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Female Gametophyte Development

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INTRODUCTION

Early in their evolution, plants acquired a life cycle that alternates between a multicellular haploid organism, the gametophyte, and a multicellular diploid organism, the sporophyte. Angiosperms have both female and male gametophytes. The female gametophyte is critical to many steps of the angiosperm reproductive process, including pollen tube guidance, fertilization, the induction of seed development upon fertilization, and maternal control of seed development after fertilization. Genetic analysis in Arabidopsis and maize has revealed mutants defective in almost all stages of female gametophyte development, and analysis of these mutants is beginning to reveal features of the female gametophyte developmental program. In addition, mutants defective in female gametophyte function have revealed regulatory genes required for the induction of endosperm development. From these studies, we are beginning to understand the regulatory networks involved in female gametophyte development and function.

Gametophytes and sporophytes differ morphologically and functionally. The major function of diploid sporophyte generation is to produce haploid spores, which are the products of meiosis. Spores undergo cell proliferation and differentiation to develop into gametophytes. The major function of gametophyte generation is to produce haploid gametes. The fusion of egg and sperm gives rise to the zygote, which is the beginning of diploid sporophyte generation, thereby completing the life cycle (Gifford and Foster, 1989).

During the angiosperm life cycle, the sporophyte produces two types of spores, microspores and megaspores, that give rise to male gametophytes and female gametophytes, respectively. The angiosperm gametophytes develop within sporophytic tissues that constitute the sexual organs of the flower. The male gametophyte, also referred to as the pollen grain or microgametophyte, develops within the stamen's anther and is composed of two sperm cells encased within a vegetative cell (McCormick, 1993, 2004). The female gametophyte, also referred to as the embryo sac or megagametophyte, develops within the ovule, which is found within the carpel's ovary. The most common female gametophyte form, 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).

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The angiosperm gametophytes are essential for the reproductive process. During sexual reproduction in angiosperms, the male gametophyte is transferred from the anther to the carpel's stigma, whereupon it forms a pollen tube that grows great distances through the carpel's internal tissues to deliver its two sperm cells to the female gametophyte. One sperm cell fertilizes the egg cell and the second sperm cell fuses with the central cell. After fertilization, the ovule gives rise to a seed; the seed's embryo, endosperm, and seed coat are derived from the fertilized egg cell, fertilized central cell, and ovule integuments, respectively (Maheshwari, 1950).

The female gametophyte plays a critical role in essentially every step of the reproductive process. During pollen tube growth, the female gametophyte participates in directing the pollen tube to the ovule (Higashiyama, 2002; Johnson and Preuss, 2002; Higashiyama et al., 2003). During fertilization, cytoskeletal components within the female gametophyte direct the sperm cells to the egg cell and the central cell (Russell, 1992, 1993; Lord and Russell, 2002). Upon fertilization, female gametophyte–expressed genes control the initiation of seed development (Chaudhury et al., 2001). During seed development, female gametophyte–expressed gene products play a role in controlling embryo and endosperm development (Ray, 1997; Chaudhury and Berger, 2001).

In this review, we describe angiosperm female gametophyte structure and development, summarize the female gametophyte's reproductive functions, and discuss the molecular and genetic approaches that are being used to understand these processes at the molecular level. Comprehensive reviews of the female gametophyte have been published previously (Maheshwari, 1950; Willemse and van Went, 1984; Haig, 1990; Huang and Russell, 1992; Russell, 2001).

DEVELOPMENT AND FERTILIZATION OF THE FEMALE GAMETOPHYTE

Female gametophyte development occurs over two phases referred to as megasporogenesis and megagametogenesis. More than 15 different patterns of female gametophyte development have been described. As summarized in Figure 2 and discussed below, the different patterns arise mainly from variations in cytokinesis during meiosis, number and pattern of mitotic divisions, and pattern of cellularization. The developmental pattern exhibited by most species is referred to as the Polygonum type (Figure 2, top row) because it was first described in *Polygonum divaricatum* (Strasburger, 1879; Maheshwari, 1950). The Polygonum-type female gametophyte

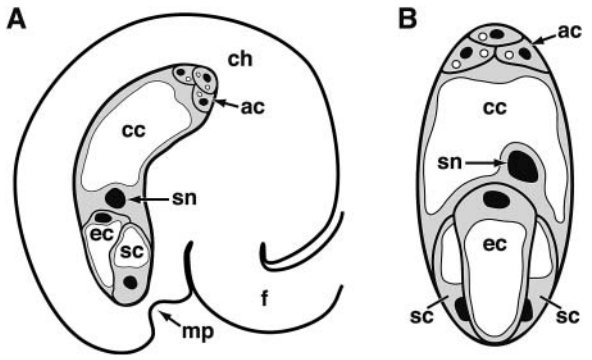

Figure 1. The Arabidopsis Female Gametophyte.

