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REVIEW ARTICLE

Genetic Analysis of Female Gametophyte Development and Function

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INTRODUCTION

The plant life cycle alternates between a multicellular haploid generation, called the gametophyte, and a multicellular diploid generation, called the sporophyte. In plants, meiosis gives rise to haploid spores, which undergo cell proliferation and differentiation to develop into gametophytes. A major function of the gametophyte generation is to produce haploid gametes. Fusion of the egg cell with the sperm cell gives rise to the sporophyte, thereby completing the life cycle (Raven et al., 1992).

In many lower plants, gametophytes are the dominant and free-living generation. Angiosperms, by contrast, have dramatically reduced gametophytes, which are comprised of very few cells and are embedded within the sexual organs of the flower (Raven et al., 1992). The angiosperm male gametophyte (i.e., the pollen grain or microgametophyte), which develops within the stamen's anther, is a three-celled structure comprised of two sperm cells encased within a vegetative cell (McCormick, 1993). The female gametophyte is also referred to as the embryo sac or megagametophyte. It develops in the ovule, which is found within the carpel's ovary. The most common female gametophyte form, which is depicted in Figure 1, consists of seven cells and four different cell types: three antipodal cells, two synergid cells, one egg cell, and one central cell (Maheshwari, 1950; Willemse and Van Went, 1984; Haig, 1990; Huang and Russell, 1992a; Reiser and Fischer, 1993).

Sexual reproduction in angiosperms is initiated when pollen is transferred from anther to stigma. Shortly thereafter, the male gametophyte germinates a pollen tube and delivers its two sperm cells to the female gametophyte to effect "double" fertilization of the egg cell and the two polar nuclei of the central cell (Maheshwari, 1950; Russell, 1992). The female gametophyte plays a critical role at many steps of the reproductive process. For example, as the pollen tube grows, the female gametophyte participates in directing the pollen tube to the ovule (Hülskamp et al., 1995; Ray et al., 1997), and during fertilization, cytoskeletal components within the female gametophyte direct one sperm cell to the egg cell and the other to the central cell (Russell, 1992, 1993; Huang and Russell, 1994). Upon fertilization, genes expressed in the female gametophyte participate in inducing seed development (Ohad et al., 1996; Chaudhury et al., 1997) and may continue to play a role in the development of the embryo and the endosperm, which are derived from the egg cell and the central cell, respectively (Ray, 1997).

Gene products required for female gametophyte development and function could be encoded by genes expressed either within the female gametophyte or in the surrounding sporophytic cells of the ovule. Several sporophytic mutations disrupting ovule development and function have been isolated (Robinson-Beers et al., 1992; Lang et al., 1994; Léon-Kloosterziel et al., 1994; Modrusan et al., 1994; Gaiser et al., 1995; Elliot et al., 1996; Klucher et al., 1996; Baker et al., 1997; Schneitz et al., 1997). In some of these mutants, female gametophyte development is altered (Robinson-Beers et al., 1992; Modrusan et al., 1994; Elliot et al., 1996; Klucher et al., 1996; Schneitz et al., 1997). However, it is not clear whether these mutations affect female gametophyte development directly or as a secondary consequence of an effect on the ovule's surrounding sporophytic tissue.

Genetic studies also suggest that female gametophyte development depends on the activities of many female gametophyte–expressed genes. For example, studies in maize have shown that most chromosomal deletions are deleterious to the female gametophyte, suggesting that genes essential for embryo sac viability are present throughout the maize genome (Patterson, 1978; Coe et al., 1988; Buckner and Reeves, 1994; Patterson, 1994; Vizir et al., 1994; Vollbrecht and Hake, 1995). Moreover, morphological analysis of a battery of embryo sacs harboring a variety of small chromosomal deletions has demonstrated that female gametophyte–expressed genes control fundamental aspects of female gametophyte development, such as nuclear migration, the coordination of developmental events, and the establishment of polarity (Vollbrecht and Hake, 1995); many of these genes may perform regulatory functions.

The identities and specific functions of the haploid-expressed genes required by the female gametophyte are

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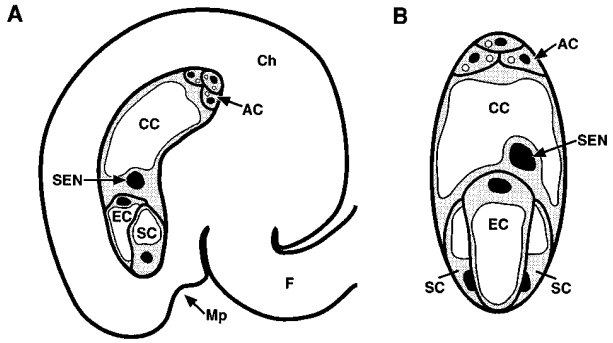

Figure 1. The Polygonum-Type Female Gametophyte of Arabidopsis.

(A) View in longitudinal section in the context of the ovule.
(B) View perpendicular to that in **(A)**.
The gray areas represent cytoplasm, the white areas represent vacuoles, and the black areas represent nuclei. AC, antipodal cells; CC, central cell; Ch, chalazal region of the ovule; EC, egg cell; F, funiculus; Mp, micropyle; SC, synergid cell; SEN, secondary endosperm nucleus.

almost completely unknown. Recently, however, screens for female gametophyte mutants have been initiated in several laboratories, and mutations affecting specific steps of female gametophyte development and function have been identified. In this review, we discuss the approaches that have been used to identify female gametophyte mutants, summarize the cellular processes that are affected in these mutants, and discuss other genetic and molecular approaches that are being used to identify genes required by the female gametophyte. Comprehensive reviews of female gametophyte structure and development have been published previously (Willemse and Van Went, 1984; Haig, 1990; Huang and Russell, 1992a; Reiser and Fischer, 1993); we emphasize recent progress made in Arabidopsis and maize in which genetic analyses of female gametophyte development and function are in progress.

