Genetics of gametophyte biogenesis in *Arabidopsis*
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The identification of several mutations and genes involved in sporogenesis and gametogenesis has initiated a genetic framework for understanding gametophyte biogenesis. Recent advances include the molecular characterization of genes required for sporocyte formation and meiosis. These studies have revealed some unexpected interactions linking development of sporophytic cells and tissues with initiation and progression of gametophyte development in angiosperms.

### Introduction

The alternation between a diploid sporophytic generation and a haploid gametophytic generation is fundamental to the life cycle of plants. In higher plants, the formation of the gametophyte from the sporophyte is the result of two sequential processes, sporogenesis and gametogenesis. The molecular and genetic mechanisms controlling these processes are as yet poorly understood. In recent years, genetic studies have begun to reveal mutations and, in some cases, the corresponding genes that control these processes. Genes controlling ovule and pollen development have been reviewed extensively [1,2•–5•,6••]. In this article, we review progress over the past year in the area of sporogenesis and gametogenesis has initiated a genetic framework for understanding gametophyte biogenesis. Recent advances include the molecular characterization of genes required for sporocyte formation and meiosis. These studies have revealed some unexpected interactions linking development of sporophytic cells and tissues with initiation and progression of gametophyte development in angiosperms.

### Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ant</td>
<td>aintegumenta</td>
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<tr>
<td>GC</td>
<td>generative cell</td>
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<td>gem1</td>
<td>gemini pollen 1</td>
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<tr>
<td>gf/gfa</td>
<td>gametophytic factor</td>
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<td>sap</td>
<td>sterile apetala</td>
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<td>SPL</td>
<td>SPOROCYTELESS</td>
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<td>VC</td>
<td>vegetative cell</td>
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### Formations of sporocytes

Sporogenesis starts with the differentiation of hypodermal cells of the ovules and anthers to form archesporial cells, which subsequently differentiate into sporocytes and undergo meiosis. In the ovule, the archesporial cell is a single cell that differentiates from the hypodermal layer at the distal end of the nucellus. In maize, the recessive *mac1* mutation results in the formation of multiple archesporial cells in ovules [7]. It is likely that *Mac1* regulates archesporial cell specification in the ovule primordium through a signal that suppresses differentiation of neighboring hypodermal cells. Thus far, no similar mutation has been identified in *Arabidopsis*.

### Interactions between the megasporocytes and sporophytic tissues

Sporophytic tissues such as integuments (Figure 1) play a role in the progression of the meiotic divisions of the megasporocyte as defects in integument development in *Arabidopsis* [8••]. In *spl* mutants, subepidermal cells of anther and ovule primordia are able to form archesporial cells but subsequently fail to form megaspores or microspores. In *spl* ovules, integument development is unaffected; however, in *spl* anthers, formation of the anther walls and tapetum is also disrupted. The archesporial cells divide to form sporogenous cells and primary parietal cells but further development of both cell layers is arrested. The *SPL* gene has been cloned and shown to encode a nuclear protein with limited similarities to MADS box transcription factors. *SPL* expression is restricted to sporogenous cells and microsporocytes in anthers and to megaspores in the ovules. Thus the primary role of *SPL* may be to promote the formation of male and female sporocytes and the defect in anther wall development is likely to be indirect. The implication is that development of the anther walls and tapetum is dependent upon signals from the microsporocytes; in their absence, sporophytic development of the anther cannot proceed.

### Figure 1

Schematic drawing of a 7-cell ovule near maturity in *Arabidopsis*.
nucellar region (archesporial cell and nucellar cells), later expression is predominantly in the developing integuments. The sap phenotype and the expression pattern of SAP could be taken to mean that signals from the integuments, regulated or mediated by the SAP gene product, are required for the meiotic progression of the megasporocyte.

In wild-type ovules, degeneration of the nucellar tissue occurs during embryo-sac development. When megasporogenesis is arrested, however, as in sap and spl mutants, the nucellus persists through ovule development, suggesting that formation of the functional megaspore may be required for nucellar degeneration. In spl mutants, the nucellus becomes arrested initially but later starts to divide to form an abnormal finger-like structure shortly after the completion of integument development. This may imply that signals from the developing integument are responsible for suppression of nucellar growth.

A role for ethylene in the ovule?

