

Pollen Tube Guidance, a Hard Journey to Complete Fertilization

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Abstract Pollen tubes growth and guidance process is a pivotal step for delivering male gametes to female gametes to complete fertilization in flowering plant. This process is very complicated and needs multiple signals (attractants) to guide pollen tubes to their target, including signals (attractants) coming from sporophyte and female gametophyte (FG) of plant. The latest decade had seen substantial process in this field, which provided the convincing evidences about the important function of FG cells in pollen tube guidance and demonstrated the attractants secreted from synergid cell. In this review, we focus on the gametophyte phase of pollen tube guidance, the function of FG cells in pollen tube guidance and the characteristics and verification of potential attractants.

Key words pollen tube guidance; female gametophyte (FG); attractants; receptor; synergid cell

花粉管导向, 完成植物双受精的艰难旅程

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摘要 在高等植物中, 花粉管生长和导向是将精子细胞传递到植物雌配子体, 完成双受精的关键步骤。这一过程非常复杂, 需要多重复杂信号的参与, 信号分子来自植物的孢子体和雌配子体细胞。过去十年中, 在花粉管导向的研究领域取得了重大突破, 各种证据证实了雌配子体各细胞在花粉管导向中的关键作用, 雌配子体分泌的信号分子也被鉴定出来。在该综述中, 将各配子体细胞在花粉管导向中的功能及信号分子的特征和潜在受体作为讨论的重点。

关键词 花粉管导向; 雌配子体; 诱导分子; 受体; 助细胞

The sperm cells of flowering plants are enwrapped in pollen and nonmotile and its target, embryo sac, containing egg cell and central cell, also deeply buried in ovules, which in turn embedded in the pistil^[1]. The sperm cells overcome these difficulties to reach the target cell and complete double fertilization via pollen tube guidance, a mechanism that delivering two sperm cells to egg cell and central cell of FG

(embryo sac) by its migration within the pollen tube (PT), a polarized cell extension of pollen that navigates through five distinct tissue of pistil^[2]. Pollen tube guidance comprised the first two major steps of double fertilization: (1) attraction and arrest of pollen tube; (2) the release of two male gametes in degenerated synergid cell^[3].

From pollen depositing and germinating on the stigma of pistil, pollen tube grew through several kinds of cells of sporophyte and gametophyte tissue to achieve its goal, including penetration of stigma, migrating the transmitting tract, grown on the inner surface of ovary after exiting the transmitting tract, then grew towards and along the funiculus and turned abruptly into the micropyle of ovules (Fig.1A,B). In

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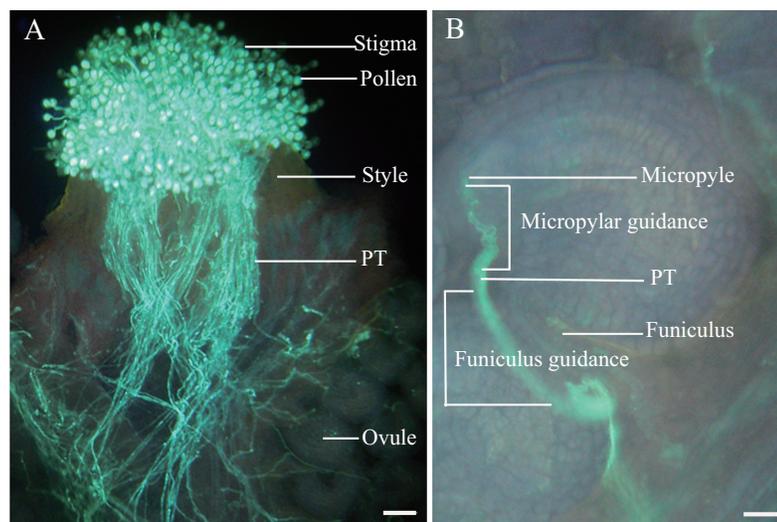
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A: aniline blue staining of pollen tube growth *in vivo*. After pollens depositing and germinating on the stigma of pistil, pollen tube penetrated stigma, migrated in the transmitting tract, grown on the inner surface of ovary, and then grew towards and along the funiculus and turn abruptly into the micropyle of ovules. Bar=100 μm ; B: in *Arabidopsis*, pollen tube guidance by female gametophyte is governed by at least two process, funicular guidance and micropylar guidance. Bar=20 μm .

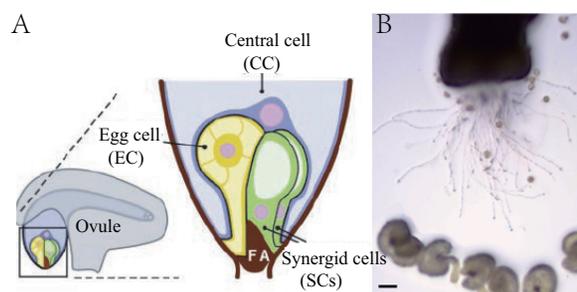
Fig.1 Pollen tube guidance in *Arabidopsis*

embryo sac pollen tube penetrated the synergid cell of embryo sac and ceased to growth; The sperm cells were discharged to complete double fertilization^[2]. In all these process, pollen tubes never lose their way. It's now widely accepted that multiple stage-specific signals mediate pollen tube guidance^[4] and various mechanisms appear to be involved in pollen tube guidance, including chemo attraction, mechanical guidance, growth stimulate, adhesion, in which the chemo attractant responsible for pollen tube guidance is the major player^[5-6].

