

# Ovule and embryo development, apomixis and fertilization

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Genetic analyses, particularly in *Arabidopsis*, have led to the identification of mutants that define different steps of ovule ontogeny, pollen stigma interaction, pollen tube growth, and fertilization. Isolation of the genes defined by these mutations promises to lead to a molecular understanding of these processes. Mutants have also been obtained in which processes that are normally triggered by fertilization, such as endosperm formation and initiation of seed development, occur without fertilization. These mutants may illuminate apomixis, a process of seed development without fertilization extant in many plants.

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## Abbreviations

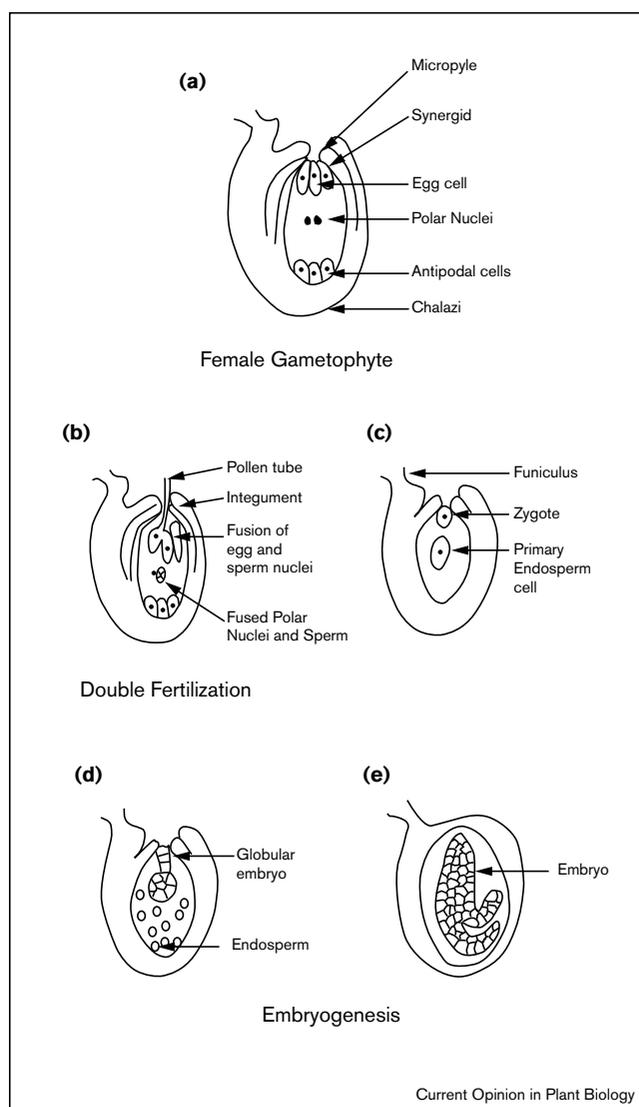
**fis** fertilization-independent seed  
**fie** fertilization-independent endosperm

## Introduction

Ovules are critical in plant sexual reproduction. They contain a nucellus (the site of megasporogenesis), one or two integuments that develop into the seed coat and a funiculus that connects the ovule to the ovary wall. The nucellar megasporangium produces meiotic cells, one of which subsequently undergoes megagametogenesis to produce the mature female gametophyte containing eight haploid nuclei [1] (Figure 1). One of these eight nuclei becomes the egg. Pollen grains are deposited on the stigma and germinate if the pollen and stigma are compatible. Germination is preceded by hydration; plants with dry stigmas, such as *Arabidopsis*, will support pollen hydration from similar species. Following hydration and compatible interaction pollen grains extend as polarized projections known as pollen tubes that invade the pistils and migrate to the eggs (Figures 1 and 2). The egg is fertilized by one of the two pollen sperm cells to produce the zygote, which subsequently becomes the embryo. Two other female gametophyte nuclei fuse to generate the central cell which is fertilized by the second sperm cell to produce the triploid endosperm. The embryo is linked to the sporophyte through the suspensor. The endosperm cells go through a free nuclear stage and then become cellular, surrounding the embryo and playing a critical role in nourishing it.

