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Green love talks; cell–cell communication during double fertilization in flowering plants

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Abstract

Background

Flowering plant seeds originate from a unique double-fertilization event, which involves two sperm cells and two female gametes, the egg cell and the central cell. For many years our knowledge of mechanisms involved in angiosperm fertilization remained minimal. It was obvious that several signals were required to explain how the male gametes are delivered inside the maternal reproductive tissues to the two female gametes but their molecular nature remained unknown. The difficulties in imaging the double-fertilization process prevented the identification of the mode of sperm cell delivery. It was believed that the two sperm cells were not functionally equivalent.

Scope

We review recent studies that have significantly improved our understanding of the early steps of double fertilization. The attractants of the pollen tube have been identified as small proteins produced by the synergid cells that surround the egg cell. Genetic studies have identified the signalling pathways required for the release of male gametes from the pollen tube. High-resolution imaging of the trajectory of the two male gametes showed that their transport does not involve the synergid cells directly and that isomorphic male gametes are functionally equivalent. We also outline major outstanding issues in the field concerned with the barrier against polyspermy, gamete recognition and mechanisms that prevent interspecies crosses.

Introduction

Flowering plants (angiosperms) have evolved a unique fertilization process, called 'double fertilization'. Two sperm cells fertilize two female gametophytic cells: the egg cell and the central cell (Fig. 1; Appendix). After fertilization, the embryo develops from the fertilized egg cell and the central cell gives rise to the endosperm, which nourishes the embryo for its development. These

two fertilization events are tightly controlled temporally and spatially, and take place in a coordinated manner to ensure successful embryogenesis. Unlike animals and lower land plants, such as bryophytes, lycophytes and ferns, flowering plant sperm cells are immotile and delivered to the female gametophyte by pollen grains. The pollen grain consists of two sperm cells inside a vegetative cell. After pollen deposition on the stigma,

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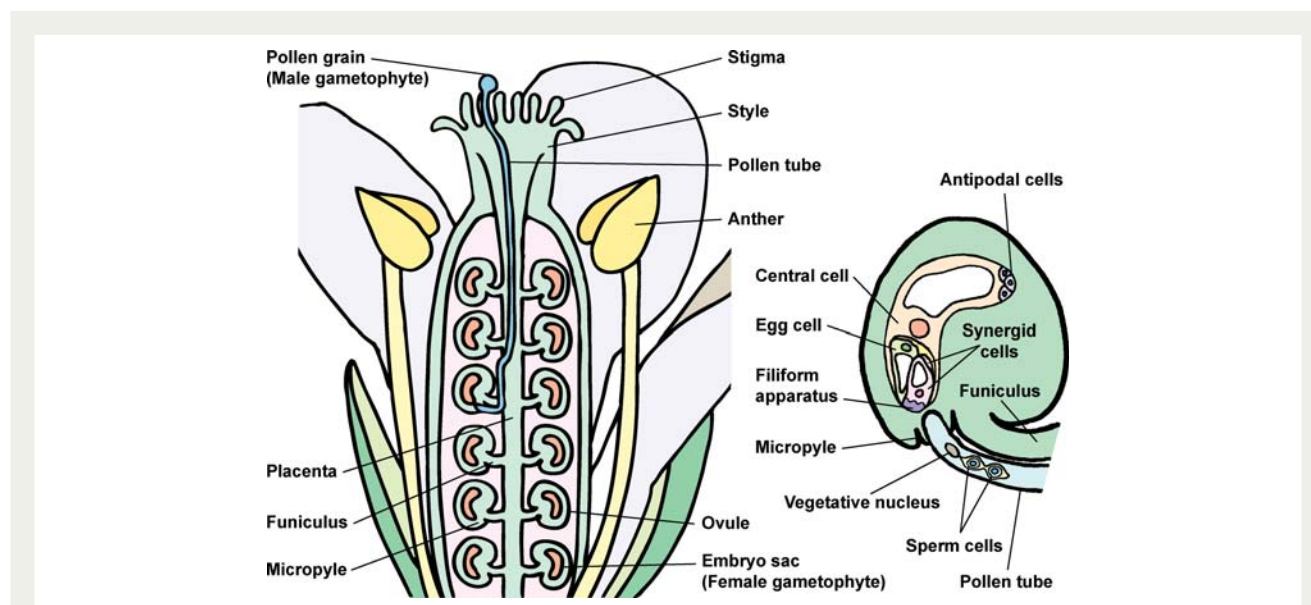