(A) Ovule.

(B) Female gametophyte.

The view in **(B)** is perpendicular to that in **(A)**. The mature female gametophyte in Arabidopsis is ~105 μm long and ~25 μm wide. 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; sn, secondary nucleus.

is exhibited by >70% of flowering plants and is the pattern found in many economically and biologically important groups, including Brassicaceae (e.g., *Arabidopsis*, *Capsella*, *Brassica*), Gramineae (e.g., maize, rice, wheat), Malvaceae (e.g., cotton), Leguminoseae (e.g., beans, soybean), and Solanaceae (e.g., pepper, tobacco, tomato, potato, petunia) (Maheshwari, 1950; Willemse and van Went, 1984; Haig, 1990; Huang and Russell, 1992).

Megasporogenesis

During megasporogenesis, the diploid megaspore mother cell undergoes meiosis and gives rise to four haploid nuclei. Angiosperms exhibit three main patterns of megasporogenesis, referred to as monosporic, bisporic, and tetrasporic. These three patterns are summarized in Figure 2. The three types differ mainly in whether cell plate formation occurs after these divisions, thus determining the number of meiotic products that contribute to the formation of the mature female gametophyte. In the monosporic pattern, both meiotic divisions are accompanied by cell plate formation, resulting in four one-nucleate megaspores. Subsequently, three megaspores, generally the micropylar-most megaspores, undergo cell death. In the bisporic pattern, cell plates form after meiosis I but not meiosis II. The result is two two-nucleate megaspores, one of which degenerates. In the tetrasporic pattern, cell plates fail to form after both meiotic divisions, resulting in one four-nucleate megaspore. Thus, these three patterns give rise to a single functional megaspore that contains one (monosporic), two (bisporic), or four (tetrasporic) meiotic nuclei. The monosporic pattern is the most common form and is represented within the Polygonum pattern (Maheshwari, 1950; Willemse and van Went, 1984; Haig, 1990; Huang and Russell, 1992).

Megagametogenesis

During megagametogenesis, the functional megaspore gives rise to the mature female gametophyte. Initially, the megaspore undergoes one or more rounds of mitosis without cytokinesis, resulting in a multinucleate coenocyte. Subsequently, cell walls form around these nuclei, resulting in a cellularized female gametophyte. For example, in the Polygonum-type pattern summarized in Figure 3, a single nucleus undergoes two rounds of mitosis, producing a four-nucleate cell with two nuclei at each pole. During a third mitosis, phragmoplasts and cell plates form between sister and nonsister nuclei, and soon thereafter, the female gametophyte cells become completely surrounded by cell walls. During cellularization, two nuclei, one from each pole (the polar nuclei), migrate toward the center of the developing female gametophyte and fuse together either before or upon fertilization of the central cell. These events result in a seven-celled structure consisting of three antipodal cells, one central cell, two synergid cells, and one egg cell (Figure 3). The monosporic, Polygonum type of female gametophyte is typically a seven-celled structure at maturity. However, this structure may be modified by cell death or cell proliferation events in various species. For example, in Arabidopsis, the antipodal cells undergo cell death immediately before fertilization (Figure 3), whereas in grasses (e.g., maize), the antipodal cells proliferate (Maheshwari, 1950; Willemse and van Went, 1984; Haig, 1990; Huang and Russell, 1992; Drews et al., 1998).

Throughout development, the female gametophyte exhibits a polarity along its chalazal-micropylar axis. During Polygonum-type megasporogenesis, the chalazal-most megaspore survives and the other three megaspores undergo cell death (Figure 3). During cell differentiation, the nuclei at the micropylar end become specified to develop into the egg cell, the micropylar polar nucleus, and the synergid cells; the chalazal nuclei develop into the three antipodal cells and the chalazal polar nucleus (Figure 3). Furthermore, all of the cells within the female gametophyte differentiate into polar structures. For example, in many species, the egg cell's nucleus is located toward the chalazal end and its vacuole occupies the micropylar end; by contrast, the synergid and central cells have the opposite polarity (Figure 3) (Willemse and van Went, 1984; Huang and Russell, 1992; Christensen et al., 1997). Thus, the establishment of polarity within the female gametophyte corresponds to the asymmetric development of the surrounding ovule layers, suggesting that female gametophyte polarity is regulated, at least in part, by the surrounding sporophytic tissues. Sporophytic factors that influence female gametophyte development have yet to be identified.

