ELSEVIER

Endosperm: an integrator of seed growth and development

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Plant reproduction relies on interactions between parental and zygotic components. Elaborate reciprocal signaling pathways enable coordination of the genetic programs between these components. A first and important step in this communication is the tight control of cell cycle events in the gametes prior to fertilization. This prepares for coordinated fertilization and the initiation of seed development. The dialog between the various actors of reproduction extends after fertilization, with the endosperm taking a central role. Importantly, the endosperm mediates a maternal input that is based on memory of the transcriptional states of imprinted genes, which is crucial for harmonious seed growth. Our current knowledge suggests that the endosperm is an integrator of the different components and genetic programs that are involved in seed development.

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Introduction

In plants, the diploid generation produces a special lineage leading to meiosis. The haploid meiotic products (spores) then develop as morphologically distinct life forms, the male and female gametophytes, which will differentiate a germ-line and produce the male and female gametes (Figure 1). In flowering plants, double-fertilization has evolved parallel to specialization of the haploid gametophyte towards reproduction. The gametophytic life form has been drastically reduced to be nearly equivalent to the germ line. In *Arabidopsis*, as in the majority of flowering plants, the pollen grain develops from an initial asymmetric division of the haploid meiotic product, Pollen Mitosis I (PMI) (Figure 1). The smaller generative cell undergoes PMII and divides again, giving rise to two sperm cells. The female embryo sac develops from only one of the meiotic products and typically undergoes three successive syncytial divisions followed by cellularization into seven cells, including the two female gametes, namely the egg cell and the central cell (Figure 1; [1]).

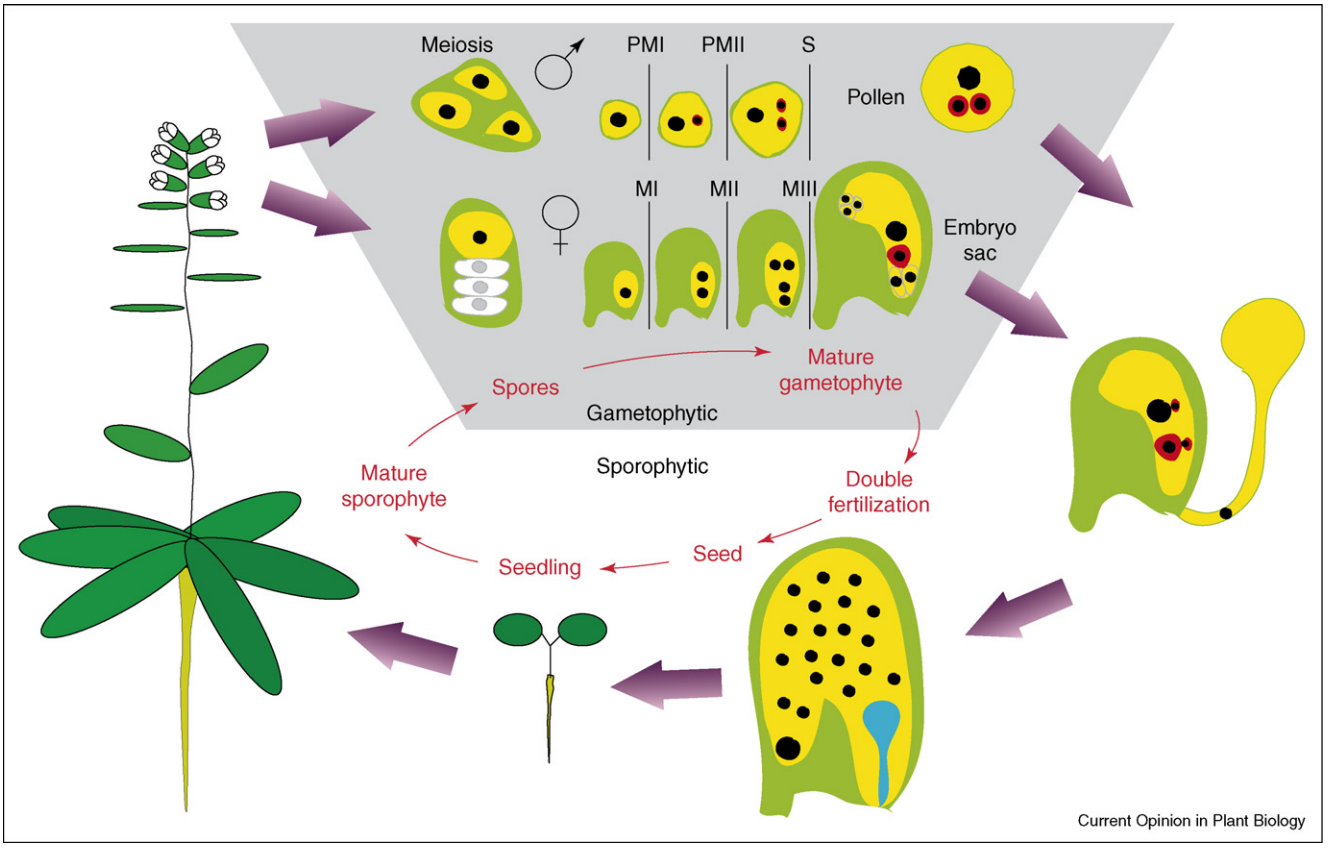
At the time of fertilization, the pollen grain grows a pollen tube that delivers the two sperm cells to the embryo sac. Despite significant advances in our understanding of guidance mechanisms for the pollen tube and the transcriptional activity of the gametes [2–4], our knowledge on the actual mechanism of fertilization remains extremely limited. A remarkable recent study has shown, however, that the fusion between the sperm cells and the female gametes involves the conserved protein GENERATIVE CELL SPECIFIC 1 (GCS1) [5••]. One sperm cell fertilizes the egg cell, giving rise to a zygote from which the embryo develops. The other sperm cell fuses with the central cell generating the endosperm, which nurtures embryo development in the seed (Figure 1).