Fig. 1 Schematic representation of sexual reproduction in *A. thaliana*. The pollen grain, once hydrated on the stigma, elongates the pollen tube into the style. After emerging onto the surface of the placenta, the pollen tube keeps elongating to reach the funiculus, which connects the placenta and the ovule. When the ovule is not fertilized, the pollen tube is guided to grow onto the surface of the funiculus to the ovule by unknown mechanisms (funicular guidance). The chemoattractants secreted from the synergid cells then guide the pollen tube to the micropyle of the ovule (micropylar guidance). The pollen tube stops its growth (pollen tube perception), and discharges sperm cells. The two sperm cells are released and one fertilizes with the egg and the other with the central cell, giving rise to the embryo and the endosperm, respectively (double fertilization).

the vegetative cell elongates the pollen tube into the ovary to deliver two sperm cells (Fig. 1). A successful double fertilization depends on (i) proper guidance of the pollen tube to the unfertilized embryo sac (Appendix), (ii) release of the two sperm cells towards the egg cell and the central cell, (iii) recognition and fusion between each pair of gametes (plasmogamy, Appendix), and (iv) fusion between gamete nuclei (karyogamy, Appendix) and zygotic activation. During the past few years, identification of gamete-specific genes and promoters made it possible to mark gametes, allowing gamete transcriptome analyses (Borges *et al.* 2008) and *in vivo* imaging of double fertilization (Berger 2011). Moreover, *in vivo* live-cell imaging with high resolution has addressed controversial questions of double fertilization (Ge *et al.* 2011; Hamamura *et al.* 2011). Here, we review recent findings pertaining to signalling events during double fertilization.

Pollen tube guidance: directional pollen tube growth to the female gametophyte

On the stigma, pollen grains hydrate and germinate to initiate pollen tube growth into the transmitting tissues of the ovary (Fig. 1). The pollen tube then emerges on the surface of the placenta and is guided to grow onto

the funiculus (funicular guidance), which connects the embryo sac and placenta. The pollen tube eventually reaches the micropyle (Appendix) of the embryo sac (micropylar guidance). Remarkable advances have been made in our understanding of micropylar guidance (Marton and Dresselhaus 2010; Okuda and Higashiyama 2010; Sprunck 2010). It was first demonstrated experimentally *in vitro* that the synergid cells (Appendix) of the female gametophyte primarily govern micropylar guidance in *Torenia fournieri* (Higashiyama *et al.* 2001). In *Arabidopsis thaliana*, MYB98 is a transcription factor expressed preferentially in synergid cells (Fig. 2A). The *myb98* mutant shows defects in the organization of the filiform apparatus of synergid cells and micropylar guidance of pollen tubes, demonstrating that proper function of synergid cells is essential for micropylar guidance (Kasahara *et al.* 2005).

Several attempts have been made to identify pollen tube attractants secreted by synergid cells. Many MYB98-dependent synergid-specific transcripts were identified, including those encoding cysteine-rich polypeptides (CRPs) that are secreted to the filiform apparatus (Punwani *et al.* 2007, 2008). Whether these secreted CRPs are required for pollen tube micropylar guidance in *A. thaliana* remains to be determined, but pollen tube attractants were identified in *Zea mays* (maize)

