Title: There are secrets along the pollen tube pathway, still in need to be discovered

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Abstract

Plant organogenesis is the charming process that embodies outstanding structures of refined control of gene expression. During this elegant process, a subtle communication occurs between neighboring cells, based on chemical signals, inducing cellular mechanisms of patterning and growth.

The path to be followed starts once the stigmatic cells recognize a compatible pollen grain, facing immediately self-incompatibility responses, and follows dealing with distinct players interacting on pollen tube growth and puzzling navigation along the transmitting tract. Pollen tube goes through a guidance process that starts at a preovular stage, with interacting players from the transmitting tissue. The ovular guidance stage considers the specific relation between the pollen tube and its ovule, divided in funicular and micropylar guidance steps, with plenty of receptors working in signalling cascades. Lately, just after the pollen tube passage beyond synergids, gametes fusion occurs and the aimed target, the developing seed, will start to mature.

The present review presents the existing knowledge of interactions and partners involved in the crucial biological process of pollen-pistil interaction giving rise to the new seed generation, the one aimed treasure.

The long lasting *abominable mystery*

When Charles Darwin found the puzzling phenomenon of angiosperms fossil record, an *abominable mystery* has been settled to be solved: how did the angiosperm dominance emerged and how flowers, their reproductive units, help them to thrive and evolve (Crepet, 2000)? And now, this long lasting mystery still needs to be solved. All the already gathered information about angiosperms life cycle, helped us to better understand this phenomenon, but every day, new players are being found, interacting during sexual plant reproduction process, to integrate the overall network of the male female crosstalk, that will lead to the generation of new seeds - the final aimed treasure.

This is, thus, a proposal to settle the overall map of this aimed treasure hunt, gather the known clues and trace the needed paths to reach this untangled mystery.

Remembering the basal, we need to retrace the scenario: angiosperms change from a sporophytic diploid generation to a gametophytic haploid generation. Inside the flowers, stamens and ovules generate two different types of spores, by meiotic divisions, the microspores and the megaspores, respectively. After mitotic divisions, these spores produce their gametophytes that will produce the gametes. The double fertilization event occurs when the two identical male gametes fuse with the two female gametes, oosphere and central cell, establishing a new diploid sporophytic generation and completing the plant's life cycle (Yadegari and Drews, 2004).

Arabidopsis thaliana is the important model plant, the well-known scenario to input all the needed quests, because it is small, with a good sequenced

genome and a rapid life cycle (Meinke, 1994). Inside *A. thaliana* carpel, ovules are enclosed within the ovary and in each ovule, there is a single megasporocyte that undergoes meiosis and produces four haploid megaspores, surviving only the functional megaspore (FM), after the others dyed by programmed cell death (PCD). After three successive mitotic divisions, the FM at the chalaza, forms a female polygonum gametophyte with seven cells and eight nuclei – the embryo sac (Yadegari and Drews, 2004; Palanivelu and Tsukamoto, 2012). At the micropylar pole near the opening of the two integuments, two synergids with their filiform apparatus, resulting from the complex invagination of the synergids cell wall, are present surrounding the egg cell. At the opposite pole, the chalazal pole, there are three antipodal cells, and the central zone is occupied by the central cell with two polar nuclei, that fuse before the arrival of the pollen tube (PT). Over 70% of the flowering plants share this type of female gametophyte (Maheshwari and Johri, 1950; Yadegari and Drews, 2004; Dresselhaus, 2006).

Inside the anther the male gametophytes are formed, also known as pollen grains. The mature pollen grain has, at the beginning, two haploid cells: a vegetative and a generative cell. The generative cell undergoes mitosis originating the two male gametes. In most plants, this mitosis occurs during the pollen tube growth, but, in Arabidopsis, it still occurs within the anther. The vegetative cell forms the PT that carries and releases the male gametes inside the female gametophyte (McCormick, 1993; Lord and Russell, 2002; McCormick, 2004).

