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Bridging the generation gap: communication between maternal sporophyte, female gametophyte and fertilization products

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In seed plants, as in placental animals, gamete formation and zygotic development take place within the parental tissues. To ensure timely onset and to coordinate the development of the new generation, communication between the parent plant with the filial tissues and its precursors is of utmost importance. During female gametogenesis the maternal tissues tightly regulate megagametophyte formation and the interplay between the sporophyte and the fertilization products, embryo and endosperm, has major implications in the formation of a viable seed. We review the current knowledge on these interactions and highlight the many questions that still remain unanswered, in particular the nature of the pathways involved in these signaling events.

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Introduction

During the plant life cycle there is an alternation of generations between diploid sporophytic and haploid gametophytic multicellular stages. In seed plants, the haploid generation is short-lived and its development takes place within the parental sporophytic tissues [1,2]. Following gamete formation, the male gametophyte (pollen grain), is delivered to the maternal tissues where it germinates and forms the pollen tube, which ensures the delivery of the paternal sperm cells to the female gametophyte (FG; or embryo sac), to form a seed.

The formation of the FG, which comprises megasporogenesis and megagametogenesis, and the development of the seed both take place within the maternal tissues, implying a high degree of communication between mother and offspring [3,4•]. In particular, the onset of megasporogenesis as well as cell fate determination in the FG are both tightly regulated by sporophytic tissues, the surrounding maternal integuments [5,6]. These protective structures engulf the FG as they develop to form the ovule and only leave a small aperture, the micropyle, through which the pollen tube enters the FG. Fertilization results in the development of the diploid embryo and triploid endosperm that are surrounded by the maternal seed coat, derived from the ovule integuments. These three genetically distinct structures have to communicate and coordinate their development in order to form a viable seed. In this review we will discuss current knowledge on how the maternal sporophyte influences and communicates with the developing FG and the fertilization products and also highlight the questions that remain to be addressed. The control of megasporogensis by the maternal sporophyte has recently been reviewed and will not be discussed in this review [4•].

Female gametophyte formation is closely linked to integument development

Following megasporogenesis, the functional megaspore undergoes several mitotic divisions after which cell walls are formed and four different cell types are established: two gametes, central and egg cell, and two types of accessory cells: synergid and antipodal cells. This process is referred to as megagametogenesis and it results in the formation of the FG, which is enclosed by the ovule integuments [7]. Similarly to what happens during sporogenesis [4•], the sporophytic tissues have a fundamental role in modulating FG development. This is apparent from the observation that mutants with defects in integument identity like *bell1* (*bel1*) [8], *aintegumenta* (*ant*) [9], and *cyp78a9* [10] fail to form a functional FG. As the affected genes were not found to be expressed in the FG but in the surrounding integuments [8–11], it seems most likely that the failure of integument formation causes failure in FG formation. The presence of the inner integument alone seems to be sufficient to ensure the formation of a functional FG [12] and, consistently, defects in the innermost integument layer alter the morphology as well as the fertility of the gametophyte [13]. Nevertheless, our understanding of how the sporophyte signals to the developing gametophyte is still lacking [6]. Recently, alterations in auxin homeostasis have been shown to influence gametophytic cell fate [2,14]. While it was initially proposed that an auxin gradient within the

FG determines gametophytic cell identity [14], reinvestigations of this model rather predict that auxin levels in the surrounding sporophytic tissues non-cell-autonomously determine cell identity in the FG [15•]. An auxin-inducible Rapid ALkalinization Factor (RALF)-like peptide produced in the integuments of *Solanum* affects mitosis and nuclei distribution in the FG [16••], suggesting that movement of small peptides plays an important role during gametogenesis. This notion is supported by the finding that a substantial amount of genes coding for cysteine-rich peptides (CRPs) are differentially expressed during megagametogenesis [17•].

Notwithstanding the role of the sporophyte in FG development it is noteworthy that integument malformation does not necessarily imply failure of FG formation. In mutants for the mitogen-activated protein kinases MPK3 and MPK6, integument elongation is compromised in a dosage-dependent manner [18]. In the *mpk3*/+ *mpk6*/− double mutant the integuments cannot keep up with FG development, causing it to protrude from the sporophytic tissues [18], similar to the half-exposed FG in wishbone flower (*Torenia fournieri*) [19]. Like in *Torenia*, the FG of *mpk3 mpk6* double mutants undergoes normal development. This observation indicates that physical support and protection of the FG is not the main function of the integuments but rather active signaling from the integuments to the FG seems to be imperative for correct FG development.

