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The Female Gametophyte

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The angiosperm female gametophyte is critical for plant reproduction. It contains the egg cell and central cell that become fertilized and give rise to the embryo and endosperm of the seed, respectively. Female gametophyte development begins early in ovule development with the formation of a diploid megaspore mother cell that undergoes meiosis. One resulting haploid megaspore then develops into the female gametophyte. Genetic and epigenetic processes mediate specification of megaspore mother cell identity and limit megaspore mother cell formation to a single cell per ovule. Auxin gradients influence female gametophyte polarity and a battery of transcription factors mediate female gametophyte cell specification and differentiation. The mature female gametophyte secretes peptides that guide the pollen tube to the embryo sac and contains protein complexes that prevent seed development before fertilization. Post-fertilization, the female gametophyte influences seed development through maternal-effect genes and by regulating parental contributions. Female gametophytes can form by an asexual process called gametophytic apomixis, which involves formation of a diploid female gametophyte and fertilization-independent development of the egg into the embryo. These functions collectively underscore the important role of the female gametophyte in seed and food production.

INTRODUCTION

Plants undergo an alternation of generations life cycle that involves a multicellular haploid generation, called the gametophyte, and a multicellular diploid generation, called the sporophyte. Sexual reproduction is initiated with sporogenesis, during which specialized cells (mother cells) within the sporophyte undergo meiosis and give rise to haploid spores. Spores undergo gametogenesis, a process of cell proliferation and differentiation, to develop into multicellular gametophytes, which then produce the gametes (sperm and egg cells). Fusion of egg and sperm to form the zygote, followed by embryo body plan development gives rise to the sporophyte, thereby completing the life cycle (Gifford and Foster, 1989).

Angiosperms, or flowering plants, are heterosporous, producing two types of spores that develop into two types of unisexual gametophytes. The first spore type is the megaspore. During megasporogenesis, diploid megaspore mother cells undergo meiosis and give rise to haploid megaspores, which then, during megagametogenesis, develop into haploid female gametophytes. The second spore type is the microspore. During microsporogenesis, diploid microspore mother cells give rise to microspores, which then undergo microgametogenesis and develop into male gametophytes (Gifford and Foster, 1989).

The angiosperm gametophytes are composed of few cells and are embedded within the sexual organs of the flower. The female gametophyte develops within the ovule and generally consists of three antipodal cells, one central cell, two synergid cells, and one egg cell (Figures 1A and 1B). The female gametophyte is also commonly called the embryo sac or megagametophyte. The male gametophyte, also called the pollen grain or microgametophyte, develops within the anther and consists of two sperm cells encased within a vegetative cell (Gifford and Foster, 1989).

Female gametophyte formation is required for sexual and asexual seed development in angiosperms. In sexually reproducing angiosperms, seed formation begins when pollen is transferred from the anther to the carpel's stigma. The male gametophyte then forms a pollen tube that grows through the carpel's internal tissues and into the ovule to deliver its two sperm cells to the female gametophyte. One sperm fertilizes the egg, and the second fuses with the central cell. Following double fertilization, the egg cell gives rise to the seed's embryo, which is the beginning of the sporophyte generation, the central cell develops into the seed's endosperm, which surrounds and provides nutrients to the developing embryo, and the surrounding sporophytic cells give rise to the seed coat (Gifford and Foster, 1989).

Plants can also produce seeds asexually by apomixis. Apomixis occurs in over 40 plant families and more than 400 genera. Apomixis does not occur in Arabidopsis but is found in a related genus, *Boechera*. Apomictic species exhibit much variation in the developmental mechanism leading to asexual seed production, and some routes bypass female gametophyte formation. A form of apomixis that involves the female gametophyte is gametophytic apomixis (Nogler, 1984; Koltunow, 1993; Koltunow et al., 1995).

During gametophytic apomixis, meiotic reduction is bypassed and diploid female gametophytes are formed by a variety of developmental routes in different species. The egg cell then forms an embryo autonomously (i.e., without fertilization; also termed

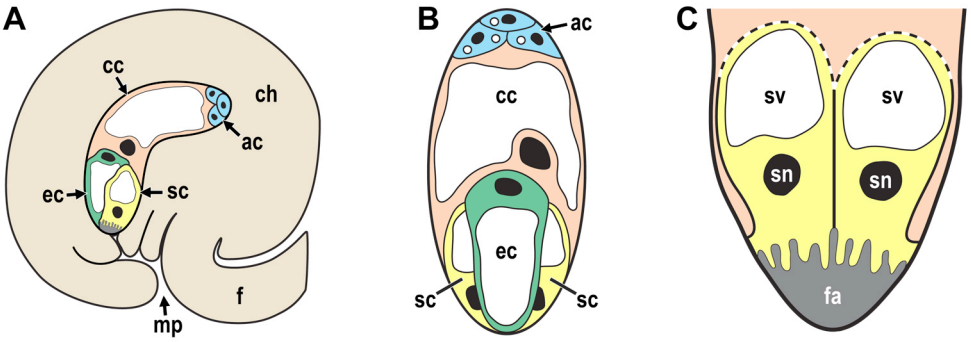

Figure 1. The Arabidopsis female gametophyte.

(A) Ovule. **(B)** Female gametophyte. **(C)** Synergid cells.

View in **(B)** and **(C)** is perpendicular to that in **(A)**. The mature female gametophyte in Arabidopsis is approximately 105 µm in length and approximately 25 µm in width. In all panels, the black circles/ovals represent nuclei and the white areas represent vacuoles. The dashed line at the chalazal ends of the synergid cells in **(C)** represents a discontinuous or absent cell wall. Abbreviations: ac, antipodal cells; cc, central cell; ch, chalazal region of the ovule; ec, egg cell; f, funiculus; fa, filiform apparatus; mp, micropyle; sc, synergid cell; sn, synergid nucleus, sv, synergid vacuole.

parthenogenesis) and endosperm formation may occur autonomously or may require central cell fertilization (termed pseudogamy; Nogler, 1984; Koltunow, 1993; Koltunow et al., 1995).

Apomixis gives rise to clonal progeny with a maternal genotype through seed. Apomixis, therefore, could be important in plant breeding to fix hybrid vigor and could significantly reduce the costs of generating high yielding hybrid seeds (Koltunow et al., 1995). The molecular basis of apomixis is not currently understood. However, recent work in Arabidopsis and other species has provided many insights and may allow engineering of this important trait.

Analysis of female gametophyte development is therefore important for many reasons. It is integral to the plant life cycle and essential for both sexual and apomictic seed formation. Furthermore, as discussed below, the female gametophyte controls many steps of the angiosperm sexual reproductive process: during pollen tube growth and fertilization, the female gametophyte guides the pollen tube to the ovule and embryo sac, controls pollen tube growth within the female gametophyte, and mediates fertilization of the egg cell and central cell; and upon double fertilization, female gametophyte-expressed genes participate in inducing embryo and endosperm formation during seed development. In the absence of fertilization in autonomous apomicts, the unreduced female gametophyte contains factors to stimulate embryo and endosperm formation.

FEMALE GAMETOPHYTE DEVELOPMENT IN ARABIDOPSIS

Angiosperms exhibit many different patterns of female gametophyte development. Arabidopsis undergoes the *Polygonum*-type pattern, which is the most common pattern and is exhibited by over 70% of flowering plants. The *Polygonum*-type pattern is also exhibited by many economically important groups including Gramineae (*e.g.*, maize, rice, wheat), Phaseoleae (*e.g.*, beans, soybean), Brassicaceae (*e.g.*, Brassica), Malvaceae (*e.g.,* cotton), and Solanaceae (*e.g.*, pepper, tobacco, tomato, potato, petunia), as well as most apomictic species (Maheshwari, 1950; Willemse and van Went, 1984; Huang and Russell, 1992).

Regardless of the specific developmental pattern, female gametophyte development occurs within the developing ovule and consists of two main phases: megasporogenesis followed by megagametogenesis. During Arabidopsis megasporogenesis, the diploid megaspore mother cell undergoes meiosis and gives rise to haploid megaspores (Figure 2). During Arabidopsis megagametogenesis, one of the megaspores develops into the mature female gametophyte (Figure 3).

Megasporogenesis

Megasporogenesis in Arabidopsis has been described (Misra, 1962; Webb and Gunning, 1990; Schneitz et al., 1995; Christensen et al., 1998; Bajon et al., 1999) and is depicted in Figure 2. Megasporogenesis comprises three major events: megaspore mother cell formation, meiosis to produce haploid megaspores, and megaspore selection (i.e., selection of the megaspore that develops into the female gametophyte).

Ovule primordia in Arabidopsis arise as finger-like projections from the placental tissue of the ovary. During early ovule development, a sub-epidermal cell at the distal end of the ovule primordium forms the archesporial cell. In Arabidopsis and most other species, the archesporial cell differentiates directly into the megaspore mother cell (also called the female meiocyte or megasporocyte); thus, in these species, there is no functional difference between an archesporial cell and a megaspore mother cell. The ovule cells that do not develop into the megaspore mother cell are sporophytic cells and are often referred to as somatic cells. Relative to the somatic cells, the megaspore mother cell is larger and has a denser cytoplasm and a larger nucleus. Just before meiosis, the megaspore mother cell is dramatically enlarged and

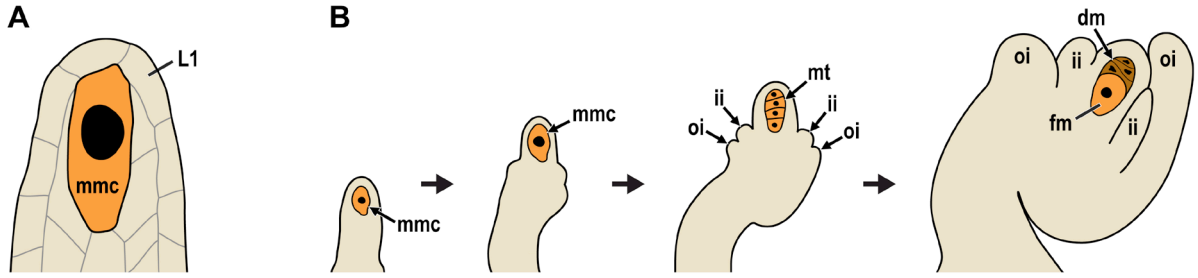

Figure 2. Megasporogenesis in Arabidopsis.

(A) Apical region of a young, finger-like ovule primordium. The megaspore mother cell forms from a sub-epidermal cell at the distal end of the ovule primordium. L1 is the outer layer of cells.

(B) Steps of megasporogenesis. Ovule primordia arise as finger-like projections from the placenta. The megaspore mother cell undergoes meiosis and forms four megaspores. Three of the megaspores undergo cell death. The chalazal-most megaspore survives, becomes the functional megaspore, and undergoes megagametogenesis.

Black circles/ovals represent nuclei.

Abbreviations: dm, degenerating megaspores; fm, functional megaspore; ii, inner integument; L1, L1 epidermal layer of the ovule primordium; mmc, megaspore mother cell; mt, meiotic tetrad; oi, outer integument.

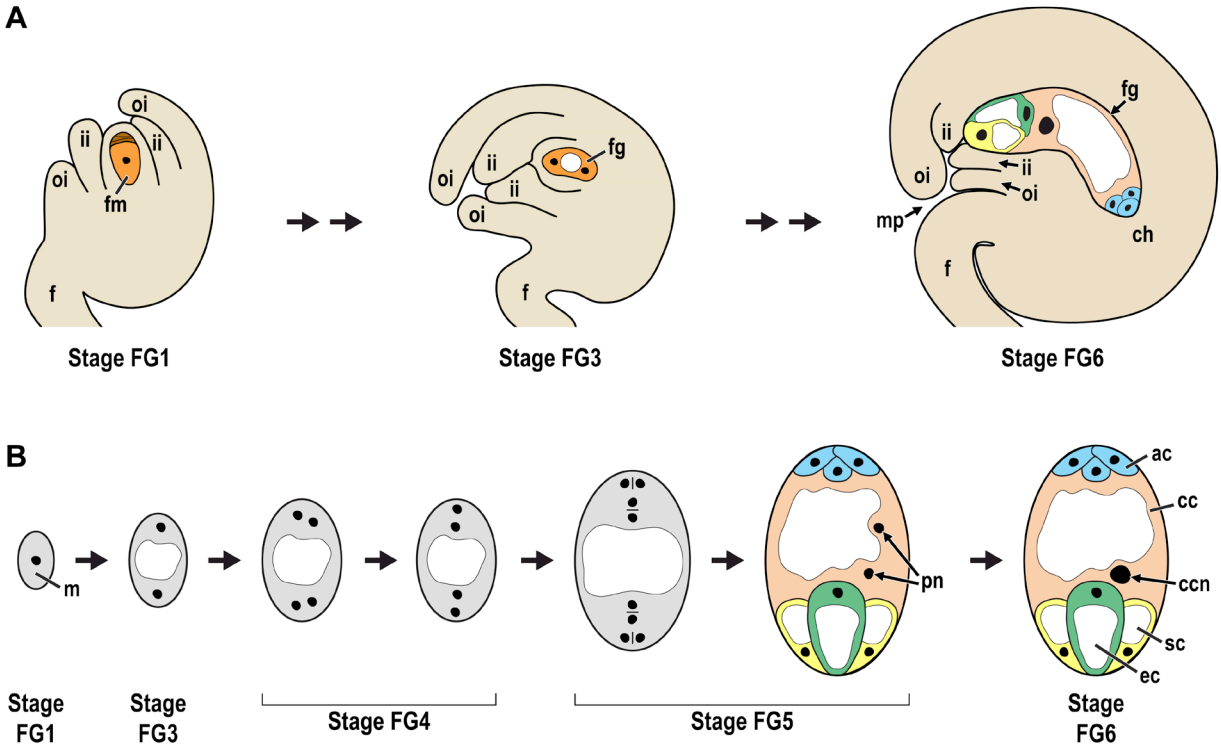

Figure 3. Megagametogenesis in Arabidopsis.

