Female Gametophyte Development in Flowering Plants

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Abstract
The multicellular female gametophyte, a unique feature of higher plants, provides us with an excellent experimental system to address fundamental questions in biology. During the past few years, we have gained significant insight into the mechanisms that control embryo sac polarity, gametophytic cell specification, and recognition between male and female gametophytic cells. An auxin gradient has been shown for the first time to function in the female gametophyte to regulate gametic cell fate, and key genes that control gametic cell fate have also been identified. This review provides an overview of these exciting discoveries with a focus on molecular and genetic data.
INTRODUCTION

A female gametophyte is a multicellular haploid structure that develops into an embryo and endosperm after fertilization. In the past decade, gametophyte development in plants has emerged as an excellent system to address fundamental questions in biology, such as cell specification, cell-cell interaction, and the developmental role of basic cellular machinery. Significant progress has been made to define the genetic components that govern gametogenesis. This review focuses on recent advances in defining genetic control of female gametophyte development.

OVULE DEVELOPMENT

An ovule is a female organ within the carpel of a flower that harbors the female gametophyte. Ovule development starts as a protrusion (primordium) on the edges of the septum of the gynoecium. As the ovule primordium elongates, a finger-like structure (nucellus) is formed. Then, a hypodermal cell at the tip of the nucellus starts to differentiate and forms an archesporial cell, which produces the germline. The archesporial cell enters meiotic development to differentiate a megasporocyte, which becomes distinct by its large size and nuclear morphology (Figure 1a). The megasporocyte then undergoes meiosis to give rise to four haploid megaspores. In most flowering plants, which include the model species Arabidopsis and rice, micropylar megaspores undergo programmed cell death, and the chalazal-most megaspore becomes functional and ultimately forms the female gametophyte, the embryo sac (Figure 1b). Concurrently, epidermal cells at the proximal third of the nucellus divide parallel to the long axis and form two primodia, which become the inner and outer integuments, respectively (Figure 1a). These enclose the functional megaspore, which becomes the embryo sac, forming a narrow opening at the micropyle where the pollen tube enters after pollination.

While nonfunctional megaspores undergo cell death, the functional megaspore increases in size and undergoes a nuclear division without cytokinesis to produce a two-nucleate embryo sac (Figure 1c). The two daughter nuclei, now separated to the poles by the formation of a central vacuole (Figure 1d), proceed to a second karyokinesis to form a four-nucleate embryo sac with nuclei in a 2n+2n configuration (Figure 1e). As the vacuole increases in size, the third karyokinesis takes place, which results in the formation of a huge coenocytic cell with eight nuclei that adopt a 4n+4n configuration—the eight-nucleate embryo sac (Figure 1f). Thereafter, two polar nuclei, one from each pole, migrate to the micropylar cytoplasm of the embryo sac and finally fuse to form a diploid central nucleus. As polar nuclei migrate, cell walls are formed simultaneously, dividing the embryo sac into seven cells with four cell types: three antipodal cells at the chalazal end, a diploid central cell, two synergids, and...
an egg cell at the micropylar end (Figure 1g). Antipodal cells degenerate shortly before fertilization in Arabidopsis (Figure 1b) or undergo further mitosis as seen in maize. Antipodal cells are likely dispensable for fertilization. Therefore, the central cell, the egg, and two synergid cells form a female germ unit—a functional unit that is able to attract a pollen tube, interact with the tube to trigger sperm release, and complete double fertilization. As mentioned above, ovule development involves both sporophytic and gametophytic processes, and is an excellent system to study the basic developmental mechanisms that control germline formation, cell growth and division, and gametic cell fate specification.

THE TRANSITION FROM SOMATIC TO GERMLINE FATE

In angiosperms, the initial cells of the germline, called archesporial cells, are formed de novo from the hypodermal L2 cell layer of the ovule primordium. Generally, in females, a single hypodermal L2 cell at the tip of the nucellus differentiates into the germline cell that enters the meiotic pathway, which ultimately gives rise to gametic cells: the egg and the central cell.

Figure 1
Development of the female gametophyte in Arabidopsis. Confocal optical section showing (a) nucleus with MMC (green) and primordia of inner (it) and outer (ot) integuments; (b) one-nucleate embryo sac (green) and degenerating megaspores (yellow) close to the micropyle; (c) an early two-nucleate embryo sac (green), inner (it) and outer (ot) integuments, and funiculus (fc); (d) a late two-nucleate embryo sac (green), inner (it) and outer (ot) integuments, and funiculus (fc); (e) a four-nucleate embryo sac (green), with antipodal nuclei (pink), polar nuclei (blue), egg nucleus (red), and synergid nuclei (yellow); (f) a late eight-nucleate embryo sac (green), with antipodal nuclei (pink), polar nuclei (blue), egg nucleus (red), and synergid nuclei (yellow); (g) a mature four-celled embryo sac (green), with the secondary nucleus (blue), egg nucleus (red), and synergid nuclei (yellow). Scalebar: 10 μm. Confocal images were modified with PhotoShop to highlight the megaspore mother cell, embryo sac, and nuclei.

The archesporial cell first becomes morphologically distinguishable from its surrounding nucellar cells by its larger size and pronounced nucleus, and it is called a megasporocyte when
its nucleus enters the prophase of meiosis. How the transition from a somatic cell fate to a germline fate is controlled remains unknown. However, several putative components that control this transition have been identified through genetic approaches. In *Arabidopsis*, the SPOROCYTELESS gene (SPL/NOZZLE) has been implicated in controlling germline cell fate. In *spl* mutants, archesporial cells are formed in both anther and ovule primodia, but they fail to develop further, which results in a complete lack of germline in male and female organs (63, 75). This indicates that SPL plays an essential role in germline formation. A recent study revealed that the floral homeotic regulator AGAMOUS (AG) can activate the SPL gene by binding to the CarG-box in its 3′ region (24). SPL expression activated by AG induces the ectopic formation of microspores on petaloid floral organs, which indicates ectopic formation of the male germline. This finding not only showed that SPL is a direct downstream target of AG but also demonstrated that SPL is sufficient to trigger male germline specification in *Arabidopsis*. Whether SPL is also sufficient for female germline specification remains unknown. SPL encodes a novel nuclear protein with limited homology to MADS-box transcription factors (63, 75). Therefore, how SPL regulates its downstream genes in germline specification is of great interest. Recently, researchers suggested that SPL may be involved in auxin homeostasis by repressing *YUCCA* genes in lateral organ development (32). It would be interesting to investigate whether auxin is also involved in germline formation.