In orchids, the ovary is immature and lacks ovules at anthesis, and pollination triggers ovule development. It was shown that pollination induces ethylene production but a direct causality between ethylene and ovule development could not be established [13]. More definitive evidence comes from tobacco, where ovule development is completed before pollination. When pistil-specific ethylene production is abolished by gene silencing of the ethylene forming enzyme, ACC (1-amino-cyclopropane-1-carboxylate) oxidase, or its action is inhibited by silver thiosulfate, ovules are arrested and megasporocytes are unable to start or complete meiosis; consequently no embryo sacs are formed [14•]. Furthermore, application of exogenous ethylene can restore ovule development in the arrested ovary. These data demonstrate that ethylene is essential for the progression of meiosis and ovule development in tobacco. It is not known whether this is also the case for Arabidopsis, although it has been noted that the constitutive triple response 1 (ctr1) ethylene-response mutant is defective in female gametophyte development [2•]. A closer examination of the flower phenotypes of other Arabidopsis mutants defective in the ethylene pathway might be informative.

Genes involved in meiosis

Several genes and mutations that affect male meiosis have been reported recently [15–18] (Figure 2). The MALE STERILE 5 (MS5) (THREE DIVISION MUTANT 1/POLLLENLESS3) gene has been cloned and found to encode a protein with limited homologies to a synaptonemal complex protein and to cell cycle regulatory proteins; the homology to cell cycle proteins is consistent with the observed phenotype, in which the microsporocytes undergo an extra division after meiosis II [15,18,19•,20]. Two mutations that affect chromosomal separation during meiosis have been identified. The Arabidopsis skp1-like (ask1) mutation is defective in chromosomal separation in anaphase I of meiosis in the anther, resulting in the formation of a variable number (two to seven) of microspores with abnormal karyotypes [21]. The ASK1 gene product shows high homology with the human and yeast S-PHASE KINASE ASSOCIATED PROTEIN 1 (SKP1) protein, which is involved in targeting of specific proteins for ubiquitin-mediated degradation. Mutations in the SYNAPSI S 1/DETERMINATE INFERTILE 1 (SYNJ1/DIFI) gene disrupt chromosomal segregation through meiosis I and II in both male and female meiosis, resulting in non-disjunction and sterility. SYNJ1/DIFI encodes a putative cohesin, which is a homolog of proteins required for sister chromatid cohesion in fission yeast, the Rec8p/Rad21p cohesins [22,23]. Mutations in MEIOSIS 1 (MEI1), are likely to act later as it is required for cytokinesis but not for the nuclear divisions in meiosis [16].

Interestingly, mutations of MS5, TES, MEI1 and ASK1 cause defects only in male meiosis, with MEI1 and ASK1 possibly involved in proteolytic reactions required for
progression through the meiotic cell cycle. These observations suggest that different genes are employed during male and female meiosis in plants. Compared to genes that specifically control male meiosis, few genes or mutants have so far been identified that specifically affect female meiosis. In the sap1 mutant discussed earlier, only a female meiotic defect is observed, but because of the failure of anther development prior to meiosis, it is not clear if the defect is actually female specific. Recently, however, a mutant called dyad has been identified which results in female specific arrest of meiosis at the dyad stage, that is after meiosis I (I Siddiqui, personal communication).

Establishment of functional megaspore
Meiosis of the megasporocytes results in the formation of four megaspores of which only the chalazal megaspore becomes functional, while the others degenerate (Figure 3). The cell polarity of the megasporocyte has long been considered important for the specification of the functional spore [24]. In Arabidopsis, polarity of the megasporocyte is evident from the accumulation of plastids at the chalazal end and endoplasmic reticulum and vesicles at the micropylar end [25]. In addition, the presence of plasmodesmata between the chalazal megaspore and its neighboring nucellar cells suggests that there is cell–cell communication between these two distinct cell types. We have recently identified a mutation, antikevorkian (akv) that prevents degeneration of the remaining megaspores (WC Yang, V Sundaresan, unpublished data). In plants carrying the akv mutation, any one of the surviving megaspores appears capable of forming an embryo sac and, in some cases, all four megaspores appear to develop equally and form multiple embryo sacs. The AKV gene may be required for positional signaling from the chalazal megaspore to promote degeneration of the other spores, resulting in a single embryo sac per ovule.

Female gametophyte development
Several mutations affecting nuclear division and polar nuclear fusion and cell specification associated with megagametogenesis have been described [2•,26]. The majority of the mutants show defects in nuclear division. These include defects in the first division as in female gametophyte 2 (fem2), fem3, gametophytic factor (gf), gametophytic factor 4 (gfa4) and gfa5, defects in the second or third division as in cell division cycle 16 (cdc16) (HS Kwee, V Sundaresan, unpublished data) and prolifera (prl) [27], or all three divisions as in hadad (hdd) [28]. It is likely that some of these mutations involve genes that are required for operation of the cell cycle machinery, as exemplified by prl and cdc16. In gfa2, gfa3 and gfa7 mutants the two polar nuclei are able to migrate but fail to fuse, while gfa3 and gfa7 mutants are also defective in nuclear division as some ovules contain five nuclei or lack an embryo sac. In fem1 mutants, the embryo sac degenerates at anthesis and the degeneration of the central vacuole precedes the cell disintegration, while the fem4 mutants seems to have pleiotropic defects in cell morphology (26; Figure 3). Further phenotypic characterization of the mutants and isolation of corresponding genes should reveal details of the mechanisms controlling megagametogenesis in plants.