(A) Steps of megagametogenesis emphasizing development within the ovule.

(B) Stages of megagametogenesis (Christensen et al., 1998). The megaspore contains a single nucleus (stage FG1). This nucleus undergoes two rounds of mitosis, producing a four-nucleate coenocyte, with two nuclei at each pole separated by a large central vacuole (stage FG4). During a third mitosis, phragmoplasts and cell plates form between sister and non-sister nuclei and the nuclei become completely surrounded by cell walls (Stage FG5). During cellularization, the polar nuclei migrate toward the center of the female gametophyte and fuse before fertilization. These events produce a seven-celled structure consisting of three antipodal cells, one central cell, two synergid cells, and one egg cell. If the female gametophyte is not fertilized, the antipodal cells eventually degenerate (Stage FG7, not shown).

White areas represent vacuoles and black circles/ovals represent nuclei.

Abbreviations: ac, antipodal cells; cc, central cell; ccn; central cell nucleus; ch, chalazal region of the ovule; ec, egg cell; f, funiculus; fg, female gametophyte; fm, functional megaspore; ii, inner integument; m, megaspore; mp, micropyle; oi, outer integument; pn, polar nuclei; sc, synergid cells.

elongated. The megaspore mother cell then undergoes meiosis and gives rise to four one-nucleate, haploid megaspores. Subsequently, three of the megaspores degenerate and one survives. In Arabidopsis and most other species, the chalazal-most megaspore survives during megaspore selection (Figure 2).

Programmed cell death is likely to be the cause of megaspore degeneration because TUNEL assays show DNA fragmentation in degenerating megaspores of alfalfa (*Medicago sativa* L) ovules (Citterio et al., 2005). Furthermore, degenerating megaspores express *MPS-ONE-BINDER* (*MOB1*) genes, which encode homologs of fly and mammalian proteins that regulate apoptosis factors (Citterio et al., 2005; Hirabayashi et al., 2008).

A histological marker associated with the megaspore mother cell is callose (β-1,3-glucan). During megasporogenesis, the megaspore mother cell and the cells undergoing meiosis accumulate callose in their cell walls. After meiosis, callose is lost in the cell walls of the selected megaspore as it enlarges and begins the transition to megagametogenesis. The role of callose in megasporogenesis and megaspore selection is not understood (Rodkiewicz, 1970; Webb and Gunning, 1990).

Megagametogenesis

Megagametogenesis in Arabidopsis is depicted in Figure 3. Megagametogenesis also involves three identifiable events: a series of mitoses without cytokinesis, followed by cellularization of the nuclei and then cell differentiation.

In Arabidopsis, the single surviving megaspore enlarges and then undergoes two rounds of mitosis without cytokinesis, resulting in a four-nucleate coenocyte with two nuclei at each pole. During a third mitosis, phragmoplasts and cell plates form between sister and non-sister nuclei; this is the beginning of the cellularization process and the female gametophyte cells quickly become completely surrounded by cell walls. During and after cellularization, one nucleus from each pole (the polar nuclei) migrates toward the center of the developing female gametophyte and they fuse. These events result in a seven-celled structure consisting of three antipodal cells, one central cell, two synergid cells, and one egg cell (Figures 1 and 3). The central cell inherits two identical haploid nuclei and is therefore homodiploid. The other cells all inherit single haploid nuclei. If the female gametophyte is unfertilized, the antipodal cells eventually disappear or undergo cell death; however, at the time of fertilization, the female gametophyte most likely is a seven-celled structure (i.e., the antipodal cells are present; Schneitz et al., 1995; Christensen et al., 1998).

Structure of the mature female gametophyte

The structure of the mature female gametophyte in Arabidopsis has been described using transmission electron microscopy (Mansfield et al., 1991; Murgia et al., 1993; Kasahara et al., 2005; Kagi et al., 2011). These studies have shown that the female gametophyte's cell types all have a plethora of structural specializations. We discuss those specializations that are important for the fertilization process in angiosperms.

The egg and central cells are polarized such that the nuclei of both cells lie very close to each other (Figure 1). This feature is important for double fertilization because these two nuclei are the targets of the two sperm nuclei. Furthermore, in the regions where the egg, synergid, and central cells meet, the cell walls are absent or discontinuous and the plasma membranes of these cells are in direct contact with each other (Mansfield et al., 1991; Kasahara et al., 2005). The absent cell walls in this region provide direct access of the sperm cells to the fertilization targets because the pollen tube releases its two sperm cells into one of the synergid cells (discussed below).

The synergid cell wall is further specialized (Figure 1C). At the micropylar pole, the synergid cell wall is thickened and extensively invaginated, forming a structure referred to as the filiform apparatus. The filiform apparatus greatly increases the surface area of the plasma membrane in this region and contains a high concentration of secretory organelles, suggesting that it may facilitate transport of substances into and out of the synergid cells. Based on cytological staining properties in species other than Arabidopsis, the filiform apparatus appears to be composed of a number of substances including cellulose, hemicellulose, pectin, callose, and protein. The filiform apparatus has at least two functions associated with the fertilization process. First, the synergid cells secrete pollen tube attractants via the filiform apparatus (discussed below). In addition, the pollen tube enters the synergid cell by growing through the filiform apparatus, suggesting that the filiform apparatus is important for pollen tube reception (Maheshwari, 1950; Willemse and van Went, 1984; Huang and Russell, 1992; Punwani and Drews, 2008).

By contrast, the antipodal cells in Arabidopsis have no dramatic specializations and no known function. In other species, the antipodal cells contain finger-like cell wall projections resembling the filiform apparatus (Maheshwari, 1950; Willemse and van Went, 1984; Huang and Russell, 1992). In cereals, the antipodal cells proliferate into as many as 100 cells (Diboll and Larson, 1966; Maeda and Miyake, 1997). These observations suggest that the antipodal cells indeed have a function and that they may function as tranfer cells, transporting substances from the surrounding ovule cells into the female gametophyte.

Female Gametophyte Polarity

Figure 1 shows that the ovule and female gametophyte are polarized structures. The ovule's micropylar pole is the end at which the integuments form a pore, and its chalazal pole is the end that joins the funiculus. Within the female gametophyte, the egg and synergid cells occupy the micropylar pole and the antipodal cells lie at the chalazal pole. This polarity is important for fertilization because the pollen tube reaches the female gametophyte by growing through the micropyle (discussed below).

This polarity is apparent throughout female gametophyte development. The megaspore mother cell's cytoplasm is polarized along the chalazal-micropylar axis (Webb and Gunning, 1990; Bajon et al., 1999). During megaspore selection, the chalazal-most megaspore survives, whereas the other three undergo cell death (Figure 2B). During cell differentiation, the three cells at the micropylar end develop into the egg and the two synergid cells, whereas those at the chalazal end develop into three antipodal cells (Figure 3). Furthermore, as discussed above, the individual cells are polarized at the sub-cellular level (Maheshwari, 1950; Willemse and van Went, 1984; Huang and Russell, 1992).

Female gametophyte polarity corresponds to the overall polarity of the ovule, suggesting that this polarity is regulated by the surrounding sporophytic tissue. As discussed below, it has recently been found that auxin gradients established by the surrounding sporophytic tissue are critical for establishing the asymmetric structure of the female gametophyte in Arabidopsis (Pagnussat et al., 2009; Bencivenga et al., 2011).

ALTERNATIVE PATTERNS OF FEMALE GAMETOPHYTE DEVELOPMENT

Although the *Polygonum*-type pattern discussed above is the most common gametophyte form, angiosperms exhibit many additional patterns of female gametophyte development. These different developmental patterns in sexual species arise due to variation in both megasporogenesis and megagametogenesis. Gametophyte development in apomictic species omits steps evident in the sexual pathway. Comprehensive discussions of the variation in female gametophyte structure in sexual species can be found in several reviews (Maheshwari, 1950; Willemse and van Went, 1984; Huang and Russell, 1992).

Variation in the formation of meiotically reduced female gametophytes

In Arabidopsis and most other species, the archesporial cell develops directly into the megaspore mother cell. In some flowering plants, the archesporial cell enlarges and undergoes a periclinal division, and subsequently the inner cell differentiates into the megaspore mother cell (Maheshwari, 1950; Willemse and van Went, 1984; Huang and Russell, 1992).

In Arabidopsis and most other species, one meiotic product contributes to formation of the mature female gametophyte and this pattern is referred to as monosporic. Angiosperms exhibit two other megasporogenesis patterns referred to as bisporic and tetrasporic. In the bisporic pattern, cell plates form following meiosis I but not meiosis II and one of the two-nucleate megaspores degenerates, resulting in a single functional megaspore. In the tetrasporic pattern, cell plates fail to form following both meiotic divisions, resulting in one four-nucleate megaspore (Maheshwari, 1950; Willemse and van Went, 1984; Haig, 1990; Huang and Russell, 1992).

Angiosperm species also exhibit significant variation in megagametogenesis. Most species undergo the same general pattern described above for Arabidopsis: a phase of nuclear proliferation without cytokinesis followed by cellularization and differentiation. Variation arises due to the number of nuclei within the megaspore that gives rise to the female gametophyte (i.e., the type of megasporogenesis), the number of mitoses prior to cellularization, the timing of fusion of the polar nuclei, and whether or not additional mitoses occur after cellularization. (Maheshwari, 1950; Willemse and van Went, 1984; Haig, 1990; Huang and Russell, 1992; Yadegari and Drews, 2004). For example, in maize, which has a *Polygonum*-type female gametophyte, the polar nuclei do not fuse until fertilization and the antipodal cells proliferate into 40 or more cells (Diboll and Larson, 1966; Diboll, 1968).

Formation of meiotically unreduced female gametophytes during gametophytic apomixis

Angiosperms exhibit two mechanistically different forms of apomixis referred to as sporophytic and gametophytic. Sporophytic apomixis occurs in *Citrus* and mango and involves direct formation of an embryo from an ovule somatic cell adjacent to a developing embryo sac. Sporophytic apomixis bypasses female gametophyte formation and, thus, is not further discussed here.

Gametophytic apomixis involves formation of a meiotically unreduced (i.e., diploid) female gametophyte. The egg cell then forms an embryo by parthenogenesis (i.e., without fertilization) and endosperm formation may be either autonomous (i.e., occurring without fertilization) or pseudogamous (i.e., occurring in response to fertilization of the central cell). Most apomictic species are facultative apomicts. In facultative apomicts, meiotically reduced gametophytes are formed at low frequency and these can be fertilized via the sexual route, giving rise to viable seed (Nogler, 1984; Koltunow, 1993; Koltunow et al., 1995).

Angiosperms exhibit two forms of gametophytic apomixis referred to as diplospory and apospory (Figure 4). Diplospory occurs, for example, in some *Boechera* species, which are relatives of Arabidopsis, and some *Tripsacum* species, which are relatives of maize. In diplospory (Figure 4B), the megaspore mother cell either undergoes an abortive meiosis that prevents meiotic reduction and recombination or directly undergoes megagametogenesis; in either case, this cell gives rise to an unreduced female gametophyte. In most diplosporous species, callose level and distribution in the megaspore mother cell differs from that in sexual relatives as the cell undergoes diplospory, indicating a possible mis-specification of this cell. For example, callose is absent in the megaspore mother cells of diplosporous *Tripsacum* (Bicknell and Koltunow, 2004).