FEMALE GAMETOPHYTE MUTATIONS

Gametophytic Mutations

Not long after the rediscovery of Mendel's laws of heredity in 1900, it was found that some mutations exhibit aberrant segregation patterns (e.g., Correns, 1902). It was later determined that these mutations affect the gametophytic phase of the plant life cycle (Brink and MacGillivray, 1924; Jones, 1924). Over the intervening years, it has become clear that plants contain two broad classes of mutations that exhibit fundamentally different segregation patterns. Sporophytic mutations affect sporophytically expressed genes and generally exhibit Mendelian 3:1 segregation patterns. Gametophytic mutations, by contrast, affect gametophytically expressed genes and are not transmitted through the egg and/or sperm. As a consequence, gametophytic mutations exhibit apparent non-Mendelian segregation patterns and can only be passed from generation to generation as heterozygotes.

Because angiosperms have two gametophytes, three classes of gametophytic mutations exist. The first class affects the female gametophyte but not the male gametophyte, the second class affects the male gametophyte but not the female gametophyte, and the third class affects both gametophytes. We refer to these classes as female gametophyte specific, male gametophyte specific, and general gametophytic, respectively.

The segregation patterns of these mutation classes are summarized in Table 1, which shows that in a self-cross of a heterozygous individual (e.g., genotype *A*/*a*), female gametophyte–specific mutations segregate 1:1 for *A*/*A*:*A*/*a* progeny (Table 1, second row). If a mutation affects both

Table 1. Segregation of Sporophytic and Gametophytic Mutations in a Cross of Two Heterozygous Individuals (*A*/*a* × *A*/*a*)

	Functional Gametes			
Mutation Class	MG[a]	FG[b]	Progeny Genotypes	KanR:KanS[c]
Sporophytic	*A* and *a* (1:1)	*A* and *a* (1:1)	*A/A A/a a/a* (1:2:1)	3:1
Gametophytic				
FG specific	*A* and *a* (1:1)	*A*	*A/A A/a* (1:1)	1:1
MG specific	*A*	*A* and *a* (1:1)	*A/A A/a* (1:1)	1:1
General gametophytic	*A*	*A*	*A/A*	0

[a] MG, male gametophyte.
[b] FG, female gametophyte.
[c] Kanamycin (Kan) resistance ratios if the mutation is caused by an insert (T-DNA or transposon) that carries a gene conferring Kan resistance.

gametophytes and is fully penetrant, it cannot be transmitted to subsequent generations (Table 1, bottom row). However, many gametophytic mutations are partially penetrant (Singleton and Mangelsdorf, 1940; Nelson and Clary, 1952; Kermicle, 1971; Niyogi et al., 1993; Kieber and Ecker, 1994; Springer et al., 1995; Chaudhury et al., 1997; Feldmann et al., 1997) and therefore can be transmitted to subsequent generations.

Identification of Female Gametophyte Mutations

Mutations affecting the female gametophyte can be identified as lethals in which female gametophytes harboring the mutation either abort development or are nonfunctional. Two basic types of screens, segregation distortion and seed-set screens, have been used to identify female gametophyte mutants.

In segregation distortion screens, the altered segregation of female gametophyte mutants is exploited to identify this mutant class. These screens are facilitated by following the segregation of either linked visible markers (Singleton, 1932; Rhoades and Rhoades, 1939; Singleton and Mangelsdorf, 1940; Redei, 1965) or linked T-DNAs/transposons (Feldmann et al., 1997; Moore et al., 1998). For example, in T-DNA–mutagenized lines in which the T-DNA carries a gene conferring Kan resistance, the ratio of Kan-resistant to Kan-sensitive seedlings (KanR:KanS) can be used to identify lines that have a T-DNA insert disrupting a gene required for gametophyte development (Feldmann et al., 1997); in the progeny of a plant heterozygous for a female gametophyte–specific mutation, KanR:KanS is 1:1 compared with 3:1 for a sporophytic mutation (Table 1). However, male gametophyte mutations also exhibit segregation distortion (Table 1); thus, additional criteria (e.g., a seed-set screen or reciprocal crosses) are necessary to determine whether a given mutation affects the female or male gametophyte.

Another criterion by which to identify female gametophyte mutants is reduced seed set. This phenotype arises because, on a plant heterozygous for a female gametophyte mutation (e.g., genotype *A*/*a*), approximately half of the female gametophytes are mutant and nonfunctional (genotype *a*) and because an ovule harboring a defective female gametophyte fails to undergo seed development. An ovule that fails to develop into a seed desiccates and forms a small white mass (Meinke and Sussex, 1979). Thus, one way to identify female gametophyte mutants is to screen for lines with siliques/ears containing 50% normal seeds and 50% desiccated ovules.

In theory, reduced seed set is the only criterion required to identify female gametophyte mutants. However, reduced seed set also can be caused by a variety of other factors, including adverse environmental conditions (e.g., high growth temperature or water stress), chromosomal rearrangements (e.g., reciprocal translocations or large inversions), and sporophytic mutations (e.g., female-sterile mutations with 50% penetrance). Thus, additional experiments (e.g., a segregation distortion screen) are required for the definitive identification of female gametophyte mutants.

The frequency of female gametophyte mutants among mutagenized lines can be estimated from preliminary screening data. In screens currently in progress, ~1% of T-DNA–mutagenized (C.A. Christensen, D. Lee, J. Gold, R. Brown, and G.N. Drews, unpublished data) and transposon-mutagenized (Moore et al., 1998) lines exhibit both segregation distortion and reduced seed set. This suggests that female gametophyte mutants occur at high frequency and that in a saturation screen (Waddington, 1940; Jürgens et al., 1991), several hundred embryo sac mutants would be identified.