In sexually developing plants these processes occur as a result of pollination and fertilization. In contrast, in

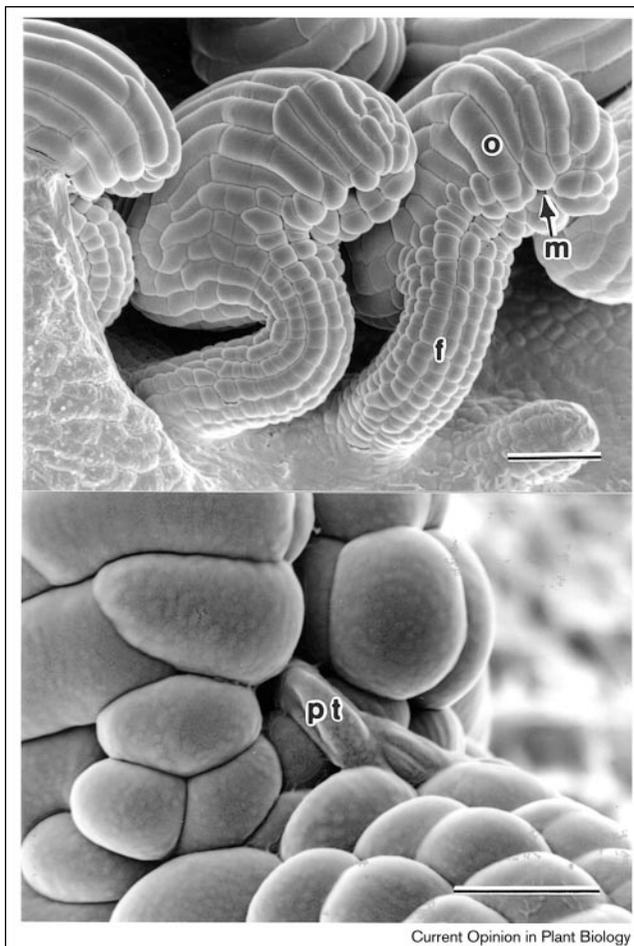
Figure 1



Female gametophyte, fertilization, and embryogenesis. **(a)** The ovule contains the female gametophyte composed of an egg, a  $2n$  central cell, two synergids next to the egg, and three antipodal cells in the chalazal end. **(b)** Pollen tube enters the ovule through the micropyle and delivers two sperm cells that fuse with the egg and the central cell. **(c)** Following fertilization a zygote and a primary endosperm cell are produced. **(d)** During embryogenesis embryo and endosperm development occurs. **(e)** At the end of embryogenesis a mature embryo is formed.

apomictic plants embryo and endosperm development occur without fertilization. Genetic analysis indicates that apomictic plants might arise from sexual plants by one or a few changes. *Arabidopsis* has been mutagenised and mutants which show fertilization-independent seed (*fis*) development isolated. In this review we shall survey recent advances that have occurred in the fields of ovule

Figure 2



*Arabidopsis* ovules and the growth of pollen tube. Mature ovules (o) attached to the ovary with funiculus (f). Micropyle (m) is the aperture through which pollen tube (pt) grows inside the ovule, initiating double fertilization. Bar=50  $\mu$ m.

ontogeny, fertilisation, embryogenesis and apomixis that have been made possible mainly by the analysis of *Arabidopsis* mutants.

### Several mutations disrupt ovule ontogeny

Genetic analyses and advanced techniques of microscopy have played an important role in the rapid advances in the understanding of ovule ontogeny [2•,3]. In recent years a number of mutants of *Arabidopsis* have been obtained that impair ovule development [2•]. In conjunction with the production of transgenic petunia lines which exhibit altered ovule development [4,5•] these mutants have illuminated the genetic regulation of this process.

Mutations in the *AINTEGUMENTA* (*ANT*) locus of *Arabidopsis* lead to an impaired or absent integument [6•,7•]. Early in ovule development *ANT* product is found to be expressed in the chalaza, the area from which integuments emerge. This result is consistent with an important role of *ANT* protein in initiating integument

formation [7•]. *ANT* product is also expressed in floral organ primordia and the mutation in the *ant* locus has a pleiotropic effect on petal shape and stamen number [6•,7•]. *ANT* encodes a protein that is similar to members of a class of transcription factors that include *AP2* [6•,7•].

Two other mutations, *aberrant testa shape* (*ats*) and *bell1* (*bel1*), produce ovules with one rather than two integuments [2•]. In *ats* mutants the two integument layers are fused while in *bel1* the integument identity is completely lost, thus producing an aberrant structure that sometimes develops into an ectopic carpel. *BEL1* encodes a putative transcription factor containing a homeodomain.