Fertilization and Early Seed Development

Soon after pollen is transferred from anther to stigma, the male gametophyte forms a pollen tube that grows via a tip-growth process through the carpel's sporophytic tissue to reach the female gametophyte. The pollen tube enters the female gametophyte by growing into one of the two synergid cells through a structurally elaborated portion of the micropylar cell wall known as the filiform apparatus. The penetrated synergid

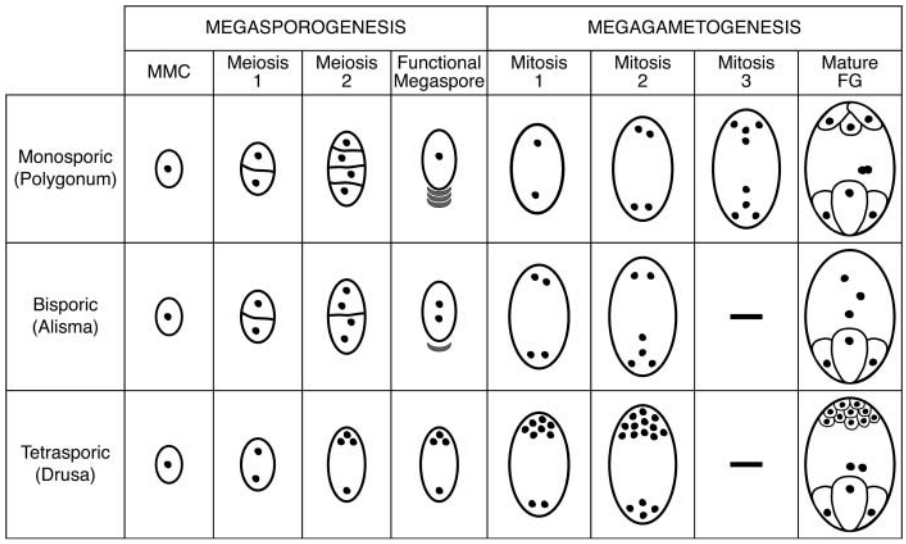

Figure 2. Patterns of Female Gametophyte Development Exhibited by Angiosperms.

Genera exhibiting these patterns are indicated in parentheses. More comprehensive descriptions of the variation among angiosperms can be found in several reviews (Maheshwari, 1950; Willemse and van Went, 1984; Haig, 1990; Huang and Russell, 1992; Russell, 2001). In this figure, the chalazal end of the female gametophyte is up and the micropylar end is down. FG, female gametophyte.

cell undergoes cell death soon before or upon pollen tube arrival. Immediately after arrival, pollen tube growth ceases, an aperture forms at or near the pollen tube tip, and the contents of the pollen tube, including the two sperm cells, are released rapidly into the degenerating synergid cytoplasm. Double fertilization occurs when the two sperm cells migrate to the egg and central cells and their plasma membranes fuse with the respective target cell to transport the sperm nuclei for karyogamy (van Went and Willemse, 1984; Russell, 1992, 1996).

ANALYSIS OF THE FEMALE GAMETOPHYTE

Identification of Mutations That Affect Female Gametophyte Development and Function

Because of their two-staged life cycle, plants possess two broad classes of mutations: sporophytic mutations and gametophytic mutations. Sporophytic mutations affect the diploid sporophyte phase of the plant life cycle and exhibit Mendelian 1:2:1 segregation patterns. Gametophytic mutations, by contrast, affect the haploid gametophyte phase of the plant life cycle and are not transmitted through egg and/or sperm. As a consequence, gametophytic mutations exhibit non-Mendelian segregation patterns and can only be transmitted from generation to generation as heterozygotes. For example, in a self cross of a heterozygous individual (e.g., genotype *A*/*a*), female gametophyte–specific mutations (i.e., mutations that affect the female gametophyte but not the male gametophyte) segregate 1:1 for *A*/*A*:*A*/*a* progeny (Moore et al., 1997; Drews et al., 1998; Drews and Yadegari, 2002; Page and Grossniklaus, 2002).

Sporophytic and gametophytic mutations affect different aspects of female gametophyte development. Sporophytic mutations affect those aspects that occur during the diploid phase, including megaspore mother cell development, meiosis, and control of female gametophyte development by the surrounding sporophytic tissue (e.g., female gametophyte polarity; discussed above). Sporophytic mutations that affect these processes are identified in screens for female-sterile mutants (Chaudhury et al., 1998; Gasser et al., 1998; Grossniklaus and Schneitz, 1998; Schneitz et al., 1998; Schneitz, 1999).