Settling the treasure hunt map – double fertilization

The aimed treasure – the new seed generation – is only granted in flowering plants due to the double fertilization process, the unique key biological process that conceded the angiosperms success. The path to be followed starts once the stigmatic cells recognize a compatible pollen grain, it becomes properly hydrated, forming the PT, that will carry the two sperm cells into the ovule embryo sac (Figure 1). The rapid and controlled navigation of the PT is granted by a series of communications and consequent signalling cascades leading to various interactions between PT, sporophytic and gametophytic female tissues. The PT grows through the extracellular matrix of cells of the female reproductive tissue: stigma, style and transmitting tissue (TT) (Yadegari and Drews, 2004), until it gets the signals that direct it into an ovule. Once close to the ovule, the PT emerges from the septum, and the double fertilization moment starts. The PT that has grown along the funiculus, finally reaches the embryo sac entrance, the micropyle, and through the filiform apparatus of one of the two existing synergids, stops growing, ruptures and releases its two sperm cells. It is time for the process of simultaneous fertilization to occur, where one sperm cell fuses with the egg cell, generating the embryo while the other fuses with the central cell to form the endosperm, the basal tissue for the embryo nourishment (Palanivelu and Tsukamoto, 2012; Beale and Johnson, 2013; Dresselhaus and Franklin-Tong, 2013; Pereira *et al.*, 2014). After PT entrance, the invaded synergid dies by PCD (Higashiyama *et al.*, 2001; Dresselhaus, 2006; Dresselhaus e Franklin-Tong, 2013), and the persisting synergid will fuse with the endosperm after successful fertilization of both female gametes.

This plant organogenesis charming process will embody new structures of refined control of gene expression, based on a subtle communication occurring between neighbouring cells and on chemical signals that will induce cellular

mechanisms of patterning and growth, tightly constrained in a complex regulatory network. Since the adhesion of the pollen grains to the stigma, every single step that will lead to the generation of the new seed, is being deeply studied, to map each player role in each tissue, in order to untangle this complex regulatory network of interacting genes.

Step by step: the clues

First interaction pollen-stigma

When a pollen grain lands at the stigma cells (Figure 2), it has to immediately face self-incompatibility (SI) responses, a pre-fertilization barrier avoiding the negative effects associated with inbreeding depression (Losdat *et al.*, 2014). Selective inhibition starts to prevent self-fertilization and also to grant the gene pool and genetic variation of plants (Kitashiba and Nasrallah, 2014). This process is controlled by the multiallelic *S*-locus in many plant species (Takayama and Isogai, 2005).

The complex exocytic subunit Exo70A1 in *A. thaliana* stigma, accepts a compatible pollen grain, acting on the polarized secretion of stigmatic cells to provide vesicles containing aquaporins, improving the water permeability, allowing the hydration of pollen and providing cell wall modifying enzymes to grant the stigma PT growth (Samuel *et al.*, 2009). Phosphatidylinositol-4-phosphate (PI4P) is crucial in this initial step, because mutants with low levels of PI4P on the stigma had slower hydration rates and maternal fertility defects due to failed pollinations (Chapman and Goring, 2010).

The VACUOLAR SORTING 41 protein (AtVPS41) is also a controlling factor, as the PTs of *atvps41* plants do not enter the female TT due to a compromised endocytic pathway (Hao *et al.*, 2016). The quiet studied signalling molecule Ca^{2+} can be an important intracellular signalling factor for the PT formation and its polarized growth (Iwano *et al.*, 2004; Pereira *et al.*, 2016a).

The 'minor' cell wall components and mysterious Arabinogalactan protein (AGP) family, always involved in distinct stages of the reproductive process, may also be essential regulatory components of PT growth (Lamport *et al.*, 2018). Apple blossom stigma receptivity depends on AGPs secretion and their disappearance is linked with PT growth (Losada and Herrero, 2012). The molecular properties and periplasmic location of the classical AGPs (Lamport *et al.*, 2006), make them the primary source of cytosolic Ca^{2+} oscillations, pectic plasticizers, working as Ca^{2+} signposts to the ovule (Lamport *et al.*, 2018). AGPs being present in the stigma cells and in growing PTs have roles in pollen–stigma interactions and pollen grain competence to initiate PT growth. AGP6, AGP11, AGP23 and AGP40, pollen specific AGPs, are included in the PT endosome machinery (Costa *et al.*, 2013; Pereira *et al.*, 2016a).

