Reproductive cross-talk: seed development in flowering plants

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Abstract

Flowering plants have evolved to be a predominant life form on earth. A common principle of flowering plants and probably one of the main reasons for their evolutionary success is the rapid development of an embryo next to a supporting tissue called the endosperm. The embryo and the endosperm are protected by surrounding maternal tissues, the integuments, and the trinity of integuments, embryo and endosperm comprise the plant seed. For proper seed development, these three structures have to develop in a highly controlled and co-ordinated manner, representing a paradigm for cell-cell communication during development. Communication pathways between the endosperm and the seed coat are now beginning to be unravelled. Moreover, recently isolated mutants affecting plant reproduction have allowed a genetic dissection of seed development, and revealed that the embryo plays a previously unrecognized yet important role in co-ordinating seed development.

Introduction

Seeds of flowering plants build a highly elaborated functional unit with the aim of propagating the plant’s offspring represented by the embryo within the seed. Seeds typically accomplish three major functions. (i) They are survival units possessing a hard shell and an extremely reduced metabolism (dormancy). (ii) They are dispersal units in space and time and, depending on the morphology of the outer cell layers, can, for instance, swim or fly. In addition, they germinate only under favourable conditions, e.g. after a long winter. (iii) Finally, they are nutritional units providing the developing embryo and often also the germinating seedling with food reserves.

The two tissues surrounding the embryo, the endosperm and the integuments, have specialized roles to fulfil these functions. The endosperm is involved in nurturing the embryo during the different stages of seed development. In addition, the endosperm contributes to other seed developmental processes, e.g. seed dormancy and germination [1]. Furthermore, the endosperm might be an instrument to execute post-zygotic reproduction barriers preventing interspecies hybridization or inbreeding [2–4]. One possible basis for this reproduction barrier is the endosperm’s sensitivity towards gene dosage effects. Regulatory processes, especially epigenetic mechanisms such as genomic imprinting, are in charge to assure the correct gene expression levels (see [5–8] for reviews).

The endosperm, like the embryo, is a sexual product, resulting from the fusion of a homodiploid central cell with one of the two sperm cells delivered by the pollen tube. The other sperm cell fuses with the haploid egg cell, giving rise to the actual embryo. Thus, in contrast with the embryo, the endosperm is typically a triploid organ in diploid species. Following a first phase of free nuclear divisions immediately after fertilization, the endosperm differentiates into distinct compartments that appear to have specific functions. This functional distinction is best seen in cereals: the ETCs (endosperm transfer cells) are in contact with maternal tissue and are responsible for the uptake of nutrients from the mother plant [9–11]. The ESR (embryo-surrounding region) mediates nutrient transfer to the embryo and might act as a site of signalling cross-talk between the embryo and the endosperm. Starchy endosperm cells represent the bulk of the endosperm and synthesize and store reserve proteins and starch until germination. Finally, the outer endosperm layer (aleurone) releases enzymes which hydrolyse the storage reserves in the starchy endosperm at seed germination [9,10,12,13].

In many dicotyledonous species, such as Arabidopsis thaliana, the main bulk of the endosperm does not persist until seed maturity. Here, the nutritive role of the endosperm might be transient storage of nutrients before they are transferred to the developing embryo [14]. Yet,

Key words: endosperm, flowering plant, integument, reproductive cross-talk, seed development.