Gametophytic mutations affect those aspects of female gametophyte development that occur after meiosis, including megagametogenesis and functioning of the mature female gametophyte (pollen tube guidance, fertilization, induction of seed development, or maternal control of seed development). Gametophytic mutants typically are identified using two criteria: reduced seed set and segregation distortion. Reduced seed set results because on a plant heterozygous for a female gametophyte mutation, approximately half of the female gametophytes are mutant and nonfunctional; thus, they fail to undergo normal seed development. Segregation distortion results because, as described above, gametophytic mutations are transmitted to subsequent generations at reduced frequency (Moore et al., 1997; Drews et al., 1998; Drews and Yadegari, 2002; Page and Grossniklaus, 2002).

Sporophytic Mutations That Affect Megagametogenesis

As discussed above, the sporophytic tissue surrounding the female gametophyte may play a role in controlling megagametogenesis. Consistent with this notion, megagametogenesis is affected in most sporophytic ovule-development mutants (Chaudhury et al., 1998; Gasser et al., 1998; Grossniklaus and Schneitz, 1998; Schneitz et al., 1998; Schneitz, 1999). Mutants

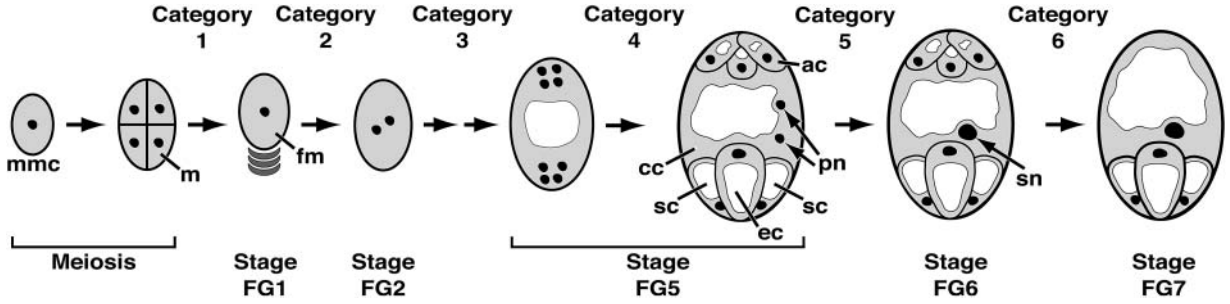

Figure 3. Female Gametophyte Development in Arabidopsis.

The steps are described in the text and by Christensen et al. (1997). Category designations show the developmental stage affected in the female gametophyte mutants. Phenotypic categories are as follows: category 1, megaspores fail to undergo cell death; category 2, megaspores do not progress beyond stage FG1; category 3, pleiotropic defects during the nuclear division phase; category 4, failure to cellularize or abnormal cell shape; category 5, polar nuclei fail to fuse; category 6, antipodal cells fail to undergo cell death; category 7 (not shown), morphologically wild-type female gametophytes at the terminal stage. The gray areas represent cytoplasm, the white areas represent vacuoles, and the black areas represent nuclei. In this figure, the chalazal end of the female gametophyte is up and the micropylar end is down. ac, antipodal cells; cc, central cell; ec, egg cell; fm, functional megaspore; m, megaspore; mmc, megaspore mother cell; pn, polar nuclei; sc, synergid cell; sn, secondary nucleus.

defective specifically in the sporophytic control of megagametogenesis should exhibit defects in megagametogenesis but not in the sporophytic parts of the ovule. Mutants with this phenotype have been reported (Schneitz et al., 1997). However, the genes affected in these mutants have not been identified; thus, the molecular basis of these sporophytic effects remains to be determined.

Gametophytic Mutations That Affect Megagametogenesis

During the last few years, many gametophytic mutants affected in female gametophyte development have been identified and analyzed (Christensen et al., 2002; Drews and Yadegari, 2002; Grini et al., 2002; Huck et al., 2003; Rotman et al., 2003). We have identified and analyzed >150 mutants, allowing genetic dissection of the female gametophyte developmental pathway (Christensen et al., 2002; D. Otsuga, C. Dever, N. Huefner, L.A. Ogden, L.G. Jones, and G.N. Drews, unpublished data). Most of these mutants are affected in megagametogenesis. As summarized in Figure 3, the megagametogenesis mutants fall into six phenotypic categories corresponding to key developmental events involved in the formation of a mature female gametophyte. Although the genes affected for most of the megagametogenesis mutants are not known yet, the use of insertional mutagens (e.g., T-DNA or transposons) should facilitate their rapid identification.