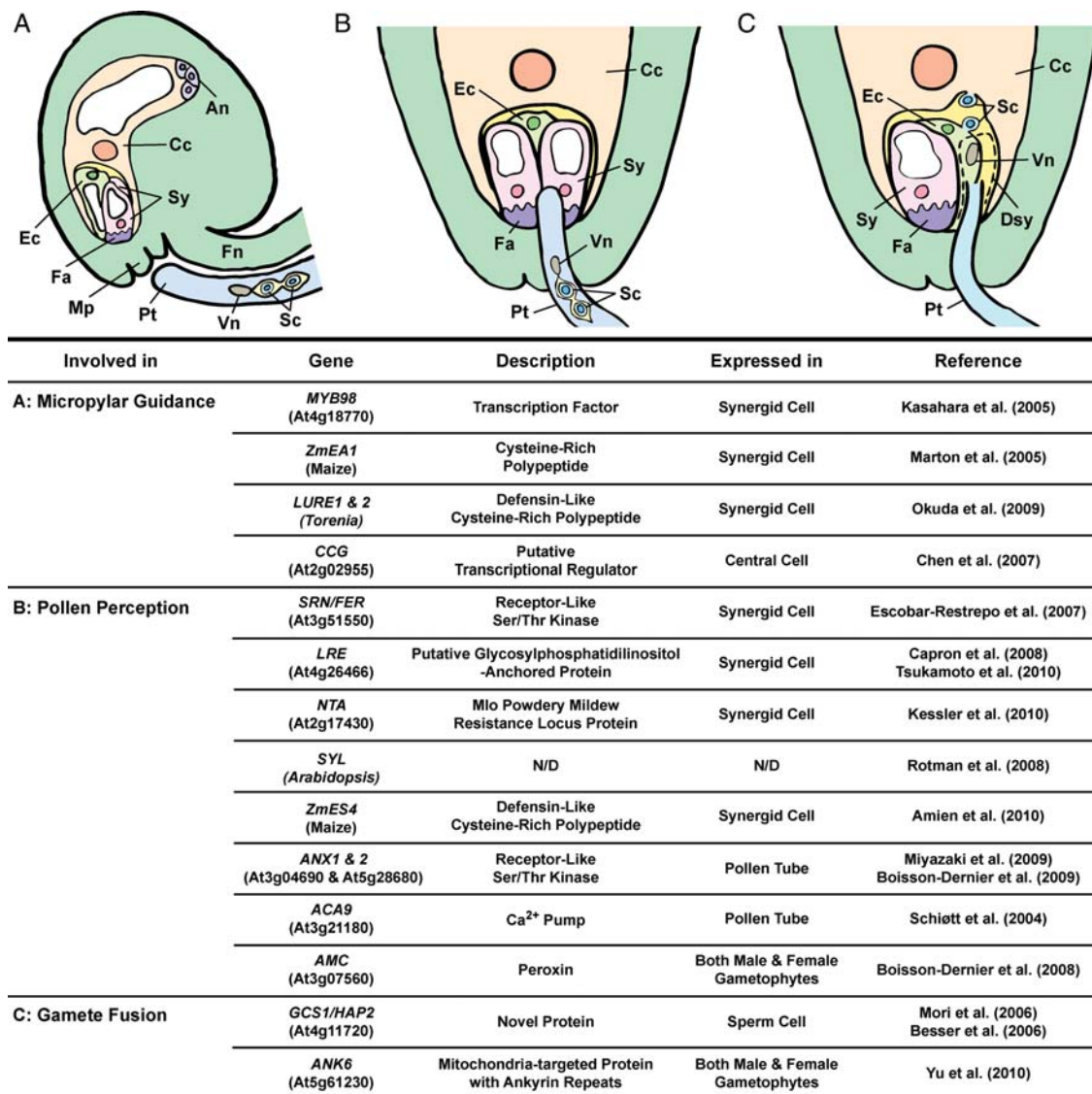


Fig. 2 Schematic representation of the role of genes involved in the early steps of double fertilization in *A. thaliana*. (A) The pollen tube on the funiculus is guided to the micropyle of the unfertilized ovule. Chemo-attractants such as LUREs in *Arabidopsis* and ZmEA1 in maize are secreted from the synergid cells through the filiform apparatus, generating a concentration gradient to conduct the directional growth of the pollen tube into the micropylar end of the embryo sac. (B) Through communication between the synergid cell and the pollen tube, the pollen tube arrests its elongation at the proper position and prepares for pollen tube discharge. (C) Pollen tube discharge generates cytoplasmic flow of the pollen tube content, which is likely to be sufficient for sperm cells to migrate directly into the inter-cellular region between the egg cell and the central cell. The ejected two sperm cells then start plasmogamy with the egg cell and the central cell. An, antipodal cells; Cc, central cell; Ec, egg cell; Dsy, degenerated synergid cell; Fa, filiform apparatus; Fn, funiculus; Mp, micropyle; Pt, pollen tube; Sc, sperm cell; Sy, synergid cell; Vn, pollen vegetative nucleus.

and *Torenia* (Fig. 2A). The maize gene *ZmEA1* encodes a polymorphic small protein and the *ZmEA1*-GFP fusion protein is detected in the cell wall that surrounds the synergid cells. Knockdown of *ZmEA1* affects the entrance of the pollen tube in the intercellular space of the micropyle (Marton et al. 2005). *In vitro* analyses

showed that the predicted mature *ZmEA1* protein can attract maize pollen tubes directly (Dresselhaus and Marton 2009; Marton and Dresselhaus 2010), further supporting the idea that the *ZmEA1* protein is the attractant for micropylar guidance. In *T. fournieri*, *LURE1* and *LURE2* encode CRPs, which belong to a subgroup of the