Female Gametophyte Mutations Fall into Two Classes

The known female gametophyte mutations are listed in Table 2. Many (11/25) of these mutations do not affect the male gametophyte and thus fall into the female gametophyte–specific class (Table 2). Female gametophyte–specific mutations are potentially important for two reasons. First, they are less likely to occur in "housekeeping" genes, because such mutations would be expected to affect the male gametophyte as well as the sporophyte. Second, they may affect female gametophyte–specific processes, such as the establishment of female gametophyte polarity, specification and differentiation of the female gametophyte cells, polar nuclei migration and fusion, antipodal cell death, pollen tube guidance, fertilization, and the induction of seed development (discussed below).

Most (14/25) of the known female gametophyte mutants also affect the male gametophyte and thus fall into the general gametophytic class (Table 2). This class of mutant is also important because many fundamental cellular processes take place during the development of both male and female gametophytes. These include mitosis, vacuole formation, cell expansion, subcellular migration, cellularization, and cell wall formation (discussed below). Furthermore, mutations that affect these critical processes in the gametophyte are unlikely to become homozygous in the sporophyte (Table 1) and can therefore be isolated only in screens for gametophytic mutants.

Phenotypic Analysis of Female Gametophyte Mutants

Phenotypic analysis of female gametophyte mutants in Arabidopsis and maize is facilitated by several factors. First, the Arabidopsis pistil contains 50 to 60 ovules (Christensen et al., 1997) and the maize ear contains 400 to 500 ovules (Dumas and Mogensen, 1993). Second, within an Arabidopsis pistil, female gametophyte development is synchronous (Christensen et al., 1997). In maize, each ear contains a gradient of embryo sacs in a developmental series, with adjacent

Table 2. The Known Female Gametophyte Mutants

Mutant	Species	Class	Defect	Reference
constitutive triple response1 (ctr1)	Arabidopsis	FGS[a]	Megagametogenesis not affected	Kieber and Ecker (1994); C.A. Christensen and G.N. Drews, unpublished data
emb173	Arabidopsis	ND[b]	Megagametogenesis not affected	Castle et al. (1993); C.A. Christensen and G.N. Drews, unpublished data
female gametophyte1 (fem1)	Arabidopsis	FGS	Female gametophyte disintegrates soon after cellularization	S. Subramanian and G.N. Drews, unpublished data
female gametophyte2 (fem2)	Arabidopsis	FGS	Developmental arrest at the one-nucleate stage	C.A. Christensen and G.N. Drews, unpublished data
female gametophyte3 (fem3)	Arabidopsis	GG[c]	Developmental arrest at the one-nucleate stage	C.A. Christensen and G.N. Drews, unpublished data
female gametophyte4 (fem4)	Arabidopsis	GG	Cellular morphology is abnormal	C.A. Christensen and G.N. Drews, unpublished data
fertilization-independent endosperm (fie)	Arabidopsis	FGS	Megagametogenesis not affected; endosperm development occurs in the absence of fertilization	Ohad et al. (1996)
fertilization-independent seed1 (fis1)	Arabidopsis	FGS	Megagametogenesis not affected; endosperm development occurs in the absence of fertilization	Chaudhury et al. (1997)
fertilization-independent seed2 (fis2)	Arabidopsis	FGS	Megagametogenesis not affected; endosperm development occurs in the absence of fertilization	Chaudhury et al. (1997)
fertilization-independent seed3 (fis3)	Arabidopsis	FGS	Megagametogenesis not affected; endosperm development occurs in the absence of fertilization	Chaudhury et al. (1997)
Gametophytic factor (*Gf*)	Arabidopsis	GG	Developmental arrest at the one-nucleate stage	Redei (1965); Christensen et al. (1997)
gametophytic factor1 (*gfa1*)	Arabidopsis	GG	ND	Feldmann et al. (1997)
gametophytic factor2 (*gfa2*)	Arabidopsis	GG	Polar nuclei fail to fuse	Feldmann et al. (1997); C.A. Christensen and G.N. Drews, unpublished data
gametophytic factor3 (*gfa3*)	Arabidopsis	GG	Polar nuclei fail to fuse	Feldmann et al. (1997); C.A. Christensen and G.N. Drews, unpublished data
gametophytic factor4 (*gfa4*)	Arabidopsis	GG	Developmental arrest at the one-nucleate stage	Feldmann et al. (1997); C.A. Christensen and G.N. Drews, unpublished data
gametophytic factor5 (*gfa5*)	Arabidopsis	GG	Developmental arrest at the one-nucleate stage	Feldmann et al. (1997); C.A. Christensen and G.N. Drews, unpublished data
gametophytic factor6 (*gfa6*)	Arabidopsis	GG	ND	Feldmann et al. (1997)
gametophytic factor7 (*gfa7*)	Arabidopsis	GG	Polar nuclei fail to fuse	Feldmann et al. (1997); C.A. Christensen and G.N. Drews, unpublished data
hadad (*hdd*)	Arabidopsis	GG	Developmental arrest at the two- to eight-nucleate stage	Moore et al. (1998)

(continued)

Table 2. (continued)

Mutant	Species	Class	Defect	Reference
indeterminate gametophyte1 (*ig*)	Maize	FGS	Pleiotropic; primary defect thought to be in nuclear division	Kermicle (1971); Huang and Sheridan (1996)
lethal ovule1 (*lo1*)	Maize	FGS	ND	Singleton and Mangelsdorf (1940)
lethal ovule2 (*lo2*)	Maize	GG	Pleiotropic; primary defect thought to be in nuclear division	Nelson and Clary (1952); Sheridan and Huang (1997)
prolifera (*prl*)	Arabidopsis	FGS	Developmental arrest at the four-nucleate stage	Springer et al. (1995)
small pollen1 (*sp1*)	Maize	GG	ND	Singleton and Mangelsdorf (1940)
small pollen2 (*sp2*)	Maize	GG	ND	Rhoades and Rhoades (1939)
trp1; *trp4*	Arabidopsis	FGS	ND	Niyogi et al. (1993)

[a] FGS, female gametophyte specific.
[b] ND, not done.
[c] GG, general gametophytic.