While the absence of a functional FG does not visibly affect integument development, in *Arabidopsis* mutants such as *sporocytless/nozzle* (*spl/nzz*) [20,21], *determinant infertile1* (*dif1*) [22], *coatlique* (*coa*) [23], and *topless* (*tpl*) [24], transcriptomic data revealed that the lack of a FG does influence gene expression in sporophytic tissues, suggesting a feedback regulation from the gametophytic to the sporophytic tissues [23,25]. A substantial amount of genes encoding for putative secreted proteins are dependent on the female gametophyte [26]. While the function of many of these proteins is yet to be tested, these observations suggest that peptide signaling is likely to play an important role in non-cell autonomous communication in the context of ovule development.

Post-fertilization: communication between endosperm and seed coat

During FG formation, communication with the integuments is believed to take place through cytoplasmic connections that connect integuments with the nucellus (the sporophytic tissue surrounding the FG inside the integuments) and the FG [3]. Following FG cellularization and degradation of the nucellus in some plant species, such as *Arabidopsis*, it is generally assumed that the FG becomes symplastically isolated from the maternal sporophytic tissues [3,27]. As a consequence, the transport of nutrients, water and signaling molecules between sporophyte and gametophyte at this transition has to undergo significant alterations. Nevertheless, whether symplastic isolation of the FG is a general phenomenon remains to be investigated. For instance, plasmodesmata were proposed to exist between the sporophyte and the aleurone layer of *Medicago truncatula* seeds [28]. However, ablation of the seed coat endothelium after fertilization has no substantial effect on seed development in *Arabidopsis* (in contrast to its important role for FG development before fertilization), supporting the view that functional symplastic communication between the zygotic and sporophytic tissues is likely limited [29]. Therefore, communication between the sexual endosperm and the sporophytic seed coat should mostly occur through routes alternative to the symplast [3,30], limiting the nature of signaling molecules to those that can traverse the cell membrane and cuticle or to solutes that can be actively transported between the two tissues.

While not being the signal itself, genes involved in the HAIKU (IKU) pathway could be part of the signaling chain between endosperm and seed coat. *IKU* genes are expressed in the endosperm and when mutated cause reduced syncytial endosperm growth and decreased final seed size [31–34]. Nevertheless, apart from molecular signaling events, mechanical cues could play an as yet underappreciated role in this process as well. The adaxial layer of the outer integument was shown to function as a mechanosensor for the expanding endosperm [35•]. This cell layer reacts to the endosperm pressure by altering microtubule dynamics, cell wall deposition and consequently its expansion. Furthermore, the mechanosensing properties of this cell layer modulate the expression of *EUI-LIKE P450 A1* (*ELA1*; coding for a cytochrome P450 oxigenase) which, together with *ELA2*, regulates organ size by controlling gibberellic acid catabolism [35•,36]. As a likely consequence of cell wall thickening in the seed coat, the maternal tissues on their turn have a substantial impact on endosperm proliferation. This notion originates from observations that mutations that influence seed coat growth lead to variations in seed size, which normally coincide with altered endosperm proliferation rates. While mutants such as *apetala2* (*ap2*) and *megaintegumenta/auxin response factor 2* (*mnt/arf2*) that positively impact on seed coat development produce large seeds with increased number of endosperm nuclei [37,38], mutants with reduced seed coat growth, such as *transparent testa glabra 2* (*ttg2*) or *enhancer of da1-1* (*eod3*) [39,40], produce smaller seeds, with fewer endosperm nuclei. Whether this effect on endosperm development is due to an undiscovered signaling pathway or purely due to spatial limitation of endosperm growth, remains to be investigated.

Notwithstanding the fact that turgor pressure by the endosperm has implications on seed coat growth, endosperm proliferation *per se* is not sufficient to trigger seed coat development. Mutants that code for gametophytic