Several genes that control the number of cells entering germline fate have also been identified. Mutation in **MULTIPLE ARCHESPORIAL CELLS 1** (MAC1) results in an excessive number of archesporial cells in maize (64). Similarly, in rice, the *multiple sporocyte 1* (msp1) mutants have increased numbers of both male and female sporocytes (45). These excess sporocytes likely result from excessive archesporial cells, which suggests that MSP1 is required for archesporial cell fate. The *MSP1* gene is expressed in nucellar cells that surround germline cells but not in the developing germline cells. This supports a role for MSP1 in determining germline cell fate by preventing the surrounding cells from becoming germline cells, similar to MAC1 in maize (64). MSP1 encodes a leucine-rich repeat containing receptor-like kinase (LRR-RLK). This implies that signaling between the archesporial cell and its neighboring cells is controlled by a ligand-receptor signaling cascade, and this plays a critical role in female germline development. These findings imply that a lateral inhibition mechanism may act in controlling the number of germline cells.

Because MSP1 is a membrane receptor kinase, it likely exerts its effects by binding to a ligand. Recently, a putative ligand OsTDL1A was shown to bind the extracellular domain of MSP1 by yeast two-hybrid and BiFC experiments (78). Similar to MSP1, TDL1A is expressed exclusively in nucellar cells but not in germline cells. Furthermore, knockdown of *OsTDL1A* expression phenocopies the *msp1* phenotype in the nucellus. A similar mechanism, mediated by TPD1-EMS1/EXS1 signaling, controls male sporocyte fate (25, 36). These data suggest that specification of germline cell fate and number in plants involves a similar ligand-receptor signaling system in both the male and female.

In animals, germline fate is controlled by a germline-specific PIWI-associated miRNA (piRNA) system (33). Emerging data suggest that miRNA also plays an important role in germline development in plants. **MEIOSIS ARRESTED AT LEPTOTENE1** (**MEL1**), a germline-specific member of **ARGONAUTE** (**AGO**) genes family, is specifically expressed in archesporial cells and sporogenous cells (SCs), and disappears when SCs enter meiosis in anther and ovule. Ovule development in *mel1* mutants is arrested at various stages from pre-meiosis to tetrad. Chromosomes remain uncondensed, and aberrant chromatin modification is detected in *mel1* germline cells, which suggests that MEL1 acts on chromatin structures, similar to those of the PIWI subfamily of...
AGOs in *Drosophila*. This implies that *MEL1* is required for female germline development, most likely by regulating cell division of pre-meiotic germline cells, modification of meiotic chromosomes, and progression of meiosis; but it does not affect the initiation, establishment, and early mitotic division of germline cells (46).

Interestingly, the *MEL1* expression domain is larger than the germline cell in anther and ovule primordia. Therefore, archesporial cells may be produced in excess of subsequent archesporial cells, and the fate of archesporial derivatives may be controlled by signals from SCs. Therefore, *MEL1* may suppress somatic gene expression during germline development (46). How does *MEL1* function? *MEL1* may modify chromatin structure to repress the somatic gene expression program in germline cells. Although the role of *MEL1* orthologs in germline formation has not been identified in other plants, this demonstrates for the first time that, as in animals, the small RNA-mediated gene silencing pathway mediated by *MEL1* plays a key role in plant germline formation (18).

**SPECIFICATION OF THE FUNCTIONAL MEGASPORE**

The female megasporocyte undergoes meiotic division to produce four haploid megaspores. Of the four megaspores, one, two, or four may participate in the formation of the final female gametophyte depending on the plant species. In the more advanced species, only one of the four megaspores is functional, and the remaining three undergo programmed cell death. The choice of functional megaspore is position dependent and also species dependent. In most flowering plants, including *Arabidopsis* and rice, the megaspore that is closer to the maternal tissue becomes the functional megaspore; distal micropylar megaspores undergo programmed cell death. Evolution seems to favor the more defined monosporic type of gametogenesis, and genetic mechanisms must have evolved to define such developmental control.

Observed in many species, polarity within the megasporocyte is manifested by the polar distribution of organelles, the dynamic deposition of callose, and the microtubule cytoskeleton. So far, the role of this polarity on megasporocyte development is unknown. The disruption of this polarity in megasporocytes was observed in *switch1 (swi1)/dyad* ovules in *Arabidopsis* (42, 68). *SWII* encodes a novel protein involved in chromatid cohesion establishment and chromosome structure during meiosis (2, 38). Interestingly, *dyad* mutation causes defective meiosis that produces two unreduced megaspores (diploid) (60). However, only the chalazal megaspore, but not the micropylar megaspore, expresses functional megaspore-specific markers. This indicates that only the chalazal megaspore is functional and suggests a position-dependent mechanism. It also implies that a decision on cell fate has been made at this stage of ovule development. In maize, a similar mutation in *aneiotic (am1)* has been identified in which meiosis is replaced by a mitotic division, as in *swi1/dyad*. Analysis of *am1* allelic mutations indicates that the division of the megasporocyte is completely blocked in some alleles, or meiosis is initiated but not completed in other alleles. However, all meiotic processes are impaired in *am1*. Together, these data suggest that SWI and AM1 are essential for the switch between meiotic and mitotic division cycles and likely regulate the transition through a novel leptotene–zygotene checkpoint. AM1, which shares 30% identity with SWI1, is a plant-specific chromatin-binding protein with as yet unknown function (54).

Surprisingly, the chalazal unreduced megaspore in *dyad* mutants occasionally proceeds to form an unreduced embryo sac that can be fertilized to produce triploid seeds (60), which may have implications for engineering hybrid seed production in agriculture. This is likely linked to the truncated SWII protein produced in *dyad*. The functional dissection of SWII and AM1 will shed light on how meiotic to mitotic transition is regulated.
PROGRESSION OF THE GAMETOPHYTIC MITOTIC CYCLE

The functional megaspore undergoes rapid growth, taking up the space left by the degenerating megaspores, and goes through three consecutive rounds of karyokinesis to form an eight-nucleate syncytial embryo sac. This sac is cellularized simultaneously to form a seven-celled female gametophyte, composed of four different cell types: egg, synergid, central, and antipodal. In the past decade, genetic studies have identified several mutations and genes that control different stages of embryo sac development (50). Meanwhile, comparative expression-profiling studies and cell-specific EST sequencing have revealed many genes that are expressed in the female gametophyte (31, 70, 74, 76, 77). We discuss progress in each developmental stage and focus on recent important findings below.

The Initiation of Female Gametogenesis

Little is known about the genetic and molecular control of the initiation of female gametogenesis in flowering plants, although many gametophytic mutations block embryo sac development at the one-nucleate stage. Mutations in \textit{AGL23}, a type I MADS-box gene, block the first nuclear division of the functional megaspore (12). \textit{AGL23} expression is first detected in the functional megaspore and persists in the embryo sac. Together these data suggest that \textit{AGL23}-regulated transcription is required for early female gametogenesis. However, \textit{AGL23} may not be required for the initiation or cell division of the functional megaspore, or cell cycle progression during subsequent embryo sac development in \textit{Arabidopsis}.