Certain key features of megagametogenesis mutant screens will enable a comprehensive analysis of gametophyte generation and also will contribute to an understanding of important cellular and developmental processes throughout the plant life cycle. First, these screens have yielded mutants with defects throughout megagametogenesis. Based on these phenotypes, the affected genes can be ordered, to some extent, within the developmental program. In addition, many mutations exhibit defects in specific cellular processes, including mitosis, vacuole formation, cell wall formation, nuclear fusion, and cell death. Significantly, many of these mutations would not be identified in typical sporophytic screens because they cannot become homozygous during sporophyte generation. Therefore, genetic analysis of these key processes likely will rely on the characterization of defective gametophytes.

Pollen Tube Guidance

During the final stages of pollen tube growth, the pollen tube grows toward an ovule and then up the surface of the funiculus until it enters the micropyle to penetrate the female gametophyte (Higashiyama, 2002; Johnson and Preuss, 2002; Higashiyama et al., 2003). This directed growth pattern suggests that the ovule and female gametophyte play a role in guiding pollen tube growth. Many studies suggest that pollen tube guidance is controlled by both sporophytically expressed and gametophytically expressed factors (Palanivelu and Preuss, 2000; Franklin-Tong, 2002; Higashiyama, 2002; Johnson and Preuss, 2002; Higashiyama et al., 2003). Recently, γ-aminobutyric acid was identified as a sporophytic signal that regulates pollen tube growth and guidance (Palanivelu et al., 2003).

To determine whether the female gametophyte plays a role in pollen tube guidance, several groups analyzed pollen tube growth patterns in Arabidopsis mutants defective in embryo sac development. Such studies have shown that ovules lacking female gametophytes fail to attract pollen tubes, suggesting very strongly that the embryo sac is the source of an attractant that guides the pollen tube to the ovule (Hulskamp et al., 1995; Ray et al., 1997; Couteau et al., 1999; Shimizu and Okada, 2000).

Analysis of pollen tube growth patterns in Arabidopsis mutants defective in female gametophyte development suggests that guidance by the female gametophyte involves multiple steps. In mutants in which female gametophyte development is affected severely (e.g., mutants in categories 1 to 3 in Figure 3), pollen tubes fail to grow along the funiculus (Hulskamp et al., 1995; Ray et al., 1997). By contrast, in mutants in which female gametophyte development is affected less severely (e.g., *magatama*

mutants, which exhibit delayed development and have unfused polar nuclei at the time of pollination), pollen tubes grow along the funiculus but do not enter the micropyle (Shimizu and Okada, 2000). These observations suggest that guidance by the female gametophyte involves at least two phases: guidance from the placenta to the funiculus (funiculus guidance phase) and guidance from the funiculus to the micropyle (micropyle guidance phase) (Shimizu and Okada, 2000).

To identify the specific cells within the female gametophyte that are the source of the pollen tube attractant, Higashiyama and colleagues (1998) performed laser ablation experiments in an in vitro pollen germination system using ovules from *Torenia fournieri*. In this species, much of the embryo sac protrudes from the ovule integuments and is accessible to experimental manipulation (Tiwari, 1982). Control ovules (no cells ablated) and ovules in which the egg cell and central cell were ablated attracted pollen tubes, whereas ovules with ablated synergid cells failed to attract pollen tubes (Higashiyama et al., 2001). These studies identify the synergid cells as the source of the pollen tube attractant. Studies in the in vitro *T. fournieri* system also indicate that the effective distance of pollen tube attraction is 100 to 200 μm and that the attraction signal is species specific (Higashiyama et al., 1998, 2003; Higashiyama, 2002).

What is the biochemical nature of the synergid cell guidance cue? Calcium has been proposed to be a guidance signal because it can attract pollen tubes in vitro in some species (Mascarenhas, 1962; Mascarenhas and Machlis, 1962, 1964; Reger et al., 1992), is present in high concentrations in synergid cells (Jensen, 1965; Chaubal and Reger, 1990, 1992a, 1992b, 1993; Tian and Russell, 1997), and is necessary for pollen tube growth (Pierson et al., 1994, 1996; Holdaway-Clarke et al., 1997; Li et al., 1999). However, the addition of calcium to the medium in the in vitro *T. fournieri* system does not affect pollen tube attraction, indicating that calcium is not a universal attractant (Higashiyama, 2002; Higashiyama et al., 2003). The *T. fournieri* in vitro pollen tube guidance assay potentially could facilitate the biochemical isolation of the guidance cue, although an inherent difficulty of this approach is likely to be the limitations on the amount of the target tissue, the synergid cells. Alternatively, screens for female gametophyte mutants with defects specifically in pollen tube guidance may facilitate the identification of the attractant(s). Female gametophyte mutants defective specifically in pollen tube guidance have yet to be reported. Thus, the chemical nature of the synergid cell guidance cue remains to be determined.