In this review, the signaling events that follow double-fertilization in flowering plants are outlined with particular emphasis on the following points. What signals control the cell cycle at fertilization? What mechanisms co-ordinate the relative growth of the three main seed components, the embryo, the endosperm and the maternal seed integuments? Finally, what roles do gametes play in embryo and endosperm development?

Control of cell cycle progression before and after fertilization

In contrast to cell cycle control in animal gametes, cell cycle control in plant gametes has remained largely unknown until recently. In *Arabidopsis*, direct measurements of DNA content suggest that sperm cells enter a new S-phase after PMII, which is presumably completed either before or during pollen tube growth [6,7]. Thus, when delivered to the embryo sac, sperm cells are likely to be in G_2 phase [8]. Further evidence of a fusion at G_2 phase comes from a mutant in the *Arabidopsis* cyclin-dependent kinase gene *CDKA;1*, which encodes a homolog of the yeast cdc2a kinase. In *cdka;1* mutant pollen, the generative cell fails to enter PMII and remains in the G_2 phase with a DNA content of about 2C [9••,10]. Importantly, this mutant pollen is able to complete fertilization [9••]. To prevent aneuploidy and allow proper development of the zygote, the two nuclei of the gametes need to fuse in the same cell cycle phase. The simplest assumption derived from the cell cycle phase of the male gamete is that female gametes also reach the G_2 phase, matching

Figure 1



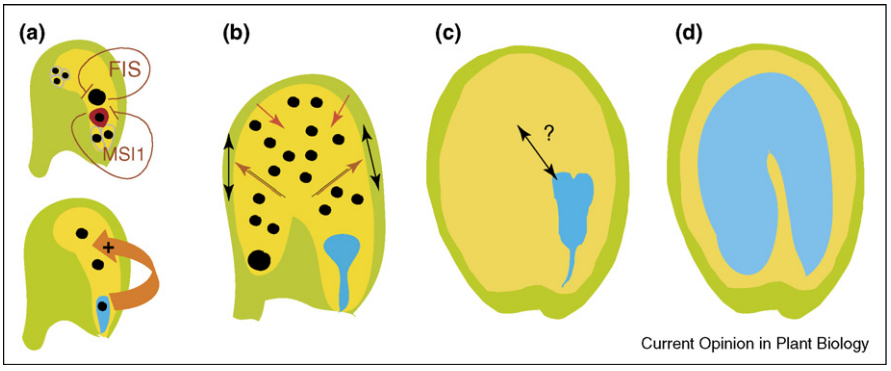
Life cycle of flowering plants. In flowering plants, the predominant life form is the sporophyte (the green plant). In the flowers, the sporophyte will asexually generate female and male spores. The male spore will undergo two rounds of cell divisions, PMI and PMII. The mature pollen comprises a large vegetative cell and embedded in this, two sperm cells, the actual gametes (shown with red cytoplasm). Female gametogenesis includes three rounds of nuclear divisions followed by cellularization, resulting in a seven-cell embryo sac. The embryo sac contains the egg cell (again shown with red cytoplasm) flanked by two synergid cells and the central cell (shown in yellow), which contains a large nucleus. During the double fertilization process, sperm cells are delivered by the pollen tube to the embryo sac. One sperm cell fuses with the egg cell and generates the zygote, whereas the second sperm cell fuses with the central cell, giving rise to the endosperm. The endosperm supports the developing embryo. Double-fertilization represents the end of the gametophytic life stage and the beginning of the sporophytic phase.