Using monoclonal antibodies against cell wall components, female reproductive lineage has been associated with distinct changes in the distribution and types of arabinogalactan protein (AGP) epitopes (11). Whether AGPs function in cell surface signaling or recognition during gametogenesis remains to be clarified. In \textit{AGP18} knockdown plants, the functional megaspore fails to enlarge and divide. \textit{AGP18} is expressed in the female germline including the functional megaspore, and it is weakly expressed in somatic nucellar cells. This suggests that a cell surface proteoglycan is required for functional megaspore development in \textit{Arabidopsis} (1).

Control of the Gametophytic Cell Cycle

Many female gametophyte (\textit{fem}) mutations, which were identified through a distorted Mendelian segregation screen, display mitotic arrest of the female gametophytic division cycle (50). This indicates that progression of the mitotic cycle is critical for the formation of a functional gametophyte. Several mutants defective in the progression of the mitotic division cycle have been identified and molecular cloning of these genes is starting to shed light on how cell cycle progression is regulated during female gametogenesis in plants.

The anaphase-promoting complex/cyclosome (APC/C) is a cell cycle–regulated, multiple-subunit E3 ubiquitin-protein ligase that controls important transitions during mitotic progression and exit by sequentially targeting for degradation many cell cycle regulators, such as cyclins (55). The knockout of APC/C components often impairs female gametophyte development. Mutations in either \textit{NOMEGA}, which encodes APC6/CDC16, or \textit{APC2}, which interacts with APC11 and APC8/CDC23, cause embryo sac arrest at the two-nucleate stage (7, 29). This indicates that the APC/C ubiquitin-mediated proteolysis pathway plays a role in female gametophytic cell cycle control. Similarly, mutations in \textit{regulatory particle triple A ATPase (RPT)} of the 26S proteasome arrest embryo sac development at the one- or two-nucleate stage in the \textit{rpt5a-4 \textit{rpt5b}} double mutant, which indicates a defect of the first or second mitosis of the female gametophyte (17). Similarly, the double mutation of two RING-finger E3 ligase genes...
RHF1a and RHF2a results in the interphase arrest of the mitotic cell cycle in the female gametophyte. RHF1a directly targets a cyclin-dependent kinase inhibitor ICK4/KRP6 for proteasome-mediated degradation (34). This suggests that the ubiquitin/26S proteasome system helps control cell cycle progression during female gametophyte development.

PRL encodes the DNA replication licensing factor subunit MCM7 that is required in all proliferating cells, including female germline cells (69). Knockout of PRL function arrests embryo sacs primarily at the one-nucleate stage. Recently, we identified the slow walker1 (swa1) mutation that causes slow progression of the gametophytic division cycle and female sterility owing to incomplete development of the female gametophyte at anthesis (65). Delayed pollination tests showed that a small fraction of swa1 ovules are able to form functional female gametophytes; however, they missed the correct time for fertilization because of the slow down of female gametophyte development when naturally pollinated, which indicates that coordinated development between the male and female gametophyte is critical for fertility in Arabidopsis. SWA1 encodes a nucleolar WD40-containing protein that is involved in the processing of pre-18S rRNA in Arabidopsis, which suggests that RNA biogenesis plays a role in the progression of the gametophytic division cycle.

Mutations in the Arabidopsis retinoblastoma-related (RBR) gene, a key negative regulator that controls G1/S transition by repressing E2F transcription factors, result in arrested mitosis and uncontrolled nuclear proliferation, which gives rise to embryo sacs with supernumerary nuclei that are irregular in size and partially enclosed by cell-wall-like structures (14). This indicates that cellularization and karyokinesis can be uncoupled, and they are regulated independently. Consistently, premature cellularization has been observed in badad (bdd) ovules after the first or second gametophytic division (41). Although most proliferating rbr female gametophytes fail to express cell-specific markers, a fraction of ovules in selfed rbr1–1/+ siliques or rbr1–1/+ siliques that are pollinated with wild-type pollen form abnormal embryos, which implies that RBR is required for a complete differentiation of all gametophytic cells (14, 26). This indicates that rbr mutant ovules are able to cellularize and form functional egg cells, which suggests that egg cell fate requires the completion of a third nuclear division, but not an arrest in the gametophytic cell cycle. Therefore, RBR connects cell cycle control to cellular differentiation processes.

In the indeterminate gametophyte1 (ig1) mutant of maize, female gametophytes have a prolonged phase of free nuclear divisions before cellularization, which leads to a variety of embryo sac abnormalities, including extra egg cells, extra synergids, and extra central cells with extra polar nuclei (16, 20). The rbr1 mutants of Arabidopsis have a similar phenotype, with additional defects in pollen development. This suggests that IG1 restricts the proliferative phase of female gametophyte development.

Together, these data suggest that timely mitotic progression of the division cycle is vital for gametogenesis. In addition to the conserved cell cycle machinery, other regulatory mechanisms may play a role in gametophytic cell fate in plants. One such mechanism is protein phosphorelay. The loss of CYTOKININ INDEPENDENT1 (CKI1) function also results in the early degeneration of gametophytic cells and excess nuclei (56). CKII encodes a histidine kinase, which suggests that His/Asp phosphorelay signaling plays a role in female gametogenesis. At the onset of megagametogenesis, RNA interference depletes CHR11, a member of the ATP-dependent SWI2/SNF2 family of chromatin-remodeling factors. This depletion arrests uncellularized embryo sac development at the one-, two-, four-, or eight-nucleate stages in Arabidopsis, which indicates a role for chromatin-remodeling in karyokinesis during female gametogenesis (22). RBR suppresses E2F-like protein activity by recruiting chromatin-remodeling factors of the SWI2/SNF2 family. Together, CHR11 and RBR regulate the E2F family proteins, and thereby promote nuclear proliferation during female gametogenesis. Furthermore, histone
acetyltransferase HAM1 and HAM2 act redundantly during ovule development because ham1
ham2 double mutant embryo sacs arrest at the one-nucleate stage with a huge nucleus (30),
which indicates that gene suppression and chromatin compaction requiring hypoacetylation of
histones in the functional megaspore block the first karyokinesis during megagametogenesis.