The gene, *O-FUCOSYLTRANSFERASE1* (*AtOFT1*) cannot be forgotten, as it plays a key role in PT penetration through the stigma-style interface and, according to genetic and cell biological evidences, a Golgi-localised fucosyltransferase system may be needed for PT growth through pistil tissues. Although yet unknown, the AtOFT1 substrates may include cell surface receptors or structural proteins secreted into the extracellular matrix. A nearly sterile *oft1* phenotype opens the path to find how putative proteins OFTs are utilized along pollen-pistil interactions (Smith *et al.*, 2018). Fasciclin-like AGPs (FLAs) are also recruited during this process as FLA3 is expressed in the pollen grains

and PTs and it is involved in the microspores development affecting cellulose deposition (Li *et al.*, 2010).

The puzzling PT navigation along the TT

The rapid and controlled growth of PT is provided by an accurate communication network and signalling cascades during pollen-pistil interactions (Figure 3-4). The PT guidance process starts at preovular guidance, where any compatible PT travels in the extracellular matrix of TT or channel, from stigma to the ovary, later turning to ovular guidance, in a specific relation between one PT and its ovule (Higashiyama and Takeuchi, 2015; Kanaoka and Higashiyama, 2015; Mizuta and Higashiyama, 2018).

Mechanical, chemotropic, geometric and physiological orientation mechanisms may explain the precise growth of PTs towards the ovary (Heslop-Harrison, 1987; Lush, 1999; Chebli and Geitmann, 2007). There is a strict balance between cell turgor and cell wall resistance to keep the shape of PT, enabling the tip growth to expand and preventing the loss of the cell integrity (Winship *et al.*, 2011). The accurate cell wall assembly are fundamental for PT growth. The polysaccharide pectin, an essential component of the primary cell wall, interferes with structure and flexibility during cell growth. Pectin methylesterases (PMEs) enzymes control spatially and temporally the de-esterification of pectin, essential for PT integrity. PTs become unstable when loosing de-esterified pectin. The Vanguard1 (VGD1)-encoded pectin methylesterase mutant helps to demonstrate the importance of pectin in cell wall for PT integrity, as it presents *in vitro* bursting and *in vivo* delayed growth (Jiang *et al.*, 2005; Bosch and Hepler, 2005). In addition, the Cl^- channel, MSL8 in Arabidopsis was shown to cause precocious PT burst before pollen arrival at the synergids (Li and Yang, 2018). Enzymatic production of reactive oxygen species (ROS) also affects PT growth. The characterization of the NADPH

oxidases RbohH and RbohJ (Respiratory burst oxidase homolog H and J) in Arabidopsis showed that the PTs tip growth was severely impaired in *rbohHrbohJ* double mutant. Both RbohH and RbohJ, whith Ca^{2+} binding EF-hand motifs, showed Ca^{2+}-induced ROS-producing activity at the PT tip of the plasma membrane. The PT slows down to couple the rate of exocytosis and cell expansion, possibly through the Ca^{2+} influx and K^{+} efflux (Lassig *et al.*, 2014; Kaya *et al.*, 2014). The affected amplitude and frequency of ion oscillation are detrimental to the precise coupling of tip growth and cell wall dynamics. The PT integrity depends on the cell wall composition and is controlled by its modifying enzymes and ROS, except for extensin (Jiang *et al.,* 2005; Woriedh *et al.*, 2013).

In *Torenia fournieri*, the PT has a unidirectional orientation (Higashiyama and Hamamura, 2008) maybe without having the need of the FG for the PT growth from stigma to style (Sogo and Tobe, 2005). With preovular guidance in mind, the chemocyanin of the stigma can be found to induce the PT chemotropism in *Lilium longiflorum,* with stigma cysteine-rich adhesins (SCAs) binding the PT, allowing the chemocyanin physical access to the plasma membrane, controlling and directing the PT growth (Park *et al.*, 2000; Kim *et al.*, 2003). The PT pectins may interact with the *A. thaliana* LIPID TRANSFER protein 5 (LTP5), facilitating its guidance (Chae *et al.*, 2009). In tomato, the CYSTEINE-RICH STIGMA SPECIFIC PROTEIN 1 (STIG1) interacts with a KINASE 2-type pollen receptor (LePRK2) and with phosphatidylinositol 3-phosphate (PI3P), as a signal peptide to promote PT growth (Tang *et al.*, 2004); In Arabidopsis, the PT growth is promoted by the pistil brassinosteroids (Vogler *et al.*, 2014), with the γ-aminobutyric acid (GABA) modulating the Ca^{2+} channels in the plasma membranes, regulating the PT signalling pathways (Palanivelu *et al.*, 2003; Yu