According to signals or attractants coming from sporophyte tissue or FG, the pollen tube guidance was divided into two phases, the sporophyte phase and the gametophyte phase, and the surface of the placental tissue in the ovary has been suggested to be the transition point of the two phases^[1,7-8] (Fig.1B).

In the latest two decades, different approaches and experimental systems were used to uncover molecules and cells that involved in the signaling of pollen tube guidance, including biochemical protocols^[9], genetics analysis^[8,10-15], transcriptomics analysis^[16-20] and laser ablation^[21]. *Torenia fournieri* naked FG (Fig.2A) and *Arabidopsis* semi *in vivo* experimental system (Fig.2B) also have made a significant contribution

to research breakthrough in this field^[19,21-26]. *Torenia fournieri* has a naked embryo sac that protrudes from the micropyle of the ovule (Fig.2A). This special structure made FG cells operation conveniently and directly in this species^[22]. Semi *in vivo* pollen tube (SIV PT) growth system has been proved to be a very useful protocol to study pollen tube guidance. Using the special structure of *Torenia fournieri* FG, synergid cells were confirmed to be the most important player in pollen tube guidance^[21,23,27]; LURE, the first exact molecule



A: schematic representation of the ovule (left) and the embryo sac (right) of *T. fournieri*. The naked embryo sac contains the egg cell (EC), two synergid cells (SCs), and the central cell (CC). The filiform apparatus (FA) is the structure formed by the thickened cell walls of two synergid cells; B: an *Arabidopsis* semi *in vivo* pollen tube guidance system. Bar=50 μm .

Fig.2 *T. fournieri* naked FG (modified from reference [21]) and *Arabidopsis* semi *in vivo* pollen tube guidance system

attracting pollen tube to synergid cells was also derived from *Torenia fournieri*^[24]. The attractants for micropylar guidance in *Arabidopsis*, *T. concolor* and *Zea mays* also had been identified recently. There are defensin-like CRPs, the AtLURE1 from *Arabidopsis*, TcCRP1 from *T. concolor* and another type of peptide, ZmEA1 from *Z. mays*^[18,26,28].

In this review, we will discuss mainly on the gametophyte phase of pollen tube guidance. The cells participated in the pollen tube guidance and the characteristics and verification of potential attractants and its receptor will be our emphases.

1 Funicular and micropylar guidance

The first step in understanding gametophytic phase PT guidance depends mainly on genetics and phenotypic analysis of several mutants.

Genetics analysis on mutants *short integuments* (*sin1*), *47H4*, *54D12*, *TI-1* indicate that the guidance by the FG is necessary for pollen tube growth towards the funiculus of ovules in *Arabidopsis*^[7-8]. In *short integuments* (*sin1*) mutants, both the sporophytic tissue and the embryo sac are affected, no embryo sac is generated. In *47H4* and *54D12*, the sporophytic tissue of the ovule and pistils is apparently normal. While no embryo sac develops in *47H4* mutants and the extent of ovule development varies in *54D12* mutants, in that either no embryo sacs or a grossly aberrant or intact embryo sac is produced. The pollen tube growth path was analyzed in all four mutants. The sporophytic growth path is normal, but pollen tubes lose their strong preference of emergence point on the surface of septum and instead of being directed toward a funiculus and the micropyle of an ovule, pollen tubes grow randomly using all available surfaces, including the ovary wall. In *54D12* with variable phenotype of embryo sacs, 92% of the ovules with the wild-type phenotype received a pollen tube. The results provide unique *in vivo* evidence for an ovule-derived, long-range activity controlling pollen tube guidance. A morphological comparison of the ovule mutants used in this study indicates that within the ovule, the haploid embryo sac

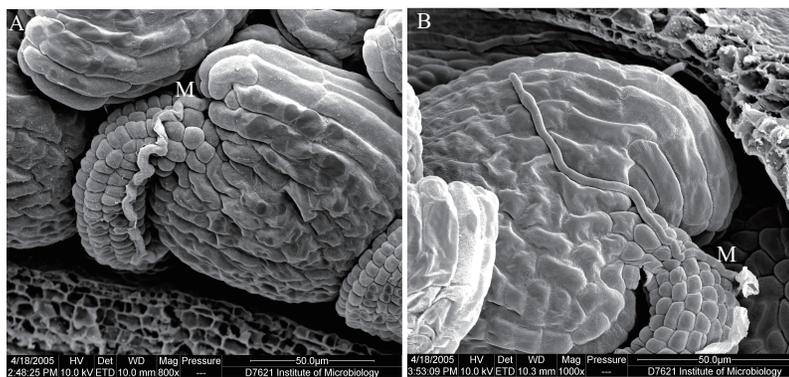
plays an important role in this long-range signaling process.

However, studies on these four mutants were not possible to unambiguously identify the signaling cells within an ovule: the haploid FG or the diploid sporophytic cells. Ray and colleague used a reciprocal chromosomal translocation mutant *TI-1* to distinguish genetically between these two possibilities^[8]. In *TI-1* of *Arabidopsis*, approximately half the ovules do not contain a functional FG but all ovules contain genotypically normal sporophytic cells. Pollen tubes are guided to the normal but not to the abnormal FGs. These results strongly suggest that the FG is responsible for pollen tube guidance.

Further study showed that guidance by the FG can be genetically divided into two steps: from the surface of the septum to the funiculus, and from the entrance of the micropyle to the embryo sac (Fig.1B; Fig.3A). In *magatama* (*maa*) mutant^[13], female gametophyte development was delayed and two polar nuclei of the central cell were not fused at stage of FG7. Pollen tube guidance is aberrant in two aspects, one is that the *maa* mutant FGs attracts more than one pollen tube; another is that pollen tubes grew on the funiculus but lost their way at the entrance to the micropyle (Fig.3B).