CELLULARIZATION OF THE EMBRYO SAC

Cellularization of the syncytial embryo sac is controlled temporally and spatially during
ovule development, and it is critical for gametophytic cell fate specification. Several genes have
been implicated in controlling this process. In gemini pollen2 (gem2), the cellularization of the
embryo sac is impaired: resulting in embryo sacs that contain five nuclei at the micropylar
pole and three at the chalazal pole. Moreover, no cell boundaries or only partial cellularizations
are observed between nuclei. Most mature mutant embryo sacs contain one or two extremely large nuclei, which might arise from the fusion of free nuclei at the micropylar pole (52). However, gem2 plants also display a variety of division defects in pollen development, which include division asymmetry and incomplete cytokinesis. Together, these data suggest that GEM2 plays a critical role in coordinating karyokinesis and cytokinesis during gametogenesis. Interestingly, the partial cellularization of gem2 ovules can develop further and form an embryo sac with a large cell that possesses vacuolar characteristics of an egg cell, which suggests that egg cell fate can occur in a partially cellularized embryo sac, and local cellularization may reinforce a gradient of cell fate determinants that are established in the coenocytic embryo sac (52). In addition, gem1/mor1 and two in one (tio)
mutants display similar or even more severe ovule phenotypes as gem2. GEM1/MOR1 encodes a microtubule-associated protein (73), and TIO encodes an essential phragmoplast-associated protein that is homologous to the-FUSED (Fu) Ser/Thr protein kinase of the

hedgehog signaling complex in animals. Both GEM1/MOR1 and TIO have a general effect on
plate formation in somatic and reproductive cells. The incomplete cell plate in tio
pollen is positioned correctly, which suggests that TIO is not required for the positioning or
establishment of the cell plate but has a specific role in cell plate expansion (47). In addition,
knockout of TUBG1 and 2 genes, which encode γ-tubulin, results in uncellularized embryo sacs
with aberrant morphology, positioning, and number of nuclei (53). In plant cells, the lateral
expansion of phragmoplast and cell plate is regulated by a kinesin-MAPKKK pathway during
cytokinesis; mutations in two kinesin-like proteins, AtNACK1 and AtNACK2, that bind and
activate the MAPKK kinase NPK1 result in the mispositioning of nuclei and the formation of
nonfunctional gametophytes (72). Therefore, they all play a role in positioning phragmoplast
and cell plate expansion, thereby controlling the cellularization of the embryo sac. However, it
is not clear how the cell plate expansion phenotype is associated with the karyokinesis defect.

EMBRYO SAC POLARITY AND GAMETOPHYTIC CELL SPECIFICATION

The embryo sac is a polarized structure with antipodal cells at the chalazal end and the egg
apparatus at the micropylar end. Gametophytic cells within the embryo sac are also highly polarized. The nucleus of the egg cell is located toward the chalazal end of the embryo sac, whereas the nuclei of the synergid and central cells are located toward the micropylar end. Therefore, the egg cell, and the central cell and synergids have opposite polarity. The opposite polarity of the central cell and egg brings their nuclei into close proximity, which may facilitate their fusion with the sperm nuclei during double fertilization. The final polarity of the embryo sac is a result of coordinated nuclear division and positioning, expansion of the central vacuole, and cellularization. This polarity can be traced back to the four-nucleate stage, when two pairs of nuclei, separated by a large
central vacuole, migrate to opposite ends of the syncytial embryo sac. At the chalazal pole, the nuclei are positioned one above the other with respect to the micropylar-chalazal axis. Meanwhile, the nuclei generally locate side by side at the micropylar end. Comparatively large nucleoli distinguish the two polar nuclei in the eight-nucleate stage. The migration and precise positioning of the nuclei and morphological differentiation of the polar nuclei suggest an early distinction between the nuclei before cellularization. Polar expression of genes has also been observed. For example, the *DEMETER (DME)* gene, a key regulator of gene imprinting during endosperm development, is polarly expressed in the micropylar domain of the embryo sac: first in the polar nuclei and synergid nuclei before cellularization and then restricted to the central cell after cellularization. *FIS2*, a downstream gene of *DME*, is expressed only in the polar nuclei but not in the future synergid or egg, or antipodal nuclei (9). In *rbr* mutant embryo sacs, *FIS2* expression is lost or occasionally deregulated, which indicates the RBR-controlled *FIS2* polarity is necessary for the differentiation of the central cell in the embryo sac (26). This polarity further suggests a role for positional cues in gametic cell specification. Compared to embryo sac polarity, the polarity within gametophytic cells is obviously established after cellularization of the eight-nucleate embryo sac. The differentiation of egg and synergid cell fate also suggests that a lateral inhibition mechanism may exist for gametophytic cell specification after cellularization (19).

Although the cellular and molecular basis of gametophytic cell specification in the embryo sac is largely unknown, emerging evidence supports the involvement of positional and lateral inhibition mechanisms in determining gametophytic cell fate. Several studies in maize and *Arabidopsis* support the idea of a positional mechanism. In the maize *ig1* mutant, embryo sacs undergo extra rounds of free nuclear divisions, which result in extra egg cells, extra central cells, and extra polar nuclei (17, 23). The final fate of the extra nuclei as either egg nuclei or polar nuclei depends on their relative position in the embryo sac. *IG1* encodes a LATERAL ORGAN BOUNDARIES (LOB) domain protein with high similarity to ASYMMETRIC LEAVES2 (17), which controls leaf symmetry in *Arabidopsis*. During female gametogenesis, *IG1* is expressed in functional, but not nonfunctional, megaspores; it is strong in antipodal cells and the egg; and no signal is detected in the synergids (16). Thus *IG1* is asymmetrically expressed in the embryo sac, which supports the existence of embryo sac polarity.

Recently, Sundaresan and colleagues discovered an asymmetric auxin gradient in the syncytial embryo sac that plays a key role in gametic cell specification (49). Using the synthetic *DR5:GFP* or *DR5:GUS* reporter that is responsive to auxin, the auxin response can be traced by monitoring reporter expression. During megasporogenesis, GFP or GUS expression is detected at the distal tip of the nucellus and increases in nucellar cells that surround the developing embryo sac at the early one-nucleate stage. As the ovule develops, auxin level increases within the micropylar domain of the embryo sac at the two- to eight-nucleate stages. Interestingly, the auxin response distribution is less polarized in the cellularized embryo sac, because the reporter is expressed in all gametophytic cells. These data suggest a micropylar-chalazal gradient of auxin in embryo sacs. Consistently, the disruption of auxin responses by downregulating *AUXIN RESPONSE FACTOR (ARF)* gene expression, or auxin synthesis by ectopic expression of the auxin biosynthetic *YUC1* gene, impairs gametophytic cell identities at the micropylar domain. Specifically, synergids adopt the fate of the egg cell in *ARF* knockdown embryo sacs, and ectopic *YUC1* expression results in the misspecification of all gametophytic cells. However, no abnormalities in embryo sac polarity or changes in central cell and antipodal cell fates are observed. This suggests that such an auxin gradient is essential for gametophytic cell specification but not for embryo sac polarity and central cell and antipodal cell fates. Furthermore, auxin polar transporters of the PIN family are not expressed in the embryo sac, suggesting that the auxin