ovules at similar developmental stages (Vollbrecht and Hake, 1995). Third, within a pistil/ear of a heterozygous female gametophyte mutant, half of the female gametophytes are genotypically wild type and half are genotypically mutant (Table 1); thus, the developmental stage of wild-type female gametophytes can be used to determine the developmental stage of mutant female gametophytes (Christensen et al., 1997). Fourth, extensive descriptive studies of female gametophyte structure and development have been conducted in Arabidopsis (Misra, 1962; Poliakova, 1964; Webb and Gunning, 1990, 1994; Mansfield et al., 1991; Murgia et al., 1993; Schneitz et al., 1995; Christensen et al., 1997) and maize (Weatherwax, 1919; Cooper, 1937; Diboll and Larson, 1966; Diboll, 1968; Russell, 1979; Huang and Sheridan, 1994; Vollbrecht and Hake, 1995). These studies, which are summarized in the following section, provide the essential framework upon which mutagenic approaches are based. Finally, confocal laser scanning microscopy (CLSM) procedures have been developed to analyze rapidly female gametophyte development in Arabidopsis (Christensen et al., 1997) and maize (Vollbrecht and Hake, 1995).

MEGAGAMETOGENESIS

Development of the Angiosperm Female Gametophyte

Before one can effectively evaluate the phenotype caused by a gametophyic mutation, it is important to understand how gametophyte development proceeds in the wild type. Over 15 different patterns of female gametophyte development have been described (Maheshwari, 1950; Willemse and Van Went, 1984; Haig, 1990). The developmental pattern exhibited by most species, including Arabidopsis (Misra, 1962) and maize (Weatherwax, 1919; Cooper, 1937), is the "normal type," which is usually referred to as the Polygonum type because it was first described in *Polygonum divaricatum* (Strasburger, 1879; Maheshwari, 1950). The Polygonum-type female gametophyte is found in ~70% of the species that have been examined (Maheshwari, 1950) and is thought to be the ancestral type (Huang and Russell, 1992a).

Polygonum-type female gametophyte development can be divided into two phases: megasporogenesis and megagametogenesis (Maheshwari, 1950; Willemse and Van Went, 1984; Haig, 1990; Huang and Russell, 1992a; Reiser and Fischer, 1993). During megasporogenesis, a diploid megaspore mother cell undergoes meiosis to produce four haploid megaspores. The chalazal-most megaspore survives, and the other three undergo cell death. During megagametogenesis, which is depicted in Figure 2, the functional megaspore (Figure 2B) undergoes three rounds of mitosis, producing an eight-nucleate cell (Figure 2C). Two nuclei (the polar nuclei), one from each pole, then migrate toward the center of the cell. In Arabidopsis and other species, the polar nuclei fuse, forming the secondary endosperm nucleus (Figures 2A, bottom, and 2D; Poliakova, 1964; Newcomb, 1973; Schulz and Jensen, 1973; Webb and Gunning, 1994; Schneitz et al., 1995; Christensen et al., 1997). By contrast, in maize and other species, the polar nuclei only partially fuse before fertilization (Figure 2A, top; Jensen, 1964; Diboll, 1968; Wilms, 1981; Bedinger and Russell, 1994; Vollbrecht and Hake, 1995).

During polar nuclei migration, the embryo sac cellularizes to form the seven-celled structure depicted in Figure 1. In some species, this is the final form (Maheshwari, 1950; Willemse and Van Went, 1984; Haig, 1990; Huang and Russell, 1992a). However, in both Arabidopsis and maize, the female gametophyte undergoes additional developmental steps

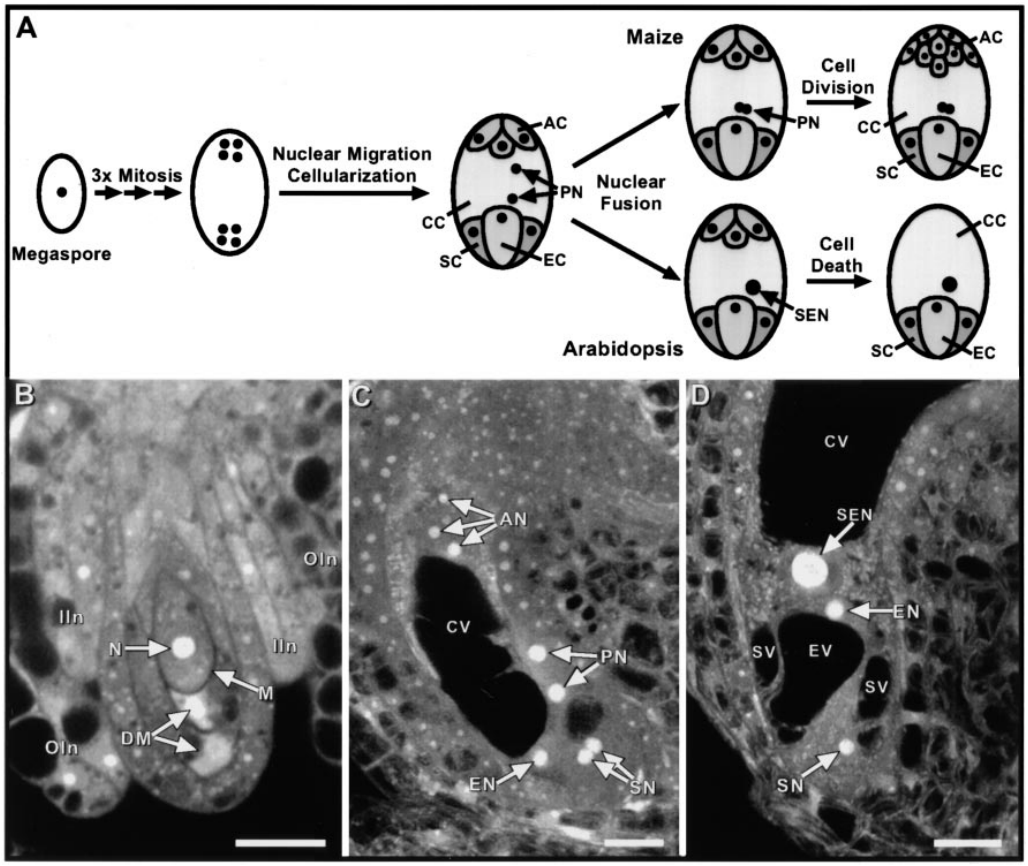

Figure 2. Megagametogenesis in Angiosperms.