Fertilization and Synergid Cell Death

The pollen tube enters the female gametophyte by growing into one of the synergid cells. Soon thereafter, the pollen tube ceases growth, ruptures at or near its tip, and releases its contents. The synergid cell penetrated by the pollen tube undergoes cell death, either before or upon pollen tube arrival; thus, the pollen tube contents are released into the degenerating cytoplasm of the synergid cell (van Went and Willemse, 1984; Willemse and van Went, 1984; Russell, 1992, 1996; Higashiyama, 2002; Lord and Russell, 2002; Raghavan, 2003). The time course of these events has been determined in Arabidopsis (Faure et al., 2002).

Synergid cell death may be a prerequisite for normal fertilization in angiosperms. For example, the process of degeneration itself could decrease resistance to both pollen tube penetration and sperm cell migration during fertilization (Willemse and van Went, 1984; Huang and Russell, 1992). In addition, synergid degeneration generally is accompanied by cytoskeletal reorganizations that may facilitate male gamete transfer from the pollen tube to the egg and the central cell (Russell, 1993; Fu et al., 2000).

In some species, synergid cell death appears to be a final step of the megagametogenesis developmental program. By contrast, in other species, including Arabidopsis, synergid degeneration is not an inherent feature of the megagametogenesis process per se, because synergid cell death does not occur if pollination is prevented (van Went and Willemse, 1984; Willemse and van Went, 1984; Russell, 1992; Christensen et al., 1997; Faure, 2001). Two general, and not necessarily mutually exclusive, mechanisms might be responsible for synergid cell death in species such as Arabidopsis. First, synergid degeneration may be a purely physical process (e.g., the release of pollen tube contents into the synergid cell may physically rupture the synergid membrane). Second, synergid cell death may be a physiological process induced by pollen. Pollen could induce synergid cell death via a diffusible signal (from the pollen tubes or from female tissue) or through direct contact with the synergid cell (van Went and Willemse, 1984; Willemse and van Went, 1984; Russell, 1992; Higashiyama, 2002). All of these mechanisms may act in angiosperms. For example, in some species, the synergid cell appears to be completely intact at the moment of pollen tube discharge, suggesting degeneration via a physical process. By contrast, in other species, synergid cell death appears to be initiated before pollen tube arrival at the female gametophyte, suggesting that a long-range, diffusible signal induces synergid cell death (van Went and Willemse, 1984; Willemse and van Went, 1984; Russell, 1992; Higashiyama, 2002). In Arabidopsis, the status of synergid cell death at the moment of pollen tube arrival at the female gametophyte has not been determined. However, as discussed below, analysis of several female gametophyte mutants suggests that synergid cell death in Arabidopsis is an induced, physiological process.

Because fertilization takes place within the embryo sac, it is likely that many female gametophyte–expressed gene products are necessary for this process. However, because the targets of double fertilization are physically inaccessible, a molecular understanding of the angiosperm fertilization process has been lagging. The development of in vitro fertilization systems (Kranz et al., 1991; Kranz and Lorz, 1994; Kranz and Dresselhaus, 1996) should allow the manipulation and dissection of double fertilization (Kranz, 2001; Lord and Russell, 2002). For example, the in vitro fertilization system could facilitate the identification of all metabolites, including mRNA, protein, and small molecules, present within pure preparations of gametophytes before, during, and after fertilization.

An alternative approach is the identification of mutants defective in the fertilization process. During the last 2 years, several female gametophyte mutants affected in the fertilization process have been reported, including *gametophytic factor2* (*gfa2*) (Christensen et al., 2002), *feronia* (*fer*) (Huck et al., 2003), and

sirene (*srn*) (Rotman et al., 2003). In all three mutants, embryo sac development is normal (*fer* and *srn*) or essentially normal (*gfa2* female gametophytes also have defects in fusion of the polar nuclei) and mutant female gametophytes attract pollen tubes but fail to become fertilized. In response to pollination, *fer* embryo sacs undergo synergid cell death (Huck et al., 2003); by contrast, *gfa2* and *srn* embryo sacs fail to undergo synergid cell death after pollination (Christensen et al., 2002; Rotman et al., 2003). The *gfa2* and *srn* mutations do not affect megaspore or antipodal cell death, suggesting that synergid cell death has unique features.