defensin-like gene superfamily, and are expressed highly in the synergid cells (Okuda *et al.* 2009). LUREs are secreted from the synergid cells to the filiform apparatus, and morpholino knockdown prevents proper pollen tube attraction. *In vitro* pollen tube attraction assay demonstrated that LURE1 and 2 indeed attract *Torenia* pollen tubes (Okuda *et al.* 2009), confirming that LURE1 and 2 are truly the attractants for micropylar guidance. LURE homologues from *Torenia concolor* are also expressed in synergid cells but are poor attractants for pollen tubes of *T. fournieri*, suggesting that LUREs might participate in the prevention of cross-fertilization between species (Kanaoka *et al.* 2011). *Arabidopsis* synergid cells also express and secrete defensin-like CRPs to the filiform apparatus (Punwani *et al.* 2007, 2008), and further analyses will be necessary to understand whether defensin-like CRPs secreted by *Arabidopsis* synergid cells are also involved in micropylar guidance.

Pollen tube perception: pollen tube growth arrest and rupture

After arriving at the micropylar end of the female gametophyte, the pollen tube stops elongation. This is followed by pollen tube rupture and sperm cell release for successful double fertilization (Fig. 2; Weterings and Russell 2004). Genetics in *Arabidopsis* have identified several gametophytic factors involved in pollen tube perception. For example, *ABSTINENCE BY MUTUAL CONSENT* (*AMC*) encodes a peroxin, which is important for protein transport into peroxisomes, and the *amc* mutation causes defects in pollen tube perception only when both the male and female gametophytes carry the mutant *amc* allele (Boisson-Dernier *et al.* 2008). By contrast, many factors found to be important for pollen perception are female or male gamete specific, and analyses on these factors revealed that communications between the pollen tube and synergid cells are essential, as detailed in the following paragraph.

How do synergid cells sense pollen tube arrival?

FERONIA (*FER*) encodes a receptor-like serine/threonine kinase (RLK) (Escobar-Restrepo *et al.* 2007). When faced with *fer* female gametophytes, wild-type pollen tubes fail to arrest growth and invade the female gametophyte without sperm release (Huck *et al.* 2003; Rotman *et al.* 2003). The *FER*-GFP fusion protein is expressed in synergid cells and localized at the filiform apparatus, presumably playing a role in sensing pollen tube arrival. Recently, other mutants exhibiting female gametophytic *fer*-like phenotype have been identified: *lorelei* (*lre*,

LRE encodes a putative glycosylphosphatidylinositol-anchored protein) (Capron *et al.* 2008; Tsukamoto *et al.* 2010), *scylla* (*syl*) (Rotman *et al.* 2008) and *nortia* (*nta*, *NTA* encodes a MILDEW RESISTANCE LOCUS O family protein) (Fig. 2B; Kessler *et al.* 2010). *LRE* and *NTA* are expressed in synergid cells, and *NTA*-GFP fusion protein localizes at the filiform apparatus which extends the membrane of the synergid cells toward the micropyle. Furthermore, the filiform apparatus localization of *NTA*-GFP fusion protein takes place upon pollen tube arrival, and is *FER* dependent (Kessler *et al.* 2010). This suggests that the *FER* pathway is important for sensing pollen tube arrival and re-localizes *NTA* in the synergid cell upon pollen tube arrival (Boisson-Dernier *et al.* 2011). It remains unclear, however, how the re-localization of *NTA* is important for pollen tube perception and whether the *FER* pathway is involved only in the pollen tube and synergid cell communication. Interestingly, the *NTA* gene belongs to the plant-specific MILDEW RESISTANCE LOCUS O family, which was first identified in the context of powdery mildew susceptibility in barley (Büschges *et al.* 1997). Furthermore, it was possible to obtain *fer* homozygous mutant plants, and these showed powdery mildew resistance (Kessler *et al.* 2010), implying that pollen tube perception and the fungal invasion pathway share some molecular components. Genes closely related to *FER* and *NTA* are found in bryophytes (Devoto *et al.* 2003; Lehti-Shiu *et al.* 2009), suggesting that *FER* and *NTA* in flowering plants might have evolved from ancestral proteins with functions unrelated to pollen tube perception. It is still unclear whether *FER* and *NTA* homologues in bryophytes have functions in fungal invasion. What the ancient functions of original *FER* and *NTA* proteins were, and how *FER* and *NTA* developed those functions in flowering plants, remain elusive.

Are other female gametophyte cells involved in pollen tube perception?

