The molecular and genetic control of ovule development
Kay Schneitz

A genetic approach has resulted in an extensive framework for the methodical analysis of ovule development. The most recent progress was accomplished in the areas of primordium formation and integument morphogenesis. Furthermore, systematic screens have identified a number of gametophytic mutations disrupting several distinct steps of embryo sac ontogenesis.

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Current Opinion in Plant Biology 1999, 2:13–17
http://biomednet.com/elecref/1369526600200013
© Elsevier Science Ltd ISSN 1369-5266

Abbreviation
MMC megaspore mother cell

Introduction
Sexual reproduction constitutes an important event in the life cycle of a plant. In seed plants the ovule represents the major female reproductive organ. The ovule carries the egg cell, it is the organ where fertilization occurs, and it eventually develops into the seed which harbors the plant embryo.

For decades, the development of the ovule was only very poorly understood at the genetic and molecular level. This situation is changing and the ovules, particularly of Arabidopsis, but also of Petunia and maize, are now under thorough investigation. This is partly due to the exciting biology of the ovule and also due to its suitability to genetic analysis, which makes the ovule an excellent model system to study organogenesis in plants. Mutations in many genes with an important function in ovule development lead to sterility and this trait is easily scorable in regular mutagenesis experiments. The current molecular and genetic work is accompanied by an extensive analysis addressing the evolution of the basal angiosperm ovule [1,2].

A generalized ovule has a simple but nevertheless highly differentiated structure (Figures 1 and 2). It develops from a protrusion of the placental tissue of the gynoecium. Within the apex or nucellus, the megaspore mother cell (MMC) develops, undergoes meiosis, and one of the resulting megaspores gives rise to the multi-cellular haploid embryo sac or female gametophyte which includes the egg cell. In addition, usually two integuments originate from a region known as the chalaza. They will grow around the nucellus and eventually become the seed coat. The connection to the placenta is made through a stalk or funiculus. The nucellus, the chalaza with the integuments, and the funiculus constitute part of the sporophyte and hence consist of diploid tissue.

This review focuses on some of the advances achieved in the past one and a half years. Thus it largely deals with the control of primordium formation, and the identification and initial genetic characterization of gametophytic genes which are important for the development of the embryo sac. By necessity this review is brief; therefore, I would like to point out a number of recent reviews which discuss many aspects of ovule development in much greater detail [3•–6•].

The formation of the ovule primordium
The initial phase of ovule development encompasses aspects such as initiation and outgrowth of a protrusion from the placental tissue, commitment to the ovule fate and pattern formation in the primordium. The molecular basis of ovule specification is best understood in Petunia hybrida where two MADS box genes are known, floral binding protein 7 (FBP7) and FBP11, that function in this process, probably in a redundant manner [7,8]. Recently progress was made in the understanding of the mechanism controlling the outgrowth of the ovule protrusion. Pattern formation is less well understood; however, some data begin to shed light on certain aspects of this process as well.

Initiation and outgrowth of the ovule protrusion
In Arabidopsis, the AINTEGUMENTA (ANT) gene acts in floral organ initiation, growth and shape in general [9•,10,11,12•]. It encodes a putative transcription factor [10,11] of the EREBP/AP2 domain class [13]. Compared to wild type, plants defective for ANT function develop fewer and more distantly spaced ovules indicating that ANT is involved in ovule primordium initiation. The ovules that are produced form a nucellus, chalaza and funiculus, but lack integuments and an embryo sac.

The typical ant mutant phenotype is caused even by putative null-alleles (the corresponding proteins would be truncated by about two-thirds) [10,11], suggesting that partially redundant factors regulate ovule primordium outgrowth. Are such activities known? The HUELENLOS (HLL) gene [12•] represents one recently described example [1•–4•] of partially redundant regulation. The phenotype of hll mutants is restricted to the ovule and is similar to the phenotype of ant mutants as far as the early block in integument development is concerned. The analysis of hll ant double mutants revealed the function of HLL in ovule primordium outgrowth. Plants defective for HLL and ANT activities bear ovules that are drastically reduced in their longitudinal or proximal–distal axis. Either the funiculus or both the funiculus and the chalaza...
fail to form. A nucellus is always present, however. This implies that *HLL* and *ANT* control ovule primordium outgrowth in a partially redundant fashion.