et al., 2014). During preovular guidance (Figure 3), the PT growth is, therefore, granted by several mechanisms (Mizuta and Higashiyama, 2018).

The TT connecting the stigma, style and ovules, supports the growth of PTs through an extracellular matrix (ECM) extremely rich in glycoproteins, polysaccharides, glycolipids and AGPs (Pereira *et al.*, 2015). The Arabidopsis TT is solid and composed of cylindrical cells (Lennon *et al*., 1998; Erbar, 2003) that facilitates PT development and nutrition (Faure *et al.*, 2002; Crawford and Yanofsky, 2008). According to hormonal studies, the establishment of TT may be controlled by both auxin and cytokinin, which are responsible for the tissue polarity (Deb *et al.*, 2018) and thus, may play a key role along the complex interactions that control PT growth.

In *Nicotiana tabacum*, the AGP highly glycosylated TRANSMITTING TISSUE SPECIFIC (TTS) proteins are promoting PT growth and guidance to the ovules (Cheung *et al.*, 1995; Wu *et al.*, 2001). Transcription factors (TFs) like NO TRANSMITTING TRACT (NTT), HECATE 1 (HEC1), HEC2, HEC3, HALF FILLED (HAF), SPATULA (SPT) and water soluble chlorophyll proteins (AtWSCP) are important for TT development, because they induce the tissue PCD, after PT passage, which is also crucial for its own growth (Alvarez and Smyth, 2002; Crawford and Yanofsky, 2011; Boex-Fontvieille *et al.*, 2015; Mizuta and Higashiyama, 2018). The *ntt* mutants show abnormal development of the TT, with defects and slower growth of the PT, and a reduced fertilization rate (Crawford *et al.*, 2007). In addition, the *ntt* mutants have lower acidic polysaccharides, possibly related with AGPs contents, as AGPs are acidic glycoproteins, belonging to the TT.

PT may be guided by distinct cues, including K^+, Cl^-, Ca^{2+} (Hepler *et al.*, 2006), glycoproteins (Sommer-Knudsen *et al.*, 1998), ROS (Foreman *et al.*, 2003),

nitric oxide (Prado *et al.*, 2016), peptides (Qu *et al.*, 2015) besides other complex signalling networks not yet elucidated (Leydon *et al.*, 2015).

In Arabidopsis, the Shaker family K^+ channel, SPIK, affects PT development. Loss of SPIK leads to precocious PT burst and consequent defected growth, suggesting a conserved role of K^+ homeostasis in PT growth and burst (Mouline *et al.* 2002).

AGPs are other guidance cues due to the evidences of their presence along the pathway from the stigma to the egg cell (Pereira *et al.*, 2014; Pereira *et al*., 2016a). If pollen tubes grown *in vitro* acidify their growth medium (Feijo *et al.*, 1995), it may indicate that *in vivo,* they can dissociate AGP-Ca^{2+} of the TT, enabling PT to create a Ca^{2+} path to the ovule. The fertilization of multiple ovules can also be granted by the cooperation of multiple PTs (Heslop-Harrison *et al.*, 1985) together with the H^+ efflux increase and the Ca^{2+} release from the locally abundant AGP-Ca^{2+} (Lamport *et al.*, 2018).