Abbreviations used: AP2, APETALA 2; ASK5, Arabidopsis thaliana sucrose transporter 5; CAP2, CAPRICE2; CDKA;1, CYCLIN-DEPENDENT KINASE A;1; CMI, cell wall invertase; DTA, diphtheria toxin A; ESR, embryo-surrounding region; ETC, endosperm transfer cell; FBL17, F-BOX-LIKE PROTEIN 17; FIE, FERTILIZATION-INDEPENDENT ENDOSPERM; FIE2, FERTILIZATION-INDEPENDENT SEED 2; GLC, GLACIER; KNU, KNUCKLE; KEA, NERICA; KUN1, MINISEED1; MNT, MINIATURE1; MINIATURE2; MINIATURE3; MNT1, MEGANUTRIMENTA; NAC, NITROGEN ASSIMILATION CONTROL; NARS, NAC-REGULATED SEED MORPHOLOGY; SHB1, SHORT HYPOCOTYLS UNDER BLUE LIGHT 1; SNI1, SHORT INTEGUMENT 1; STIP, sugar transporter; TFG2, TRANSPARENT TESTA GLABRA 2.

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also in Arabidopsis, a single-cell aleurone layer persists in mature seeds, and has key functions during seed dormancy, germination and seedling sustenance [1,15,16].

The maternally derived integuments build the seed coat in angiosperm seeds. The seed coat not only provides a sheltered environment for embryo development, but also is involved in the transfer of nutrients from the mother plant to the embryo and the endosperm. In many plant species, the mature seed coat (testa) forms a tough layer protecting the seed contents from damaging environmental influences, such as UV radiation or pathogens, and represents a barrier to precocious germination [1,17,18].

Seed coat development is initiated upon fertilization and is marked by rapid growth and differentiation of ovule integuments into seed integument layers. In Arabidopsis, the innermost endothelium layer accumulates proanthocyanidin flavonoids that are later oxidized, which causes the brown colour of the ripe seeds [19]. Two other layers of the inner integument die and are compressed early during seed development without apparent differentiation. The two layers of the outer integuments accumulate starch containing amyloplasts, followed by the deposition of large quantities of pectinaceous mucilage in the outermost cell layer. At last, as the seed matures, the outer integumental cells also die [1,17,18]. In Arabidopsis, the outer integuments form a symplastic domain with the maternal phloem, but are only poorly symplastically connected with the inner integuments. Nutrient transport therefore has to occur centripetally from the outer through the inner integuments via the apoplast to the endosperm and the embryo on routes that are beginning to be unravelled [20,21].

To form a functional whole, not only does growth and development within the individual seed components have to be controlled (e.g. the differentiation of the endosperm into an ESR and the generation of ETCs), but also the different tissues have to harmonize their development (e.g. endosperm growth and embryo growth have to be co-ordinated). In addition, it is likely that the mother plant also actively communicates with the developing seed. Recent experiments are now providing genetic, and in some cases molecular, evidence for the existence and function of co-ordinating signals and communication pathways in the angiosperm seed. In the present review, we discuss these recent findings and address the cross-talk during seed development by looking at the reciprocal communication pathways that may exist between the embryo, the endosperm and the seed coat.

Communication during early stages of seed development: signals from the embryo to the endosperm and to the integuments

Co-ordinating signals emerging from the embryo were unknown for a long time. Seed growth, for instance, did not appear to be influenced by the size of the embryo. Moreover, there are mutants in which seed development was found to start even without fertilization and in absence of an embryo [22–28]. However, recently identified mutants in CDKA;1 (CYCLIN-DEPENDENT KINASE A;1) and FBL17 (F-BOX-LIKE PROTEIN 17) in Arabidopsis initially suggested two novel signalling cascades resulting from the fertilization of the egg cell and/or the developing embryo. (One signal pathway operating early after fertilization is discussed in this section; one putative signal operating at later stages is described below.)

In cdka;1 and fbl17 mutants, pollen containing a single sperm cell instead of the usual pair of sperm cells is formed at anthesis [29–32]. Remarkably, pollen with a single sperm could accomplish fertilization, but appeared to be exclusively fused with the egg cell.