Additional evidence suggested that *FER* might also be involved in the communication between female gametophytic cells for pollen tube perception (Rotman *et al.* 2008). In addition to a *fer*-like phenotype, *syl* triggers autonomous endosperm development without fertilization, a trait associated with the *FERTILIZATION INDEPENDENT SEED* (*FIS*) class mutants. This discovery led to re-investigations of the *fer* mutant using the *sirene* (*srn*) allele. *srn/fer* mutants also showed autonomous endosperm development and further demonstrated synergistic interactions with the *fis2* mutant. *fis2* also displayed defects in pollen tube discharge as in *srn/fer*, demonstrating that the central cell is also important for pollen tube perception and that *FER* might also play a role in pollen tube perception, which involves

the communication between synergid cells and the central cell (Fig. 2B; [Rotman et al. 2008](#)). A possible involvement of the central cell for pollen tube perception is also pointed out by the analysis of *CENTRAL CELL GUIDANCE* (CCG), encoding a putative transcriptional regulator expressed in the central cell. The *ccg* mutation causes a loss of pollen tube micropylar guidance (Fig. 2A; [Chen et al. 2007](#)). Consistently, the communication between the central cell and other female gametophytic cells for proper development has also been recently reported ([Kägi et al. 2010](#)). Further studies are now required to identify FER ligands and where these are secreted (from the central cell and/or the pollen tube) to activate the FER pathway in the synergid cell for pollen tube perception.

How does the pollen tube control its growth and rupture?

The closest paralogues of *SRN/FER*, *ANXUR1* and 2 (*ANX1* and 2), expressed in the pollen tube, are also critical for pollen tube perception (Fig. 2C; [Boisson-Dernier et al. 2009](#); [Miyazaki et al. 2009](#)). The double knockout mutant pollens are able to germinate and elongate the pollen tube, but rupture prematurely without reaching the female gametophyte, indicating that the ANX pathway is preventing premature pollen tube rupture ([Miyazaki et al. 2009](#)). The possible function of ANX for pollen tube perception has been postulated; the ANXs might function in a cell-autonomous manner ([Berger 2009](#)). In this scenario, the pollen tube would maintain ANXs activation in a positive feedback loop until the tip reaches the synergid cell where the extracellular environment would contain factors involved in quenching the ANXs pathway. Further analyses are anticipated that will reveal more of the precise molecular mechanisms by which ANXs control pollen tube growth arrest and rupture.

Defensin-like *Zea mays* Embryo Sac 4 (*ZmES4*) protein was identified as a putative signalling molecule controlling pollen tube rupture (Fig. 2C; [Amien et al. 2010](#)). *ZmES4* is expressed in maize synergid cells, and is capable of triggering pollen tube rupture *in vitro*. Furthermore, the authors demonstrated that *ZmES4* is able to open a potassium channel (K^+ -channel *Zea mays* 1) expressed in the pollen tube in a heterologous system. Previously, the Ca^{2+} pump ACA9 in the *Arabidopsis* pollen tube has been shown to be involved in pollen tube discharge ([Schjøtt et al. 2004](#)). The cellular and molecular mechanisms by which ions such as K^+ and Ca^{2+} play roles in pollen tube perception remain to be determined.

Sperm cell dynamics: sperm cell release and gamete recognition

The lack of high-resolution *in vivo* methods to analyse sperm dynamics during fertilization prevented an understanding of how sperm cells migrate and many hypotheses arose to explain the cellular mechanisms of double fertilization ([Berger et al. 2008](#)). However, rapid advances in technology and methodology, such as spatiotemporal high-resolution confocal microscopy coupled with cell-specific fluorescent markers, now allow the process of double fertilization to be dissected in much greater detail ([Berger 2011](#)).

When and where are the two sperm cells released?