A number of other mutants affecting different aspects of ovule development have been described recently. They include *HUELLENLOS* (*HLL*), *INNER NO OUTER* (*INO*), *UNICORN* (*UCN*), *STRUBBELIG* (*SUB*), *BLASIG* (*BAG*), *MOLLIG* (*MOG*), *LAELLI* (*LAL*) and *LUENIG* (*LUG*) [8•]. In the *hll* mutants the defects are restricted to the ovules. The mutant ovules are small and lack an embryo sac, both integuments and a mature vascular strand. The inner integument does not develop beyond the first cell division, in the outer integument cell enlargement occurs but cell divisions are not detected. Gametogenesis is arrested after the formation of tetrads. In the *ino* mutants the outer integuments are missing but the inner integuments develop normally. The development of the embryo sac is also abnormal leading to sacs containing one and four nuclei. In the *ucn* mutants a horn-like structure protrudes from the outer integument. The embryo sac is often missing giving rise to semi-sterility. In the *sub* mutants the outer integument forms several protrusions at the distal tip and in extreme cases the outer integument does not encapsulate the inner integument, but forms a sheet-like structure. Plants with the *bag* mutation are smaller than wild-type and bear flowers that open prematurely. In the ovule, the mutation causes very dramatic changes; except for the funiculus no other morphological features are developed properly. In the *mog* mutants ovules have large balloon-like cells in both the inner and outer integuments and the cell layers are often not regular in appearance. The effect on embryo sac development varies from a patch of tissue to occasional eight nuclei containing embryo sacs. The ovules of *lal* mutants resemble those of *sin1* mutants; however, the mutants are not allelic. The ovules of *lal* mutants lack an embryo sac and show a reduced outer integument with the nucellus and inner integument protruding. The inner integument appears normal.

Two pivotal genes controlling ovule development have been isolated from petunia [4]. The closely related *Petunia hybrida* *MADS* box genes *FBP7* and *FBP11* are expressed in the ovule primordia, integuments and funiculus. The *MADS* box class of genes contains a conserved domain and includes many genes that define meristem and organ identity in floral development. Suppression of *FBP7* and

*FBP11* in transgenic plants resulted in the development of carpel-like structures in positions normally occupied by ovules. When the *FBP11* gene was overexpressed using the *35S* promoter the transgenic plants formed normal ovules on sepals and petals. The surface of the sepals expressing *FBP11* also formed placental tissues, suggesting that both ovule and placental tissue formation can be initiated by *FBP11*. These results have led to the suggestion that ovules represent a separate floral organ, where the identity of the ovule is controlled by the MADS box genes *FBP7* and *FBP11*. The ABC model of floral identity postulates that the identity of the four whorls of flowers (sepals, petals, stamens and carpels) can be specified by three classes of genes: class A expressed in whorls 1 and 2, B expressed in 2 and 3, and C expressed in 3 and 4. As an extension of this model *FBP7* and *FBP11* have been put forward as class D genes; the class D homeotic genes determining the identity of the central meristem which is the progenitor tissue of placenta and ovules [4].

### Fertilization

In flowering plants pollen grains are deposited on the stigmal surface during anthesis. If the pollen and stigma are compatible a pollen tube grows that enters the intercellular space of the ovary [9]. The pollen tube then grows through a specialised tissue of the ovary called the transmitting tract and emerges at random points on the inner surface of the carpel. It then grows towards the micropyle of the ovule where it delivers two sperm cells and initiates the double fertilization event within the embryo sac.

The pollen tube changes direction many times in order to reach the ovule. Thus it needs a guidance mechanism to take it through all the twists and turns that it must undergo from the stigmal surface to the micropyle. A recent study found that the directional guidance of the pollen tube is a tip-focused  $\text{Ca}^{2+}$  gradient [10•]. By combining ion imaging with confocal microscopy Malho and Trewavas [10•] showed that increasing  $\text{Ca}^{2+}$  concentration to one side of the pollen tube apex induces reorientation of the pollen tube growth axis. Local domains of elevated intracellular  $\text{Ca}^{2+}$  concentration were obtained through the use of nitr-5, a caged compound that releases  $\text{Ca}^{2+}$  when irradiated with 360 nm wavelength light. Although these procedures have certain limitations, the overall conclusion of the paper indicates a critical role for calcium in pollen tube guidance.