It is possible to find AGP1, AGP4 (JAGGER) and AGP19 in Arabidopsis TT, with high levels of expression along this tissue (Yang *et al*., 2007; Pereira *et al.*, 2014; Pereira *et al*., 2016a, Pereira *et al.*, 2016b). AGP19 has high expression in the style, ovary walls, TT and siliques, and the a*gp19* mutants have low flower production and smaller siliques (Yang *et al.*, 2007). AGP12 and AGP15 also located along the PT pathway, can contribute nutritionally to the PT growth, from the top of the stigma to the base of the pistil, facilitating its movement, guiding to its targets, or even making it competent for being received by the embryo sac (Pereira *et al.*, 2014).

An AGP sidechain fragment designated AMOR, is the PT activation molecule for response to the synergids LURE guidance peptides in Torenia (Okuda *et al.*, 2009). AMOR was identified as a methyl-Oglucuronosyl-b-D-galactose

(Mizukami *et al.*, 2016), possibly a key component of the AGP Ca^{2+}-binding motif, a Ca^{2+} carrier and good company for the AGP and Ca^{2+} couple (Lamport *et al.*, 2018). AMOR has a differential localization in AG sugar chains specific structures, indicating that AG biosynthesis enzymes are controlling AGPs functions and their sugar chains (Su and Higashiyama, 2018).

However, it is still not entirely clear how all TT components interact with PT and besides the importance of these interactions, the different components present in the TT, point to a contact-mediated ability provided by the stigma and style, for the PT to become aware of the signals that direct it to the ovule (Palanivelu and Preuss, 2006). The TT is thus, a key tissue for the quick growth of the PT throughout the pistil, but also to grant it the ability to move accurately to the ovule (Figure 4).

Ovular guidance: funicular and micropylar guidance

The PT has to emerge, exiting from the TT through a very narrow space between the cells of the septum (Palanivelu and Tsukamoto, 2012), facing all the quests and tissues that trigger its emergence (Higashiyama and Takeuchi, 2015), during a crucial transition phase from preovular to ovular guidance (Mizuta and Higashiyama, 2018). When PT emerges from the septum, it faces a funicular guidance phase from the placenta, along the funiculus and afterwards a micropylar guidance, where it navigates from the funiculus to the micropyle (Shimizu and Okada, 2000; Mizuta and Higashiyama, 2018). This pathway is dependent on signals from sporophytic tissues and from the female gametophyte (Pereira *et al.*, 2016a) (Figure 5).

In Arabidopsis, two potassium (K^+) transporters CATION HYDROGEN EXCHANGER 21 (CHX21) and CHX23 present in PTs, are able to block their growing along the funiculus. The *chx21* and *chx23* mutants show normal PT growth through the TT, but are unable to grow along the funiculus and into the

ovule, without perceiving the signals that come from the ovules (Lu *et al.*, 2011). PT competence for signalling is also dependent on the MITOGEN-ACTIVATED PROTEIN KINASE (MPK3) and MPK6 (Guan *et al.*, 2014), with defective PTs in the *mpk3mpk6* mutant along the funicular guidance phase. PTs can only pass through the TT with the controlling help of the PHYTOSULFOKINE (PSK), as its mutants show very low fertilization rate and loss of funicular orientation (Stührwohldt *et al.*, 2015). The knowledge path on the mechanisms that regulate this stage is now clearer, but sparse, and multiplying quests are trying to find new cues stage, especially under female control.

AAn ABNORMAL POLLEN TUBE GUIDANCE 1 (APTG1), a mannosyltransferase (Dai *et al.*, 2014), acts in the glycosylphosphatidylinositol (GPI) synthesis of the COBRA-LIKE 10 protein (COBL10) (Li *et al.*, 2013). The COBL10 protein, located at the PT end, plays a crucial role in its orientation and growth. Mutations in COBL10 generate gametophytic male sterility due to low PT growth and difficult directional sensing in the female TT. The apical pectin cap deposition and cellulose microfibrils were broken in *cobl10* PTs. (Li *et al.*, 2013).