**Patterning the primordium**  
Pattern formation deals with the spatial control of cell fate. On the basis of morphological criteria, one can recognize a distinct pattern along a proximal–distal axis of the ovule [15] (Figure 1). Distally, the nucellus is characterized by the presence of an MMC, and eventually an embryo sac. Centrally, the chalaza is recognized as the region that initiates the two integuments at its flank. Proximally, the funiculus harbors the vascular strand. From an evolutionary point of view, this is a very conserved pattern and is thus a typical feature of a generalized seed plant ovule [16].

What mechanism creates the proximal–distal arrangement? We hypothesized that one important aspect is the setup of a corresponding prepattern, with distal, central and proximal pattern elements, during the early proliferative phase of primordium formation [15] (for a detailed discussion see [5,6]). Progress on the identification of genes that directly function in the establishment of the pattern elements has been scarce. The present data, however, allow one to infer some conclusions about the patterning process. For example, the *BEL1* (*BEL1*) expression pattern provides molecular evidence for the existence of the central pattern element [17]. In addition, the region-specific defects of many early-acting ovule genes, for example *BEL1*, *ANT*, or *INNER NO OUTER* (*INO*), argue in favor of the presence of such a pattern [12•]. Furthermore, in *hll ant* double mutants a nucellus is present even in the absence of a chalaza and/or funiculus, which indicates that the distal pattern is independently formed. This finding also raises the possibility that pattern formation takes place progressively, beginning at the distal end of the primordium [14•]. It does not imply, however, that the formation of the distal element is a prerequisite for establishing the central and proximal pattern elements.

**The control of integument development**  
A number of genes have been identified that are important for the growth and morphogenesis of the integuments [4•–6•]. The results indicate that at least two levels of control exist; some genes, such as *HLL*, *ANT* or *INNER NO OUTER* (*INO*) are required for the initiation of integuments, others, including for example *SHORT INTEGUMENT1* (*SIN1*), *SUPERMAN* (*SUP*), *STRUBBELIG* (*SUB*) and *TSO1*, for their morphogenesis. The functions of members of the first group are likely to precede the activities of members of the second class and in some instances this is corroborated by genetic analysis [9•]. For example, in corresponding double mutant studies it was shown that *ant* is epistatic to *sin1*, *sup* and *isoli* and *ino* is epistatic to *sup*.

The analysis of the later aspects of integument morphogenesis is still in its infancy; however, the results already foreshadow a complex scenario of multiple distinct steps. The genes being implicated in these events appear to be important for cell proliferation and/or cell shape [4•–6•], and the corresponding mutations lead to an altered morphology of the integuments, rather than to their absence. As might be expected for genes with a general function in morphogenesis, members of this class usually exhibit pleiotropic activities. One example is *TOUSLED* (*TSL*) [18]. Among other functions, *TSL* appears to control leaf development, and it promotes specific cell divisions within the floral meristem as well as the growth of the ovule integuments [18,19]. *TSL* is likely to be part of a signal transduction mechanism as this gene encodes a serine/threonine kinase [19,20•]. A second example is *TSO1*, which is also required for flower and ovule development, and which appears to have a role in cell division and/or directed cell expansion during floral organogenesis [21,22].

**From megaspore mother cell to mature embryo sac**  
Gametogenesis can be subdivided into two phases [3•,5•]. Sporogenesis encompasses processes such as megaspore
Sporogenesis was identified [12•]. Mutations in this group of genes with a function apparently restricted to gametophyte development prior to fertilization (Figure 2). Under normal circumstances endosperm and embryo development would already be well underway. The salient features are indicated. Within the central cell, the two polar nuclei have fused to yield a large, di-haploid nucleus. By this stage the antipodal cells are already degenerated. The 211E6 mutant [12•]. This sporophytic mutant exhibits a block at the four-nuclear embryo sac stage (stage 3-IV [15] or FG4 [39•]). No cellularization occurred. The sporophytic tissue is apparently normal. The ovules developed within the same gynoecium of a plant heterozygous for the hdd mutation. In the wild-type ovule to the left, fertilization occurred and an eight-nucleate endosperm is present (arrow). In the hdd-mutant ovule to the right, development did not proceed beyond the four-nuclear embryo sac stage (arrow head). Abbreviations: ap, antipodal cells; cc, central cell; ec, egg cell; fu, funiculus; ii, inner integument; oi, outer integument; syn, synergid; vs, vascular strand. Scale bars, 20 µm