Apospory occurs, for example, in *Hieracium* subgenus *Pilosella* species, relatives of sunflower, and grass genera such as *Pennisetum* and *Brachiaria*. In apospory (Figure 4C), a megaspore mother cell forms and initiates meiosis. In parallel, somatic cells of the ovule, termed aposporous initials (AIs), enlarge near developing megaspores and form unreduced embryo sacs. In some aposporous species (e.g., *Hieracium* subgenus *Pilosella,* and *Pennisetum*), the adjacent sexual megaspores degenerate during aposporous embryo sac formation. In other species, sexual female gametophyte development is not affected, and meiotically reduced and unreduced aposporous gametophytes co-exist in the same ovule. In contrast to the sexual megaspore mother cell, *Hieracium* AIs lack callose in their cells walls (Bicknell and Koltunow, 2004) and do not express a marker gene that is expressed in the Arabidopsis megaspore mother cell (Tucker et al., 2003). Marker genes for female gametophyte, embryo, and endosperm development show a similar expression pattern in sexual and aposporous *Hieracium*. These observations suggest that AI cells bypass megasporogenic events and embark on a megametogenic program where fertilization is also bypassed to give rise to viable seeds (Tucker et al., 2003).

Polygonum-type embryo sac formation is common in gametophytic apomicts. However, they can deviate from this pattern.

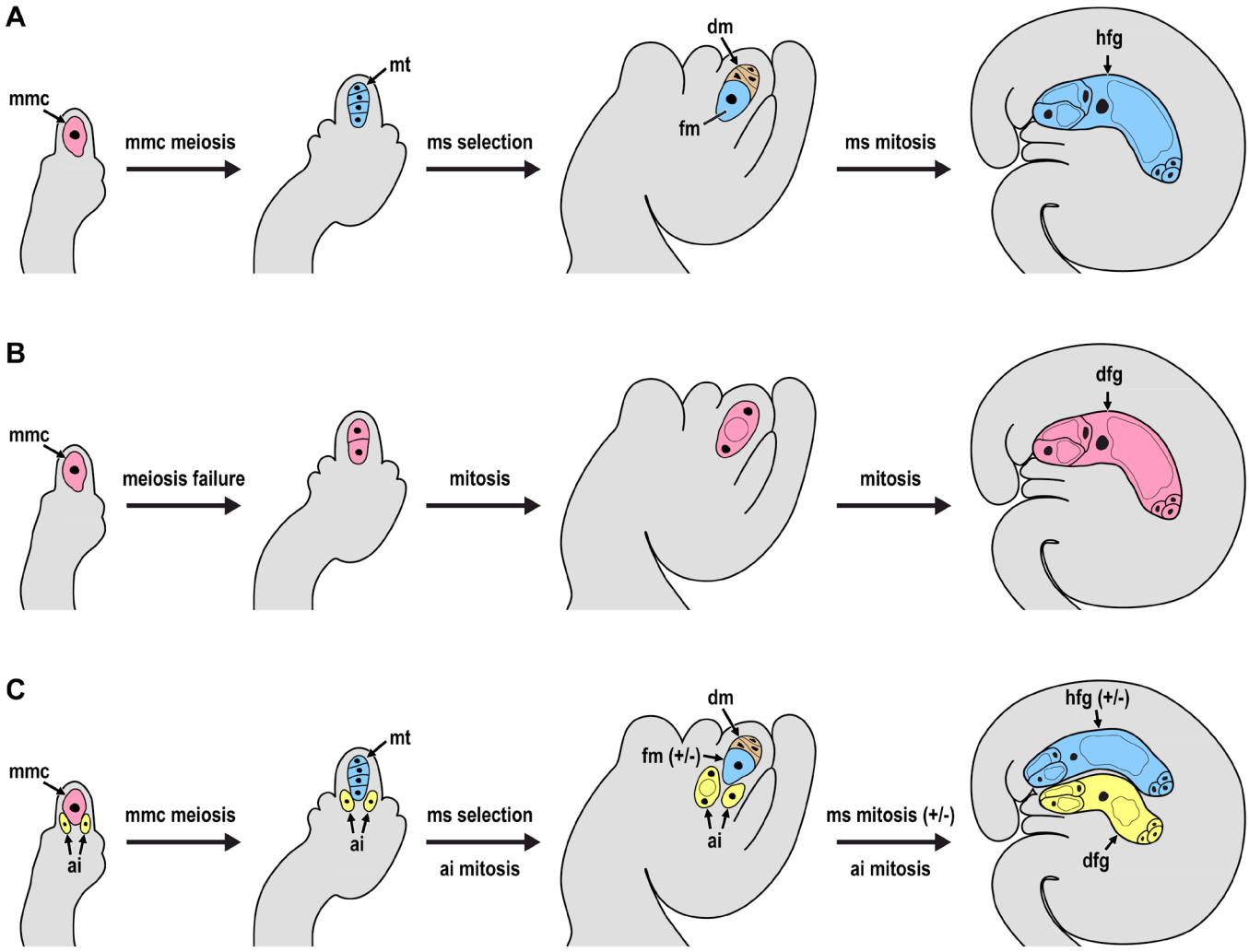

Figure 4. Female gametophyte development in diplosporous and aposporous apomicts compared with Arabidopsis.

(A) Steps of megasporogenesis and megagametogenesis in Arabidopsis ovules. Red and blue colors represent diploid and haploid cells, respectively.

(B) Steps in diplosporous female gametophyte development. The megaspore mother cell enters meiosis and the process fails with the resultant diploid cell undergoing mitosis to form a diploid female gametophyte (red). Alternatively, the megaspore mother cell may directly undergo mitosis to form the diploid female gametophyte. In the latter it is unclear if the cell initiating diplospory is a functional megaspore mother cell.

(C) Steps in aposporous female gametophyte development. Megaspore mother cell differentiatiation (red) occurs and it can undergo megasporogenesis and megagametogenesis to form a haploid female gametophyte (blue). Diploid aposporous initial cells differentiate during the events of megasporogenesis close to sexually programmed cells and undergo mitosis forming a diploid female gametophyte (yellow). Aposporous initial cell formation begins at different times in different apomictic species, either soon after megaspore mother cell formation, during meiotic tetrad development or functional megaspore selection. In some species, both haploid and aposporous gametophytes can co-exist in ovules while in others the sexual pathway terminates, usually during early mitotic divisions of the aposporous initial cell. Abbreviations: ai, aposporous initial cells; dfg, diploid female gametophyte; dm, degenerating megaspores; fm, functional megaspore; hfg, haploid female gametophyte; mmc, megaspore mother cell; mt, meiotic tetrad; (+/-), may be present or absent.

Both mitosis and cell specification seem to be perturbed in some apomicts. For example, only two rounds of mitosis occur in the AI cells of aposporous *Pennisetum*, leading to a four-nucleate embryo sac, commonly containing an egg cell, one polar nucleus and two synergid cells. In aposporous *Hieracium* species, antipodals may not form and multiple embryos may develop in an embryo sac (Koltunow et al., 2000; Koltunow et al., 2011b).

FERTILIZATION IN ANGIOSPERMS

Many aspects of the fertilization process in Arabidopsis have been characterized. The pollen tube growth pathway, from stigma to ovule, was described using light microscopy and transmission electron microscopy (Pruitt et al., 1993; Kandasamy et al., 1994; Hulskamp et al., 1995; Lennon et al., 1998). Pollen tube growth

within the female gametophyte was characterized using transmission electron microscopy and real-time imaging (Rotman et al., 2003; Palanivelu and Preuss, 2006; Sandaklie-Nikolova et al., 2007). Finally, the events following pollen rupture were observed using high-resolution time-lapse imaging (Hamamura et al., 2011). These observations are summarized below.

Soon after pollination, the Arabidopsis male gametophyte becomes hydrated and then germinates a pollen tube. The pollen tube initially penetrates and grows through the intercellular spaces between the papillar cells of the stigma and then grows through the transmitting tract of the carpel's style and ovary. The pollen tube then emerges from the transmitting tract and grows along the surface of the placenta toward an ovule. Upon reaching an ovule, the pollen tube grows along the surface of the ovule's funiculus, through the micropyle, and into the female gametophyte (Pruitt et al., 1993; Kandasamy et al., 1994; Hulskamp et al., 1995; Lennon et al., 1998).

The Arabidopsis pollen tube enters the female gametophyte by growing through the filiform apparatus of the synergid cells (Figure 1C). The pollen tube then comes in contact with the synergid cells and ceases growth. One of the synergid cells then undergoes cell death. Finally, soon after synergid degeneration is initiated, the pollen tube ruptures and releases its contents (i.e., the two sperm cells, vegetative nucleus, and pollen cytoplasm) into the degenerating synergid cytoplasm (Rotman et al., 2003; Sandaklie-Nikolova et al., 2007). The pollen tube ruptures within 20 seconds after entering the female gametophyte (Rotman et al., 2003).

Real-time imaging of pollen tube growth within the female gametophye has also been performed in *Torenia* (Higashiyama et al., 2000). Here, the pollen tube grows through the filiform apparatus and then between the two synergid cells. Immediately after contacting the synergid cells, the pollen tube ceases growth and discharges. This is followed by synergid degeneration, possibly due to explosive pollen tube discharge (Higashiyama et al., 2000).

Following pollen tube discharge in Arabidopsis, the two sperm cells move rapidly (within 10 seconds) to the chalazal-most region of the degenerated synergid cell, in the area between the egg cell and the central cell. Sperm movement, most likely, is propelled by cytoplasmic flow ejected from the pollen tube. The two sperm cells then are immobile in that region for approximately seven minutes. The two sperm cells then move toward and fuse with the egg cell and central cell. Each of the sperm cells is able to fertilize either female gamete, indicating that the two sperm cells are functionally equivalent (Ingouff et al., 2009; Hamamura et al., 2011).

IDENTIFICATION OF FEMALE GAMETOPHYTE MUTANTS

Segregation of female gametophyte mutations

Because of their alternation of generation life cycle, plants possess two broad classes of mutations: sporophytic and gametophytic mutations, which affect the diploid and haploid phases of the plant life cycle, respectively. Gametophytic mutations can affect the female and/or male gametophyte, giving rise to three classes of gametophytic mutations referred to as female gametophyte-specific, which affect the female gametophyte but not the male gametophyte, male gametophyte specific, which affect the male gametophyte but not the female gametophyte, and general gametophytic, which affect both gametophytes.

Sporophytic and gametophytic mutations exhibit fundamentally different segregation patterns that are summarized in Table 1. Sporophytic mutations typically exhibit Mendelian segregation patterns. By contrast, gametophytic mutations exhibit altered segregation patterns due to gametophytic lethality. For example, female gametophyte-specific mutations transmit through the male gametophyte but not the female gametophyte and, as a consequence, cannot become homozygous in the sporophyte generation and transmit from generation-to-generation as heterozygotes (Table 1). Mutations affecting both gametophytes (general gametophytic mutations) cannot transmit to subsequent generations unless partially penetrant (Table 1).

Identification of Female Gametophyte Mutants

Female gametophyte mutants have been identified using the criteria of reduced seed set and/or segregation distortion. The siliques of heterozygous female gametophyte mutants exhibit reduced seed set because 50% of the female gametophytes are mutant and nonfunctional. The ovules containing the nonfunctional female gametophytes fail to become fertilized and/or undergo seed development; these eventually desiccate and degenerate. Thus, the siliques of heterozygous female gametophyte mutants contain only half the normal number of seeds.

Female gametophyte mutations exhibit segregation distortion because they fail to transmit through the female gametophyte (Table 1). Segregation analysis can be facilitated by mutagenesis with transposons or T-DNAs containing an antibiotic- or herbicide-resistance gene**.** Resistant:sensitive (R:S) ratios then can be used to rapidly identify lines containing a female gametophyte mutation. For example, in the progeny of a self-pollinated heterozygous plant, the R:S ratio is 1:1 for lines with female gametophyte-specific mutations, as compared to 3:1 for most lines with sporophytic mutations (Table 1; Feldmann et al., 1997).

One disadvantage of insertional mutagenesis in female gametophyte mutant screens is that chromosomal rearrangements can result and occur at a frequency of 10-20% (Curtis et al., 2009; Clark and Krysan, 2010). Some chromosomal rearrangements (e.g., reciprocal translocations or large inversions) can cause a condition referred to as semi-sterility (Belling, 1914; Blakeslee and Cartledge, 1926; Brink, 1927; Brink and Burnham, 1929; Burnham, 1930; Ray et al., 1997). In semi-sterile lines, half of the meiotic products (i.e., the male and female gametophytes) are defective, resulting in reduced seed set, which is one of the criteria used to identify female gametophyte mutants.

Male gametophyte mutations segregate in a pattern similar to that of female gametophyte mutations (Table 1). Whether a mutation affects the female gametophyte or male gametophyte can be resolved using two criteria. First, male gametophyte mutants generally do not result in reduced seed set because pollen is not usually limiting. Second, as summarized in Table 2, female gametophyte and male gametophyte mutations segregate differently in crosses of heterozygous females with wild-type males.