However, recent experiments showed that, in many cases, a second male mitotic division was executed during pollen tube growth. Thus, instead of a single sperm, two sperm-cell like cells arrive at the embryo sac in the majority of cases. However, only the egg cell was properly fertilized, giving rise to a zygote that developed into an embryo. Intriguingly, karyogamy of the central cell with the second sperm-cell-like cell failed and the male pronucleus was excluded from the central cell (S.J. Aw, Y. Hamamura, C. Zhong, A. Schnittger and F. Berger, unpublished work). Although the central cell remained unfertilized, it started to proliferate. On the one hand, nuclear proliferation of the central cell, resembling the beginning of endosperm development, could be triggered by a developing embryo, possibly hinting at a positive signal that emanates from the fertilization of the egg cell (Figure 1, Signal A) [29,30]. However, not all seeds after pollination with cdka;1 or fbl17 mutants that displayed a developing embryo showed endosperm proliferation [29,34]. Thus, and in the light of recent results (S.J. Aw, Y. Hamamura, C. Zhong, A. Schnittger and F. Berger, unpublished work), alternatively or in addition to a signal from the fertilization of the egg cell, the fusion of a sperm-cell-like cell with the central cell could be sufficient to trigger nuclear proliferation even without karyogamy.

In any case, not only nuclear proliferation was promoted, but also the diploid central cell nuclei that were generated died. In a second mitotic division during pollen tube growth, thus, two sperm-cell like cells arrive at the embryo sac in the majority of cases. However, only the egg cell was properly fertilized, giving rise to a zygote that developed into an embryo. Intriguingly, karyogamy of the central cell with the second sperm-cell-like cell failed and the male pronucleus was excluded from the central cell (S.J. Aw, Y. Hamamura, C. Zhong, A. Schnittger and F. Berger, unpublished work). Although the central cell remained unfertilized, it started to proliferate. On the one hand, nuclear proliferation of the central cell, resembling the beginning of endosperm development, could be triggered by a developing embryo, possibly hinting at a positive signal that emanates from the fertilization of the egg cell (Figure 1, Signal A) [29,30]. However, not all seeds after pollination with cdka;1 or fbl17 mutants that displayed a developing embryo showed endosperm proliferation [29,34]. Thus, and in the light of recent results (S.J. Aw, Y. Hamamura, C. Zhong, A. Schnittger and F. Berger, unpublished work), alternatively or in addition to a signal from the fertilization of the egg cell, the fusion of a sperm-cell-like cell with the central cell could be sufficient to trigger nuclear proliferation even without karyogamy.

In any case, not only nuclear proliferation was promoted, but also the diploid central cell nuclei that were generated adopted endosperm-specific characteristics as well [34]. Furthermore, even in occasionally occurring cases where the central cell did not divide, it started to express endosperm-specific reporter genes, indicating that proliferation was not necessary for the central cell to adopt endosperm fate [34].

Endosperm proliferation of cdka;1-fertilized seeds was restricted to four to five rounds of free nuclear divisions resulting in a maximum of 32 nuclei, followed by the eventual abortion of the seeds [29,34]. Mutants in the PRC2 (polycomb-repressive complex 2) components, mea (medea), fis2 (fertilization-independent seed 2) and fie (fertilization-independent endosperm), display autonomous endosperm development when left unfertilized, and an overproliferation phenotype of the endosperm when fertilized, resulting in embryo lethality later in seed development [22–25]. When fertilizing these FIS-class mutants with cdka;1 or fbl17 mutant pollen, viable embryos developed that gave rise to fertile plants, underlining the functionality of a diploid
unfertilized endosperm, i.e. without any contribution from the paternal genome [30,35].

Conversely, in ferontia/sirene mutants, in which the pollen reaches the ovule, but does not release sperm cells, autonomous endosperm development was not reported, indicating that either cell fusion of the central cell with one sperm or the fusion of the other sperm with the egg cell triggers endosperm development and proliferation [36–38]. Future work will be required to untangle central cell fusion from the possibility of a non-autonomous acting signal originating from the fertilization of the egg cell. Even if a signal from the fertilization of the egg cell is involved in instructing the central cell, the exact path of this signalling cascade could be indirect, e.g. it is not clear whether this could be a direct zygote/embryo–endosperm interaction or rather an indirect communication pathway, for example via the integuments.