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As discussed above, embryo sac polarity and an auxin gradient play a key role in gametophytic cell fate. The manipulation of auxin responses or synthesis results in the switching of gametic and nongametic cell fates (49). To identify genes that control egg cell fate, Gross-Hardt and colleagues (19) mutagenized an egg cell-specific marker line and then screened for expression changes of the egg cell-specific marker. They identified three mutants, lachesis (lis), datbo (do) and atropos (ato), that showed deregulation of the marker (19, 40). In lis embryo sacs, the expression of the egg–specific marker is expanded to the synergids and central cell. Consistently lis synergids display egg cell morphology defects and downregulate synergid-specific gene expression. Pollen tube attraction is compromised, and reduced as well. These results indicate that the synergids have adopted an egg cell fate in lis embryo sacs. Interestingly, the ET884 synergid-specific marker is ectopically expressed throughout lis embryo sacs. Similarly, the central cell in lis embryo sacs also adopts an egg cell fate. Polar nuclei rarely fuse and cellularize separately to give rise to small uninucleate cells that are morphologically indistinguishable from the egg cell. Compromised central cell fate is further suggested by the downregulation of expression from the central cell-specific MEA promoter. Surprisingly, the antipodal cells of lis embryo sacs adopt a central cell fate as evidenced by their fusion, and the downregulation of an antipodal-specific marker, and the activation of a central cell-specific marker (19). A similar phenotype is also found in the clo/gfa1 mutant (40). Unlike that of the igl mutant in maize, the accessory cell (synergid and antipodal cell) fates have been mis-specified because no supernumerary nuclei or cells are observed in lis or clo/gfa1 embryo sacs. One idea that accounts for these phenotypes is that accessory cells are gradually recruited as gametic cells. This demonstrates a central role for LIS and CLO/GFA1 in gametic cell fate specification during female gametophyte development. Furthermore, LIS nuclear localization requires the CLO/GFA1 protein. The findings above have several profound implications, as pointed out by Gross-Hardt and colleagues (19). First, all gametophytic cells are competent to adopt gametic cell fate, and LIS is involved in a mechanism that represses gametic cell fate in accessory cells. Second, there might be an intracellular signaling mechanism that senses the number of nuclei in a given cell. Third, there are two levels of cell fate regulation: one between the gametic cell and accessory cells, and the other between the egg cell and central cell. Together, these genes might be involved in an as yet unknown signaling pathway that operates in gametic cells and prevents accessory cells from adopting a gametic cell fate.

Interestingly, LIS, CLO/GFA1, and ATO all encode components of RNA splicing machinery. LIS is homologous to the yeast splicing factor PRP4; CLO/GFA1 is a plant homolog of yeast Smn114p, likely a component of the U5 snRNP of the spliceosome, and is required for the cell-specific expression of LIS gene (35, 40). This implies that LIS is downstream of CLO/GFA1 in the pathway controlling gametic cell specification. In addition, LIS and CLO/GFA1 are colocalized to nuclear speckles; this suggests they may form a complex as well. ATO is homologus to SF3a60, a protein that is implicated in prespliceosome formation. These findings suggest that the RNA splicing machinery plays an important role in gametic cell specification in the female gametophyte, although the underlying mechanisms remain to be elucidated.

The LIS gene is expressed strongly in reproductive tissues and remains high in gametic cells but downregulated in accessory cells shortly after cellularization. Combined with its mutant phenotype, this has led to a lateral inhibition model in which, upon differentiation, gametic cells generate an inhibitory signal that is transmitted to adjacent cells to prevent excess gametic cell formation (19). This model can explain the maintenance of only one egg cell in the embryo sac after the initial specification of
cell fate. However, once the egg cell is differentiated, a lateral inhibition mechanism may be necessary to maintain cell fates because accessory cells can differentiate into gametic cells later if this inhibitory mechanism is not present (19).

EOSTRE, which encodes a BELL-like homeodomain protein (BLH1), plays a role in restricting synergid cell fate. In the eostre mutant, the female gametophyte is arrested at multiple stages that range from one-nucleate to mature embryo sacs, and some mutant embryo sacs collapse completely. The mutant embryo sacs often display mispositioned nuclei during the syncytial stage. Interestingly, a portion of eostre embryo sacs displays abnormal cell specification, in which one of the two synergids exhibits polarity characteristic of the egg cell and expresses an egg cell-specific marker. This indicates that one synergid has adopted an egg cell fate, and therefore that there are two egg cells in a single embryo sac, whereas the initial central cell and the egg cell fate are not affected (51). Furthermore, the extra egg cell can be fertilized after pollination, which indicates that it is fully functional. Molecular analysis showed that the eostre phenotype is caused by the misexpression of the EOSTRE gene, which is not expressed in the ovule in wild type. BELL functions by forming a BELL-KNAT heterodimer whose activity is regulated by ovate family proteins (OFPs). Consistently, the eostre phenotype can be reversed by a mutation in the class II knox gene KNAT3 and phenocopied by disruption of AtOFP5, a regulator of BLH1-KNAT3 heterodimers (51). Together, these data suggest a role for BLH-KNAT3 complex and AtOFP in gametic cell specification. Other BELL-like genes may substitute the EOSTRE function to promote egg cell fate in female gametophytes because the loss of EOSTRE function has no effect on female gametophyte development. In addition, it would be interesting to know whether the aberrant nuclear configuration observed in eostre syncytial embryo sacs or the polarity change in one of the synergid cells has any role in gametic cell specification.

THE FUNCTIONAL FEMALE GERM UNIT

The Egg Cell

Egg cell specification is a result of the interplay between the auxin gradient and the LIS-mediated mechanism as discussed above. Although many egg-specific transcripts have been identified through single cell library and EST analysis, key genes that control egg cell function have not been identified. GAMETE EXPRESSED3 (GEX3) is expressed specifically in the egg cell of the female gametophyte and in pollen, and encodes a plasma membrane-localized protein with unknown function that has homologs in other plants. Both knockdown and overexpression of GEX3 impair the female control of micropylar pollen tube guidance (3). Although the underlying mechanism is still unknown, this demonstrates a role for the egg cell in the female control of pollen tube guidance, in addition to fertilization.