In addition to male gametophyte mutations, several sporophytic-mutant classes segregate in a pattern similar to that of female gametophyte mutations, even in crosses of heterozygous females with wild-type males. As summarized in Tables 1 and 2, these classes include mutations affecting paternally imprinted genes

Table 1. Segregation of gametophytic and sporophytic mutations in a self-cross of a heterozygous individual ("A/a" x "A/a")

	Functional gametes			
Mutation Class	MG[a]	FG[b]	Progeny Genotypes[c]	R/S[d]
Gametophytic				
FG specific	A & a	A	A/A, A/a	1.0
MG specific	A	A & a	A/A, A/a	1.0
General gametophytic	A	A	A/A	0
Sporophytic				
Typical (not lethal)	A & a	A & a	A/A, A/a, a/A, a/a	3.0
Embryo defective	A & a	A & a	A/A, A/a, a/A, a/a*	2.0
Endosperm defective	A & a	A/A & a/a	A/A/A, A/a/a, a/A/A, a/a/a*	2.0
Haplo-insufficient endosperm defective	A & a	A/A & a/a	A/A/A, A/a/a*, a/A/A, a/a/a*	1.0
Paternally imprinted embryo defective	A & a	A & a	A/A, A/a*, a/A, a/a*	1.0
Paternally imprinted endosperm defective	A & a	A/A & a/a	A/A/A, A/a/a*, a/A/A, a/a/a*	1.0
Maternally imprinted embryo defective	A & a	A & a	A/A, A/a, a/A*, a/a*	1.0
Maternally imprinted endosperm defective	A & a	A/A & a/a	A/A/A, A/a/a, a/A/A*, a/a/a*	1.0

Abbreviations: MG, male gametophyte; FG, female gametophyte.
[a]Genotypes of the sperm cells. Underlined alleles are imprinted.
[b]Genotypes are of the egg cell except for endosperm-defective mutations, in which the genotypes are of the homodiploid central cell. Underlined alleles are imprinted.
[c]Genotypes are of the embryo except for endosperm-defective mutations, in which the genotypes are of the triploid endosperm. Genotypes with asterisks are lethal. Underlined alleles are imprinted and inactive.
[d]Seedling resistance ratios if the mutation is caused by a T-DNA or transposon that carries an antibiotic/herbicide resistance gene.

required for seed development and haplo-insufficient endosperm-defective mutations. Because of these similarities, one must be cautious in concluding that a given mutation affects the female gametophyte based on genetic analysis alone. In practice, additional criteria must be applied to definitively conclude that a mutation affects the female gametophyte. For example, microscopic analysis showing abnormal embryo sac structure clearly demonstrates an effect on the female gametophyte. However, even this criterion becomes ambiguous in cases where female gametophyte structure appears to be unaffected (e.g., gametophytic maternal-effect mutants, discussed below); in these cases, molecular analysis such as expression analysis of the affected gene must be carried out.

Female gametophyte mutants

Large-scale screens for female gametophyte mutants have been performed by many groups (Feldmann et al., 1997; Bonhomme et al., 1998; Christensen et al., 1998; Howden et al., 1998; Christensen et al., 2002; Pagnussat et al., 2005; Brukhin et al., 2011). In these screens, mutant frequency generally was 0.5-1.0% and most mutants also exhibited defects in the male gametophyte (Yadegari and Drews, 2004; Pagnussat et al., 2005).

Collectively, these screens have identified hundreds of female gametophyte mutants and phenotypic analysis of these mutants has revealed genes required for each step of megagametogenesis (Yadegari and Drews, 2004; Pagnussat et al., 2005). Most of the identified genes mediate essential functions. However, mutant screens have been successful in identifying regulatory genes important for cell specification, fertilization, and the inhibition of endosperm development (discussed below).

IDENTIFICATION OF FEMALE GAMETOPHYTE-EXPRESSED GENES

Genes functioning in the female gametophyte also have been identified using reverse-genetics approaches in which female gametophyte-expressed genes were identified. In *Arabidopsis*, most of these genes were identified through three main approaches. First, several groups performed differential-expression screens using wild-type ovules and mutant ovules lacking female gametophytes. Collectively, these screens identified >1,000 genes exhibiting reduced expression in mutant ovules and potentially expressed in the female gametophyte. Expression within the female gametophyte was confirmed with 75 genes using *in situ* hybridization or through analysis of transgenic plants containing promoter::reporter constructs (Kasahara et al., 2005; Yu et al., 2005; Johnston et al., 2007; Steffen et al., 2007; Steffen et al., 2008; Wang et al., 2011). Second, laser-capture microdissection (LCM) followed by microarray hybridization identified genes expressed in individual female gametophyte cell types (Wuest et al., 2010). Finally, gene family analysis has identified many additional transcription factor genes expressed in the female gametophyte including 28 Type I MADS-box genes (Bemer et al., 2010), two WOX genes (Haecker et al., 2004), and three Class IV Homeodomain Leucine Zipper (HD-ZIP) genes (Nakamura et al., 2006).

Table 2. Segregation of gametophytic and sporophytic mutations in a cross of a heterozygous female ("A/a") with a wild-type ("A/A") male

	Functional gametes			
Mutation Class	MG[a]	FG[b]	Progeny Genotypes[c]	R/S[d]
Gametophytic				
FG specific	A	A	A/A	0
MG specific	A	A & a	A/A, A/a	1.0
Sporophytic				
Haplo-insufficient endosperm defective	A	A/A & a/a	A/A/A, A/a/a*	0
Paternally imprinted embryo defective	A	A & a	A/A, A/a*	0
Paternally imprinted endosperm defective	A	A/A & a/a	A/A/A, A/a/a*	0
Maternally imprinted embryo defective	A	A & a	A/A, A/a	1.0
Maternally imprinted endosperm defective	A	A/A & a/a	A/A/A, A/a/a	1.0

Abbreviations: MG, male gametophyte; FG, female gametophyte.
[a]Genotypes of the sperm cells. Underlined alleles are imprinted.
[b]Genotypes are of the egg cell except for endosperm-defective mutations, in which the genotypes are of the homodiploid central cell. Underlined alleles are imprinted.
[c]Genotypes are of the embryo except for endosperm-defective mutations, in which the genotypes are of the triploid endosperm. Genotypes with asterisks are lethal. Underlined alleles are imprinted and inactive.
[d]Seedling resistance ratios if the mutation is caused by a T-DNA or transposon that carries an antibiotic/herbicide resistance gene.

An additional approach not yet reported in Arabidopsis is to generate cDNA libraries and EST collections from dissected mature female gametophytes or isolated live gametophyte cell types. The approach has been used in other species such as maize (Dresselhaus et al., 1994; Cordts et al., 2001; Dresselhaus et al., 2005; Le et al., 2005; Marton et al., 2005; Yang et al., 2006), barley (Vrinten et al., 1999), *Torenia* (Okuda et al., 2009), rice (Ohnishi et al.), and wheat (Sprunck et al., 2005).

Many of these approaches are also being used to characterize the transcriptomes of apomictic embryo sacs. Dissection procedures to isolate female gametophytes have now been developed. In addition, the application of laser capture microdissection and transcriptome sequencing to examine gene expression in cells undergoing apospory is underway in *Hieracium* (Koltunow et al., 2011a).

Collectively, these studies have identified large collections of genes expressed specifically in each of the female gametophyte's cell types. These genes provide a rich collection of markers for analysis of female gametophyte development and function. Functional analysis has not been performed with most of these genes. However, functional analysis of a few of these genes has already identified regulatory genes important for cell differentiation during megagametogenesis and pollen tube attraction by the mature female gametophyte.

REGULATION OF MEGASPOROGENESIS

Megasporogenesis comprises the sequential events of megaspore mother cell differentiation, meiosis, and megaspore selection. Many genes have been identified that influence these steps. This phase of female gametophyte development is under sporophytic control, as the identified mutants discussed below are all sporophytic in action. Most of the mutants also affect microsporogenesis, but the discussion below considers only their roles in female spore formation.

Establishment of megaspore mother cell fate

In Arabidopsis and most other species, the megaspore mother cell develops from a sub-epidermal cell within a young ovule primordium (Figure 2A). Most ovule primordia contain just a single megaspore mother cell (Figure 2). However, ~5-6% of Arabidopsis ovules form additional enlarged cells (Olmedo-Monfil et al., 2010; Armenta-Medina et al., 2011). These enlarged cells resemble megaspore mother cells, but markers have not been utilized to establish cell identity. In ovules containing multiple enlarged cells, only one cell undergoes the subsequent events of megasporogenesis and megagametogenesis (Armenta-Medina et al., 2011). The observation that several cells within the pre-meiotic ovule may form megaspore mother-like cells suggests a model in which megaspore mother cell fate is established in response to local signaling events (Sheridan et al., 1996).

Recent studies in Arabidopsis, rice, and maize have identified a group of genes that promote megaspore mother cell development. The genes identified include the Arabidopsis *NOZZLE/SPOROCYTELESS* (*NZZ/SPL*; Schiefthaler et al., 1999; Yang et al., 1999), *WUSCHEL* (*WUS*), *WINDHOSE1* (*WH1*), *WH2*, and *TORNADO2* (*TRN2*) genes (Lieber et al., 2011); the rice *MEIOSIS ARRESTED AT LEPTOTENE* (*MEL1*) gene (Nonomura et al., 2007); and the maize *ARGONAUTE104* (*AGO104*) gene (Singh et al., 2011).

In Arabidopsis *nzz/spl* mutants, most ovules do not form megaspore mother cells, suggesting that *NZZ/SPL* is required to specify megaspore mother cell fate (Schiefthaler et al., 1999; Yang et al., 1999). However, *NZZ/SPL* is also required for proximal-distal pattern formation within the ovule, suggesting that the

megaspore mother cell defect may be a secondary consequence of a failure to form the distal ovule region (Schiefthaler et al., 1999; Balasubramanian and Schneitz, 2000). *NZZ/SPL* encodes a nuclear protein with some similarity to MADS-box transcription factors (Yang et al., 1999) and is expressed in a range of ovule tissues including the megaspore mother cell. NZZ/SPL also acts to repress expression of *YUCCA* genes that are required for auxin biosynthesis (Li et al., 2008), suggesting a link between auxin and megaspore mother cell fate during ovule development.

WUS is a stem cell regulator in the shoot meristem that also functions in the ovule downstream of *NZZ/SPL*. Megaspore mother cell development is defective in *wus* ovules and *WUS* expression in the nucellus is required to activate *WH1* and *WH2*. *WH1* and *WH2* encode small peptides that are required together with the tetraspanin-type protein TRN2 for megaspore mother cell function and megasporogenesis. Thus *NZZ*, *WUS*, *WH1*, *WH2*, and *TRN2* define a pathway promoting female gametophyte formation from somatic precursor cells in Arabidopsis (Lieber et al., 2011).

Rice *mel1* mutants form megaspore mother cells, but these either fail to undergo meiosis or arrest at various stages in meiosis. This leads to absence of female gametophytes in some ovules. *MEL1* encodes a member of the ARGONAUTE (AGO) protein family with highest similarity to Arabidopsis AGO1, which is required for miRNA-directed mRNA cleavage. *MEL1* is first expressed in the sub-epidermal cells in ovule primordia during archesporial cell differentiation. Later, expression becomes restricted to the megaspore mother cell. Expression then declines and is not evident at meiosis. These observations suggest that *MEL1* is required in the megaspore mother cell for functional megaspore mother cell formation. It may be involved in cleaving mRNAs that function to suppress somatic gene expression or meiosis to enable a gametophytic fate (Nonomura et al., 2007).

Maize *ago104* loss-of-function mutants are dominant. In *ago104* mutants, megaspore mother cells form normally, but these fail to undergo meiosis and instead undergo mitosis and megagametogenesis. This leads to the formation of functional unreduced (diploid) embryo sacs. Maize *AGO104* encodes an AGO protein that falls within a different clade than rice MEL1 (discussed above) but within the same clade as Arabidopsis AGO9 (discussed below). This AGO clade is important for heterochromatin silencing and AGO104 appears to perform this function within young ovule primordia. AGO104 protein is present in the sub-epidermal cells surrounding the developing megaspore mother cell but is not present in the megaspore mother cell. These observations suggest that the surrounding cells produce an AGO104-dependent mobile signal influencing megaspore mother cell differentiation and/or behavior. This mobile signal may function to either promote meiosis or repress somatic cell fate in the megaspore mother cell (Singh et al., 2011).

Inhibition of megaspore mother cell formation during early ovule development

The formation of multiple megaspore mother cells is generally a rare event in sexually reproducing species. Genes that play a role in restricting the number of megaspore mother cells to just one per ovule include the maize *MULTIPLE ARCHESPORIAL CELLS1* (*MAC1*) gene (Sheridan et al., 1996; Sheridan et al., 1999), and the rice *MULTIPLE SPOROCYTE1* (*MSP1*; Nonomura et al., 2003) and *OsTDL1A* (Zhao et al., 2008) genes.