Evidence for communication between the embryo and the integuments is scarce. One example might be the production of flavonoids in the endothelium layer of the integuments that marks fertilized and developing seeds (Figure 1, Signal B) [39]. Flavonoid production in the endothelium layer was also found in plants single-fertilized with cdka;1 mutant pollen and producing only an embryo and no endosperm [34]. Remarkably, the production of flavonoids started in the endothelium layers that were closest to the developing embryo. However, it is unclear whether the differentiation of the endothelium layer is a direct interaction between the embryo and the integuments, or whether this could be mediated via the endosperm, especially since autonomously developing endosperm was found to induce flavonoid production in the absence of an embryo (see below) [40].

Communication during early stages of seed development: signals from the endosperm to the embryo

Owing to its nutritive function for the embryo, it is assumed that embryo development relies on proper endosperm growth and differentiation. Early experiments in alfalfa and Solanaceous species led to the suggestion that a poorly developing endosperm after inbreeding and inter-species crosses can cause the arrest of embryo development and seed abortion [41,42].

The first direct evidence for the crucial role of the endosperm came roughly 60 years later when Weijers et al. [43] ablated endosperm during early seed development in Arabidopsis by tissue-specific expression of DTA (diphtheria toxin A). DTA expression led to endosperm degeneration after a few rounds of nuclear proliferation without harming the embryo directly, but resulting in subsequent embryo arrest and seed abortion. Furthermore, as the embryos did not arrest immediately after endosperm ablation, these results also suggested a certain independence of embryo development. However, the promoter used in these studies typically does not confer expression before four to eight endosperm nuclei are formed, and the presence of this initial endosperm nuclei might allow initiation and continuation of embryo development even after the endosperm was ablated.
Figure 2 | A putative globular stage checkpoint in seed development

Different experiments and mutant analyses hint at the existence of a checkpoint in seed development. In many cases of early endosperm failure, the embryo continues to develop until the globular stage regardless of the amount of endosperm nuclei. (A) In cap2 mutants, endosperm development is terminated precociously and occasionally cellularizes. (B) After fertilization by cdka;1 mutant pollen (exclusively accomplished egg-cell fertilization and absence of karyogamy of the second sperm with the central cell), various numbers of autonomous diploid endosperm nuclei can develop (without any contribution from the paternal genome). (C) Ablation of the early endosperm by DTA expression after approximately the second to third nuclear division round. (D) In glc mutant seeds, the endosperm completely fails to proliferate.

Nonetheless, a restricted independence of embryo development from endosperm formation was corroborated by two subsequent reports. The female gametophytic glc (glauce) mutant in Arabidopsis specifically affects central cell differentiation since the central cell cannot be fertilized [44]. Thus, when glc mutants were pollinated with wild-type pollen, only the egg cell fused with a sperm cell and, although no endosperm was formed, an embryo developed reaching late globular stage before glc mutant seeds eventually aborted.

Further evidence came again from analysing cdka;1 mutants. Ungru et al. [34] found that there exists a large natural genetic variation with respect to the onset of autonomous central cell proliferation (development of an endosperm without a contribution of the paternal genome): upon cdka;1 mutant fertilization, some Arabidopsis accessions generated up to 32 nuclei in the central cell, whereas, in other accessions, the central cells did not divide in the majority of seeds. Analysing embryo development in cases where the central cell did not divide showed that embryos reached a globular stage that was independent of central cell proliferation. Nevertheless, a positive correlation between the amount of autonomous endosperm proliferation and the growth rate of the embryo was observed during the first few hours after fertilization by cdka;1 pollen (Figure 1, Signal C) [34].