Once the pollen tube reaches the micropylar end of the female gametophyte, its growth arrest and release of the two sperm cells take place by rupture of the pollen tube tip through interaction with the synergid cells (Fig. 2C). *In vivo* observations suggested that degeneration of the synergid cell takes place at the time of pollen tube arrival in *Arabidopsis* ([Rotman et al. 2003](#); [Sandaklie-Nikolova et al. 2007](#)) or at pollen tube rupture in *Torenia* ([Higashiyama et al. 2000](#)). However, the mechanisms responsible for the transport of the two sperm cells remain unclear. It was proposed that the two sperm cells are released into the degenerated synergid cell and are transported to the gamete fusion site by an active mechanism involving the cytoskeleton ([Huang and Russell 1994](#)). An alternative hypothesis proposes that the two sperm cells are transported passively by the cytoplasmic flow of the pollen tube content discharged between the synergid and the egg cell. This issue was addressed by [Hamamura et al. \(2011\)](#) using high temporal resolution imaging of *Arabidopsis* pollen tube rupture. With a sampling rate of 0.20–0.33 s, the authors demonstrated that the maximum velocity of the sperm cells right after pollen tube discharge is $10 \mu\text{m s}^{-1}$ and the duration of the rapid migration phase is of the order of 10 s. After sperm cell release is completed, the two sperm cells apparently become wedged between the central cell and the egg cell, and do not change position until gamete fusion (plasmogamy). If sperm cells were deposited in synergids and transferred to the female gametes by cytoskeletal elements, a pause after the initial pollen burst should be observed, followed by migration at a rate of $0.1\text{--}1 \mu\text{m s}^{-1}$ (the typical range of speed of transport along the cytoskeleton) ([Staiger 2000](#)). Hence, live imaging observations indicate that the cytoplasmic flow by pollen tube rupture is sufficient for the sperm cells to reach the site of gamete fusions. This finding is consistent with the observation in *Torenia* ([Higashiyama et al.](#)

2000), and this may indicate that the sperm cell delivery by cytoplasmic flow is conserved among flowering plants. The pollen tube cytoplasm could be injected between the egg cell and one synergid cell, creating a channel for the delivery of sperm cells between the plasma membranes of the synergid cell and the egg cell. The end of this channel reaches the contact between the egg cell and the central cell plasma membranes where sperm cells remain immobile until plasmogamy (Hamamura *et al.* 2011). Synergid cell degeneration would be a secondary event, resulting either from a specific signalling mechanism or from the mechanical stress caused by the burst of pollen tube cytoplasm (Fig. 2C).

Do dimorphic sperm cells prevent polyspermy in double fertilization?

Successful double fertilization relies on two independent gamete cell fusions. One sperm cell must fuse with the egg cell and the other with the central cell. As Hamamura *et al.* (2011) demonstrated, the two sperm cells are released at the site of gamete fusion simultaneously. If one sperm cell is able to fuse with the egg cell while the other is able to fuse with the central cell, coordination of the two fertilization events is secured and polyspermy (Appendix) is prevented. *Plumbago zeylanica* produces dimorphic sperm cells, which express different sets of transcripts (Gou *et al.* 2009). Each sperm cell shows preferential fertilization to the egg cell or the central cell, depending on its identity (Russell 1985). *Arabidopsis*, like most flowering plant species, produces isomorphic sperm cells that cannot be distinguished by gene expression—so far (Ge *et al.* 2011). However, only one of the two sperm cells is associated in closer vicinity with the vegetative nucleus of the pollen (Fig. 2A and B) (McCue *et al.* 2011). This association involves the outer endocytic membrane that surrounds both sperm cells. It is thus possible that one sperm cell receives specific signals from the vegetative nucleus that would determine its capacity to fuse preferentially with one female gamete (McCue *et al.* 2011). Therefore, it has been an issue of debate whether sperm cells are functionally equivalent.

The question of sperm functional differentiation was initially tested in *Arabidopsis* mutants that produce multiple egg cells or a single sperm cell (Berger 2011). Mutants *eostre* (Pagnussat *et al.* 2007) and *retinoblastoma related 1* (Ingouff *et al.* 2006, 2009) generate two egg cells, which can be fertilized by both sperm cells delivered by a single pollen tube. Furthermore, mutants *chromatin assembly factor 1* (Chen *et al.* 2008) and *cyclin dependent kinase α ;1* (*cdka;1*) (Aw *et al.* 2010) produce a single sperm cell per pollen

grain, which shows the ability to fertilize either the egg cell or the central cell. Only a single sperm-like cell formed by translational inhibition in the male germ cell shows preferential fertilization to the central cell (Frank and Johnson 2009). Although these studies supported an equivalent functional identity for both sperm cells, they were not conclusive because they did not involve wild-type sperm cells and wild-type female gametes. To end this controversy, Hamamura *et al.* (2011) used a photo-convertible fluorescent protein to mark each sperm cell differently and examined the preferentiality of fertilization of two sperm cells delivered by a wild-type pollen tube to a wild-type ovule. Both sperm cells in either position (associated with the vegetative nucleus or not) fertilize the egg cell and the central cell with a similar probability, demonstrating that *Arabidopsis* sperm cells are functionally equivalent.