Downregulation of these three genes by mutation (*mac1* and *msp1*) or RNAi (*OsTDL1A*) produces ovules with multiple megaspore mother cells that that are derived from sub-epidermal cells. The multiple megaspore mother cells can undergo meiosis and megagametogenesis, resulting in ovules with multiple reduced female gametophytes. The *mac1* and *msp1* mutants are partially female fertile, indicating that at least some of these female gametophytes are functional (Sheridan et al., 1996; Nonomura et al., 2003). Some *msp1* seeds contain multiple embryos (Nonomura et al., 2003).

The identity of maize *MAC1* is unknown. Rice *MSP1* encodes a leucine-rich repeat receptor-like kinase (RLK; Nonomura et al., 2003) and *OsTDL1A* encodes a putative ligand for MSP1 (Zhao et al., 2008). OsTDL1A and MSP1 interact in yeast two-hybrid and bimolecular fluorescence complementation (BiFC) assays. *MSP1* and *OsTDL1A* are both expressed in pre-meiotic ovules, in all cells except the megaspore mother cell. These observations suggest that MSP1 and OsTDL1A may be part of a pathway that establishes or maintains somatic cell fate (or inhibits megaspore mother cell fate) during early ovule development.

Inhibition of megaspore formation during early ovule development

Megaspores normally arise from meiosis of the megaspore mother cell (Figure 2). Recent genetic analysis suggests that the formation of megaspores from somatic cells in pre-meiotic ovules is actively repressed by a group of genes that includes *ARGONAUTE9* (*AGO9*), *RNA-DEPENDENT RNA POLYMERASE6* (*RDR6*), and *SUPPRESSOR OF GENE SILENCING3* (*SGS3*) from Arabidopsis (Olmedo-Monfil et al., 2010), and *DOMAINS REARRANGED METHYLTRANSFERASE103* (*DMT103*) from maize (Garcia-Aguilar et al., 2010).

Loss-of-function *ago9* mutants are dominant. Arabidopsis *ago9*, *rdr6*, and *sgs3* mutants have similar ovule phenotypes. All form multiple megaspore-like cells in the pre-meiotic ovule in addition to a megaspore mother cell. The megaspore mother cell contains callose in its walls and is functional; that is, it undergoes megasporogenesis and megagametogenesis and the resulting embryo sacs can be fertilized, producing viable seed. The additional enlarged cells do not appear to be megaspore mother cells, as they do not undergo meiosis and do not contain callose. These enlarged cells express an Arabidopsis functional megaspore marker, suggesting that they may be programmed as functional megaspores. Multiple embryo sacs appear to form in some ovules but functional unreduced embryo sacs have not been reported in these mutants (Olmedo-Monfil et al., 2010).

Arabidopsis *AGO9* encodes an AGO protein within the same AGO clade as maize AGO104, which is discussed above (Olmedo-Monfil et al., 2010). This clade is important for heterochromatin silencing and RNA-directed DNA methylation (RdDM). AGO9 preferentially interacts with 24-nt small RNAs derived from transposable elements (TEs) and is required for transposable element silencing in the female gametophyte, particularly the egg appa-

ratus prior to fertilization. RDR6 and SGS3 are required for the biogenesis of trans-acting siRNAs (tasiRNAs), which can move to adjacent cells and cause gene silencing at distant sites. Localization of RDR6 and SGS3 within young ovules has not been characterized. AGO9 protein is present only in the external L1 epidermal cells of the ovule primordium adjacent to the sub-epidermal cells that give rise to the megaspore mother cell. These observations suggest that AGO9, RDR6, and SGS3 are necessary to produce a mobile signal in the L1 epidermal cells that moves to the sub-epidermal cells, where it restricts megaspore fate (Olmedo-Monfil et al., 2010).

Maize *DMT103* encodes a DOMAINS REARRANGED METHYLTRANSFERASE, which is involved in DNA methylation. It is expressed in ovules in a restricted zone in and around the specified megaspore mother cell. When *DMT103* activity is downregulated using RNAi, additional cells enlarge near the megaspore mother cell and their nuclei undergo mitosis similar to that found in *ago 9* mutants discussed above. The megaspore-like cells also appear to arise at later stages. The additional embryo sacs that develop near the sexually programmed cells are not functional. Progression of the megaspore mother cell through subsequent events of female gametophyte formation is not apparently impeded as fertile seeds form after fertilization (Garcia-Aguilar et al., 2010). The DNA methylation pathway involving DM103 appears to play a role in regulating competency of cells to undergo gametophyte formation and in the restriction of cell fate in the ovule so that a single gametophyte forms. It may also function in regulatory processes determining decisions to form reduced or unreduced gametophytes in maize.

Regulation of meiosis

Most of the known genes required for meiosis in plants exhibit a high degree of conservation compared with those in animals and their roles in meiosis have been reviewed (Mercier and Grelon, 2008). Most meiosis mutations in plants impact upon both male and female spore formation, resulting in both male and female sterility. However, some meiotic mutations lead to viable unreduced gametophytes and these are discussed in the section on apomixis.

Regulation of megaspore selection

In the normal course of events following meiosis in *Polygonum*-type embryo sac formation, one of the megaspores is selected to undergo megagametogenesis and the other three megaspores degenerate (Figure 2B). A number of processes influence megaspore degeneration. The Arabidopsis *ANTIKEVORKIAN* (*AKV*) gene is involved in regulating megaspore survival, as 10% of *akv* mutant ovules contain all four megaspores (Yang and Sundaresan, 2000). The identity of *AKV* is unknown. In rice ovules, the S5 aspartic protease may regulate megaspore fate because it is expressed in megaspores and cells adjacent to megaspores. Loss-of-function mutations give rise to reduced female fertility (Chen et al., 2008). Changes in Ca^{2+} concentration have been observed during megaspore degeneration and within the functional megaspore (Qiu et al., 2008). Changes in Ca^{2+} concentration are implicated in programmed cell death in plant and animal cells (Yamaguchi et al., 1999; Canzoniero et al., 2004). Finally, loss of callose is associated with the selected megaspore but not the degenerating megaspores (discussed above). The roles of both Ca^{2+} and callose in megaspore selection, if any, remain unclear.

Summary

Signaling from somatic cells in premeiotic ovules plays a major role in the promotion and restriction of cells that can undergo female gametophyte development. These signaling pathways involve both genetic and epigenetic networks (see also Armenta-Medina et al., 2011; Bencivenga et al., 2011). Disruption of these pathways can result in failure of megaspore mother cell formation, or alternatively, in the development of multiple female gametophyte precursor cells. Their disruption can also lead to a change in megaspore mother cell fate so that it skips meiosis and undergoes mitosis developing into a diploid embryo sac. Orchestration of genetic and epigenetic pathways appears to be essential for restricting the number of gametophyte precursor cells that form, so that a single female gametophyte develops in the angiosperm ovule. These networks also appear to be important in regulating the sequential progression of female gametophyte formation so that megasporogenesis follows megagametogenesis.

MOLECULAR ANALYSIS OF APOMIXIS

Hybrids resulting from the crossing of two parental plant lines often exhibit hybrid vigor, which can lead to large increases in yield in crops such as maize and rice. Hybrid vigor is not stable and declines in subsequent generations due to allele segregation during sexual reproduction. Apomixis could potentially fix hybrid vigor because it produces clonal seed. Potential benefits of apomixis include decreased cost of hybrid seed production, rapid fixation of complex genotypes, rapid development of new cultivars, and decreased loss in crop failure arising from poor pollination. Although apomixis occurs in wild species and in some forage and fruit crops, it is absent in vegetable, cereal and grain crops that significantly contribute to the world food supply (Koltunow et al., 1995).

Two complementary approaches are being used with the goal of introducing apomixis into crops. One is to identify the molecular basis of apomixis in wild species by isolating causal genes. The second is to identify and analyze apomixis-like mutants in sexual model organisms including Arabidopsis, maize, and rice. Studies in these model organisms are also directed toward building functional apomixis in sexual species by diverting the sexual pathway to an apomictic mode of reproduction. Information obtained from both strategies should lead to a biotechnological solution for the introduction of apomixis in crops.

Control of gametophytic apomixis

Genetic segregation analysis of the apomixis trait has been carried out in many species. Such studies have shown that apomixis is controlled by one to five dominant loci. In some species, the loci controlling the three key components of apomixis, meiotic avoid-

ance during gametophyte formation and autonomous embryo and endosperm formation (if the latter occurs), are encoded by a single locus. In other species the components segregate and additional loci influence the process. Gene identification has been hindered in some species because the identified loci are often associated with large regions where recombination is suppressed (Ozias-Akins and van Dijk, 2007).

An example of gametophytic apomicts under study are *Hieracium* subgenus *Pilosella* species that undergo aposporous apomixis (Figure 4C). Sexual megasporogenesis initiates and then somatic cells of the ovule, termed AIs, develop directly into female gametophytes (discussed above). During this process, the sexual pathway is suppressed. Embryo and endosperm formation within aposoporous gametophytes is fertilization-independent. In *Hieracium praealtum*, apomixis is controlled by two dominant loci that have been further dissected using deletion analysis. These analyses identified two loci referred to as *LOSS of APOMEIOSIS* (*LOA*) and *LOSS of PARTHENOGENESIS* (*LOP*). *LOA* activity requires the initiation of sexual megasporogenesis (Koltunow et al., 2011b). *LOA* functions sporophytically in the ovule and is required for both AI cell differentiation and suppression of the adjacent sexual pathway. Gametophytic expression of *LOP* in the aposporous embryo sac enables fertilization independent embryo and endosperm formation (Catanach et al., 2006; Koltunow et al., 2011b). These loci do not contain genes essential for sexual reproduction because their deletion results in a reversion to sexual reproduction. The sexual mode of reproduction is therefore the ground state in *H. praealtum* and the action of these loci suppresses sex. These loci presumably contain information to deregulate or alter an intact, default sexual pathway (Koltunow et al., 2011b). Given that most apomicts retain a capacity to form seeds via the sexual route, the role of apomixis loci deregulating a default sexual pathway may extend to apomictic species in general.

Apomixis-like mutants in model organisms

An alternative strategy to understanding apomixis is to identify apomixis-like mutants in sexual model organisms. This approach could lead to molecular insights into apomixis but also may be used to engineer apomixis in sexual species by combining mutations that induce an aspect of apomixis. Recent work in Arabidopsis, maize, and rice has identified several apomixis-like mutants and the corresponding genes. Here we focus on some mutants that give rise to viable unreduced gametophytes.

Several of the mutants discussed above exhibit apomixis-like phenotypes. In Arabidopsis *ago9*, *rdr6*, and *sgs3* mutants and the maize *dmt103* mutant, somatic cells of the ovule appear to adopt megaspore fate and develop into unreduced female gametophytes. This phenotype resembles AI cell formation and gametophyte development in aposporous apomicts. In maize *ago104* mutants, megaspore mother cells fail to undergo meiosis and instead undergo megagametogenesis and produce functional unreduced female gametophytes. This phenotype resembles diplospory in apomicts. However, in contrast to true apomicts, seed initiation requires fertilization (Garcia-Aguilar et al., 2010; Olmedo-Monfil et al., 2010; Singh et al., 2011).

Other mutants that produce apomixis-like phenotypes include the Arabidopsis *dyad/switch* (*swi*; Ravi et al., 2008) and maize *elongate* (*el*; Rhoades and Dempsey, 1966) mutants. In *dyad* mutants, the megaspore mother cell exhibits meiotic arrest but also produces a small percentage of viable unreduced female gametophytes resembling diplospory in apomicts (Ravi et al., 2008). The maize *el* mutant produces viable unreduced female and male gametophytes due to a failure of meiosis II (Rhoades and Dempsey, 1966). However, recombination occurs in *el* mutants and the egg cells are not identical in genotype to the mother plant (Barrell and Grossniklaus, 2005). The *EL* gene has not been identified. The *DYAD*/*SWI* gene encodes a meiosis-specific chromatin-associated protein and is expressed in female meiotic cells (Mercier et al., 2001).

An additional approach is to combine mutants in an effort to generate apomixis-like phenotypes. This has been achieved in Arabidopsis by generating a triple mutant that combines the absence of recombination (*Atspo11*), the absence of chromatid segregation (*Atrec8*), and avoidance of meiosis II (*osd1*). In this triple mutant, termed *MiMe*, the megaspore mother cell avoids meiosis and instead undergoes mitosis and gives rise to functional unreduced female gametophytes at very high frequency, resembling diplospory (d'Erfurth et al., 2009).

In a further study, *MiMe* was combined with a mutant line called *GEM*. *GEM* contains an altered centromere-specific histone H3 gene that enables paternal genome elimination after fertilization. Lines containing both *MiMe* and *GEM* produce seed progeny with a maternal genotype at low frequency (Ravi and Chan, 2010; Marimuthu et al., 2011). This system relies on crossing and fertilization to obtain progeny and, thus, the plants are not self-propagating like apomicts. However, this study provides proof of concept that manipulation of two to four genes in the sexual pathway controlling meiosis and chromosome segregation can give rise to clonal seed.