(A) Depiction of megagametogenesis in maize (top) and Arabidopsis (bottom).
(B) to **(D)** CLSM images of specific stages in Arabidopsis. **(B)** One-nucleate stage. This image consists of one 1.5-μm optical section. **(C)** Mid–eight-nucleate stage. This image is a projection of four 1.5-μm optical sections. **(D)** Micropylar region of the mature female gametophyte. This image is a projection of four 1.5-μm optical sections.
In the CLSM images, the white spots are nuclei, the gray areas are cytoplasm, and the black areas are vacuoles. AC, antipodal cells; AN, antipodal cell nucleus; CC, central cell; CV, central cell vacuole; DM, degenerate megaspores; EC, egg cell; EN, egg nucleus; EV, egg vacuole; IIn, inner integument; M, megaspore; N, nucleus; OIn, outer integument; PN, polar nuclei; SC, synergid cell; SEN, secondary endosperm nucleus; SN, synergid nucleus; SV, synergid vacuole. Bars = 10 μm.

before fertilization. In Arabidopsis, the three antipodal cells degenerate (Figure 2A, bottom; Poliakova, 1964; Murgia et al., 1993; Schneitz et al., 1995; Christensen et al., 1997). By contrast, in maize, the antipodal cells proliferate into as many as 100 cells in the mature embryo sac (Figure 2A, top; Diboll and Larson, 1966; Diboll, 1968; Russell, 1979; Huang and Sheridan, 1994; Vollbrecht and Hake, 1995).

As shown in Figures 1A and 1B, polarity establishment is an important feature of female gametophyte development. The ovule has a distinct axis of polarity. The micropylar pole is the end at which the integuments form a pore, and the chalazal pole is the end that joins the funiculus. The female gametophyte also has a distinct polarity, with the egg and synergid cells at the micropylar pole and the antipodal cells at the chalazal pole.

Thus, to understand how female gametophyte development is regulated, many important questions must be addressed: How is female gametophyte polarity within the ovule established? How is the number of mitoses regulated? Which cytoplasmic elements mediate and control nuclear migration? How is cellularization initiated and controlled? What controls cell specification during megagametogenesis? What factors mediate fusion of the polar nuclei? How are antipodal cell death (in Arabidopsis) and proliferation (in maize) controlled? As discussed below, genetic approaches

have begun to identify some of the genes involved in these processes.

Mutations Affecting Specific Steps of Megagametogenesis

It is not known which developmental steps during megagametogenesis are controlled by haploid-expressed genes, nor is it known when these genes are required during megagametogenesis. However, a relatively large number of female gametophyte mutants with defects in megagametogenesis are now available to begin to address these questions. CLSM images and schematics of some of these mutants are shown in Figure 3.

Megagametogenesis is affected in 14 of the female gametophyte mutants listed in Table 2. Some of these mutants (*fem2*, *fem3*, *Gf*, *gfa4*, and *gfa5*) are affected at the earliest possible step, the one-nucleate stage (Table 2, and Figures

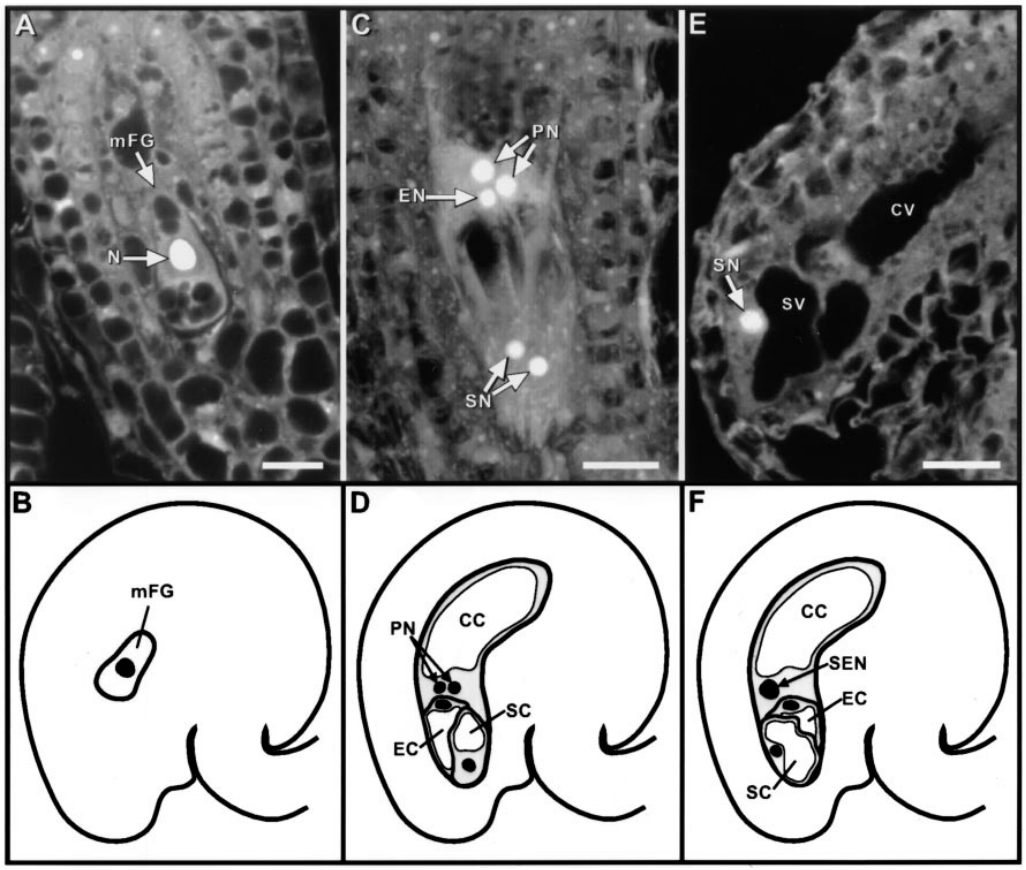

Figure 3. Images of Mutants with Defects in Megagametogenesis.