The Synergid Cell

Synergid cells are key components of the female germ unit (FGU) and are located, side by side with the egg cell, in the micropylar portion of the embryo sac. Typically, the synergid cells display an opposite polarity compared to the egg cell, they have no or discontinuous cell wall at the chalazal end, and they are sealed by a specialized cell wall-like structure—the filiform apparatus at the micropylar opening. Often, one of the two synergids undergoes cell death upon arrival of the pollen tube. The synergids likely play important roles in the attraction and recognition of the pollen tube, sperm release, and transportation. Therefore, genes that are involved in those processes may be expressed in synergids. However, the mechanisms by which synergid cells develop their unique features are poorly understood.

Mutations that affect synergid development and function and genes that are specifically expressed in the synergids have been identified (70). MYB98, which encodes a R2R3-type MYB
transcription factor, is specifically expressed in the synergids. Mutation in the MYB98 gene specifically abolishes the formation of the filiform apparatus and has no effect on other aspects of ovule development (28). Thus, MYB98 is required specifically for the formation of the filiform apparatus during synergid cell differentiation. Consistently, recent studies show that MYB98 binds to a specific DNA sequence (TAAC) and regulates a subset of genes that encode secreted proteins targeted to the filiform apparatus (59). Most synergid-expressed genes that are downregulated in myb98 encode small defensin-like cysteine-rich proteins (CRPs) that are secreted into the filiform apparatus, which suggests that they play a role in either the formation or the function of the filiform apparatus (59). Many of these genes are also weakly expressed in egg and/or central cells. Interestingly, myb98 female gametophytes that lack the filiform apparatus also lose their ability to guide the pollen tube to the micropyle; therefore, MYB98 may also play a role in the production of the guidance cue. Together, these data strongly suggest that MYB98 acts as a synergid-specific transcriptional regulator to activate downstream genes that are required for pollen tube guidance and filiform apparatus formation.

To attract and recognize the pollen tube, synergids need the ability to secrete pollen attracting signals and receive the pollen tube. Recent studies have provided strong evidence of this. Using laser ablation, synergids, but not any other gametophytic cells, were shown to be required for pollen tube guidance in Torenia fournieri (21). Also in T. fournieri, CRPs named LUREs have been identified and are secreted by synergids to attract pollen tubes in a semi–in vivo assay (48). In Arabidopsis, CRPs are encoded by a large gene family and expressed in the synergids and secreted into the filiform apparatus as discussed above. However, genetic data that support their role as the guidance cue are still lacking.

Synergid cells also play an essential role for pollen tube reception. FERONIA (FER) (allelic to SIRENE, SRN) is a key gene that controls the recognition between the synergids and the pollen tube. In fer/srn ovules, the pollen tube enters a synergid and overgrows within the embryo sac (23, 61), which suggests that FER/SRN plays a role in pollen tube reception by the synergid. FER encodes a LRR-RLK that accumulates specifically on the plasma membrane of the synergids (15). Therefore, FER may be a synergid-specific membrane receptor which, upon binding to a signal from the pollen tube, triggers a signaling cascade within the synergids to prepare for fertilization and also sends a signal to stop pollen tube growth (15, 37). In support of this idea, additional mutations are also reported in Arabidopsis. In the lorelei (lre) mutant, pollen tubes that reach embryo sacs often do not rupture but continue to grow in the embryo sac, reminiscent of the fer/srn phenotype. Moreover, lre embryo sacs often attract additional pollen tubes. LRE encodes a small plant-specific putative glucosylphosphatidylinositol-anchored protein and is expressed in synergid cells prior to fertilization (76). Pollen tubes of anxur1/anxur2 double mutants rupture before arriving at the synergid cells (39). Both ANXUR1 and 2 genes encode receptor-like kinases that are specifically expressed in pollen tubes. In addition, mutations in ABERRANT PEROXISOME MORPHOLOGY2/ABSTINENCE BY MUTUAL CONSENT (APM2/AMC), which is required for peroxisome transport, impair pollen tube reception (5). This suggests another signaling cascade, independent of the FER/SRN-ANXUR kinase pathway that requires intact peroxisome function in both male and female gametophytes, although the underlying mechanism remains to be elucidated.

Synergid cell death associated with fertilization is a common phenomenon, which has been described in many plant species and invariably involves the collapse of vacuoles, a dramatic decrease in cell volume, and complete disintegration of the plasma membrane and most organelles. The underlying mechanisms have not been revealed so far. In Arabidopsis,
synergid cell death is initiated upon pollen tube arrival but before pollen tube discharge, which suggests that pollen tube-synergid contact triggers a signaling cascade that induces synergid cell death (62). In \textit{gfa2} mutant ovules, the polar nuclei fail to fuse and the synergid persists after pollination. These ovules can attract pollen tubes but are not fertilized. This indicates that synergid cell death associated with fertilization did not occur, which suggests a role for \textit{GFA2} in promoting synergid cell death (10). The \textit{GFA2} gene encodes a mitochondrion-located DnaJ domain–containing protein similar to yeast Mdj1p that functions as a chaperone in the mitochondrial matrix. Consistently, \textit{GFA2} partially complements a yeast \textit{mdj1} mutant, which suggests a role for mitochondria in synergid cell death in plants. How \textit{GFA2} acts in the mitochondria in promoting synergid cell death remains unknown. Interestingly, cell deaths of nonfunctional megaspores and antipodal cells are not affected in \textit{gfa2} mutants; this suggests that cell death pathways are different between synergids and antipodal cells.

The Central Cell

Molecular mechanisms that control the specification and differentiation of the central cell are poorly understood. Genetic evidence suggests that Type I MADS-box genes play an important role in central cell development. In the \textit{diana} (\textit{dia}, \textit{agl61}) mutant, polar nuclei of the central cell are not fused, and central cell morphology is aberrant. The mutant embryo sac is able to attract a pollen tube but fertilization of the central cell does not occur (4, 71). Egg- and synergid-specific markers, but not central cell-specific markers, are expressed in \textit{dia} ovules, which indicates that egg and synergid fates are specified, and that central cell fate is impaired in the mutant. \textit{DIA} is expressed exclusively in the late central cell, and the \textit{DIA} protein is localized in the polar nuclei and the central cell nucleus. All these data suggest that \textit{DIA} is required for central cell differentiation and function. \textit{DIA} forms a heterodimer with \textit{AGL80}, and its nuclear localization requires \textit{AGL80} because \textit{DIA} nuclear localization is lost in the \textit{agl80} mutant. \textit{AGL80} is also expressed in the polar nuclei and the secondary nucleus of the central cell, and a mutation in the \textit{AGL80/FEM111} gene in \textit{Arabidopsis} specifically affects central cell maturation. Polar nucleoli and vacuole maturation fail and lead to endosperm development arrests after fertilization. The egg, synergid, and antipodal cells are correctly specified (57). Therefore, both \textit{DIA} and \textit{AGL80}, most likely forming a heterodimer, are required for polar nuclear fusion and central cell differentiation. Furthermore, \textit{AGL80}, by interacting with another Type I MADS-box protein \textit{AGL62}, also plays a critical role in endosperm development. \textit{AGL62} is expressed in the syncytial endosperm and is suppressed by the FIS Polycomb complex just before endosperm cellularization. Mutation in \textit{AGL62} causes precocious cellularization of the endosperm (13, 27). Together, these Type I MADS-box transcription factors are critical for central cell and endosperm development in \textit{Arabidopsis}. It would be interesting to know the downstream genes controlled by the DIA-AGL80 complex. Likely candidates would be \textit{DD46} and \textit{DME}, which are not expressed in \textit{agl80} mutant ovules (57). However, there is no MADS-box binding site, the CarG box \([CC(A/T)_{6}GG]\) found in either gene, which suggests that they may activate these downstream genes indirectly. Therefore, the DIA-AGL80 complex may ultimately activate the transcription of Polycomb group genes in the central cell to repress endosperm development prior to fertilization.