the cell cycle stage of sperm cells. This assumption was recently supported by measurements of DNA content in tobacco gametes [8].

Conserved chromatin remodeling Polycomb group (Pc-G) complexes control the arrest of the *Arabidopsis* female gametophyte. The Pc-G complex FERTILIZATION INDEPENDENT SEED (FIS) contains the SET domain protein MEDEA (MEA) [11], the VEFS domain protein FIS2 [12], and the WD40 domain protein FERTILIZATION INDEPENDENT ENDOSPERM (FIE) [13]. Loss-of-function mutations of *FIS* genes cause autonomous onset of cell division in the central cell in the absence of fertilization [14,15]. The FIS complex also includes the WD40 protein MULTICOPY SUPPRESSOR OF IRA 1 (MSI1) [16,17]. Unexpectedly, when compared to the *fis* mutants, *msi* mutants show additional autonomous division in the egg cell that leads to a non-viable parthenogenetic embryo [18•]. This suggests that partially distinct mechanisms control the arrest of the egg cell and the central cell (Figure 2a).

MSI1 interacts with plant homologs of the mammalian tumor suppressor protein Retinoblastoma (Rb) [19]. Autonomous cell proliferation in the embryo sac has been reported in plants that carry null alleles of the *Arabidopsis* Rb homolog *RBR1* [20]. However, the pleiotropy of the phenotype has prevented unambiguous identification of the origin of proliferating cells in *rbr1*. The *rbr1* mutant phenotype is likely to be compound. RBR1 can interact with components of the FIS-complex [21], but could also act upstream of the FIS Pc-G complex and together with MSI1 to directly control cell cycle progression in the female gametophyte. In analogy to its function in root stem cells, RBR1 might also be involved in specifying gamete identity [22].

Figure 2



Interactions between seed components. **(a)** Before fertilization, a negative signal embodied by the action of the chromatin remodeling complex FIS restricts the premature proliferation of the central cell. An equivalent repression depends on MSI1 and targets the egg cell. After fertilization, a positive signal is derived from the fertilization of the egg cell that triggers endosperm development if the central cell is not fertilized. The origin and nature of this positive signal is currently not known. **(b)** During later stages, reciprocal signaling between the endosperm (yellow with black nuclei), the embryo (blue) and the seed integuments (green) is required to achieve proper seed growth. The endosperm takes a central role as an integrator and mediator of these signals. The endosperm controls cell elongation (black double arrows) in the seed integuments (dark red arrows). Reciprocally, the seed integuments restrict the potential growth of the endosperm (red arrows). As a result, the final seed size is determined. **(c)** Finally, endosperm trophic inputs and potential signals to the embryo sustain cell proliferation in the embryo. **(d)** At the mature stage, the embryo occupies most of the space initially created by the interactions between the endosperm and the seed integuments.