It has also been shown that the re-emergence of the pollen tube within the ovary and its targeted arrival at the micropyle requires genes that are expressed in the maternal sporophyte in the male and female gametophyte [11•,12,13,14••]. An *Arabidopsis* mutant that disrupts the guidance of pollen tubes without altering pistil structures has been identified [11•]. The mutant is defective in two

functionally redundant genes, *POP2* and *POP3*, and is self-sterile, indicating a role for both male and female tissues in the signalling [11•,12,13].

The female gametophyte has also been implicated in the guidance mechanism. In an elegant set of experiments ovules were generated that contained genotypically wild-type sporophytic cells and either a functional or nonfunctional female gametophyte [14••]. Pollen tubes were shown to be guided only to an ovule with a functional female gametophyte. Thus, the final phase of pollen tube growth seems to be controlled by the female gametophyte. It seems likely that the guidance signal is produced by the gametophyte or by a sporophytic tissue that is induced by the female gametophyte [14••].

### Embryogenesis

During embryogenesis there is a transition from the fertilised egg to the multicellular embryo (Figure 1). An apical–basal pattern is established along the main body axis of the embryo that contains the shoot meristem, cotyledons, hypocotyl, root and root meristem. Later the two primary meristems, root and shoot, elaborate the postembryonic development giving rise to the adult plant [15].

The basic body plan of the plant is established early during embryogenesis. The apical–basal axes are aligned according to the chalaza–micropyle axis of the ovule, suggesting maternal influence in this orientation. Genetic evidence also indicates a role for maternal influence in *Arabidopsis* [16••] and in petunia [17••]. The precise nature of maternal influence that dictates this polarity is not known. As well as the maternal tissue, the endosperm cells might also have a role in embryogenesis. Endosperm appears to control embryo size [18] and some aspects of seed development [19•,20••]. The zygote consists of the embryo proper and the suspensor, which is responsible for providing nutrient to the embryo. Suspensor cells can develop into an embryo if normal embryo development is arrested or aborted and in certain mutants [21,22].

Many mutants have been isolated that are altered in embryonic pattern formation. In *KNOLLE* mutants the rate and plane of cell divisions are affected during embryogenesis resulting in mutant embryos with enlarged cells and polyploid nuclei. The KN protein is homologous to syntaxins, a protein family involved in vesicular trafficking. It has been suggested that KN plays an important role in cytokinesis during embryogenesis [23•]. In the *GNOM/EMB30* mutant the apical–basal cell polarity of the embryo is disturbed; cells divide symmetrically rather than asymmetrically as in wild-type division. The predicted *GNOM* protein has a conserved domain in common with the yeast secretory protein Sec7p and its gene sequence is closely related to the yeast *YEC2* gene [24]. The position-specific expression of a lipid-transfer protein (*AtLTP1*) is also altered in these mutants [25].

Mutants in the *GURKE* gene give rise to seedlings with highly reduced or no cotyledons [26]. The *GK* gene is required mainly for the apical region of the embryo. Mutants with an impaired *MONOPTEROS* gene lack roots and hypocotyls and are also defective in the vascularization of the cotyledons [27]. Root tissue is formed from two distinct types of cells. The center and the initials of the central root cap originate from the basal region of the embryo (hypophysis). The initials of the remaining root tissue originate from apical daughter cells. No root meristem is formed in mutants impaired in hypophysis. These mutants are also impaired in adventitious root formation. Other mutants impair postembryonic, but not embryonic, root formation [28], indicating their separate genetic control.

Mutants have been isolated that define genes required for the formation of shoot apical meristems during embryogenesis. *Petunia* embryos carrying the *no apical meristem*, or *nam*, mutations fail to form a shoot apical meristem [29•] and *nam* mRNA accumulates at the boundaries of meristems and primordia. In *Arabidopsis shoot meristemless (stm)* mutants no shoot meristems are formed [30••]. The *STM* gene encodes a protein homologous to *KNOTTED*, a homeodomain protein of maize. Mutations *wus* [31] and *zwille* [32] result in defective organization and premature termination of shoot meristem. In *wus-1* plants shoot and floral meristems terminate prematurely. In *zll* mutants shoot meristem organization is defective in embryos but post-embryonic development is relatively normal.