Male gametophyte development
After meiosis, the microspore undergoes two mitotic divisions in Arabidopsis, to produce the male gametophyte with two sperm cells and a vegetative cell. Curiously, disruptions of the cell cycle genes cdc16 and prl (see above) do not seem to affect male gametophyte development detectably. This could be due to functional redundancy of these genes but it may also reflect the fewer number of cell divisions that are required for male gametogenesis, so that the mutations are rescued by persistence of the parental gene product.

The first division of the microspore is highly asymmetric and results in a large transcriptionally active vegetative cell (VC) and a small transcriptionally repressed generative cell (GC) within the VC. The GC undergoes one more division resulting in the formation of tricellular pollen (Figure 2). The asymmetric division is important for the fate of daughter cells as revealed by differences in cell cycle progression.
and cell-specific gene expression [29,30••,31]. When the asymmetry is disrupted by in vitro colchicine treatment in tobacco, both daughters adopt the VC fate, which can be regarded as a default state [29]. Similar results have been obtained using the gem1 (gemini pollen 1) mutation, which reduces the asymmetry of the first microspore division [30••]. The altered division patterns in microspores and reduced female transmission suggest that GEM1 plays a role in cytokinesis, and may also act in megagametogenesis.

Analysis of the gem1 mutation has also provided insights into how the asymmetry and cell fate might be established in pollen mitosis [30••]. The smaller daughter cell resulting from an occasional partially asymmetric division in gem1 pollen possesses an intermediate or mixed cell fate, manifested by vegetative gene expression (VC-like) and dispersed chromatin condensation (GC-like). In some gem1 pollen, there is also failure of cytokinesis after the first mitosis, yet increased chromatin condensation of only one of the two nuclei is observed within the same cell. Therefore it is proposed that the initial transcriptionally-repressed state of the GC nucleus is a result of differential segregation of transcriptional repressors or activators prior to cell division, which may be mediated through polarized transport coupled to nuclear migration. Once the repressed state is established, it can be maintained through chromatin remodeling or through factors that remain bound to the chromosomes [6••,30••]. Consistent with the idea that establishment of polarity must precede asymmetric division, the mutant sidecar pollen undergoes a premature pollen mitosis which is symmetric, presumably because polarity has not yet been established [32]. One of the daughter cells goes on to divide asymmetrically to form VC and GC, ultimately resulting in the formation of a pollen grain with an additional cell.

**Cell cycle synchrony of the gametes**

Coordinated cell cycle progression of male and female gametes prior to fertilization is likely to be essential for their successful fusion. In most eukaryotes, gametes remain predominantly in the G1 phase of the cell cycle through karyogamy. As a result, the zygotic nucleus contains 2C DNA content. But in higher plants, the cell cycle progression of the gametes is quite variable between species, and pollen with either one or two sperm cells, arrested at either G1 or G2, can be found. During pollination and pollen tube growth, further cell cycle progression of the male gametes may occur for cell cycle synchrony with the female gametes at fertilization. A recent study shows that Arabidopsis male gametes belong to tricellular-G2 type where sperm cells have entered S phase at anthesis, progress through S phase during pollen tube growth, and are in the G2 phase at fertilization [33]. These results also imply that although Arabidopsis has distinct advantages as a model system for a molecular genetic approach, it may not be amenable for in vitro fertilization studies, as nuclei from isolated pollen require further cell cycle progression before successful fertilization.

**Conclusions**

Genetic studies have identified a large number of mutations that control gametophyte development from initiation to maturity. Molecular cloning of some of the genes involved has provided us some insights about sporocyte formation and meiosis; however, genes for many gametophytic mutations have not been isolated, and the detailed phenotypic and molecular characterization of the mutants remains a big challenge. Strategies such as combinations of confocal microscopy and cell type markers are starting to be used more extensively [26,28], and will be important in future studies.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


Blocking of ethylene biosynthesis in tobacco pistil by silencing ACC oxidase or by application of an ethylene inhibitor results in arrest of meiosis in the ovule. Application of exogenous ethylene to the silenced ovary rescues the meiotic arrest.


Results of extensive screen for male-sterile mutants and the cloning of the POLLENLESS3 gene. Also includes a detailed description of anther development.


Mutation in GEM1 causes aberrant cytokinesis during pollen mitosis I, resulting in reduced asymmetry of this cell division. The phenotypic analysis of this mutation provides important insights into pollen development.