Similarly, an embryo arrest at the late globular stage was found in seeds of the maternal effect mutant cap2 (capulet 2) [45]. In cap2, the degree of endosperm division and differentiation is variable, ranging from one to five syncytial divisions, occasionally involving precocious cellularization (after three to five division rounds). The cap2 embryo does, however, not develop further than the globular stage in any of these situations.

Taken together, these analyses substantiate the existence of a general checkpoint for seed development around the globular stage of embryo development that is activated in case of an early defect in endosperm proliferation (Figures 1, Signal D, and 2). One might speculate that the rapid proliferation of the syncytial endosperm, as seen, for example, in live observations in Arabidopsis wild-type plants [46,47], might create a first powerful sink tissue in the young seed. Instead of the initially slowly growing embryo, the endosperm would recruit nutrients from the mother plant and store it for later stages of seed development. A failure to establish this sink function might trigger the globular stage checkpoint and might even involve an active abortion mechanism of maldeveloping seeds by the mother plant (see also below).

Little is known about how nutrients are passed from the endosperm to the embryo. The sugar transporter AtSUC5 (Arabidopsis thaliana sucrose transporter 5) is expressed specifically in the endosperm surrounding the embryo and might contribute directly to embryo nutrition. Loss of AtSUC5 function, however, only results in a slight delay of embryo
development, arguing for a redundancy of nutrient transport mechanisms [48]. Furthermore, the mechanism of nutrient uptake is likely to be dynamic and might change during development, since analyses with radioactively labelled carbon atoms (^14C) in Brassicae revealed that, in later stages of seed development, sucrose was not hydrolysed in the endosperm but was taken up directly by the embryo, corroborating an important role of the endosperm, especially during early seed development [20].

Communication during early stages of seed development: signals from the endosperm to the maternal layers

The significance of the endosperm for seed growth and development, particularly its influence on the integuments, has become clear over the last few years (Figure 1, Signal E) [49]. One example is the above-mentioned production of flavonoids in the endothelium layer of the integuments in mutants that produce an autonomous endosperm, such as fis2 in Arabidopsis [40]. In addition, in FIS-class mutant seeds with an autonomously developing endosperm and without an embryo, the integuments were found to elongate, a typical feature of progressing seed development [22–24,27].

Communication between endosperm and seed coat is well known from studies in cereals; for example, the mn1 (miniature1) mutant in maize or mn1-like mutant in barley generate little endosperm and display much smaller seeds than wild-type plants [50,51]. MN1 is an endosperm-specific cell wall invertase that appears to metabolize the incoming sucrose in the endosperm and thus might have a key role in establishing the endosperm as a sink tissue.

A predominant role of endosperm during seed development was also seen in interploidy crosses in maize. A higher paternal genomic contribution increased endosperm proliferation and accompanying seed size, whereas a higher maternal contribution decreased endosperm size and seed growth [52–54]. A similar effect was also observed in interploidy crosses in Arabidopsis in which endosperm is not as prominent as in maize or other cereals [55].

Genetic screens for mutants phenocopying the effects of dosage imbalance in the endosperm in Arabidopsis led to the isolation of iku (haiku) mutants with reduced endosperm nuclei number, early endosperm cellularization, decreased integument growth and small seed size [56]. Subsequent identification and molecular characterization revealed that IKU2 encodes a LRR (leucine-rich repeat) kinase and MINI3 (MINISEED3) for a WRKY transcriptional regulator [57]. IKU2 and MINI3 are both expressed in the endosperm shortly after fertilization. The observation that IKU2 expression is reduced in a mini3 mutant background suggests a common pathway for these two genes.

Recently, it was found that the ZZ-type zinc finger protein SHB1 (SHORT HYPOCOTYLS UNDER BLUE LIGHT 1) acts upstream of IKU2 and MINI3 and promotes, probably in a direct manner, the expression of IKU2 and MINI3 [58]. Whereas mutants in SHB1 produce smaller seeds, the dominant allele shb1-D produces larger seeds, an effect that is dependent on the presence of IKU2 and MINI3.