The *srn* mutant has an additional defect also exhibited by the *fer* mutant: wild-type pollen tubes enter mutant female gametophytes but fail to cease growth, rupture, and release their contents (Huck et al., 2003; Rotman et al., 2003). With *srn*, it is unclear whether the synergid cell death defect is a secondary consequence of the pollen tube tip rupture defect or vice versa. Regardless, the phenotypes of these two mutants clearly indicate that they are defective in some aspect of pollen–embryo sac interaction.

GFA2 encodes a chaperone that functions in the mitochondrial matrix, and the yeast ortholog is required for mitochondrial function. These data suggest that synergid cell death requires functional mitochondria, which also are required for cell death in animals (Christensen et al., 2002). Molecular aspects of the synergid cell death process remain to be determined. The proteins encoded by *FER* and *SRN* are unknown.

Analysis of the *gfa2*, *fer*, and *srn* mutants reveals several aspects of the fertilization process. First, *gfa2* and *srn* do not affect pollen tube attraction and should not prevent the physical rupture of the synergid cell, suggesting that synergid cell death in Arabidopsis is not a purely physical process. Second, *gfa2* and *srn* embryo sacs fail to undergo synergid cell death and yet attract pollen tubes, suggesting that synergid cell death is not required for pollen tube attraction. Third, the abnormal pollen tube behavior within *fer* and *srn* female gametophytes suggests that the presence of synergid cells per se does not ensure normal pollen tube termination and discharge within the female gametophyte (Christensen et al., 2002; Huck et al., 2003; Rotman et al., 2003).

Control of Seed Development

Recent genetic data indicate that the female gametophyte controls seed development at several levels. First, the female gametophyte controls the initiation of seed development by expressing a set of proteins that repress this process in the absence of fertilization. Second, the female gametophyte contains factors before fertilization that are required for embryo and endosperm development after fertilization. Third, the female gametophyte plays a role in controlling the imprinting of genes required for seed development.

Female gametophyte mutations that affect the initiation of seed development include *fertilization-independent endosperm* (*fie*) (Ohad et al., 1996; Chaudhury et al., 1997), *medea* (*mea*) (Chaudhury et al., 1997; Grossniklaus et al., 1998), and *fertilization-independent seed2* (*fis2*) (Chaudhury et al., 1997). In all three mutants, endosperm development occurs in the absence of fertilization. The FIE, MEA, and FIS2 proteins are related to Polycomb group proteins involved in the heritable silencing of homeotic gene expression in *Drosophila* and mammals (Grossniklaus et al., 1998; Kiyosue et al., 1999; Luo et al., 1999; Ohad et al., 1999). The *FIE*, *MEA*, and *FIS2* genes are expressed in the female gametophyte, primarily in the central cell, before fertilization (Vielle-Calzada et al., 1999; Luo et al., 2000; Spillane et al., 2000; Yadegari et al., 2000). Together, these data suggest that the female gametophyte expresses a set of proteins that repress endosperm development before fertilization (Gehring et al., 2004). By this scenario, fertilization could lead to the initiation of endosperm development by inactivating the FIE/FIS2/MEA repressive complex (Ohad et al., 1999). How fertilization accomplishes this task remains an unanswered question.

The FIE/FIS2/MEA complex most likely represses endosperm development by preventing the transcription of target genes involved directly in this process. One such target gene, *PHERES1* (*PHE1*), was identified recently (Kohler et al., 2003). *PHE1* encodes a MADS domain–containing protein. Chromatin immunoprecipitation assays have shown that MEA and FIE interact directly with the *PHE1* promoter. In the wild type, *PHE1* expression occurs in the early stages of endosperm development and is not detected in the female gametophyte before fertilization. *PHE1* also is expressed in preglobular-stage embryos, suggesting a function in both embryo and endosperm. However, *PHE1* expression is upregulated strongly in *mea* and *fie* seeds and is activated inappropriately in *fie* female gametophytes. After fertilization, *mea* seeds exhibit endosperm overproliferation and embryo abortion (Grossniklaus et al., 1998; Kiyosue et al., 1999). These postfertilization defects are attributable, in part, to the activity of *PHE1*, because reduced levels of *PHE1* expression partially rescue the *mea* seed phenotype (Kohler et al., 2003).