The equivalence of *Arabidopsis* sperm cells raises the issue of polyspermy block (Appendix). Circumstantial evidence has suggested the presence of polyspermy block in plants (Spielman and Scott 2008). *Arabidopsis* mutants *tetraspore* (Scott *et al.* 2008) and *retinoblastoma related 1* (Ingouff *et al.* 2009) also indicated the presence of polyspermy block in the egg cell. Live-cell imaging in wild type has provided support to these results (Hamamura *et al.* 2011). The two plasmogamies take place simultaneously within less than 1 min, and in spite of simultaneous fusions of the sperm cells, the authors did not observe two fusion events with the egg cell or with the central cell. Because the two sperm cells are both equally able to fuse with either female gamete, these observations suggest that polyspermy is prevented not only in the egg cell but also in the central cell. It remains unknown what cellular and molecular mechanisms prevent polyspermy (polyspermy block) in the egg cell and the central cell, and whether there are any differences in polyspermy block between the two female gametes.

A few factors essential for plasmogamy have been identified. *GENERATIVE SPECIFIC CELL 1/HAPLESS 2* (*GCS1/HAP2*) was first discovered in *Lilium longiflorum* to be sperm cell specific and affect plasmogamy when mutated (Mori *et al.* 2006). The *GCS1/HAP2* protein localizes at the sperm cell membrane and its function is essential for gamete fusion and is highly conserved among major eukaryotic taxa (Besser *et al.* 2006; Mori *et al.* 2006; Wong and Johnson 2010). Although there is no known functional domain identified in *GCS1/HAP2*, the putative extracellular N-terminus, containing the conserved *HAP2-GCS1* domain, seems critical for its function (Mori *et al.* 2010; Wong *et al.* 2010). In *Chlamydomonas reinhardtii*, *GCS1/HAP2* proteins are targeted for rapid degradation immediately after gamete fusion,

presumably involved in polygamy block (Liu *et al.* 2010). What molecular mechanisms control the GCS1/HAP2 pathway for gamete fusion and whether rapid degradation of GCS1/HAP2 occurs in flowering plants for polyspermy block remain to be determined.

ANKYRIN REPEAT PROTEIN 6 (ANK6) has recently been identified as another factor essential for gamete fusion in *Arabidopsis* (Yu *et al.* 2010). ANK6 encodes a mitochondria-targeted protein with ankyrin repeats which are believed to act in protein–protein interaction. ANK6 is highly expressed in both the male and female gametophytes and gamete fusion is impaired between mutant *ank6* gametes (Yu *et al.* 2010). ANK6 interacts with SIG5, a mitochondrial transcriptional initiation factor, implying that ANK6 controls gamete fusion by regulating mitochondrial gene expression. Further analyses will be awaited to reveal molecular mechanisms by which mitochondria play a role in gamete fusion through ANK6.

Post-plasmogamy

After plasmogamy, each sperm nucleus joins and fuses with either the egg cell nucleus or the central nucleus. This step, called karyogamy (Appendix), is followed by zygotic activation and embryo and endosperm development. Mutations in a chaperone involved in karyogamy in yeast affect polar nuclei (Appendix) fusion in the *Arabidopsis* central cell but do not impair karyogamy directly (Maruyama *et al.* 2010), similar to other mutants affecting polar nuclear fusion in the female gametophyte (Porteiko *et al.* 2006). The molecular mechanisms controlling karyogamy remain unknown. Similarly, little is known concerning zygotic activation. Karyogamy in the central cell is immediately followed by the onset of mitosis and translational activation, whereas it takes ~6–8 h in the zygote to detect the first sign of *de novo* zygotic transcription and translation (Aw *et al.* 2010; Ingouff *et al.* 2010). Surprisingly, it has recently been discovered that plasmogamy is sufficient to activate mitosis in the central cell (Aw *et al.* 2010). The *cdk4;1* mutant produces pollen with one or, more often, two sperm cells. When wild-type ovules are fertilized by *cdk4;1* pollen that delivers two sperm cells, embryogenesis is initiated correctly although the developing seed eventually aborts. The embryo abortion results from endosperm development arrest after a few mitotic divisions. Endosperm arrest is caused by failure of karyogamy after fertilization of the central cell. These results imply that plasmogamy in the central cell is sufficient to activate mitotic division, but the paternal genome is required for subsequent endosperm development. By contrast, mitotic activation by sperm entry is not observed in the egg cell (Aw *et al.* 2010).