The results proposed that FG provides two different guidance signals, i.e., a funiculus guidance and micropyle guidance signal^[13]. The funiculus guidance signal would guide a PT from the transmitting tissue onto the surface of a funiculus. When the PT reaches the distal end of the funiculus, another guidance signal, the micropyle guidance signal, would lead the PT to the micropyle. In *maa* mutants, it seems that the funiculus guidance phase was normal, but micropyle guidance phase was absent.

Funiculus guidance and micropyle guidance signal are all most likely a gradient of guiding molecule(s) made by a diffusible chemoattractant. *In vitro* studies using naked FGs of *Torenia* suggest that the PT guidance is also mediated by diffusible chemotropic molecule^[22]. In *Torenia*, the embryo sac is naked (without integument enwrapped) and protruding, pol-



A: scanning electron micrograph of wild-type ovule showing that pollen tube grows along the funiculus and then turns abruptly to enter the micropyle; B: mutant ovule is defective in micropylar pollen guidance. Pollen tube can reach the vicinity of funiculus, but fails to find the micropylar opening of the ovule. A pollen tube bypasses the micropyle and grows on the surface of the integument. M: micropyle. Bar=50 μ m.

Fig.3 Micropylar guidance is impaired in *csg* mutant ovules (modified from reference [36])

len tubes precisely control their direction of growth in the medium and need not contact any cells of FG, they are directly attracted to the micropylar end and never leave. Such behavior of pollen tubes suggests that some diffusible attractant is derived from the micropylar end of the embryo sac.

2 FG cells and other cells function in micropylar PT guidance

Until now, the mechanism of funicular guidance is unknown: some attractant might be emitted directly from the developing and mature FG, or some signal from the FG may evoke attraction in ovular sporophytic cells indirectly. On the contrary, study on micropylar guidance is flourishing in the latest decades.

2.1 Roles of synergid cell in pollen tube guidance

Evidences from every aspects strongly suggest that micropylar guidance is governed by the synergid cell.

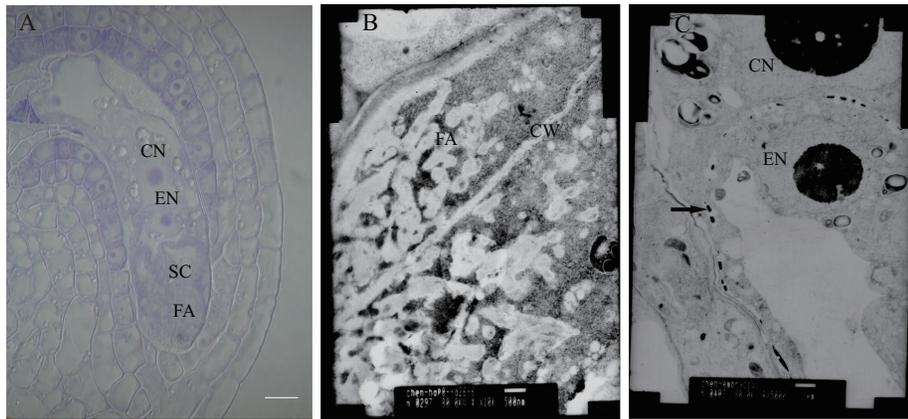
2.1.1 Synergid localization and special cell structure

Firstly, the position of synergid cells determines its importance in pollen tube guidance. The synergid cells are located at the chalazal pole of embryo sac and lie adjacent to the egg cell (Fig.4A,B); it's the first cell contacting with pollen tube during fertilization. Secondly, the mature synergid cells have several specialized structure, which favor its function in pollen tube guidance and reception. (1) Synergid cells are polarized; their nuclei and most of their cytoplasm

are located at the micropylar pole and a single, large vacuole is located at the chalazal pole (Fig.4A,B); (2) The synergid cells have an unusual and specialized cell wall. At the chalazal pole, adjacent to the egg cell and central cell, the cell wall is absent or discontinuous (Fig.4C) in most species including *Arabidopsis*^[23,29]. The partial cell wall may facilitate communication and/or nutrient transfer between these cells; (3) The most importance is filiform apparatus, a thickened cell wall structure at the micropylar pole of synergid cell, which includes numerous finger-like projections extending deeply into the synergid cytoplasm (Fig.4A,B). The filiform apparatus greatly increases the surface area of the plasma membrane at the micropylar end of the synergid cell and the cytoplasm near the filiform apparatus contains a high concentration of secretory organelles (endoplasmic reticulum, Golgi stacks, and vesicles). Based on these observations, it has been proposed that the filiform apparatus facilitates transport of substances into and out of the synergid cells. For example, the possibility of export of the pollen tube attractant(s). In most species, the pollen tube enters the synergid cell by growing through the filiform apparatus, suggesting that the filiform apparatus is important for pollen tube reception. For example, the filiform apparatus may play a role in directing pollen tube growth to the synergid cells^[1,30].

2.1.2 Cell ablation experiment

In the *in vitro Torenia* system, laser cell ablation indicated that the syn-



A: historesin section of *Arabidopsis* ovule. Bar=20 μ m; B: transmission electron microscopy images of filiform apparatus from wild-type synergid cells. Bar=0.5 μ m; C: transmission electron microscopy images of connection region between egg cell, central cell and synergid cell. Arrow shows the special structure between FG cells. Bar=1 μ m. CN: central cell nuclear; EN: egg cell nuclear; SC: synergid cell; FA: filiform apparatus; CW: cell wall between the synergid cells.