After the initiation step, the female gametophyte controls seed development by providing maternal cues required for this process; specifically, the female gametophyte contains maternal factors before fertilization that are required for embryo and endosperm development after fertilization. Female gametophyte–expressed genes required for embryo and endosperm development are referred to as gametophytic maternal-effect genes (Ray, 1997; Drews et al., 1998; Drews and Yadegari, 2002). This is in contrast to sporophytic maternal-effect genes (e.g., Arabidopsis *SHORT INTEGUMENTS1*, barley *SHRUNKEN ENDOSPERM*, and petunia *FLORAL BINDING PROTEIN FBP7* and *FBP11*), which are expressed in sporophytic tissue (Felker et al., 1985; Ray et al., 1996; Colombo et al., 1997).

The clearest examples of gametophytic maternal-effect mutants described to date include the Arabidopsis *capulet1* (*cap1*) and *cap2* mutants (Grini et al., 2002), the Arabidopsis *prolifera* (*prl*) mutant (Springer et al., 1995, 2000), and the maize *maternal effect lethal1* (*mel1*) mutant (Evans and Kermicle, 2001). With the exception of *prl*, which shows arrest predominantly at the four-nucleate stage of development (Springer et al., 1995), female gametophytes in these mutants appear normal, whereas the development of the embryo or endosperm or both is affected severely very early during seed development. For example, embryos arising from *cap1* female gametophytes exhibit defects as early as the zygote stage and fail to progress beyond the

one-cell proembryo stage (Grini et al., 2002). The *CAP1*, *CAP2*, and *MEL1* genes have not been isolated; thus, the molecular basis for the gametophytic maternal effects observed in these mutants remains to be determined. However, *PRL* encodes a highly conserved homolog of the DNA replication licensing factor Mcm7, whose accumulation in the female gametophyte appears to be required for normal embryo development (Springer et al., 1995, 2000).

A third level at which the female gametophyte influences seed development is through the control of genomic imprinting. An important aspect of seed development is the parent-specific expression of genes required during this process. Parent-specific expression is achieved by imprinting the alleles inherited from the male or female gametophyte. For example, with *FIS2* and *MEA*, the maternal alleles are active but the paternal alleles are inactive during endosperm development (Kinoshita et al., 1999; Luo et al., 2000).

The imprinting is achieved via epigenetic modification of the maternal or paternal alleles, a process generally associated with the methylation of cytosine residues within and flanking the coding region of the target gene (Jaenisch and Bird, 2003). An important question is when the inhibitory modifications are established during development. With paternally imprinted genes, the simplest model is that the inhibitory modifications are established during the male gametophyte lineage. An alternative possibility is that both alleles become modified at some point during the life cycle and the inhibitory modifications are removed during the female gametophyte lineage. The latter possibility appears to be the case with the *MEA* gene. Recent evidence suggests that inhibitory modifications of the maternal allele of *MEA* are removed in the female gametophyte's central cell by a protein called DEMETER (DME). DME is a DNA glycosylase/lyase related to the superfamily of base excision DNA repair proteins (Choi et al., 2002). *DME* is expressed in the female gametophyte's central cell before fertilization. *MEA* expression is reduced in *dme* female gametophytes and the endosperm of seeds derived from *dme* embryo sacs. DME likely activates the *MEA* gene by modifying chromatin structure through the removal of inhibitory methylated cytosine residues from its gene-regulatory sequences and counteracting *MEA*'s imprinted/silenced state in the female gametophyte. In effect, the female gametophyte marks the maternal allele of *MEA* and presumably other regulatory genes for continued activity after fertilization. The function of *DME* in derepressing gene expression may not be unique, because mutations in a related gene, termed *REPRESSOR OF SILENCING1*, have been shown to cause transcriptional silencing of a transgene and a homologous endogenous gene (Gong et al., 2002).

SUMMARY

The female gametophyte represents an essential portion of the plant life cycle mediating several reproductive processes, including pollen tube guidance, fertilization, the induction of seed development, and maternal control of seed development. During the past few years, genetic approaches in Arabidopsis and maize have provided us with molecular and genetic information about megagametogenesis and the induction of seed development. However, we still know little about pollen tube guidance, fertilization, and the maternal control of seed development at the molecular level. In addition, a comprehensive profile of the genes expressed in the female gametophyte has not been obtained. In light of the fact that a large proportion of female gametophytic genes are expected to have partially or completely redundant functions (Drews and Yadegari, 2002), a combination of forward- and reverse-genetics approaches will be required to completely understand the development and reproductive functions of the female gametophyte.

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