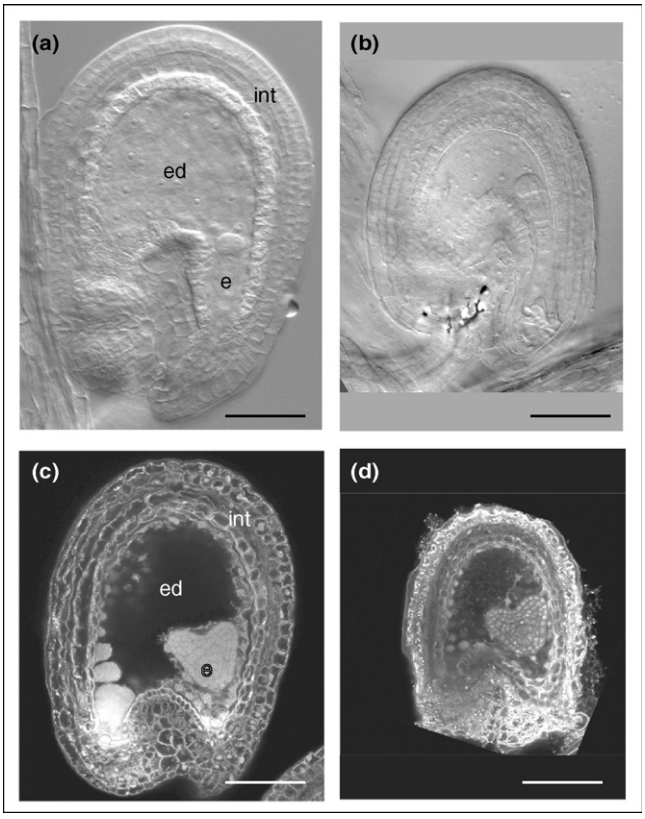
In conclusion, the cell cycle arrest of the central cell is controlled by the *FIS* pathway, whereas the egg cell arrest probably depends on a MSI1-dependent action that is independent of the Polycomb Group *FIS* pathway. Proper differentiation of both female gametes might involve the function of RBR1. A recent report [9••] on a functional knock-out of the *Arabidopsis CDKA1;1* has provided evidence for mechanisms that might be involved in cell-cycle control prior to fertilization.

Co-ordination of growth between the different seed components

During seed growth, co-ordination is necessary between the embryo, the endosperm, the maternally contributed seed integuments and the nucellus, which is also of maternal origin. No experimental data have yet been obtained for the role of the nucellus, but recent advances have demonstrated that interplay between endosperm and seed integument growth is essential for seed size determination in *Arabidopsis* (Figure 2). Mutations in the *HAIKU* (*IKU*) genes decrease endosperm size and eventually embryo and seed size [23]. The *HAIKU* genes *IKU2* and *MINISEED3* (*MINI3*) encode a leucine-rich-repeat kinase and a WRKY transcription factor, respectively [24]. These genes are expressed in endosperm immediately after fertilization. The decreased endosperm size of *iku* mutants is accompanied by a decrease in cell elongation in the seed integuments, indicating communication between these two genetically distinct seed components (Figure 3c–f; [25•]). Similarly, reducing the degree of cell elongation in the seed integuments reduces endosperm growth. Conversely, increasing the number of seed integument cells causes a symmetrical increase in endosperm growth [26,27]. In *Arabidopsis*, the final size of the seed is determined before the embryo initiates the major phase of cell proliferation after the heart embryo stage (Figure 2). These results indicate the capacity of seed integuments to regulate endosperm growth by a maternal sporophytic effect (Figure 2b). In cereal species, the embryo remains confined to a small volume in the mature seed and the endosperm extends its development until seed maturity and stores reserves. Hence, the model of seed size control established in *Arabidopsis* is likely to be applicable to seed development in cereals. The mechanistic nature of the communication between the endosperm and the integuments remains unknown. A likely factor could be biophysical forces; a growing endosperm might exert mechanical tensions on the integument cells [25•,28].

How the embryo adjusts its degree of cell proliferation to the space available (as seen in *iku* seeds) is not yet understood. However, this observation indicates some kind of communication from the endosperm to the embryo. Communication from the embryo to the endosperm has been revealed by analysis of the mutant for the *Arabidopsis* homolog of the cyclin dependent kinase *cdc2a*, *CDKA;1* [9••]. In heterozygous *cdka;1* null mutants, 50% of the pollen contain a single sperm cell [9••,10] that exclusively fertilizes the egg cell. The development of the zygotic embryo has a dramatic effect because it triggers nuclear divisions of the central cell and initiates endosperm development (Figures 3a,b; [9••]). Thus, either the fertilization process or the developing embryo provides a signal that counteracts cell cycle arrest in the central cell and stimulates endosperm development

Figure 3



Phenotypes of mutants in which seed development is affected. **(a,b)** The effects of pollination of wildtype ovules by *cdka;1*/+ plants. (a) One and a half days after self-fertilization, the seeds contain an octant stage embryo (e) and stage VI (26–30 nuclei) endosperm (ed) surrounded by seed integuments (int). (b) By contrast, wildtype ovules that are fertilized with *cdka;1* mutant pollen produce seeds that have a two- or four-cell-stage embryo one day after pollination. **(c,d)** In comparison to (c) wildtype seeds, (d) *iku* seeds contain an endosperm (ed) that is reduced in size and whose seed integuments (int) do not elongate, whereas embryo growth is not altered.