REGULATION OF MEGAGAMETOGENESIS

Many genes required for megagametogenesis have been identified. Comprehensive discussions of the roles such genes play during megagametogenesis have been reviewed recently (Kagi and Gross-Hardt, 2010; Liu et al., 2010; Yang et al., 2010; Sprunck and Gross-Hardt, 2011). Here, we focus on the factors that control overall female gametophyte pattern or differentiation of the female gametophyte cell types.

Regulation of female gametophyte polarity

The female gametophyte is structurally polarized along its chalazal-micropylar axis and this polarity corresponds to the overall polarity of the ovule. These observations suggest that female gametophyte polarity may be established by factors provided by the surrounding sporophytic cells. For example, a morphogen gradient provided by surrounding cells may specify nuclear fate according to position within the embryo sac coenocyte, as occurs during early development in *Drosophila* (Ephrussi and St Johnston, 2004).

Mutant analysis supports the idea that the developing female gametophyte contains a gradient of positional information. The Arabidopsis *retinoblastoma related* (*rbr*) and Maize *indeterminate*

gametophyte1 (*ig1*) mutants undergo extra rounds of nuclear divisions during megagametogenesis, resulting in supernumerary nuclei (Huang and Sheridan, 1996; Ebel et al., 2004; Guo et al., 2004; Ingouff et al., 2006). Arabidopsis *eostre* mutants have the normal number of nuclei but one of the micropylar nuclei has an abnormal position (Pagnussat et al., 2007). In all three mutants, the extra or abnormally positioned nuclei differentiate according to their positions in the embryo sac. For example, in *eostre* mutants at the eight-nucleate stage, one of the micropylar nuclei is abnormally positioned near the presumptive egg cell nucleus and this results in embryo sacs containing two egg cells (Pagnussat et al., 2007).

Recent studies in Arabidopsis suggest that an auxin gradient may provide positional information during megagametogenesis. During the one-nucleate stage (stage FG1), high auxin is detected exclusively in the sporophytic cells at the embryo sac's micropylar pole. As megagametogenesis progresses (stages FG2 to FG5), high auxin is detected within the embryo sac and is highest in the micropylar region. Manipulation of auxin levels or responses in the female gametophyte affects female cell fate: increased auxin levels (by expressing *YUCCA1* in the whole female gametophyte) causes the cells at the chalazal end to adopt micropylar identity, and reduction of auxin responses (by downregulation of *AUXIN RESPONSE FACTOR* expression) causes the synergid cells to adopt egg cell fate. These results suggest that auxin provides positional information within the developing female gametophyte and that the cells differentiate according to their position within this gradient: highest auxin leads to synergid cell fate and the lowest amount of auxin leads to antipodal cell fate (Pagnussat et al., 2009).

In this model, nuclear position at the time of cell/nuclear specification is an important aspect of cell specification. Notably, the manipulation of auxin levels or responses discussed above did not affect nuclear positioning. As shown in Figure 3, nuclear positioning during Arabidopsis megagametogenesis seems to involve nuclear migrations and positioning of the division planes. The mechanisms controlling these processed during megagametogenesis are not understood. However, in maize, a diSUMO-like protein called ZmDSUL that contains two head-to-tail SUMO-like domains is required for nuclei positioning and cell specification during female gametophyte maturation (Srilunchang et al., 2010).

Regulation of nuclear proliferation during megagametogenesis

Two aspects of the cell cycle are regulated during megagametogenesis. The first is the number of cell cycles, which is limited to three. The second is cytokinesis (cell wall formation). During the first two divisions, cykokinesis does not occur. The third division is accompanied by cell plate formation followed by complete cellularization (Figure 3B). The molecular processes controlling cytokinesis during megagametogenesis are not understood. However, several genes regulating the number of cell cycles have recently been characterized.

The extent of nuclear proliferation during megagametogenesis is regulated by *RBR*. RBR is a cell cycle regulator that inhibits cell cycle entry by repressing E2F transcription factors, which activate genes required for the G1/S transition (Shen, 2002; Dimova and Dyson, 2005). In the female gametophyte, *rbr* mutants undergo extra rounds of mitosis, which gives rise to embryo sacs with supernumerary nuclei. *rbr* female gametophytes occasionally become cellularized and these often contain central cells that continue to undergo nuclear proliferation (Ebel et al., 2004; Ingouff et al., 2006). When cellularized, the nuclei at the micropylar-most region of the embryo sac give rise to one or more egg cells that can become fertilized but give rise to abnormal embryos (Ingouff et al., 2009). These observations suggest that RBR functions to inhibit the cell cycle during female gametophyte development.

RBR has additional functions during female gametophyte development. *rbr* embryo sacs fail to express or mis-express several cell-specific markers, suggesting that *RBR* is also required for differentiation of the female gametophyte cells (Ingouff et al., 2006; Johnston et al., 2008; Ingouff et al., 2009). Gene mis-expression is in part due to RBR (and MSI1) inhibition of *DNA METHYLTRANSFERASE1* (*MET1*) expression. MET1 is responsible for maintenance of inhibitory gene methylation and MET1 absence (along with DME activity) leads to removal of the silencing methylation marks and activation of gene expression (e.g., *FIS2* and *FWA*; Johnston et al., 2008; Jullien et al., 2008; Johnston and Gruissem, 2009).

Nuclear proliferation during megagametogenesis also may be regulated by *IG1* and *AGL23*. Maize *IG1* encodes a LATERAL ORGAN BOUNDARIES (LOB) domain protein. *ig1* mutant female gametophytes undergo extra rounds of mitosis before cellularization, which leads to embryo sacs containing extra egg cells, extra synergid cells, or central cells with extra nuclei. These phenotypes suggest that *IG1*, like *RBR*, may function to restrict the extent of nuclear proliferation during megagametogenesis (Huang and Sheridan, 1996; Guo et al., 2004; Evans, 2007).

The Arabidopsis *AGL23* gene encodes a type I MADS-box transcription factor. *agl23* mutant embryo sacs arrest at the one-nucleate stage. *AGL23* is expressed throughout megagametogenesis, from the one-nucleate stage (stage FG1) to the mature female gametophyte (stage FG7). The cause of the female gametophyte defect is not known. However, occasional *agl23* homozygous mutants have defects in chloroplast biogenesis during embryo development, suggesting that the nuclear proliferation defect may result indirectly from defects in chloroplast biogenesis (Colombo et al., 2008).

The nuclear proliferation phase of megagametogenesis is also affected by mutations affecting several chromatin-remodeling or histone-modifying factors including CHR11 (Huanca-Mamani et al., 2005), and HAM1 and HAM2 (Latrasse et al., 2008). CHR11 is a chromatin-remodeling protein within the ISWI subfamily (Huanca-Mamani et al., 2005). *HAM1* and *HAM2* are redundant genes encoding histone acetyltransferases (HATs) within the MYST subfamily (Latrasse et al., 2008). All of these mutants arrest megagametogenesis during the nuclear proliferation phase (1- to 8-nucleate stages, FG1 to early FG5), indicating that chromatin regulation is an important aspect of nuclear proliferation during megagametogenesis.

In summary, these observations suggest that *RBR* and *IG1* function to restrict the proliferative phase of megagametogenesis. *AGL23* and chromatin modifiers may be required for promotion of nuclear division during female gametophyte development.

Regulation of cell specification and differentiation during megagametogenesis

Analysis of the Arabidopsis *fiona* mutant suggests that non-cell autonomous processes may be important for establishment of cell fate during female gametophyte development. *FIONA* is expressed exclusively in the central cell and encodes a mitochondrial localized cysteinyl t-RNA synthetase. In *fiona* central cells, mitochondria lack cristae and the polar nuclei fail to fuse. *fiona* female gametophytes are also defective in antipodal cell death. These observations suggest that the central cell influences the behavior (i.e., cell death) of the adjacent antipodal cells during the late stages of female gametophyte development (Kagi et al., 2011).

A group of genes required for cell specification or differentiation during female gametophyte development have been identified. These include *LACHESIS* (*LIS*; Gross-Hardt et al., 2007), *GAMETOPHYTIC FACTOR1* (*GFA1*; Coury et al., 2007), *CLOTHO* (*CLO*; Moll et al., 2008), *ATROPOS* (*ATO*; Moll et al., 2008), *MYB98* (Kasahara et al., 2005), *AGL80* (Portereiko et al., 2006), and *AGL61/DIANA* (Bemer et al., 2008; Steffen et al., 2008) from Arabidopsis and *DMT102* from maize (Garcia-Aguilar et al., 2010).

Arabidopsis *MYB98* is required for synergid cell differentiation. *MYB98* is expressed predominantly in the synergid cells and encodes an R2R3-MYB transcription factor. The synergid cells in *myb98* female gametophytes are mostly normal, indicating that *MYB98* is not required for establishment of synergid cell fate. *myb98* embryo sacs have a defect in a specific aspect of synergid cell structure: the filiform apparatus lacks the extensive projections characteristic of a wild-type filiform apparatus (Figure 1C; Kasahara et al., 2005). *myb98* female gametophytes also are defective in pollen tube guidance (discussed below). *MYB98* is required for the expression of >83 genes, most of which encode cysteine-rich peptides (CRPs). Several of the CRPs tested localize to the filiform apparatus. These data suggest that MYB98 functions as a transcription factor within the synergid cells to regulate the expression of genes required for filiform apparatus formation (Kasahara et al., 2005; Jones-Rhoades et al., 2007; Punwani et al., 2007; Punwani et al., 2008).

AGL61/DIANA and *AGL80* from Arabidopsis are required for central cell development. Both genes encode Type I MADS transcription factors that interact. These two genes have similar expression patterns and mutant phenotypes. *AGL61/DIANA* and *AGL80* are expressed exclusively in the central cell and early endosperm. Mutations in these genes affect the central cell specifically: mutant central cells exhibit an overall reduction in size, a reduced or absent vacuole, and fail to give rise to endosperm when fertilized with wild-type pollen. *agl61/diana* and *agl80* central cells fail to express several central cell-expressed genes and ectopically express several synergid- and antipodal-expressed genes, indicating a defect in cell fate. Together, these data suggest that an AGL61-AGL80 heterodimer functions to both activate and repress genes during central cell development (Portereiko et al., 2006; Bemer et al., 2008; Steffen et al., 2008).

Maize *DMT102* is required for antipodal development. *DMT102* encodes a DNA methyltransferase related to Arabidopsis CHROMOMETHYLASEs, which are required for cytosine methylation at CNG sites. *dmt102* female gametophytes develop normally until mature, but then the antipodal cells undergo abnormal growth and degenerate. *DMT102* is expressed in the megaspore mother cell and throughout the female gametophyte during early megagametogenesis. However, at the end of megagametogenesis, expression becomes restricted to the chalazal region of the female gametophyte, which is the region occupied by the antipodal cells (Figure 1A). These observations suggest that *DMT102* is required in the antipodal cells to maintain antipodal cell fate (Garcia-Aguilar et al., 2010).

Development of several female gametophyte cell types is affected in the Arabidopsis *lis* (Gross-Hardt et al., 2007), *gfa1* (Coury et al., 2007), *clo* (Moll et al., 2008), and *ato* (Moll et al., 2008) mutants. *gfa1* and *clo* are allelic (Moll et al., 2008). In *lis*, *gfa1/clo*, and *ato* embryo sacs, the synergid cells and the central cell adopt egg cell fate, and the antipodal cells adopt central cell fate. The *LIS*, *GFA1/CLO*, and *ATO* genes encode proteins required for RNA splicing: LIS is a homolog of yeast PRP4 (Precursor mRNA Processing 4), which is required for splicing via the U4/U6 spliceosome; GFA1/CLO is a homolog of yeast Snu114p, a spliceosome component; and ATO is a homolog of SF3a60, which is required for prespliceosome formation. These findings suggest that the RNA splicing machinery plays an important role in female gametophyte cell specification. However, the precise role of *LIS*, *GFA1/CLO*, and *ATO* in specifying cell fate during megagametogenesis is unknown (Gross-Hardt et al., 2007; Moll et al., 2008).

REGULATION OF POLLEN TUBE GUIDANCE

Control of pollen tube guidance by the female gametophyte

The mechanisms controlling pollen tube growth through the carpel's internal tissues to the ovule and female gametophyte involve multiple signals from both sporophytic and gametophytic maternal tissues of the carpels. These signals are discussed comprehensively in the review by Higashiyama et al. in The Arabidopsis Book. Here, we focus on the role of the female gametophyte in pollen tube guidance.

Several groups analyzed pollen tube growth in Arabidopsis sporophytic mutants defective in ovule and female gametophyte development. In these mutants, all or most of the ovules are defective in homozygous mutants. With some mutants analyzed, only the embryo sac is affected. In these mutants, wild-type pollen tubes grow normally during the initial phases, from pollen hydration to tube emergence from the transmitting tract, but then fail to grow towards mutant ovules lacking female gametophytes. These studies suggest that the female gametophyte does not influence the early steps of pollen tube growth (from the stigma to emergence from the transmitting tract) but is required for pollen tube growth to the ovule (Hulskamp et al., 1995; Elliott et al., 1996; Hauser et al., 1998; Couteau et al., 1999).