These findings indicate that (i) plasmogamy signals mitotic activation in the central cell and (ii) the molecular mechanisms associated with zygotic activation could differ between the egg cell and the central cell. This hypothesis is also supported by earlier findings showing that activation of the central cell, but not the egg cell, takes place in the absence of the Polycomb group pathway (Ohad *et al.* 1996; Chaudhury *et al.* 1997; Guitton *et al.* 2004) while autonomous egg cell activation depends on the WD40 protein MSI1 but not on the Polycomb group pathway (Guitton and Berger 2005). What activates the mitotic onset in the egg cell and the central cell remains elusive. Factors involved in plasmogamy such as the GCS1/HAP2 pathway (Besser *et al.* 2006; Mori *et al.* 2006), cytoplasmic activation signals in the sperm cell (Bayer *et al.* 2009) or calcium signalling as shown in the brown alga *Fucus* and maize (Roberts *et al.* 1994; Antoine *et al.* 2000) might be contributing to the central cell mitotic activation.

Conclusions and forward look

Many factors controlling successful double fertilization have been identified in recent years, including important signalling pathways. It is now obvious that communication between the female and male gametophytes is essential. Furthermore, communication within the gametophyte, such as the central cell and the synergid cell, seems critical for double fertilization. Chemical visualization of LUREs will allow an understanding of the spatio-temporal dynamics of pollen tube attraction (Goto *et al.* 2011). Yet, there are still many pieces missing in the signalling pathway jigsaw puzzle. What are the receptors of LUREs and ZmEA1 in the pollen tube for micropylar guidance? What are the ligands of SRN/FER and ANXs, and what is the receptor of ZmES4 for pollen tube perception? How do these pathways coordinate a successful double fertilization? These questions await answers.

Identification of factors associated with fertilization processes and high-resolution live-cell imaging now enable us to investigate other aspects of fertilization such as the interspecies barrier and the polyspermy block. Micropylar guidance attractants such as LUREs and ZmEA1 belong to highly polymorphic families, and indeed LUREs show species preferentiality on pollen tube guidance (Okuda and Higashiyama 2010; Kanaoka *et al.* 2011). In addition, species specificity of GCS1/HAP2 has also been explored (Mori *et al.* 2010; Wong *et al.* 2010), indicating that the interspecies barrier even exists in the last steps of fertilization. Polyspermy block also appears to be present in flowering plants to reinforce successful double fertilization (Scott *et al.* 2008; Spielman and Scott 2008; Ingouff *et al.* 2009; Hamamura *et al.* 2011).

The fact that there is a considerable time gap between sperm cell release and plasmogamy suggests cell–cell communication among gametes, presumably involved in polyspermy block and preparation for plasmogamy. We have just started to lend an ear to the conversation between male and female gametophytes, ‘green love talks’, and, surely, we will soon acquire deeper mechanistic insights into double fertilization.

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Contributions by the authors

Both authors wrote the review.

Conflicts of interest statement

None declared.

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Appendix: Glossary of terms

Central cell

The female gametophytic cell that develops as the endosperm after fertilization. In contrast to the haploid egg cell, the central cell is a homodiploid cell.

Double fertilization

Flowering plants have evolved a unique fertilization process, which involves two sperm cells and two female gametophytic cells, the egg and the central cell. Each sperm cell fertilizes with the egg or the

central cell, giving rise to the embryo and the endosperm, respectively.

Egg cell

The female gametophytic cell that develops as the embryo after fertilization.

Embryo sac

This is the mature female gametophyte. The two terms are somehow synonymous.

Karyogamy

Fusion of the nuclei of the male and female gametes.

Micropyle

The part of the ovule where the pollen tube accesses the embryo sac.

Plasmogamy

The fusion of the plasma membrane between a male and a female gamete.

Polar nuclei

The central cell inherits two haploid nuclei, which localize to the micropylar pole of the cell and are called polar nuclei. The polar nuclei fuse during central cell maturation and the central cell thus become homodiploid.

Pollen tube guidance

In order to deliver immotile sperm cells to the female gametophyte in flowering plants, the pollen tube carrying the sperm cells grows towards the unfertilized female gametophyte. This directional growth of the pollen tube is controlled by the maternal tissue and the female gametophyte.

Polyspermy block

The process that prevents the egg cell or the central cell from being fertilized by more than one sperm cell. Because the pollen tube delivers two sperm cells at the position where the fertilization takes place, it has been suggested that a polyspermy block is required for successful double fertilization.

Synergid cell

The female gametophytic cell that secretes small peptides that attract the pollen tube. In *A. thaliana*, there are two synergid cells positioned on either side of the egg cell at the micropylar end of the female gametophyte.