(Figure 2a). The developing endosperm in *cdka;1*-fertilized seeds arrests after a few rounds of the cell cycle, whereas the embryo develops until the globular stage. Such seeds remain small because they do not expand the seed integument. These observations support the essential role played by endosperm and the comparatively limited role played by the embryo in controlling seed size.

Coordination of maternal and zygotic cues during seed development

Although extensive screens have been performed, only a handful of mutations that cause maternal effects have been isolated over the past few years [29,30]. However, using a transposon-based mutagenesis approach, a recent study discovered a larger number of such mutants [31], and suggests that there might be a larger diversity of maternal effects than previously thought.

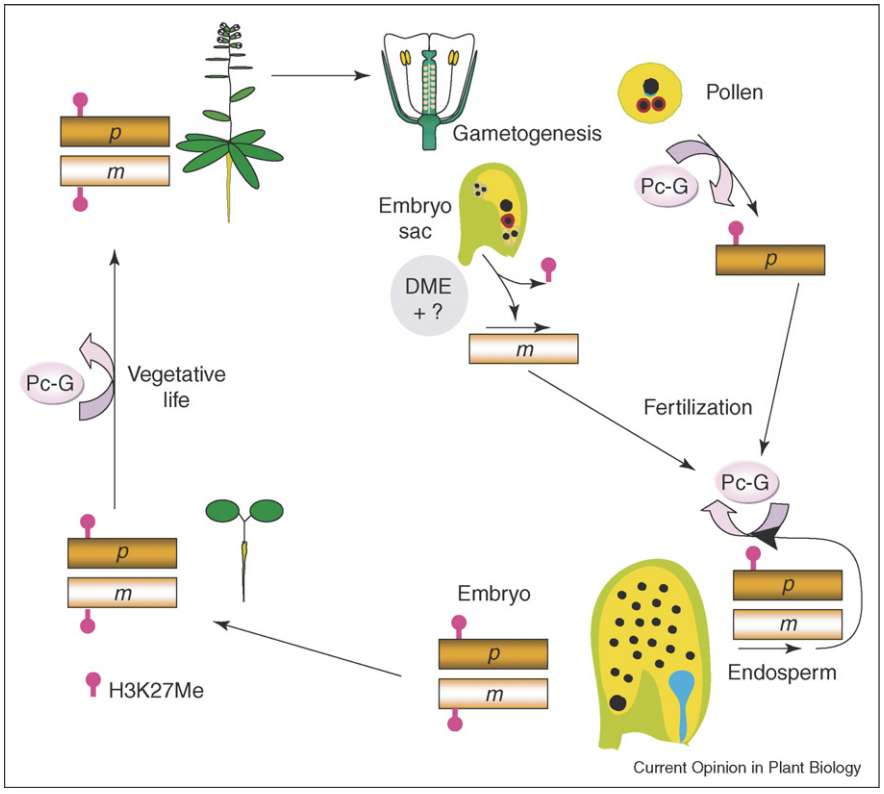
The maternal influence might be inherited by the endosperm by two routes. First, the central cell as progenitor of the endosperm contributes much cytoplasm to the endosperm. The endosperm could inherit maternally derived mRNA and proteins that are located at specific subdomains in the central cell. Indeed, the central cell shows cytological evidence of polarity, which could prefigure the future endosperm antero-posterior organization [32]. Direct maternal effects that originate from the central cell constitute an attractive hypothesis, but one that is supported by little, if any, direct evidence to date. The origin of the maternal effect of DICER-LIKE 1 remains mysterious [33], and the maternal effects that are associated with PROLIFERA (a homolog of MINICHROMOSOME MAINTENANCE 7) are complex and might involve several mechanisms [34].

The second origin of maternal influence on seed development appears to be a specific activation of maternal alleles and silencing of paternal alleles, resulting in maternal genomic imprinting. Two of the components of the FIS complex are subject to maternal genomic imprinting [35,36]. The paternal allele of *MEA* and *FIS2* is silenced. As a consequence, the maternal inheritance of a null allele, *mea* or *fis2*, cannot be rescued by a paternal wildtype *MEA* or *FIS2* allele and is sufficient to cause maternal effects on endosperm development [11,14,15,17].