Fig.4 Special structure of synergid cell and the connection between FG cells

ergid cells are source of pollen tube attractants^[21]. As we discussed above, some diffusible attractants is derived from the micropylar end of the embryo sac. Four gametophytic cells are close to the micropylar end of the embryo sac: the egg cell, two synergid cells and the central cell. These cells generally form a female germ unit, which is defined as the minimum number of cells required to accept the contents of the pollen tube and to accomplish double fertilization. To investigate the contribution of each gametophytic cell to pollen tube attraction, laser ablation was performed. When the egg cell or the central cell was ablated, attraction was also not affected; When both the egg cell and the central cell were ablated, the attraction frequency did not change; When one synergid cell was ablated, the attraction frequency decreased 30%, and combinations that included the ablation of one synergid cell and egg/central cell decreased the frequency; When two synergid cells were ablated, no embryo sac attracted a pollen tube^[21]. These results of laser ablation indicate that one synergid cell is necessary and sufficient to attract the pollen tube.

2.1.3 Genes function in synergid were required for micropylar pollen tube guidance Genetic studies have revealed several synergid-expressed genes controlling the micropylar guidance of the synergid cells. MYB98, a member of MYB family proteins, is required for the

pollen tube guidance and synergid cell differentiation in *Arabidopsis*^[23]. In the embryo sac, the putative transcription factor gene is exclusively expressed in the synergid cells. Mutation of *MYB98* leads to defects in filiform apparatus formation and micropylar pollen tube guidance, in ovules containing *myb98* FGs, wild-type pollen tubes grow onto the funiculus but fail to enter the micropyle^[23]. The defect in pollen tube guidance may result from two possibilities: secretion of the pollen tube attractant(s) was reduced due to the filiform apparatus defect, or alternatively, MYB98 could be required for synthesis of the pollen tube attractant(s).

Transcriptome analysis and reverse genetics revealed a small (94 amino acids) transmembrane protein, *Z. mays* EGG APPARATUS 1 (*ZmEA1*). *ZmEA1* is expressed in the synergid cells and the egg cell. Knockdown of *EAI* using RNA interference technology in transgenic plants leads to female sterility caused by loss of pollen attraction signal to the micropyle. A fusion protein is initially localized to the filiform apparatus and later is present in the cell wall of the surrounding nucellus. These results suggest that *ZmEA1* is released from the plasma membrane and then diffuses to the surrounding cells, consistent with a role in pollen tube guidance^[18]. However, it is still unknown if purified *ZmEA1* has the ability to attract pollen tubes.

Okuda and his colleague have discovered two

exact attractants, LURE1 and LURE2 from synergid cells of *Torenia fournieri*^[24]. Transcripts encoding for CRPs (cysteine-rich small proteins with a putative signal peptide) were most abundant in the synergid cells of *Torenia fournieri*. TfCRP1 (LURE1) and TfCRP3 (LURE2) are the two largest CRP contigs. Purified LURE1 and LURE2 have ability *in vitro* to attract pollen tube of their own species. When the production of these proteins was blocked in the embryo sacs, the pollen tubes attraction was significantly impaired.

2.1.4 Synergid cells in preventing polysperm After fertilization in *T. fournieri*, pollen tube attraction ceases, despite the persistence of one synergid cell^[21]. Some active mechanism must halt the attraction, such as the attractant being no longer being secreted, degradation of the attractant, and/or repulsion of the additional pollen tubes. In *Arabidopsis*, live imaging of wild-type ovules suggested that blocking the penetration of supernumerary pollen tubes occurs just after the first pollen tube enters the micropyle^[15,31]. The mechanism of blocking is impaired in *sirene* (*srn*)^[15] and *feronia* (*fer*)^[14] mutants, which are defective in the same receptor-like kinase gene expressed in the synergid cell^[32]. Phenotype of mutants *sirene* and *feronia* indicate that the embryo sac also controls the arrest of PT growth and PT rupture, which suggest that the attraction signal is normally rapidly lost or degraded after fertilization. The phenotype of two other *Arabidopsis* mutants, *abstinence by mutual consent* (*amc*)^[33] and *lorelei*^[34] is reminiscent of the *feronia*/*sirène* mutation. Mutants display an impairment of fertilization caused by an inability of the pollen tube to release the sperm cells upon arrival at the embryo sac. The pollen tube subsequently experiences a continuous growth, resulting in an invasion of the embryo sac. However, *amc* mutant differs from *feronia* and *lorelei* in that the defect appears only when a pollen tube from an *amc* pollen grain encounters an *amc* embryo sac, and mutant embryo sacs are fully competent to receive wild-type pollen. The *AMC* gene encodes a peroxin and may be involved in the processing of a ligand or some other signaling steps in the recognition pathway for

both the pollen and the embryo sac^[33]. The *LORELEI* gene encodes a putative plant-conserved glucosylphosphatidylinositol (GPI)-anchored protein (GAP) and is expressed in the synergid cells of the embryo sac^[34]. Analysis on these three mutants showed the signaling pathway in pollen tube reception and pollen tube discharge is very complicated.