Further analysis of pollen tube growth patterns in Arabidopsis female gametophyte mutants suggests that guidance by the female gametophyte involves multiple steps. Pollen tubes fail to grow onto the funiculus in mutants lacking female gametophytes (Hulskamp et al., 1995; Ray, 1997; Shimizu and Okada, 2000).

By contrast, in less severely affected mutants, pollen tubes grow along the funiculus but do not enter the micropyle (Shimizu and Okada, 2000). These observations suggest that the female gametophyte produces two pollen tube guidance signals: a funicular guidance signal, which attracts the pollen tube from the placenta to the funiculus, and a micropylar guidance signal, which directs pollen tube growth from the funiculus to the micropyle (Shimizu and Okada, 2000).

To determine which cells of the female gametophyte produce the pollen tube attractant(s), Higashiyama and colleagues developed an *in vitro* pollen tube guidance system utilizing ovules from *Torenia fournieri* (Higashiyama et al., 1998). In this species, much of the embryo sac protrudes from the ovule integuments (Higashiyama, 2002), which allows for laser ablation of individual female gametophyte cells. Ovules in which the egg cell and/or central cell are ablated attract pollen tubes. By contrast, ovules in which the synergid cells are ablated fail to attract pollen tubes (Higashiyama et al., 2001). These studies identify the synergid cells as the source of the pollen tube attractant(s).

Additional cells may contribute to pollen tube guidance. The egg cell appears to play a role in maize (Marton et al., 2005) and Arabidopsis (Alandete-Saez et al., 2008). In Arabidopsis, the central cell has been proposed to play a role because pollen tube guidance is defective in the *central cell guidance* (*ccg*) mutant, which is a female gametophyte mutant that is affected in the central cell specifically (Chen et al., 2007). However, several other central cell-specific mutants are not affected in pollen tube guidance (Portereiko et al., 2006; Steffen et al., 2008). Thus additional work needs to be done to assess the possible role of the central cell in pollen tube guidance.

Pollen tube attractants produced by the female gametophyte

The first female gametophyte pollen tube attractant, ZmEA1, was identified in maize. *ZmEA1* is expressed in the synergid cells and the egg cell and encodes a 94-amino acid transmembrane pre-protein. A ZmEA1-GFP fusion protein is initially localized to the filiform apparatus and later is present in the cell wall of the surrounding nucellus. Transgenic maize plants in which *ZmEA1* expression is reduced by RNAi exhibit defects in pollen tube guidance but are otherwise normal (Marton et al., 2005). A putative mature protein of 49 amino acids attracts maize pollen tubes in vitro and Arabidopsis female gametophytes expressing *ZmEA1* in the synergid cells attract maize pollen tubes in vitro (Marton and Dresselhaus, 2010).

Female gametophyte pollen tube attractants, LURE1 and LURE2, have also been identified in Torenia. LUREs are cysteine-rich proteins (CRPs) within the defensin-like (DEFL) family. The *LURE* genes are expressed in the synergid cells and the encoded proteins are secreted into the filiform apparatus. LURE downregulation reduces pollen tube attraction, and recombinant mature proteins attract pollen tubes in vitro and in a species-specific manner (Okuda et al., 2009).

An Arabidopsis pollen tube attractant produced by the female gametophyte has not yet been identified. However, pollen tube guidance is affected in the Arabidopsis *myb98* mutant (Kasahara et al., 2005). As discussed above, the *MYB98* gene encodes a Myb-type transcription factor expressed predominantly in the synergid cells and the *myb98* mutation affects the filiform apparatus within the synergid cells. In addition, *MYB98* is necessary for the expression of at least 83 genes encoding CRPs similar to the LURE1 and LURE2 pollen tube attractants identified in Torenia (Punwani et al., 2007; Punwani et al., 2008). Many of the CRPs exhibit a localization and diffusion pattern similar to that of ZmEA1; that is, they are secreted into the filiform apparatus and subsequently diffuse into the micropylar region (Punwani et al., 2007). These observations suggest that CRPs may also function as pollen tube attractants in Arabidopsis.

REGULATION OF POLLEN TUBE RECEPTION

The pollen tube enters the female gametophyte by growing through the filiform apparatus of the synergid cells. The pollen tube then ceases growth and bursts, releasing its contents including the two sperm cells. These events are collectively referred to as pollen tube reception (Huck et al., 2003; Dresselhaus and Marton, 2009).

Control of pollen tube growth arrest

Soon after the pollen tube contacts the receptive synergid cell, it ceases growth and bursts. In principle, pollen tube growth arrest could be a consequence of pollen tube burst. However, the phenotype of the Arabidopsis *aca9* mutant, discussed below, indicates that pollen tube growth arrest and pollen tube discharge are separable processes (Schiott et al., 2004).

First insights into the processes regulating pollen tube growth arrest came from analysis of pollen tube growth in interspecific crosses within the *Rhododendron* genus (Williams et al., 1982; Williams et al., 1986). In some crosses, the pollen tube entered the female gametophyte but failed to cease growth and discharge its contents. More recently, similar results have been obtained with interspecific crosses between *Arabidopsis* and other Brassicaceae species (Escobar-Restrepo et al., 2007). These observations suggest that the female gametophyte contains species-specific factors that control pollen tube growth arrest.

A group of Arabidopsis gametophytic mutants defective in pollen tube growth arrest have been identified, including *feronia* (*fer*; Huck et al., 2003), *sirène* (*srn*; Rotman et al., 2003), *lorelei* (*lre;* Capron et al., 2008; Tsukamoto et al., 2010), *scylla* (*syl*; Rotman et al., 2008), *nortia* (*nta*; Kessler et al., 2010), and *abstinence by mutual consent* (*amc*; Boisson-Dernier et al., 2008). *fer* and *srn* are allelic (Escobar-Restrepo et al., 2007). The *fer/srn*, *lre*, *nta*, and *syl* are female gametophyte-specific mutations; in these mutants, wild-type pollen tubes enter mutant female gametophytes but fail to cease growth and rupture. This results in a pollen tube overgrowth phenotype (Huck et al., 2003; Rotman et al., 2003; Capron et al., 2008; Kessler et al., 2010; Tsukamoto et al., 2010). The *amc* mutation, by contrast, affects both gametophytes. *amc* mutants also exhibit a pollen tube overgrowth phenotype but do so only when both gametophytes are mutant.

The *SYL* gene has not been identified. *FER/SRN* encodes a receptor-like kinase (RLK) within the *Catharanthus roseus* RLK1-like (CrRLK1L) subfamily (Escobar-Restrepo et al., 2007). *NTA* is a member of the Mildew Resistance Locus O (MLO) gene family (Kessler et al., 2010). *LRE* encodes a puta-

tive glycosylphosphatidylinositol (GPI)-anchored protein (Capron et al., 2008). In the context of the ovule, the *FER/SRN*, *NTA*, and *LRE* genes are all expressed predominantly or exclusively in the synergid cells and the encoded proteins are localized to the plasma membrane (Escobar-Restrepo et al., 2007; Capron et al., 2008; Kessler et al., 2010). The FER/SRN and NTA proteins are concentrated in the region of the filiform apparatus, but in the case of NTA, this polar localization occurs only upon pollen tube arrival and does not occur in *fer* mutant female gametophytes (Escobar-Restrepo et al., 2007; Kessler et al., 2010). *AMC* encodes a peroxin necessary for protein import into the peroxisomes and AMC protein localizes to peroxisomes. *AMC* is expressed in the pollen and all cells of the mature female gametophyte (Boisson-Dernier et al., 2008).

These observations suggest that FER/SRN, LRE, NTA, SYL, and AMC lie in a pathway that mediates pollen tube-synergid recognition and is required for arrest of pollen tube growth. This pathway may not be specific to pollen tube reception because some of the genes are required during other phases of plant development: *FER/SRN* is broadly expressed and has functions throughout the plant (Guo et al., 2009b; Guo et al., 2009a; Deslauriers and Larsen, 2010; Duan et al., 2010; Kessler et al., 2010), *LRE* is required during early seed development (Tsukamoto and Palanivelu, 2010; Tsukamoto et al., 2010), and *syl* and *fer/srn* undergo autonomous endosperm development (Rotman et al., 2008).

It still is not clear how these proteins cooperate to control pollen tube growth arrest. However, analysis of FER function in root hairs may provide some insights. In root hairs, FER regulates a RAC/ROP pathway that activates NADPH Oxidase and leads to production of reactive oxygen species (ROS; Duan et al., 2010). In root hairs, ROS is required for polarized root hair growth (Carol and Dolan, 2006). In synergid cells, an analogous pathway could lead to pollen tube-dependent ROS production, which then could, for example, trigger pollen tube growth arrest (and/or synergid cell death; discussed below). In support of this model, ROS are produced in peroxisomes (Nyathi and Baker, 2006), which are affected in *amc* mutants (Boisson-Dernier et al., 2008).

Control of pollen tube discharge

Immediately after ceasing growth, the pollen tube ruptures at or near its tip. Rupture leads to release of the pollen tube's contents, including its two sperm cells. In Arabidopsis and Torenia, rupture occurs within one minute following entry of the pollen tube into the female gametophyte (Higashiyama et al., 2000; Rotman et al., 2003).

Pollen tube discharge is affected in the Arabidopsis *aca9* mutant (Schiott et al., 2004) and maize RNAi lines in which the *ZmES1-ZmES4* genes are downregulated (Amien et al., 2010). *aca9* is a male gametophyte-specific mutation. *aca9* mutant pollen tubes enter wild-type female gametophytes and cease growth but fail to discharge. This phenotype indicates that *ACA9* is required for pollen tube growth discharge but not pollen tube growth arrest and, thus, that these two steps are distinct. *ACA9* encodes an autoinhibited Ca^{2+} ATPase (ACA). *ACA9* is expressed specifically in pollen and ACA9 protein is localized to the plasma membrane (Schiott et al., 2004).

ZmES1-ZmES4 are a group of closely related genes that encode defensin-like (DEFL) proteins. These genes are expressed predominantly in the synergid cells of mature female gametophytes. Before fertilization, ZmES4 protein is localized near the filiform apparatus, probably in regulated secretory vesicles, but is not secreted into the filiform apparatus. After pollen tube arrival, ZmES4 protein is not detectable and may be released. In RNAi lines in which all four *ZmES* genes are downregulated, wild-type pollen tubes enter the mutant female gametophytes but fail to rupture. In vitro, ZmES4 protein induces pollen tube rupture, opening of the KMK1 K^+ channel, and pollen membrane depolarization. These observations suggest that pollen tube contact induces the synergid cell to release ZmES1-4, which then leads to pollen tube rupture by stimulating KMK1-mediated K^+ influx. K^+ influx could possibly lead to water uptake and osmotic tube burst (Amien et al., 2010).

REGULATION OF SYNERGID CELL DEATH

The pollen tube discharges and releases its contents into one of the synergid cells, which is referred to as the receptive synergid cell. The receptive synergid cell undergoes cell death. It is likely that synergid degeneration is necessary to provide the sperm cells direct access to the egg cell and central cell for double fertilization. Synergid degeneration may also be required for pollen tube entry into the synergid cell (van Went and Willemse, 1984; Russell, 1992, 1996; Higashiyama, 2002; Punwani and Drews, 2008).

In Arabidopsis, synergid cell death requires pollination (Christensen et al., 1997), indicating that it is not part of the female gametophyte developmental program and suggesting that the cell death process is induced by pollen tubes. Real-time imaging of pollen tube growth and synergid degeneration in Arabidopsis has shown that the pollen tube contacts the synergid cell before synergid degeneration is observed (Rotman et al., 2003; Sandaklie-Nikolova et al., 2007). These observations suggest two models. First, the pollen tube could induce a physiological cell death program following pollen tube-synergid cell contact. Second, pollen tube penetration and/or discharge could trigger mechanical breakdown of the synergid cell, as appears to occur in *Torenia* (Higashiyama et al., 2000).

Synergid degeneration is defective in several Arabidopsis mutants, including *gfa2* (Christensen et al., 2002), *srn* (Rotman et al., 2003), *amc* (Boisson-Dernier et al., 2008), and *lre* (Tsukamoto et al., 2010), as well as in maize lines in which the four *ZmES* genes are downregulated using RNAi (Amien et al., 2010). With all of these mutants, the synergid cells appear normal but fail to undergo cell death following growth of the pollen tube into the female gametophyte. These observations argue against mechanical breakdown being the cause of synergid degeneration in Arabidopsis. GFA2 is a J-domain-containing protein required for mitochondrial function (Christensen et al., 2002), suggesting that synergid cell death in Arabidopsis requires functional mitochondria, as is the case for cell death in animals (Jiang and Wang, 2004). As discussed above, FER/SRN, LRE, and AMC may be part of a pathway leading to ROS production in the receptive synergid cell in response to pollen tube contact, suggesting that

synergid cell death may result from ROS in the synergid cell, as occurs in other cells in plants (Van Breusegem and Dat, 2006). Together, these observations support a model in which pollen tube-synergid contact induces a physiological cell death program within the synergid cell.