The mechanisms that lead to *MEA* and *FIS2* imprinting are in part distinct ([35,36]; Figure 4). *FIS2* is silenced throughout most of the plant life cycle by DNA methylation on a specific region in its promoter [36]. DNA methylation is maintained by the methyltransferase MET1 during vegetative development and male gametogenesis. At the end of female gametogenesis, however, *FIS2* silencing is relieved in the central cell by the DNA glycosylase DEMETER (DME). Hence, a transcriptionally active maternal *FIS2* allele is provided to the endosperm whereas the paternal *FIS2* allele remains silenced by MET1. By contrast, *MEA* silencing depends on the repressive methylation marks on lysine residue 27 of HISTONE 3 (H3K27) [37••,38••]. H3K27 methylation of the *MEA* locus depends on the activity of Pc-G complexes that are active during the vegetative phase and male gametogenesis. In endosperm, a loop involving negative feedback by the Pc-G complex, including *MEA* and *FIS2* maintains the silencing of the paternal *MEA* allele ([37••,38••]; Figure 4).

The activation of the maternal allele of *MEA* in the central cell depends on several mechanisms. Real-time quantitative PCR has shown that *MEA* expression in the central cell is inhibited by *MEA* itself [39]. DME is required for *MEA* activation [40] and is able to remove methylated cytosine from domains linked to the *MEA* locus [38••]. However, the degree of DNA methylation of *MEA* varies depending on natural *Arabidopsis* accessions

Figure 4



A model for dual control of parental genomic imprinting of *MEA* in *Arabidopsis*. The gene *MEA* is silenced during vegetative development and male gametogenesis by the continuous action of Pc-G complexes that are responsible for maintenance of H3K27 methylation (pink circles) at the *MEA* locus. *MEA* expression is activated in the central cell at the end of female gametogenesis by the DNA glycosylase (DME) and potentially by other activities that lead to the removal of H3K27 methylation. After fertilization, the *MEA* maternal allele (m) is expressed in endosperm and produces the MEA subunit of the Pc-G complex, which contains FIS2, FIE and MSI1 and is responsible for maintaining the silencing of the paternal *MEA* allele (p).

[38••,41]. Moreover, DNA methylation does not play a major role in silencing *MEA* in vegetative tissues or in pollen [37••], and loss of DNA methylation on *MEA* paternal allele does not alter their silenced state in endosperm [36,38••]. Hence, DNA methylation probably plays a limited regulatory role in *MEA* imprinting. What mechanism is responsible for the removal of H3K27 methylation that is essential for *MEA* imprinting is unclear. DME DNA glycosylase activity causes single-strand breaks in DNA [38••]. It is possible, therefore, that DNA repair mechanisms that follow DME action involve the deposition of new chromatin and that methylated H3K27 are removed, causing loss of repression of *MEA* transcriptional activity (Figure 4).

Although accumulating evidence on epigenetic regulation mechanisms has contributed to our understanding of how differential genetic programs are set up in the different components of the seed, little is known about the downstream targets that are controlled by, for example, the Pc-G FIS complex. Small-scale microarray analyses using combinations of *fis* mutants have to date discovered two genes that are repressed by the FIS-complex, and these genes encode the MADS-box protein PHERES1 (PHE1) and the SKP1-like protein MEIDOS [16]. What functions these proteins have in seed development and growth is not known, but *PHE1* has been shown to be expressed exclusively from the paternal genome after fertilization [42•]. In contrast to the gametophytic maternal effects discussed previously, genes such as *PHE1* might thus be responsible for activating paternal-specific gene sets in endosperm.

Conclusions

Our current knowledge on seed development underlines the importance of the endosperm not only as a source of nutrients but also as an integrator of seed growth and development. Different signaling routes are now emerging between the embryo, the endosperm and seed integuments. A reciprocal signaling process between seed integuments and endosperm appears to coordinate the proper course of seed growth. Signals between embryo and endosperm are probably also important for proper seed development but remain to be characterized.

Significant progress has been made in the understanding of maternal effects and the role of the endosperm as a relay between the mother and the embryo. Here, a possible role of gametophytic maternal factors that are already present in the unfertilized egg and central cell calls for attention.

A major constraint to isolate these signaling components is the small size of reproductive structures in *Arabidopsis*. This will be overcome in the future, however, by utilizing recent advances in 'genomics' tools, including new sampling techniques, such as laser-assisted micro-dissection, or mRNA-tagging approaches [43,44]. The establishment of general as well as spatial and temporal transcriptomes or proteomes is likely to lead to the generation of specific profiles for each reproductive cell type and will be a new source of information [45,46].

Note added in proof

Three important papers have appeared since we drafted this review [47•–49•].

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- •• of outstanding interest

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