However, how this and possibly other cascades communicate endosperm size to the integuments is not understood. Recent findings showing the importance of mechanical forces in growth control introduce the possibility that, in addition to an active signalling process, or perhaps even as a sole mechanism, the seed coat might passively follow the size of the endosperm [59].

Communication during early stages of seed development: signals from the maternal layers to the endosperm and the embryo

In addition to the effect that endosperm proliferation exerts on seed growth, seed size also appears to be strongly influenced by maternal factors expressed in the integuments (Figure 1, Signals F and G). In crosses between two Arabidopsis accessions with different seed sizes, Alonso-Blanco et al. [60] found that the final seed size was predominantly determined by the maternal genotype. This genetic determinant promoted both the size and the number of integument cells.

The analyses of mutants with seed phenotypes also revealed communication between integuments and zygotic parts of the seed. Cell proliferation in the integuments was found to be repressed by MNT (MEGAINGUMENTA), as mnt mutants displayed extra cell divisions in the integuments in comparison with wild-type plants coinciding with the development of enlarged seeds that also contained larger embryo [61]. MNT was found to be allelic with ARF2 (AUXIN-RESPONSE FACTOR 2) belonging to a class of transcription factors that bind to auxin-responsive elements in the promoters of auxin-regulated genes [62].

Conversely, compromised integument development as seen in the sin1 (short integument 1) mutant was found to result in severely disorganized embryos with no apical meristem being formed [63]. A second example are the NAC (NITROGEN ASSIMILATION CONTROL) transcription factors NARS (NAC-REGULATED SEED MORPHOLOGY) 1 and 2 (also called NAC2 and NAM respectively), which are expressed in the outer integuments and are redundantly required for their differentiation [64].

Mutants in the WRKY transcription factor TTG2 (TRANSPARENT TESTA GLABRA2) have a partially overlapping mutant phenotype with the nars1/nars2 mutant in respect of a missing mucilage deposition in the outer integuments in both mutants [65]. In addition, ttg2 mutants show differentiation defects of the endothelium layer and mutant seeds are also smaller than wild-type, with reduced cell size in the integuments and reduced endosperm proliferation [66]. Interestingly, reduced activity of TTG2 was mapped as a major quantitative trait that suppressed the lethality of interploidy crosses [67].

The integuments appear to play a key role in directing nutrients from the mother to the developing embryo and it is plausible that the defects seen in nars1/nars2, sin1 or ttg2 derive, at least partially, from compromising this function.
Cell–Cell Communication in Plant Reproduction

Figure 3 | Signalling at the heart stage of embryo development
The embryo influences endosperm differentiation during mid-seed development. (A) After fertilization of a FIS-class mutant ovule by wild-type pollen, the endosperm fails to terminally differentiate and seeds abort with an embryo arrested in the late heart stage. (B) In unfortified FIS-class mutant ovules, autonomous endosperm develops. This autonomous endosperm may show features of early endosperm differentiation, but does not mature and aborts after a few days of development (indicated by a discontinuous seed coat). (C) When fertilized by cdkα1 pollen (successful egg cell fertilization and lacking karyogamy of the second sperm with the central cell), FIS-class mutant ovules complete seed development (green seed coat). In these seeds, a fertilized embryo develops alongside an autonomous diploid endosperm (without any contribution from the paternal genome). This phenotype indicates that the embryo can trigger terminal differentiation of the endosperm (yellow arrow) and so directly or indirectly sustain seed development and survival (blue arrow).

Early in seed development, CWIs (cell wall invertases) and STPs (sugar transporters) are expressed at the maternal-filial interface of inner integument and endosperm (see above). CWIs and STPs catalyse the rapid hydrolysis of maternally unloaded sucrose, followed by uptake of hexoses into the endosperm [68]. Apart from the trophic aspect, the rapid uptake of hexoses is thought to increase the osmotic potential of the central cell, thereby creating the turgor necessary for the massive expansion of the syncytial endosperm that might, for instance, as discussed above, mechanically feed back to the integuments [20,21,68].