2.2 Roles of egg and central cells in pollen tube guidance

Although evidences we list above support that synergid cells play a major role in attracting the pollen tubes and pollen reception, molecular genetic analyses have shown that the egg and central cells are also involved in guiding the pollen tube^[35]. The *central cell guidance* (*cgc*) mutant identified in *Arabidopsis* from a transferred DNA insertion population showed 44% ovule abortion, suggesting a female gametophytic defect^[36]. Detailed morphological analysis shows that the *cgc* mutant ovule is defective in micropylar pollen guidance. Pollen tubes can reach the vicinity of funiculus, but fail to find the micropylar opening of the ovule (Fig.3B). *CCG* encodes a nuclear protein with an N-terminal conserved zinc-ribbon domain that is functionally interchangeable with that of TFIIB (transcription factor IIB) in yeast. This suggests that *CCG* might act as a transcription regulator for pollen tube guidance. In the FG, *CCG* is exclusively expressed in the central cell. Expression of *CCG* under the control of central cell-specific *FIS2* promoter of the *cgc* plant is sufficient to rescue the mutant phenotype^[36].

The central cell may either control pollen tube guidance directly or via the synergid and/or the egg cell. Although morphological evidence support the first possibility (the micropylar portion of the central cell surrounding the synergid and the egg cell is in close proximity to the micropyle, such that signals from the central cell could diffuse readily to the micropylar opening), we prefer that the central cell exerts its role by modulating synergid function. The junction region of the egg cell, central cell and synergid cell, the cell wall is absent or discontinuous, and the plasma membranes of these cells are in direct contact with one an-

other (Fig.4C). This eliminates the cell wall barrier that obstructing signal transduction^[30,37]. Cell-cell communication between the egg apparatus and the central cell can be achieved preferentially by plasmadesmata^[38], by which CCG in the central cell indirectly controls the expression of attractants in the synergid cell, so central cell is required for full function of the synergid cell.

It is interesting to know what kind of downstream signals are induced by CCG to attract the pollen tube to the embryo sac. Consistent with CCG, several evidences imply that other gametophytic cells, in addition to the synergid cells, may play a role in pollen tube guidance. Stronger evidence is also found in the *Arabidopsis* mutant, *magatama*, which is also defective in micropylar pollen tube guidance. This mutation affects the polar nuclei causing a fault that prevents their fusion, suggesting that a functional central cell is essential for pollen tube guidance^[13]. In maize, for example, the *ZmEAL* gene is implicated in the attraction of pollen tubes to the embryo sac, and although this gene is expressed in the synergids^[18], it is also expressed in the egg cell where it was initially isolated^[39]. Therefore, a role for this gene in pollen tube guidance resulting from its expression in the egg cell cannot be excluded. A newly reported gene *GEX3* encodes a plasma membrane-localized protein and is expressed in both the vegetative and sperm cells of the male gametophyte and in the egg cell of the FG. Transgenic lines in which *GEX3* was down-regulated or overexpressed had reduced seed set. Both in the antisense and overexpression lines, micropylar pollen tube guidance failed, which block the fertilization of mutant ovules^[40].

These data cannot exclude the possibility that cells of the FG, in addition to the synergids, may play a critical role in micropylar pollen tube guidance in *Arabidopsis*.

2.3 Roles of sperm cells in pollen tube guidance

Several mutants were reported with defects in sperm cell development and function. In *Arabidopsis*, the *cdkal* mutant, defective in one of the cyclin-dependent kinases (CDKs), produces only one sperm cell (generative-like cell)^[41]. The mutation of DUO POLLEN1(DUO1) results in a single mutant germ

cell that is unable to undergo fertilization^[42]. In lily, the *generative cell specific1 (gcs1)* mutant, defective in a plasma membrane protein of the sperm cell required for gamete fusion was reported^[43]. In all these mutants, the defect in pollen tube guidance has not been reported. In contrast, the *Arabidopsis hapless 2 (hap2)* mutants, alleles of GCS1, showed impaired pollen tube guidance; in mutant pollen tubes, attraction to the FG decreased by about 50%, and abnormal growth path and growth arrest were observed in the ovary^[44]. The findings in *hap2* suggest that sperm, long considered to be a passive cargo, is involved in directing the pollen tube to its target^[44].

3 Characteristics of the pollen tube attractant(s) from synergid

For more than a century, plant biologists have tried to identify the pollen tube attractants. Studies of many years have revealed the nature of attractants from *Torenia fournieri*, *Z. mays*, *T. concolor* and *Arabidopsis*, and also several characteristics true attractants should possess^[18,24,26,28].

3.1 True attractant is diffusible molecule and can form a concentration gradient in a given distance

The pollen tubes guidance and the guidance of axons to neural synapses are classic examples of targeted cell growth. Despite the evolutionary time that separates animals and plants, axon and pollen tube guidance share remarkable mechanistic similarities^[45]. In both cases, the growing tips are influenced by long- and short-range guidance cues that are presented by the target cell or by cells in the path leading to the target. Netrins are diffusible attractive cues for axon growth that meet following criteria: (1) form a concentration gradient in a direction that determines the orientation of growth; (2) elicit a specific response; (3) remain stable over a defined period of time; (4) vary in effectiveness with distance to the target^[45]. So attractants for pollen tube guidance in plant should have similar traits.

3.2 True attractant is species specific and evolved rapidly

First evidence comes from *in vivo* crossing^[13],

when pollen grains of brassicaceous species were crossed to *Arabidopsis* pistils, and only a fraction of the PT emerged from the transmitting tissue but failed to arrive at the FG. This suggests that the micropyle guidance signal did not attract PTs of the different species.

The *in vitro* *Torenia* system is suitable for analyzing the species difference of the attractant because the attractant from the synergid cell spreads directly through the medium, and thus the possibility of sporophytic cells contributing along the path can be excluded. Plant species having a protruding embryo sac and suitable for cultivation, such as the *in vitro* *T. fournieri* system, were surveyed^[46]. When *T. fournieri* ovules were mixed with those of another species, the pollen tubes preferentially grew toward the embryo sac of their own species and interference between their respective attraction signals was not observed. These results suggest that the attraction is species-specific, and that each species uses a different molecule(s)^[46]. Such species preferentiality likely contributes to the reproductive barrier during *in vivo* crossing^[46].