In \textit{magatama3} (\textit{maa3}) mutant ovules, the polar nuclei fail to fuse at pollination and contain a smaller nucleolus that lacks the vacuolar-like internal structure as compared to that of the wild-type. Consequently, central cell development is arrested in the mutant, which ultimately results in defective micropylar pollen tube guidance in \textit{Arabidopsis} (57). The \textit{MAA3} gene encodes a homolog of yeast SLICING ENDONUCLEASEASE1 (\textit{SEN1}) helicase involved in processing
of a variety of RNA species in yeasts. Therefore, MAA3 may regulate the RNA metabolism that is responsible for nucleolar organization of the central cell and micropylar pollen tube guidance.

The fusion of the polar nuclei is an important step in central cell development. Electron microscopy revealed that the fusion of the polar nuclei begins with contact with endoplasmic reticulum (ER) membranes that are continuous with the outer nuclear membranes of the polar nuclei. First, the fusion with the ER membranes gives rise to a continuous outer nuclear membrane that brings the inner nuclear membranes into close contact, which leads to its final fusion. So far, little is known about the molecular basis of polar nuclear membrane fusion, although many mutations and genes have been identified (10, 50, 58). Interestingly, most of the genes whose mutation blocks polar nuclear fusion encode mitochondrial proteins. Similarly, mutation in GLUCOSE 6-PHOSPHATE/PHOSPHATE TRANSLOCATOR1 (GPT1) also blocks polar nuclear fusion in the central cell (44). One possibility is that high respiratory activity is required for central cell development because the central cell has such a large cytoplasm. Alternatively, an intracellular feedback mechanism among the organellar and nuclear genomes may coordinate central cell development. GFA2 has been implicated in the membrane fusion. In gfa2 mutants, the polar nuclei fail to fuse; their outer nuclear membranes come into contact but do not fuse (10). This suggests that GFA2, either directly or indirectly, is required for the membrane fusion of the polar nuclei in Arabidopsis.

As the polar nuclei fuse, central cell specification and functional differentiation occur. In glauce (glc) mutant embryo sacs, gametophytic cells develop and are specified normally as manifested by the correct expression of gametophytic cell-specific markers; however, the central cell cannot be fertilized (43).

Genetic analysis suggests that GLC is epistatic to MEA and plays an essential role in the maternal control of embryo and endosperm development, which suggests that it might play a role in central cell competence for fertilization. The molecular nature of GLC remains to be revealed.

In addition to its role in fertilization, the central cell also plays a role in pollen tube guidance. Pollen tube guidance is abolished in several mutants that disrupt central cell development. This includes maa1 and maa3 in which the polar nuclei fail to fuse (66, 67), which indicates a defect in central cell development or/and the maturation of the female gametophyte. In contrast, in the central cell guidance (ccg) mutant, central cell development is not affected because it does not display any morphological abnormality and expresses a central cell-specific marker (8). CCG is expressed in the central cell of the mature embryo sac specifically and encodes a nuclear protein that might play a role in regulating the expression of a subset of genes that are necessary for pollen tube guidance in the central cell.

CONCLUSIONS

Molecular mechanisms that control the germline, gametic cell specification, and cell-cell interactions in plant gametogenesis are beginning to be revealed. Genes that control these processes have been identified (Table 1). The haploid female gametophyte provides us with an exciting system to investigate developmental roles of the RNA splicing machinery; signaling pathways mediated by membrane receptor kinases like MSP1, FER, and ANUXR; and genetic control of cell fates. Emerging combinatory approaches that employ genetics, single-cell based genomics, and biochemistry will undoubtedly facilitate deciphering the genetic complexity and molecular mechanisms that control female gametogenesis in angiosperms.
Table 1  List of genes discussed in the text