### Apomixis

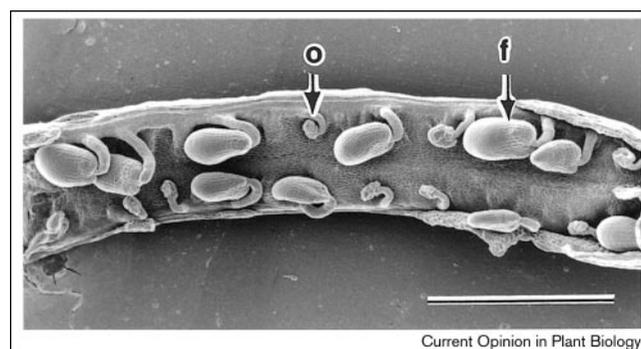
In certain plants seed development occurs without fertilization by a process called apomixis [33]. In autonomous apomixis, seed development occurs without pollination and thus without fertilization of either the egg cell or the polar cell. In pseudogamous apomixis pollination is required and in some cases pollination leads to the fertilization of the polar cell, giving rise to a fertilization-dependent endosperm but autonomous embryo.

Genetic data suggest that apomixis might be caused by a single genetic locus. In *Tripsicum*, a relative of maize, a chromosome arm has been identified that confers the apomictic phenotype [34]. Attempts are underway to transfer this chromosomal region to maize for breeding purposes. Comparison of embryo-sac-specific gene expression between apomictic buffelgrass and its sexual relatives led to the identification of one cDNA specific only to sexual ovaries and two cDNAs specific only to apomictic ovaries [35]. The significance of these cDNAs in the genetic control of apomixis remains to be determined.

Since apomixis has been found in a close relative of *Arabidopsis*, *Arabis*, and since some genetic data suggest control of apomixis by one or two genes, attempts have been made by us [20••] and others [19•] to

find mutants that form full or partial seed without fertilization. Three mutants in which different aspects of seed development occur without fertilization have been identified as mutagen-induced pseudorevertants of *Arabidopsis* male sterile mutants *cer2* [19•] or *pistillata* [20••]. Although the *pistillata* mutants have short siliques devoid of seeds the *fis* mutants in the *pi* background have long seed pods with developing seeds (Figure 3). In *fis1* and *fis2* seeds the autonomous endosperm nuclei are diploid and endosperm develops to the point of cellularization. In these two mutants pro-embryos were also detected at a low frequency but did not develop beyond the globular stage. In *fis3* mutants, no cellular endosperm or pro-embryos were detected. Endosperm development remained at the free nuclear stage. A mutant *fie* in which autonomous endosperm development occurs without fertilization has also been described [19•]. Both *fis3* and *fie* lie on chromosome 3 and might be allelic.

**Figure 3**



Seed development in a *fis* mutant. Undeveloped ovules (o) and fertilization-independent seed (f) are produced inside the silique of the *fis2* mutant at a ratio of 1:1. Bar=1 mm

The female gametophytic nature of these mutants indicates that the *FIS/FIE* genes act downstream from the point at which apomictic processes occur naturally in plants. Thus the *FIS/FIE* genes are not likely to be the genes controlling for apomictic seed development. However, these genes trigger autonomous development of many processes that are normally controlled by pollination in sexual plants. Thus endogenous *FIS/FIE* genes must be mutated or downregulated in naturally occurring apomictic plants. Presumably a number of *FIS*-like genes are simultaneously regulated by an as yet unidentified master regulatory gene that triggers apomixis. Cloning and characterization of the *FIS* genes will illuminate this process further.

### Conclusions

Recent genetic and in some cases molecular analyses have led to the identification and characterization of genes that control four important phenomena in reproductive biology; ovule ontogeny, fertilization, embryogenesis and apomixis.

These findings have led to the identification and molecular isolation of genes which are critical for ovule initiation and ontogeny. Strong experimental support has been found for a critical role of the female gametophyte in pollen tube guidance and genes critical for the control of pollen tube guidance have been identified. Several genes that define pattern in the embryo have been identified and some of them have been isolated. A remaining problem, however, is the description of how the function of these gene products is related to embryogenesis. While a molecular understanding of apomixis is not yet available, genes have been identified in *Arabidopsis* that promise to shed light on some critical aspects of the apomictic phenomenon. Overall recent years have seen impressive progress in these important areas of reproductive biology.

## Acknowledgements

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genes encodes homeodomain proteins that play an important role in the function of shoot meristems. All earlier mutants in this class of genes described in plants were gain-of-function type, making it difficult to assess the physiological function of the wild-type gene. In this work clear molecular and phenotypic work establishes the role of a class 1 *KNOTTED*-like protein in plant embryogenesis.

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