Remarkably, in mutants in the transcriptional regulator AP2 (APETALA 2), which belongs to the class of ethylene-responsive-element-binding proteins, seed size is increased and a higher ratio of glucose to sucrose was found [69]. However, whether AP2 regulates the expression of CWIs or STPs is not yet clear. Direct evidence for the importance of CWIs comes from CWI-deficient mutants in maize (mn1, as discussed above) that show a reduction of final seed mass by 70% [50,68].

However, the mother plant might not only pave the road for nutrient transport, but also have a way to actively block transport, inducing seed abortion. In incompatible interspecies crosses, embryo and endosperm development was initiated, but development slowed down shortly after initiation [70]. Remarkably, it was found that the maternally derived nucellar tissue overproliferated before seed degeneration, possibly indicating an active mechanism executed by the mother to shut off the nutrient supply for the developing seeds.

Furthermore, the mother plant appears to distribute her resources equally to its developing offspring, and a trade-off between seed number and seed mass has been observed, for instance in a RIL (recombinant inbred line) population in Arabidopsis [60,71]. This trade-off might obscure the true effect of mutants affecting seeds growth and fertility, especially since the extent of this trade-off was found to depend on the growth conditions, thus underlining the complexity of communication pathways during seed development.

Communication during later stages of seed development
During mid-seed development, the relationships of seed components are changing. In species with transient endosperm, the embryo starts a massive growth phase while consuming all or most of the endosperm and becoming the dominant element in the seed. In cereal seeds with a persistent endosperm bulk, the starchy endosperm cells start to die, and only the aleurone layer remains alive until seed maturity [9].

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Whether transient or persistent, the endosperm terminally differentiates at this stage. In *Arabidopsis*, endosperm cellularization starts in the vicinity of the embryo and proceeds in a wave-like fashion to the opposite chalazal pole of the endosperm [72]. A similar pattern can also be observed for the expression of several endosperm differentiation markers [73], hinting at a role of the embryo as an endosperm differentiation impulse emitter.

Terminal endosperm differentiation is inhibited in fertilized FIS-class mutant seeds as seen in an overproliferation phenotype and the lack of cellularization [22–28]. In addition, fertilized FIS-class mutants fail to express marker genes that are indicative of late endosperm stage, while the expression of marker genes of juvenile endosperm persist [73]. This differentiation failure is associated with an embryo arrest in the late heart stage, followed by seed abortion. These observations point to the existence of an embryo development checkpoint around late heart stage that depends on proper differentiation of the endosperm (Figure 3A).

Conversely, when FIS-class mutants are not fertilized, the developing endosperm does not overproliferate and was even occasionally found to cellularize, but nonetheless these seeds eventually aborted (Figure 3B) [22].

However, when FIS-class mutants such as mea, fis2 or fie, are fertilized by cdka1 mutant pollen, differentiation of the endosperm is restored, leading to successful cellularization and restoration of marker gene expression (Figure 3C) [35]. Since the genetic composition of the unfertilized and developing endosperm does not overproliferate and was even occasionally found to cellularize, but nonetheless these seeds eventually aborted (Figure 3B) [22].

### Conclusions

Understanding seed growth and development is important for many different research fields, such as ecology or evolution. Moreover, seeds are receiving a growing interest as a source of renewable energy and as a food source for livestock and mankind, especially since food shortage is more than ever a pressing problem for humanity. Yet, we are only at the very beginning of understanding the general principles of seed growth and the co-ordination and cross-talk between the different seed tissues. The mutants identified recently have provided a genetic framework to handle this problem and it is now crucial to elucidate the biomolecular basis of the signalling cascades involved to eventually put the pieces of the puzzle together into a comprehensive picture.

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