In *Arabidopsis*, stigma, style and ovule-derived signaling were separately analyzed in semi *in vivo* system. Crossing between *A. thaliana* and its relatives separated by ~5, 10, or 20 MY (million years) revealed that the ovule-derived attractant diverged and evolved rapidly, and the frequencies of targeting the ovules of *Arabidopsis* consistently decrease as the target plant species diverges^[31].

One exception is that pollen tube of *M. hybridas* can sense the attractant of *T. fournieri* and arrive at the embryo sac of *T. fournieri*^[47].

3.3 True attractant is developmentally regulated

Short-range guidance signals from *A. thaliana* ovules are developmentally regulated. Previously it was shown that pollen tubes grow randomly or failed to elongate in immature *Arabidopsis* pistils^[48]; *Maa* FGs with delayed developmental embryo sac lose the micropylar guidance activity^[13]. These studies suggested that the production of attractants is related to developmental stage of FG. In *Arabidopsis*, experiment in semi *in vivo*

system showed that guidance factors of embryo sac are completely absent at ~32 hrs (stage 12a) and lower at ~16-24 hrs (stages 12b-c) before flowers mature^[31].

3.4 True attractants might be functional redundant

Until now, mutant with specifically defective in synthesis of a single attractant has not been reported, implying the possibility that substances might be redundant^[5]. Attractant is encoded by gene families which consist of many highly homologous genes.

17 synergid specific genes were identified by genechip analysis using *determinant infertile1 (dif1)* ovules^[20]. In 17 genes, 16 genes were downstream regulated in *myb98* mutant, which suggested that MYB98 is required for the expression of these 16 genes during synergid cell development or attractants secretion^[19]. In *T-DNA* lines of 16 genes, FG is not affected, mutant plants exhibited full seed set. The absence of mutant phenotypes for these *T-DNA* lines is likely due to functional redundancy, as all are members of large gene families^[19]. This phenomenon is consistent with the functional redundancy characteristics of true attractants. RNAi technique using consensus sequence would knock down a set of these functional redundancy genes and reveal pollen tube guidance defective phenotype controlled by these genes, which may help to shed light on the discovery of the nature of attractants^[49].

The attractants from three species were newly discovered. There are defensin-like CRPs, the AtLURE1 from *Arabidopsis*, LUREs from *T. fournieri* and TcCRP1 from *T. concolor*. All three attractants possess four characteristics we described above.

4 Potential receptors for FG secreted attractants

How does the pollen tubes receive the attraction signals from the ovule and precisely respond to them? Recent researches showed that receptor-like kinases orchestrate love songs in plants^[50]. Many RLKs are involved in pollen-pistil interactions and pollen tube discharge of plants, and some of them interact with CRP ligands. For example, SRK for self-incompatibility

of Brassicaceae, LePRK2 for pollen germination and growth of *Solanum lycopersicum* and ANXUR1 and 2, FERONIA for pollen tube discharge^[32,51-53]. Two cysteine-rich extracellular proteins, LeSTIG1 and LAT52, interact with the extracellular domain of the pollen receptor kinase LePRK2^[54-55], but the ligands for ANXUR1, ANXUR 2 and FERONIA have not been found. To our knowledge, receptors for micropylar chemoattractants secreted from pollen tubes have not been reported in flowering plants until now. Semi *in vivo* pollen tube (SIV PT) growth system has been proved to be a very valuable protocol to study pollen tube guidance^[26,56], and comparative transcriptome using this system demonstrated the potential role of many receptors (RLK, LRR-RLK and TIR-NBS-LRR) in pollen tube guidance (unpublished data). Further reverse genetics analysis will uncover their function in pollen tube guidance.

5 Conclusion and prospect

During the past decade, great progress has been made in micropylar pollen tube guidance. Several mechanisms involved in pollen tube guidance had been revealed and some candidate molecules had been identified. Scientists also determined the key FG cells functionally in this process and deduced four characteristics true attractants should possess. With the verification of the three kinds of attractants from *T. fournieri*, *Arabidopsis* and *T. concolor*, it's urgent to identify their partner molecules, including the receptor of LURE, which is important for understanding the mechanism of speciation and plant reproduction.