(A) and **(B)** *fem2* mutant phenotype at the terminal developmental stage (compare with Figure 2D). **(A)** CLSM image consisting of one 1.5-μm optical section. **(B)** Schematic representation. This mutant does not progress beyond the one-nucleate stage. The *fem3*, *Gf*, *gfa4*, and *gfa5* mutants also exhibit this phenotype (Table 2).

(C) and **(D)** *gfa2* mutant phenotype at the terminal developmental stage (compare with Figure 2D). **(C)** CLSM image consisting of a projection of five 1.5-μm optical sections. **(D)** Schematic representation. In this mutant, the polar nuclei fail to fuse. The *gfa3* and *gfa7* mutants also exhibit this phenotype (Table 2).

(E) and **(F)** *fem4* mutant phenotype at the terminal developmental stage (compare with Figure 2D). **(E)** CLSM image consisting of one 1.5-μm optical section. Only one synergid is shown in this image. **(F)** Schematic representation. In this mutant, the cell shape and the number, location, or shape of vacuoles are abnormal (Table 2).

In these CLSM images, the white spots are nuclei, the gray areas are cytoplasm, and the black areas are vacuoles. CC, central cell; CV, central cell vacuole; EC, egg cell; EN, egg nucleus; mFG, mutant female gametophyte; N, nucleus; PN, polar nucleus; SC, synergid cell; SEN, secondary endosperm nucleus; SN, synergid nucleus; SV, synergid vacuole. Bars = 10 μm.

3A and 3B). In these mutants, it is unclear which cellular processes are affected because developmental arrest at the one-nucleate stage can result from defects in a variety of processes, including mitosis, cell metabolism, and developmental control. Nevertheless, the existence of this mutant class clearly demonstrates that haploid-expressed genes are required very early in megagametogenesis.

Many of the megagametogenesis mutations listed in Table 2 affect steps that follow the one-nucleate stage. The main events that take place subsequent to the one-nucleate stage are nuclear division, polar nuclei migration, polar nuclei fusion, cellularization, and antipodal cell death or proliferation (Figure 2A). Nuclear division is affected in the *hdd*, *ig*, *lo2*, and *prl* mutants (Table 2). *hdd* mutant female gametophytes arrest at the two-, four-, or eight-nucleate stage. Some *hdd* mutant embryo sacs exhibit asynchrony of nuclear divisions at the two poles, suggesting that the *HDD* gene plays a role in coordinating nuclear division at the micropylar and chalazal poles (Moore et al., 1998). *ig* mutant female gametophytes undergo asynchronous and extra cycles of nuclear division, resulting in excessive numbers of nuclei (Kermicle, 1971; Lin, 1978, 1981; Huang and Sheridan, 1996). *lo2* mutant embryo sacs arrest at the one-, two-, or four-nucleate stage, and in some cases, the nuclei enlarge dramatically (Sheridan and Huang, 1997). The *hdd*, *ig*, and *lo2* mutations also cause secondary defects in nuclear migration, nuclear positioning, and/or microtubule organization, suggesting a relationship between nuclear behavior and cytoskeletal organization (Kermicle, 1971; Lin, 1978, 1981; Huang and Sheridan, 1996; Sheridan and Huang, 1997; Moore et al., 1998). The *prl* mutation causes developmental arrest, most often at the four-nucleate stage (Springer et al., 1995); the *PRL* gene is related to the yeast MCM2-3-5 genes that are required for initiation of DNA replication (Springer et al., 1995).

The remaining megagametogenesis mutations affect steps other than nuclear division. Polar nuclei fusion is affected in the *gfa2*, *gfa3*, and *gfa7* mutants (Table 2). In these mutants, the polar nuclei migrate appropriately and come to lie side by side but remain unfused (Figures 3C and 3D; C.A. Christensen and G.N. Drews, unpublished data). Cellularization appears to be affected in the *fem4* mutant (Table 2). In this mutant, the shapes of the egg cell and synergids are abnormal, and their vacuoles show alterations in number, size, and shape (Figures 3E and 3F; C.A. Christensen and G.N. Drews, unpublished data). Maintenance of the central vacuole may be affected in the *fem1* mutant (Table 2). *fem1* female gametophytes develop normally until the time of cellularization, upon which the embryo sac disintegrates. In some *fem1* mutant embryo sacs, most aspects of morphology are normal except that the central vacuole is not present (S. Subramanian and G.N. Drews, unpublished data).

Thus, mutations affecting specific steps of megagametogenesis have already been identified. With the complement of megagametogenesis mutants already available, molecules mediating some of these steps should soon be identified. For example, isolation of the *GFA2*, *GFA3*, and *GFA7* genes should identify molecules required for fusion of the polar nuclei.

POLLEN TUBE GUIDANCE AND FERTILIZATION

Fertilization in Angiosperms

Soon after pollination, the male gametophyte forms a pollen tube, which grows great distances through the sporophytic tissue of the carpel to reach its final destination, the female gametophyte (Heslop-Harrison, 1987; Bedinger et al., 1994; Pruitt and Hülskamp, 1994; Preuss, 1995; Cheung, 1996; Smyth, 1997). The pollen tube enters the female gametophyte by growing through the ovule's micropyle and pushing into one of the synergid cells. The receptive synergid cell undergoes cell death either before or upon pollen tube arrival. In several species, including Arabidopsis, it has been demonstrated that synergid cell death is triggered by pollination or the presence of pollen tubes within the female tissue (Jensen et al., 1983; Huang and Russell, 1992b; Christensen et al., 1997). During synergid cell death, the synergid cytoskeleton undergoes a reorganization, resulting in the formation of two bands of F-actin (Huang et al., 1993; Huang and Russell, 1994; Huang and Sheridan, 1994).