<table>
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<tr>
<th>Gene name</th>
<th>Protein function</th>
<th>Biological function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SPOROCYTELESS (SPL/NOZZLE)</strong></td>
<td>Transcription regulator</td>
<td>Germline cell fate</td>
<td>24, 63, 75</td>
</tr>
<tr>
<td><strong>AGAMOUS (AG)</strong></td>
<td>MADS-box protein binds to CArG-box DNA sequence</td>
<td>Activation of the SPL gene, meristem determinacy and floral organ identity</td>
<td>24</td>
</tr>
<tr>
<td><strong>MULTIPLE ARCHESPORIAL CELLS 1 (MAC1)</strong></td>
<td>Not available</td>
<td>Germline cell number</td>
<td>64</td>
</tr>
<tr>
<td><strong>MULTIPLE SPOROCYTE 1 (MSP1)</strong></td>
<td>Leucine rich repeat–containing receptor protein kinase</td>
<td>Numbers of male and female sporocytes</td>
<td>45</td>
</tr>
<tr>
<td>Oo/TDL1A</td>
<td>Putative ligand of MSP1</td>
<td>Numbers of male and female sporocytes</td>
<td>78</td>
</tr>
<tr>
<td><strong>MEIOTIC ARRESTED AT LEPTOTENE1 (MEL1)</strong></td>
<td>A germline-specific member of ARGONAUTE genes family</td>
<td>Germline development; regulator of early meiosis</td>
<td>46</td>
</tr>
<tr>
<td><strong>SWITCH1 (SWI1)/DEAD</strong></td>
<td>Novel protein</td>
<td>Chromatid cohesion establishment and chromosome structure in meiosis</td>
<td>2, 38</td>
</tr>
<tr>
<td><strong>AMEIOTIC (AMI)</strong></td>
<td>A plant-specific chromatin-binding protein, with 30% identity with SWI1</td>
<td>Regulator of leptotene to zygotene transition in meiosis</td>
<td>54</td>
</tr>
<tr>
<td><strong>AGL23</strong></td>
<td>Type I MADS-box family transcription factor</td>
<td>Early female gametogenesis</td>
<td>12</td>
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<tr>
<td><strong>ARABINOGALACTAN PROTEIN 18 (AGP18)</strong></td>
<td>A cell surface arabinogalactan proteoglycan</td>
<td>Functional megaspore development</td>
<td>1</td>
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<tr>
<td><strong>ANAPHASE-PROMOTING COMPLEX/CYCLOSONE (APC/C)</strong></td>
<td>Multiple-subunit E3 ubiquitin-protein ligase</td>
<td>Mitotic progression</td>
<td>7, 17, 29, 34</td>
</tr>
<tr>
<td><strong>NOMEGA/APC6/CDC16</strong></td>
<td>A component of the Anaphase Promoting Complex</td>
<td>Cell cycle control</td>
<td>7, 29</td>
</tr>
<tr>
<td><strong>REGULATORY PARTICLE TRIPLE A ATPASE (RPT)</strong></td>
<td>A regulatory subunit of the 20S proteosome; a member of the AAA superfamily</td>
<td>Cell cycle control</td>
<td>17</td>
</tr>
<tr>
<td><strong>RING-FINGER E3 LIGASE 1a and 1b (RHF1a and RHF2a)</strong></td>
<td>Ring-finger E3 ligase that targets a cyclin-dependent kinase inhibitor ICK4/KRP6 for proteosome-mediated degradation</td>
<td>Cell cycle control</td>
<td>34</td>
</tr>
<tr>
<td><strong>SLOW WALKER 1 (SWA1)</strong></td>
<td>A nucleolar WD40-containing protein controlling pre-18S rRNA processing</td>
<td>Cell cycle progression</td>
<td>65</td>
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<tr>
<td><strong>RETINOBLASTOMA-RELATED (RBR)</strong></td>
<td>Negative regulator of G1/S transition</td>
<td>Cell cycle control</td>
<td>14, 26</td>
</tr>
<tr>
<td><strong>HADAD (HDD)</strong></td>
<td>Not available</td>
<td>Cell cycle control</td>
<td>41</td>
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<tr>
<td><strong>INDETERMINATE GAMETOPHYTE1 (IG1)</strong></td>
<td>LATERAL ORGAN BOUNDARIES (LOB) domain protein</td>
<td>Cell cycle control</td>
<td>16, 20</td>
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<tr>
<td><strong>CYTOKININ INDEPENDENT1 (CKI1)</strong></td>
<td>Histidine kinase</td>
<td>Cytokinin signaling</td>
<td>56</td>
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<tr>
<td><strong>CHROMATIN-REMODELING FACTOR 11 (CHR11)</strong></td>
<td>A member of the ATP-dependent SWI2/SNF2 family of chromatin-remodeling factors</td>
<td>Cell division control</td>
<td>22</td>
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<tr>
<td><strong>GEMINI POLLEN2 (GEM2)</strong></td>
<td>Not available</td>
<td>Cell division control</td>
<td>52</td>
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(Continued)
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<th>Biological function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEM1/MOR1</td>
<td>A microtubule-associated protein</td>
<td>Cell plate formation</td>
<td>73</td>
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<tr>
<td>TWO IN ONE (TIO)</td>
<td>A phragmoplast-associated protein</td>
<td>Cell plate formation</td>
<td>47</td>
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<tr>
<td>AtNACK1 and AtNACK2</td>
<td>M-phase-specific kinesin-like protein</td>
<td>Phragmoplast and cell plate expansion</td>
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<td>Gene imprinting during endosperm development</td>
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<td>FERTILIZATION-INDEPENDENT SEED2 (FIS2)</td>
<td>C2H2 zinc finger–containing polycomb group protein</td>
<td>Suppressor of endosperm development</td>
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</tr>
<tr>
<td>LACHESIS (LIS)</td>
<td>RNA splicing factor</td>
<td>Gametic cell specification</td>
<td>19</td>
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<tr>
<td>CLOTHO (CLD)/GEA1</td>
<td>RNA processing</td>
<td>Gametic cell specification</td>
<td>35, 40</td>
</tr>
<tr>
<td>ATROPOS (ATO)</td>
<td>A component of prespliceosome and RNA splicing machinery</td>
<td></td>
<td></td>
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<tr>
<td>EOSTRE</td>
<td>A BELL-like homeodomain protein</td>
<td>Synergid cell fate</td>
<td>51</td>
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<tr>
<td>GEMETE EXPRESSED3 (GEX3)</td>
<td>A plasma membrane protein</td>
<td>Pollen tube guidance</td>
<td>3</td>
</tr>
<tr>
<td>MYB98</td>
<td>MYB family transcription factor</td>
<td>Filiform apparatus formation and pollen tube guidance</td>
<td>59</td>
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<tr>
<td>FERONIA (FER) or SIRENE (SRN)</td>
<td>Leucine rich repeat–containing receptor protein kinase</td>
<td>Synergid-pollen tube interaction</td>
<td>15, 23, 37, 61</td>
</tr>
<tr>
<td>LORELEI (LRE)</td>
<td>Putative glucosylphosphatidylinositol-anchored protein</td>
<td>Pollen tube attraction</td>
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<td>ANXUR1 and 2</td>
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<tr>
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<td>Central cell differentiation and function</td>
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<tr>
<td>AGL80/FEM111</td>
<td>Type I MADS-box transcription factor, forming a heterodimer with DIA</td>
<td>Central cell differentiation and function</td>
<td>57</td>
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<td>AGL62</td>
<td>Type I MADS-box transcription factor</td>
<td>Central cell and endosperm development</td>
<td>13, 27</td>
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<td>MAGATAMA3 (MAA3)</td>
<td>RNA helicase involved in RNA splicing</td>
<td>Central cell maturation and pollen tube guidance</td>
<td>57</td>
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<td>GLUCOSE 6-PHOSPHATE/PHOSPHATE TRANSLOCATOR1 (GPT1)</td>
<td>Transmembrane glucose 6–phosphate translocator</td>
<td>Central cell development</td>
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<tr>
<td>GLAUC (GLC)</td>
<td>Not available</td>
<td>Central cell function</td>
<td>43</td>
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<td>CENTRAL CELL GUIDANCE (CCG)</td>
<td>Putative transcription regulator</td>
<td>Pollen tube guidance</td>
<td>8</td>
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<tr>
<td>MAGATAMA 1 (MAA1)</td>
<td>Not available</td>
<td>Central cell development and pollen tube guidance</td>
<td>66</td>
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Errata

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