References

- Higashiyama T, Kuroiwa H, Kuroiwa T. Pollen-tube guidance: Beacons from the female gametophyte. *Curr Opin Plant Biol* 2003; 6(1): 36-41.
- Johnson MA, Preuss D. Plotting a course: Multiple signals guide pollen tubes to their targets. *Dev Cell* 2002; 2(3): 273-81.
- Berger F. Double-fertilization, from myths to reality. *Sex Plant Reprod* 2008; 21(1): 3-5.
- Johnson MA, Lord E. Extracellular guidance cues and intracellular signaling pathways that direct pollen tube growth. *Plant Cell Monographs* 2006; 3: 223-42.
- Higashiyama T, Hamamura Y. Gametophytic pollen tube guidance. *Sex Plant Reprod* 2008; 21(1): 17-26.
- Dresselhaus T. Cell-cell communication during double fertilization. *Curr Opin Plant Biol* 2006; 9(1): 41-7.
- Hulskamp M, Schneitz K, Pruitt RE. Genetic evidence for a long-range activity that directs pollen tube guidance in *Arabidopsis*. *Plant Cell* 1995; 7(1): 57-64.
- Ray S, Park SS, Ray A. Pollen tube guidance by the female gametophyte. *Development* 1997; 124(12): 2489-98.
- Zinkl GM, Zwiebel BI, Grier DG, Preuss D. Pollen-stigma adhesion in *Arabidopsis*: A species-specific interaction mediated by lipophilic molecules in the pollen exine. *Development* 1999; 126(23): 5431-40.
- Drews GN, Yadegari R. Development and function of the angiosperm female gametophyte. *Annu Rev Genet* 2002; 36(1): 99-124.
- Christensen CA, Gorsich SW, Brown RH, Jones LG, Brown J, Shaw JM, *et al.* Mitochondrial GFA2 is required for synergid cell death in *Arabidopsis*. *Plant Cell* 2002; 14(9): 2215-32.
- Pagnussat GC, Yu HJ, Ngo QA, Rajani S, Mayalagu S, Johnson CS, *et al.* Genetic and molecular identification of genes required for female gametophyte development and function in *Arabidopsis*. *Development* 2005; 132(3): 603-14.
- Shimizu KK, Okada K. Attractive and repulsive interactions between female and male gametophytes in *Arabidopsis* pollen tube guidance. *Development* 2000; 127(20): 4511-8.
- Huck N, Moore JM, Federer M, Grossniklaus U. The *Arabidopsis* mutant *feronia* disrupts the female gametophytic control of pollen tube reception. *Development* 2003; 130(10): 2149-59.
- Rotman N, Rozier F, Boavida L, Dumas C, Berger F, Faure JE. Female control of male gamete delivery during fertilization in *Arabidopsis thaliana*. *Cur Biol* 2003; 13(5): 432-6.
- Pina C, Pinto F, Feijó JA, Becker JD. Gene family analysis of the *Arabidopsis* pollen transcriptome reveals biological implications for cell growth, division control, and gene expression regulation. *Plant Physiol* 2005; 138(2): 744-56.
- Sprunck S, Baumann U, Edwards K, Langridge P, Dresselhaus T. The transcript composition of egg cells changes significantly following fertilization in wheat (*Triticum aestivum* L.). *Plant J* 2005; 41(5): 660-72.
- Márton ML, Cordts S, Broadhvest J, Dresselhaus T. Micropylar pollen tube guidance by egg apparatus 1 of maize. *Science* 2005; 307(5709): 573-6.
- Punwani JA, Rabiger DS, Drews GN. MYB98 positively regulates a battery of synergid-expressed genes encoding filiform apparatus-localized proteins. *Plant Cell* 2007; 19(8): 2557-68.
- Steffen JG, Kang IH, Macfarlane J, Drews GN. Identification of genes expressed in the *Arabidopsis* female gametophyte. *Plant J* 2007; 51(2): 281-92.
- Higashiyama T, Yabe S, Sasaki N, Nishimura Y, Miyagishima SY, Kuroiwa H, *et al.* Pollen tube attraction by the synergid cell. *Science* 2001; 293(5534): 1480-3.
- Higashiyama T, Kuroiwa H, Kawano S, Kuroiwa T. Guidance *in vitro* of the pollen tube to the naked embryo sac of *Torenia fournieri*. *Plant Cell* 1998; 10 (12): 2019-31.
- Kasahara RD, Portereiko MF, Sandaklie-Nikolova L, Rabiger DS, Drews GN. MYB98 is required for pollen tube guidance and synergid cell differentiation in *Arabidopsis*. *Plant Cell* 2005; 17(11): 2981-92.