After the pollen tube arrives at the synegrid, the contents of the pollen tube, including the two sperm cells, are released into the degenerating synergid cytoplasm. Next, one sperm cell migrates to the egg cell and the other to the central cell, most likely along the F-actin bands (Huang et al., 1993; Huang and Russell, 1994; Huang and Sheridan, 1994). When the male gametes reach their destinations, their plasma membranes fuse with those of the egg cell and central cell, and the sperm nuclei are transmitted into these cells for karyogamy (Van Went and Willemse, 1984; Willemse and Van Went, 1984; Russell, 1992, 1996).

This description of the angiosperm fertilization process raises several important questions: How does the pollen tube find its way to the female gametophyte? How does the male gametophyte induce the receptive synergid cell to undergo cell death? What controls reorganization of the synergid cytoskeleton and formation of the F-actin bands? What triggers release of the pollen tube contents into the female gametophyte? How is sperm cell migration along the F-actin bands initiated and controlled? What mediates sperm–egg and sperm–central cell recognition and fusion?

Genetic Analysis of Pollen Tube Guidance

Female gametophyte mutations affecting pollen tube guidance and fertilization have yet to be identified; however, female gametophyte mutations affecting megagametogenesis have been used to establish that the embryo sac plays a role

in pollen tube guidance. Descriptive studies of pollen tube growth patterns in many species, including Arabidopsis and maize, suggest that a chemotropic guidance signal directs pollen tube growth to the ovule (Heslop-Harrison, 1987; Bedinger et al., 1994; Pruitt and Hülskamp, 1994; Preuss, 1995; Cheung, 1996; Smyth, 1997).

A likely source of a chemoattractant is the female gametophyte itself, because this is the pollen tube's ultimate target. Evidence supporting this hypothesis initially came from the analysis of pollen tube growth in Arabidopsis ovule mutants in which female gametophytes do not form. In such mutants, pollen tube growth on the placental surface was random and pollen tubes were rarely found on mutant ovules (Hülskamp et al., 1995). However, because the ovule mutants were sporophytic, it could not be ruled out that the loss of pollen tube guidance was due to a defect in the sporophytic tissue. More recently, it has been shown that ovules harboring defective female gametophytes arrested at the one-nucleate stage (because of chromosomal imbalance) fail to receive pollen tubes (Ray et al., 1997). Furthermore, we have shown that pollen tube guidance is lost in a battery of Arabidopsis female gametophyte mutants that show no defect in the ovule's sporophytic tissue and in which the embryo sac does not progress beyond the one-nucleate stage (C.A. Christensen and G.N. Drews, unpublished data). Although these data are consistent with the suggestion that the female gametophyte actively attracts pollen tubes, the nature of the proposed chemoattractant(s) and the identity of the female gametophyte cell(s) in which it may be produced remain unclear.

INDUCTION OF SEED DEVELOPMENT

Induction of Seed Development in Angiosperms

During seed development in angiosperms, the fertilized egg cell develops into the embryo, the fertilized central cell gives rise to the endosperm, and the ovule's integuments form the seed coat. During sexual reproduction, these three processes are dependent upon double fertilization of the egg cell and central cell. By contrast, in apomictic species, one or more of these developmental pathways are activated in the absence of fertilization. For example, during diplosporic gametophytic apomixis, an unreduced megaspore mother cell gives rise to a diploid female gametophyte, and embryo development (and sometimes endosperm development) is initiated in the absence of fertilization. Apomixis also may involve activation of embryo development in inappropriate cell types. For example, during sporophytic apomixis (i.e., adventitious embryony), embryo development is activated in somatic cells of the ovule that are external to the female gametophyte (Maheshwari, 1950; Nogler, 1984; Koltunow, 1993; Koltunow et al., 1995).

This discussion of sexual and asexual seed development in angiosperms raises several important questions: What controls activation of embryo, endosperm, and seed coat development in response to fertilization? How are embryo and endosperm development induced to undergo seed development in the absence of fertilization in some species? How is embryo development activated in inappropriate cell types in some species? Does the female gametophyte play a role in controlling the induction of seed development, and if so, what is its role?

Mutations Affecting the Induction of Seed Development

To gain insight into the molecular processes controlling the induction of seed development, genetic screens were performed to identify mutants in which seed development occurs in the absence of fertilization. These screens led to the identification of four mutants, *fie*, *fis1*, *fis2*, and *fis3*, that undergo endosperm and seed coat development in the absence of fertilization (Table 2; Ohad et al., 1996; Chaudhury et al., 1997). Genetic analysis showed that these four mutants segregate gametophytically and that they fall into the female gametophyte–specific class (Ohad et al., 1996; Chaudhury et al., 1997). These mutations are either loss-of-function alleles in genes whose normal functions are to prevent seed development before fertilization or gain-of-function alleles in genes that promote seed development (Ohad et al., 1996; Chaudhury et al., 1997). The phenotypes of the *fie* and *fis* mutants suggest that the induction of seed development is controlled by a signal transduction pathway, some components of which are encoded by female gametophyte genes; their isolation provides solid proof that the female gametophyte plays a role in the induction of seed development.

Another mutation that may affect the induction of seed development is *ctr1* (Table 2). The *ctr1* mutation was first identified as a sporophytic mutation affecting ethylene signal transduction (Kieber et al., 1993) and was later shown to exhibit reduced transmission through the female gametophyte (Kieber and Ecker, 1994). The *CTR1* gene encodes a Raf-like Ser/Thr protein kinase that is required for ethylene signal transduction (Kieber et al., 1993). The *ctr1* phenotype (Table 2) suggests a requirement for *CTR1* during female gametophyte function. Pollen tube guidance is normal in this mutant, suggesting that this gene is required for either fertilization or the induction of seed development (C.A. Christensen and G.N. Drews, unpublished results).