Figure 2

Mid-optical sections through mature, whole-mount ovules from (a) a male-sterile, (b) an emd-class mutant and (c) hdd. (a) A post-fertilization ovule in the absence of fertilization. Under normal circumstances endosperm and embryo development would already be well underway. The salient features are indicated. Within the central cell, the two polar nuclei have fused to yield a large, di-haploid nucleus. By this stage the antipodal cells are already degenerated. (b) The 211E6 mutant [12•]. This sporophytic mutant exhibits a block at the four-nuclear embryo sac stage (stage 3-IV [15] or FG4 [39•]). No cellularization occurred. The sporophytic tissue is apparently normal. (c) The ovules developed within the same class of sterile sporophytic mutants, the embryo sac-defective (emd) mutants [12•], with the corresponding defects apparently restricted to gametophyte development prior to fertilization (Figure 2). It may be that some of the EMD-class genes control the establishment of cues in the megaspore mother cell which are required later during embryo sac development. Thirdly, the haploid or gametophytic genome regulates megagametophyte development [29–31].

Sporogenesis

In many species, an early step in sporogenesis is the singling out of the prospective MMC from a group of cells in the nucellar L2 (the first subepidermal) layer. How is this event regulated? In maize, the mac1 gene is central to this process [24] — it appears to suppress the commitment to the gametogenesis pathway in L2 cells other than the prospective MMC [24].

The genetic control of the events following MMC commitment is being elucidated as well. Processes, such as establishment of MMC polarity, meiosis and cytokinesis, are affected in a number of mutants including switch1 (sw1) in Arabidopsis [25] (C Horlow, personal communication) and ameiotic 1 (am1) in corn [26,27]. Recently, a large group of genes with a function apparently restricted to sporogenesis was identified [12•]. Mutations in this MEGASPOROGENESIS-DEFECTIVE (MSD) class of genes lead to a block in gametogenesis at or before the mono-nuclear embryo sac stage.

Megagametogenesis

The emerging picture of the genetic and molecular control of female gametophyte ontogenesis is already quite complex. Firstly, embryo sac development appears to be influenced by signals coming from the integuments, as many mutants with a primary defect in integument development also show altered female gametophyte development [10,11,12•,17,28]. Secondly, it is also possible that the sporophytic genome controls embryo sac formation through a mechanism not involving the integuments. In Arabidopsis, there exists a large number of genes with a function during embryo sac development include members of the GAMETOGENETIC FACTOR (GFA) and FEMALE GAMETOPHYTE (FEM) group of genes [3•,32•], Gf [39•,40], PROLIFERA (PRL) [35], ANDARTA (ADA), TISTRYA (TYA) and HADAD (HDD) (Figure 2) in Arabidopsis [33•,34•] as well as lethal ovule2 mother cell differentiation and meiosis and eventually results in a tetrad of megaspores. In most species, only the proximal or chalazal-most spore will continue development. Megagametogenesis proceeds from this single spore (monospor) through a syncytial phase, encompassing three mitoses and elaborate nuclear positioning, followed by cellularization, to produce a seven-celled Polygonum-type embryo sac [23] (Figure 2).

Female gametophytic mutants

Until recently, very few female gametophytic mutations had been described [3•,5•]. In the last few years however, several laboratories have been successful in isolating and characterizing a number of gametophytic mutations causing aberrant embryo sac ontogenesis ([3•,5•,32•–34•]; M Hülskamp, personal communication).

The successful screens took advantage of two properties of gametophytic mutations. Usually, female gametophytic mutations result in an aberrant or unfunctional embryo sac. As a consequence, 50% of the ovules of a plant heterozygous for a female gametophytic mutation will not develop into seeds upon fertilization, and thus the plant exhibits semisterility. Furthermore, the segregation pattern of markers closely linked to the gametophytic mutation deviates from a typical Mendelian 3:1 ratio. The segregation pattern can be analyzed in insertion mutants generated by either T-DNA or transposons, carrying for example a resistance marker, or, in experiments involving multiply-marked chromosomes. Additional methods for isolating genes expressed in the gametophyte include enhancer-trap screens [5•,35,36] or screens of cDNA libraries prepared from isolated cells of the embryo sac [37,38].

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(lo2) and indeterminate gametophyte1 (ig1) in maize [41,42,43*]. Some of these genes also have a function during male gametophyte development (for example FEM3 or HDD) and could only be identified because the corresponding mutations are incompletely penetrant.

The different mutations lead to blocks at various steps during female gametophyte development. For example, embryo sacs in the fem2 or ada mutants fail to develop beyond the mono-nuclear embryo sac stage. Genes like HDD, PRL or lo2 appear primarily to control nuclear division, as the corresponding mutants show embryo sacs markedly arrested at the two-, four or eight-nuclear stage. In the case of PRL, this interpretation is corroborated by sequence information, because PRL shows homology to genes from yeast encoding DNA replication initiation factors [35]. Very late aspects of female gametophyte development are affected in several mutants [3*]. For example, in gfa2 mutants the two polar nuclei within the central cell fail to fuse and remain located side by side. In fem4 mutants, cellularization appears to be defective, as indicated by the abnormal shapes of the egg cells and synergids, as well as alterations in number, size and shape of the vacuoles of these cells.