- 24 Okuda S, Tsutsui H, Shiina K, Sprunck S, Takeuchi H, Yui R, *et al.* Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. *Nature* 2009; 458(7236): 357-61.
- 25 Qin Y, Leydon AR, Manziello A, Pandey R, Mount D, Denic S, *et al.* Penetration of the stigma and style elicits a novel transcriptome in pollen tubes, pointing to genes critical for growth in a pistil. *PLoS Genet* 2009; 5(8): e1000621.
- 26 Takeuchi H, Higashiyama T. A species-specific cluster of defensin-like genes encodes diffusible pollen tube attractants in *Arabidopsis*. *PLoS Biol* 2012; 10(12): e1001449.
- 27 Punwani JA, Drews GN. Development and function of the synergid cell. *Sex Plant Reprod* 2008; 21(1): 7-15.
- 28 Kanaoka MM, Kawano N, Matsubara Y, Susaki D, Okuda S, Sasaki N, *et al.* Identification and characterization of TcCRP1, a pollen tube attractant from *Torenia concolor*. *Ann Bot* 2011; 108(4): 739-47.
- 29 Mansfield S, Briarty L, Erni S. Early embryogenesis in *Arabidopsis thaliana*. I. The mature embryo sac. *Can J Bot* 1991; 69(3): 447-60.
- 30 Huang BQ, Russell SD. Female germ unit: Organization, isolation, and function. *Int Rev Cytol* 1992: 233-8.
- 31 Palanivelu R, Preuss D. Distinct short-range ovule signals attract or repel *Arabidopsis thaliana* pollen tubes *in vitro*. *BMC Plant Biol* 2006; 6(1): 7.
- 32 Escobar-Restrepo JM, Huck N, Kessler S, Gagliardini V, Gheyselinck J, Yang WC, *et al.* The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception. *Science* 2007; 317(5838): 656-60.
- 33 Boisson-Dernier A, Frietsch S, Kim TH, Dizon MB, Schroeder JI. The peroxin loss-of-function mutation abstinence by mutual consent disrupts male-female gametophyte recognition. *Curr Biol* 2008; 18(1): 63-8.
- 34 Capron A, Gourgues M, Neiva LS, Faure JE, Berger F, Pagnussat G, *et al.* Maternal control of male-gamete delivery in *Arabidopsis* involves a putative GPI-anchored protein encoded by the *LORE-LEI* gene. *Plant Cell* 2008; 20(11): 3038-49.
- 35 Fan YF, Jiang L, Gong HQ, Liu CM. Sexual reproduction in higher plants I: Fertilization and the initiation of zygotic program. *J Integr Plant Biol* 2008; 50(7): 860-7.
- 36 Chen YH, Li HJ, Shi DQ, Yuan L, Liu J, Sreenivasan R, *et al.* The central cell plays a critical role in pollen tube guidance in *Arabidopsis*. *Plant Cell* 2007; 19(11): 3563-77.
- 37 Christensen CA, King EJ, Jordan JR, Drews G. Megagametogenesis in *Arabidopsis* wild type and the Gf mutant. *Sex Plant Reprod* 1997; 10(1): 49-64.
- 38 Han YZ, Huang BQ, Zee SY, Yuan M. Symplastic communication between the central cell and the egg apparatus cells in the embryo sac of *Torenia fournieri* Lind. before and during fertilization. *Planta* 2000; 211(1): 158-62.
- 39 Dresselhaus T, Lörz H, Kranz E. Representative cDNA libraries from few plant cells. *Plant J* 1994; 5(4): 605-10.
- 40 Alandete-Saez M, Ron M, McCormick S. GEX3, expressed in the male gametophyte and in the egg cell of *Arabidopsis thaliana*, is essential for micropylar pollen tube guidance and plays a role during early embryogenesis. *Mol Plant* 2008; 1(4): 586-98.
- 41 Iwakawa H, Shinmyo A, Sekine M. *Arabidopsis CDKA1;1*, a *cdc2* homologue, controls proliferation of generative cells in male gametogenesis. *Plant J* 2006; 45(5): 819-31.
- 42 Rotman N, Durbarry A, Wardle A, Yang WC, Chaboud A, Faure JE, *et al.* A novel class of MYB factors controls sperm-cell formation in plants. *Curr Biol* 2005; 15(3): 244-8.
- 43 Mori T, Kuroiwa H, Higashiyama T, Kuroiwa T. GENERATIVE CELL SPECIFIC 1 is essential for angiosperm fertilization. *Nat Cell Biol* 2006; 8(1): 64-71.
- 44 von Besser K, Frank AC, Johnson MA, Preuss D. *Arabidopsis* HAP2 (GCS1) is a sperm-specific gene required for pollen tube guidance and fertilization. *Development* 2006; 133(23): 4761-9.
- 45 Palanivelu R, Preuss D. Pollen tube targeting and axon guidance: Parallels in tip growth mechanisms. *Trends Cell Biol* 2000; 10(12): 517-24.
- 46 Higashiyama T, Inatsugi R, Sakamoto S, Sasaki N, Mori T, Kuroiwa H, *et al.* Species preferentiality of the pollen tube attractant derived from the synergid cell of *Torenia fournieri*. *Plant Physiol* 2006; 142(2): 481-91.
- 47 Kikuchi S, Kino H, Tanaka H, Tsujimoto H. Pollen tube growth in cross combinations between *Torenia fournieri* and fourteen related species. *Breeding Sci* 2007; 57(2): 117-22.
- 48 Kandasamy MK, Nasrallah JB, Nasrallah ME. Pollen-pistil interactions and developmental regulation of pollen tube growth in *Arabidopsis*. *Development* 1994; 120(12): 3405-18.
- 49 Lu Y, Last RL. Web-based *Arabidopsis* functional and structural genomics resources. *Arabidopsis Book* 2008; 6: e0118.
- 50 Berger F. Reproductive biology: Receptor-like kinases orchestrate love songs in plants. *Curr Biol* 2009; 19(15): R647-R9.
- 51 Boisson-Dernier A, Roy S, Kritsas K, Grobei MA, Jaciubek M, Schroeder JI, *et al.* Disruption of the pollen-expressed FERONIA homologs ANXUR1 and ANXUR2 triggers pollen tube discharge. *Development* 2009; 136(19): 3279-88.
- 52 Higashiyama T. Peptide signaling in pollen-pistil interactions. *Plant Cell Physiol* 2010; 51(2): 177-89.
- 53 Miyazaki S, Murata T, Sakurai-Ozato N, Kubo M, Demura T, Fukuda H, *et al.* *ANXUR1* and 2, sister genes to *FERONIA/SIRENE*, are male factors for coordinated fertilization. *Curr Biol* 2009; 19(15): 1327-31.
- 54 Tang W, Kelley D, Ezcurra I, Cotter R, McCormick S. LeSTIG1, an extracellular binding partner for the pollen receptor kinases LePRK1 and LePRK2, promotes pollen tube growth *in vitro*. *Plant J* 2004; 39(3): 343-53.
- 55 Tang W, Ezcurra I, Muschietti J, McCormick S. A cysteine-rich extracellular protein, LAT52, interacts with the extracellular domain of the pollen receptor kinase LePRK2. *Plant Cell* 2002; 14(9): 2277-87.
- 56 Márton Mihaela L, Fastner A, Uebler S, Dresselhaus T. Overcoming hybridization barriers by the decretion of the maize pollen tube attractant ZmEA1 from *Arabidopsis* ovules. *Curr Biol* 2012; 22(13): 1194-8.