GAMETOPHYTIC MATERNAL CONTROL OF SEED DEVELOPMENT

Maternal Control of Seed Development in Angiosperms

In many animal systems, maternal control is a significant factor in embryo development (Gilbert, 1994). Maternal control

is likely to be more complex in plants than it is in animals for two reasons. First, angiosperms undergo double fertilization, which allows for maternal control over both embryo and endosperm development. Second, maternal control can be exerted by either gametophytic (i.e., the female gametophyte) or sporophytic (e.g., ovule cells that surround the female gametophyte; Ray, 1997) tissue. Because of this complexity, it is important to distinguish between two possible classes of maternal mutations: sporophytic maternal mutations and gametophytic maternal mutations.

For both classes of maternal mutations, defects in embryo and/or endosperm development are not dependent on the genotypes of these two structures. Indeed, in sporophytic maternal mutants such as the Arabidopsis *short integument* (*sin1*; Ray et al., 1996) and barley *shrunken endosperm* (*seg*; Felker et al., 1985) mutants, embryo and/or endosperm development is dictated by the genotype of the maternal sporophytic tissue (e.g., the ovule integuments). Similar effects are also caused by *FLORAL BINDING PROTEIN7* (*FBP7*)/*FBP11* cosuppression in petunia (Colombo et al., 1997). By contrast, embryo and/or endosperm development in gametophytic maternal mutants is dictated by the genotype of the haploid tissue of the female gametophyte.

Gametophytic Maternal Mutations

It should be possible to identify gametophytic maternal mutations in the reduced seed-set and segregation distortion screens described above. However, female gametophyte mutations affecting gametophytic maternal control of embryo development should produce a slightly different seed-set phenotype than those affecting megagametogenesis, pollen tube guidance, fertilization, or the induction of seed development. This is because mutations affecting embryo and/or endosperm development result in defective (white or collapsed) seeds (Meinke and Sussex, 1979; Clark and Sheridan, 1991; Miller and Chourey, 1992). Thus, heterozygous gametophytic maternal mutants should exhibit a 50% defective seeds phenotype compared with the 50% desiccated ovules phenotype exhibited by the other classes of female gametophyte mutants.

The only known mutations that exhibit segregation distortion and cause the 50% defective seeds phenotype are the Arabidopsis *fie* (Ohad et al., 1996), *fis* (Chaudhury et al., 1997), and *emb173* (Castle et al., 1993) mutations (Table 2). These mutations all cause embryo arrest at the heart or torpedo stage; therefore, it is unlikely that the corresponding genes encode maternal factors required for embryo development. Instead, these mutations could affect gametophytic maternal-effect genes required for endosperm development. Alternatively, these mutations could affect genes required zygotically (i.e., post-fertilization) for endosperm development in at least two wild-type copies; mutations in such genes can also result in a 50% defective seeds phenotype (Castle et al., 1993; Ohad et al., 1996; Chaudhury et al., 1997). Because these two possibilities cannot be distinguished genetically, none of the known female gametophyte mutations clearly falls into the gametophytic maternal class. Thus, the extent to which the maternal gametophytic tissue exerts an influence on embryo and endosperm development in plants remains an important and largely unexplored question.

IDENTIFICATION OF GENES EXPRESSED IN THE FEMALE GAMETOPHYTE

Currently, very few genes expressed in the female gametophyte have been isolated (Springer et al., 1995; Kranz and Dresselhaus, 1996; Nadeau et al., 1996; Perry et al., 1996). However, several of the female gametophyte mutations listed in Table 2 were created by T-DNA (e.g., the *fem* and *gfa* mutations) or transposon (e.g., the *hdd* mutation) inserts. This will facilitate the rapid cloning of the corresponding genes, which appear to control specific steps of megagametogenesis including cellularization and the fusion of polar nuclei.

Identifying genes expressed in specific cells of the embryo sac has been hampered by difficulties in isolating female gametophytes, which are small and relatively inaccessible. These limitations have also frustrated molecular and biochemical experiments. However, new approaches in maize and Arabidopsis are beginning to overcome these problems.

For example, in maize, procedures for isolating large numbers of viable female gametophytes and egg cells have been developed (Wagner et al., 1988; Kranz et al., 1991). This advance has allowed the construction of cDNA libraries from unfertilized egg cells (Dresselhaus et al., 1994) and the isolation of several clones that appear to be expressed exclusively in the egg cell (Kranz and Dresselhaus, 1996). These procedures could also be applied to isolate genes expressed specifically in the other female gametophyte cells (Dumas and Mogensen, 1993).

In Arabidopsis, a large collection of gene-trap and enhancer-trap lines has been generated recently using transposable elements (Sundaresan et al., 1995). Among these lines are some that exhibit reporter gene expression in specific female gametophyte cells, including the synergid cells, the antipodal cells, and the central cell (Grossniklaus et al., 1995). These lines will be extremely useful as markers for the different female gametophyte cell types. For example, each of these lines could be crossed with the existing group of megagametogenesis mutants to determine whether the mutant phenotypes are associated with inappropriate cell specification. Furthermore, the trapped genes could serve as entry points into the female gametophyte gene regulatory circuitry.

SUMMARY

The female gametophyte is an absolutely essential structure for angiosperm reproduction. It produces the egg cell and central cell (which give rise to the embryo and endosperm, respectively) and mediates several reproductive processes including pollen tube guidance, fertilization, the induction of seed development, and perhaps also maternal control of embryo development. Although much has been learned about these processes at the cytological level, specific molecules mediating and controlling megagametogenesis and female gametophyte function have not been identified.

A genetic approach to the identification of such molecules has been initiated in Arabidopsis and maize. Although genetic analyses are still in their infancy, mutations affecting female gametophyte function and specific steps of megagametogenesis have already been identified. Large-scale genetic screens aimed at identifying mutants affecting every step of megagametogenesis and female gametophyte function are in progress; the characterization of genes identified in these screens should go a long way toward defining the molecules that are required for female gametophyte development and function.

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