporic or diplosporic seed development (3).

The *FIS* genes are not likely to be the initial controlling genes for apomictic seed development but are likely to be inactivated so that full development of apomictic seeds can proceed. The gene(s) that regulate the initiation of apomixis might therefore be positive regulators of a number of *FIS*-like genes. In this model, an apomictic mutant would be one that is simultaneously impaired in a number of *FIS* functions. We predict that naturally occurring apomictic plants would have their endogenous *FIS* functions either mutated or down-regulated. Cloning of the *FIS* genes, currently underway in our laboratory, followed by the characterization of the *FIS* genes and their products in natural apomicts related to *Arabidopsis*, such as *Arabis holboellii*, will enable us to determine whether this is the case.

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