REGULATION OF SEED INITIATION

Regulation of seed initiation in Arabidopsis

In sexually reproducing angiosperms, the differentiated female gametophyte arrests at maturity and double fertilization is required to initiate seed development. In Arabidopsis, the unfertilized central cell contains a set of proteins that suppresses endosperm development until fertilization occurs. These proteins are part of a protein complex called the FIS (FERTILIZATION-INDEPENDENT SEED) complex. In addition to this pre-fertilization function, the FIS complex also has several post-fertilization functions in the endosperm including gene imprinting (Bauer and Fischer, 2011), discussed below, and regulation of cellularization (Kang et al., 2008).

The FIS complex contains proteins related to those found in the Drosophila Polycomb Group Repressive Complex 2 (PRC2). The FIS complex comprises four known proteins: FIE, a WD-repeat polycomb protein related to Drosophila EXTRA SEX COMBS; MEA, a SET-domain polycomb protein related to Drosophila ENHANCER OF ZESTE; FIS2, a zinc-finger protein related to Drosophila SUPPRESSOR OF ZESTE 12; and MSI1 (discussed above) a homolog of Drosophila p55 (Rodrigues et al., 2010a).

Mutants containing lesions in any of the four FIS genes are generally referred to as *fis* mutants. *fis* mutants undergo central cell proliferation in the absence of fertilization but the resulting seeds are not viable. This phenotype is referred to as autonomous endosperm development, in parallel with the term used for the similar apomixis phenotype. When *fis* mutants are fertilized, embryogenesis also initiates but the embryo aborts and endosperm pattern formation is defective (Ohad et al., 1996; Chaudhury et al., 1997; Grossniklaus et al., 1998; Vielle-Calzada et al., 1999; Luo et al., 2000; Spillane et al., 2000; Yadegari et al., 2000).

The FIS complex mediates the trimethylation of lysine 27 in histone H3 (H3K27), which is associated with compact, silent chromatin (Raissig et al., 2011). Presumably, FIS-mediated H3K27 methylation is involved in silencing genes in the central cell that are required for post-fertilization endosperm proliferation. Thus far, only two targets of the FIS-complex have been identified: *PHERES1* (*PHE1*), which encodes a MADS domain-containing protein (Kohler et al., 2003), and *FORMIN5* (*AtFH5*), which encodes an actin polymerization regulator (Fitz Gerald et al., 2009). Both genes are expressed during early endosperm development. Mutations in *AtFH5* result in endosperm formation defects (Ingouff et al., 2005; Fitz Gerald et al., 2009) while *phe1* mutants show no detectable seed phenotype (Kohler et al., 2003; Kohler et al., 2005). If FIS-mediated H3K27 methylation is involved in silencing endosperm genes, then these histone marks must be reversed upon fertilization. It is not clear how the complex is inactivated upon fertilization.

The Arabidopsis egg cell also contains factors that inhibit embryo development before fertilization. In addition to autonomous endosperm development, *msi1* mutations cause the initiation of embryo development in the absence of fertilization, but the haploid embryo aborts early in development (Guitton and Berger, 2005). This is not observed in mutated *fie*, *fis2*, and *mea* alleles and this aspect of MSI1 function is likely to be involved in a pathway distinct from that involving the FIS complex. MSI1 does participate in another complex involving the cell cycle control protein RBR (discussed above; Ebel et al., 2004; Guitton and Berger, 2005; Ingouff et al., 2006; Johnston et al., 2008; Jullien et al., 2008).

Regulation of seed initiation in other species

An important question is whether other sexual species have a FIS complex that inhibits endosperm development before fertilization. Furthermore, the similarity of autonomous seed initiation in apomicts with fertilization-independent embryo and endosperm initiation in Arabidopsis *fis* mutants raises the question of whether FIS complex function is altered or inactive in apomicts.

Homologs of the Arabidopsis FIS genes have been isolated from rice, maize, and *Hieracium*. Maize FIS gene function in repressing endosperm initiation has not been examined. FIE is the central linking protein of the FIS complex. Downregulation of *FIE* in rice, which contains two *FIE* homologs (Luo et al., 2009), and sexual *Hieracium*, which contains a single *FIE* gene (Rodrigues et al., 2008), does not lead to autonomous endosperm initiation which is the pre-fertilization defect in Arabidopsis *fis* mutants (Rodrigues et al., 2010a).

However, *FIE* downregulation in sexual *Hieracium* produces the post-fertilization defects (i.e., embryo abortion and failure of the endosperm to cellularize) found in fertilized Arabidopsis *fis* mutants. These observations suggest that the *Hieracium* FIS complex lacks the pre-fertilization function of repressing central cell proliferation. This function may have been lost in the evolutionary lineage leading to *Hieracium*. Alternatively, the post-fertilization function may have been the ancestral function of this complex (Rodrigues et al., 2010a).

In autonomous apomicts, both embryo and endosperm initiation occur independently of fertilization. FIS complex function has currently only been studied in apomictic *Hieracium*. In the autonomous apomict *Hieracium praealtum*, one dominant locus *LOP*, controls autonomous embryo and endosperm formation and is gametophytic in action. The number of molecular components at *LOP* is unknown. *LOP* may remove a repressive block or simulate pathways induced by the products of fertilization (Koltunow et al., 2011b).

When *FIE* is downregulated in apomictic *Hieracium* ovules, initiation of autonomous seed development is inefficient and seed abortion is observed (Rodrigues et al., 2008). These observations indicate that *Hieracium* FIE is required for autonomous seed development. *Hieracium FIE* and *MSI1* homologs of the Arabidopsis FIS-complex are present in deletion mutants where *LOP* function is lost, confirming they are not *LOP* candidates (Rodrigues et al., 2010b; Koltunow et al., 2011b). As *FIE* function is required for seed initiation in apomictic Hieracium (Rodrigues et al., 2008) it may function downstream of *LOP* activity (Rodrigues et al., 2010a).

MATERNAL CONTROL OF SEED DEVELOPMENT

Genetic data indicate that the female gametophyte influences post-fertilization seed development at two levels. First, the female gametophyte plays a role in the imprinting of specific genes so that one parental allele is transcribed and the other is silenced during seed development. Second, the female gametophyte sequesters factors prior to fertilization that are required for embryo and endosperm development following fertilization.

Genomic imprinting

Maternal and paternal alleles inherited after fertilization are usually equivalently (biallelically) expressed during development. However, a subset of genes is transcribed preferentially or exclusively in a uniparental manner. These are defined as imprinted genes. A combination of epigenetic processes are involved in repressing transcription from the imprinted parental allele (Bauer and Fischer, 2011; Raissig et al., 2011).

Imprinted genes expressed predominantly or exclusively from the maternal allele are referred to as paternally imprinted genes. By contrast, imprinted genes expressed predominantly or exclusively from the paternal allele are called maternally imprinted genes. All known imprinted genes in plants are expressed during early seed development (Bauer and Fischer, 2011; Raissig et al., 2011).

The function of imprinting is not known. Current theories suggest that imprinting may have evolved to manage parental genome conflicts related to nutrient partitioning during seed formation, to regulate dose sensitive gene expression, to promote hybridity in outcross situations, or to prevent parthenogenesis. Alternatively, imprinting may be a byproduct of defense against foreign DNA such as transposable elements (Dilkes and Comai, 2004; Ishikawa and Kinoshita, 2009; Bauer and Fischer, 2011; Raissig et al., 2011).

Until recently, fewer than 20 imprinted loci had been identified in Arabidopsis, rice and maize (Bauer and Fischer, 2011; Raissig et al., 2011). Allele-specific profiling of embryo and endosperm transcriptomes has identified a further 170 candidate genes in Arabidopsis (Hsieh et al., 2011) and 262 candidate loci in rice (Luo et al., 2011). Few imprinted genes are common to Arabidopsis and rice, suggesting that imprinting has evolved independently in eudicots and monocots (Luo et al., 2011).

The known imprinted genes comprise large numbers of both maternally and paternally expressed loci and the vast majority (all except two genes) are expressed in the endosperm. Functions for most imprinted genes are unknown, but gene identities suggest that they have roles in diverse processes (Hsieh et al., 2011; Luo et al., 2011; Raissig et al., 2011). Arabidopsis imprinted genes with known functions include *MEA*, *FIS2*, and *FIE*, and *AtFH5*, which are discussed above (Raissig et al., 2011).

Parent-of-origin-specific expression is achieved by differentially marking, with epigenetic marks, the alleles inherited from the male and female gametophytes. One route is through methylation of cytosine residues in DNA within and flanking the coding regions to silence expression from a particular allele. Other marks to silence an allele include histone methylation or acetylation (Bauer and Fischer, 2011; Raissig et al., 2011).

In Arabidopsis, imprinting of many endosperm-expressed genes appears to result from removal of methylation in the central cell. Specific genes are methylated before gametogenesis. During megagametogenesis, global demethylation occurs in the central cell and maternal alleles transmitted to the endosperm lack methylation marks. By contrast, demethylation does not occur during microgametogenesis and paternal alleles transmitted to the endosperm remain methylated. Thus, for some genes, the endosperm inherits alleles of different methylation states. This leads to maternal-allele-specific expression of some genes in the endosperm. Silencing of paternal alleles in the endosperm can occur by DNA methylation, histone K27 tri-methylation mediated by the FIS complex or a combination of both (Bauer and Fischer, 2011).

Demethylation in the Arabidopsis central cell occurs by two routes. First, passive demethylation occurs due to the activity of the RBR-MSI complex, which represses *MET1* expression. Because MET1 is the key *de novo* maintenance methytransferase required for CpG dinucleotide methylation, its absence leads to progressive loss of methylation marks (Jullien et al., 2008). Second, active demethylation in the central cell occurs by the activity of DEMETER (DME), a DNA glycosylase/lyase that removes 5-methylcytosine and replaces it with cytosine in DNA (Bauer and Fischer, 2011). The accompanying downregulation of VIM5, a protein that recruits methyltransferases to hemi-methylated DNA, is also thought to contribute to the global genome-wide demethylation of the Arabidopsis central cell (Hsieh et al., 2011). Genome hypomethylation has also been observed in the rice central cell. However, cereals do not contain DME, thus another deglycosylase or an alternative mechanism may be involved in this process (Zemach et al., 2010).

In Arabidopsis, a complex population of more than 100,000 different PolIV-derived small interfering RNAs (PolIV-siRNAs) is transcribed from maternal loci in the mature female gametophyte and during early seed development in the endosperm. This was the first described case of imprinting in non-coding regions. While the function of this si-RNA population is not clear and the conservation of this phenomenon has yet to be examined in other plants, it has been proposed that the maternal-chromosome-specific expression of these PolIV-siRNAs may provide a link between genomic imprinting and mRNA silencing in plants through *de novo* RNA directed DNA methylation (RdDM) of non-CG methylation. Thus the RdDM pathway may also be involved in imprinting by guiding *de novo* methylation (Mosher et al., 2009).

Gametophytic maternal control of seed development

In Arabidopsis, downregulation of RNA Polymerase II in the fertilization products is deleterious to endosperm development but does not block early embryo development. These data suggest that the egg cell and zygote contain maternal products to sustain early embryo development (Pillot et al., 2010). Genes that are expressed in the embryo sac but whose gene products are stored and utilized post-fertilization during seed development are referred to as gametophytic maternal-effect genes (Ray, 1997; Drews et al., 1998; Drews and Yadegari, 2002).

Mutations in gametophytic maternal-effect genes segregate as female gametophyte mutations (Table 1). Gametophytic maternal-

effect mutants described include the Arabidopsis *capulet1 (cap1)* and *capulet2 (cap2)* mutants (Grini et al., 2002), the maize *maternal effect lethal1 (mel1)* mutant (Evans and Kermicle, 2001) and the maize *baseless1* (*bsl1*) mutant (Gutierrez-Marcos et al., 2006).

With the exception of *bsl1*, female gametophytes in these mutants appear normal, whereas development of the embryo and/or endosperm is affected during seed development. *bsl1* female gametophytes have subtle defects, where the polar nuclei are abnormally situated. 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). *bsl1* affects development of the basal endosperm transfer layer (BETL). *BSL1* is thought to be required in the central cell prior to fertilization for correct endosperm patterning (Gutierrez-Marcos et al., 2006). Progeny of *mel1* mutant gametophytes have defective embryo and endosperm development. Most seeds fail to germinate and germinating seedlings show twinning and other developmental abnormalities (Evans and Kermicle, 2001). The *CAP1*, *CAP2*, *MEL1* and *BSL1* genes have not been isolated; thus, the molecular basis for the gametophytic-maternal effects observed in these mutants remains to be determined.

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