Conclusions
Recent years have witnessed a remarkable interest in the molecular and genetic analysis of ovule development. Much of the present challenge lies in the elucidation of the molecular structure of the identified sporophytic and gametophytic genes and the analysis of their genetic interactions. Besides these immediate concerns a number of general issues stand out. What genes specify the identity of the Arabidopsis ovule? Although a number of MADS box genes are candidates [44-46], and APETAL2 (AP2) appears to be involved [47], this significant aspect is not understood. It will also be important to identify the genes that regulate patterning in the early ovule primordium and to understand how primordium outgrowth and pattern formation are orchestrated. Integument morphogenesis is in part characterized by complicated patterns of cell divisions and cell shape changes. As indicated by the analysis of TSL, signaling pathways are important in this process. Do genes such as STRUBBELIG (SUB) [12*], SHORT INTEGUMENTS (SIN1) [48,49], TSO1 and others also encode components of a signaling mechanism? What connects the signaling machinery to the cytoskeleton and cell wall, which probably play a crucial role in the regulation of cell shape? With respect to gametogenesis, the study of the mechanism underlying megasporogenesis will certainly gain more attention. The analysis of gametophytic mutants may be complemented by the study of the interplay between sporophytic and gametophytic factors, which is likely to occur during female gametophyte development.

Acknowledgements
I thank the members of my lab for stimulating discussions and comments on the manuscript. I also thank Martin Hülskamp and Christine Horlow for allowing me to cite unpublished work, Robi Dudler for comments on the manuscript and Jay Moore and Ueli Grossniklaus for the kudau mutant picture. I apologize to the colleagues whose work I could not cite directly due to space constraints. Work in my laboratory is supported by the Swiss National Science Foundation (NF 31-42175.94 and NF 31-53032.97) and by the Kanton of Zürich.

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
10. Schneitz K, Hülskamp M, Kopczak SD, Pruitt RE: Dissection of sexual organ ontogenesis: a genetic analysis of ovule development in Arabidopsis thaliana. Development 1997, 124:1387-1396. A description of a systematic genetic approach to ovule development. The authors present a survey of a large number of ovule mutants, and, on the basis of this analysis, introduce a genetic model of ovule development. They also present indirect evidence for the presence of proximal-distal pattern formation in the ovule primordium.
12. Schneitz K, Baker SC, Gasser CS, Redweik A: Pattern formation and growth during floral organogenesis: HUELLENLOS and AINTEGUMENTA are required for the formation of the proximal...

An important paper which directly addresses the genetic control of ovule primordium outgrowth. It shows that at least two genes, HUL and ANT, regulate the outgrowth of the ovule primordium in a partially redundant manner. The data provide further evidence for proximal–distal pattern formation in the ovule primordium and indicate that the distal pattern element forms in an independent fashion. They also raise the possibility that proximal–distal patterning takes place progressively and in a distal–proximal direction.


The authors provide evidence that TSL encodes a nuclear serine/threonine kinase. Activation of the protein kinase seems to require interaction between TSL molecules.


24. Sheridan WF, Avalkina NA, Shamrov II, Batygina TB, Golubovskaya: The authors provide evidence that HLL identifies genes functions in cell division.


A similar study as in [22]. It includes a phenotypic description of two female gametophytic mutants. In both cases the viable megasporogenesis does not initiate the nuclear division cycles.

27. Golubovskaya I, Avalkina N, Sheridan WF, Shamrov II, Batygina TB, Golubovskaya: The authors describe the isolation of the gametophytic hadad mutation by a transposon-induced (gene-trap) mutagenesis screen. The hadd mutants exhibit an aberrant segregation ratio and reduced seed set. The embryo sac is predominantly affected and the results indicate that H2D is required for the correct progression through the mitotic division cycles.


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An excellent description of embryo sac development in Arabidopsis using confocal laser scanning microscopy. Analysis of the gmf mutant indicates a very early role for the corresponding gene in wild-type embryo sac ontogenesis.


A careful analysis of the mutant phenotype using fluorescent microscopy. The results indicate that lethal ovule2 is essential for nuclear division, migration and the accompanying tubulin cytoskeleton behavior during maize embryo sac development.