After a funicular guided navigation period, the PT steps into the micropylar guidance stage. When reaching the micropylar region, PTs permeate through the opening between the ovules integuments until one of the synergid cells, an active and typically secretory cell (Huang and Russell, 1992). The *wuschel-7* (*wus-7*) mutant has a short inner integument (Lora *et al.*, 2018) and 35% of its ovules have defected embryo sacs. In 65 % of wus-7 ovules, PTs do not emerge in the micropyle in 65%, suggesting a partial effect of the inner integument in PT guidance. It is granted that the functional FG influences the PT attraction to the ovule (Ray *et al.*, 1997; Shimizu and Okada, 2000), thus being the structure that produces the ovular attracting molecules (Takeuchi and Higashiyama, 2016). Based on *Torenia fournieri* evidences, synergids are the female cells

responsible for the ovular PT attraction. *Torenia* has a bare embryo sac, which is projected to the outside of the ovule through the micropyle when mature, thus allowing an easy access to the embryo sac cells. It was shown that, *in vitro* PTs were attracted directly to the bare embryo sacs and no longer leaving the micropylar region, due to the embryo sac production of attraction signals (Higashiyama *et al.*, 2001). Later, a laser ablation performed on different gametophytic cells found that the synergids were providing the PT guidance by diffusible chemical signals (Higashiyama *et al*., 1998; Higashiyama *et al.*, 2001; Higashiyama and Hamamura, 2008; Horade *et al.*, 2013).

An equivalent scenario happens in Arabidopsis with the MYB98 TF expressed in the synergids. The *myb98* mutant has an abnormal filiform apparatus, which blocks the secretion of attractive molecules, thus the PT cannot find the micropyle (Kasahara *et al.*, 2005; Punwani *et al.*, 2008). It is necessary to consider that the interaction between the egg and central cells is also important for the ovular guidance, since a laser disruption of the egg cell or the polar nucleus of the central cell can severely inhibit the PT attraction (Susaki *et al.*, 2015). Synergids are, therefore, together with the egg and central cell, regulating the PT attraction during the last stage of its journey, (Dresselhaus, 2006; Pereira *et al.*, 2016b; Mizuta and Higashiyama, 2018).

So there are attractive molecules produced by the synergids, identified by several studies in different plant species. In *T. fournieri*, cysteine-rich peptides (CRPs) from the defensin-like proteins subgroup, TfLURE1 and TfLURE2 were identified as PTs attractive molecules inducing micropylar guidance (Okuda *et al.*, 2009), capable of attracting PTs also *in vitro* (Higashiyama, 2010). LURES were also identified in *A. thaliana* (AtLURES), *A. lyrata* (AlLURES) (Takeuchi and Higashiyama, 2012) and *T. concolor* (TcCRP1) (Kanaoka *et al.*, 2011). These small *A. thaliana* proteins, only expressed in the synergids, are secreted

through the filiform apparatus (Takeuchi and Higashiyama, 2012). The mutants *magatama3* (Shimizu and Okada, 2000), *myb98* (Kasahara *et al.*, 2005) and *central cell guidance* (Chen *et al.*, 2007) were found to be defective in micropylar guidance due to no AtLURE1 expression, giving to the AtLURE1 the control for the PT attraction (Takeuchi and Higashiyama, 2012). The novel interaction partner of CCG, the CCG-binding protein CBP1 seems to co-regulate the expression level of a subset of CRPs, including LURES, by recruiting the Mediator complex and RNA Pol II with CCG in the central cell (Li *et al.,* 2015). Diverse genes expressed in the central cell and synergid, are downregulated in *ccg* ovules, such as *MYB98,* responsible for *LUREs* transcription regulation. Mutation of MAA3 affects PT attraction in *A. thaliana*. *MAA3* encodes a Sen1p-like RNA helicase and is expected to control target mRNA molecules, whose products are needed to guide PTs (Shimizu *et al*., 2008). Moreover, recombinant AtLURE1 peptides expressed in *Torenia* are capable of attracting Arabidopsis PTs, when *in vitro Torenia* ovules are placed close to the tip of *A. thaliana* PTs (Takeuchi and Higashiyama, 2012). These peptides are thus, enough to overcome reproductive barriers in ovule orientation, even among distant species (Mizuta and Higashiyama, 2018).

The unfertilized *A. thaliana* ovules send diffusible attractive molecules (Palanivelu and Preuss, 2006; Takeuchi and Higashiyama, 2012), but when the ovules are invaded by PTs, these molecules are no longer produced, so this may be enough to prevent the attraction of more PTs. But it may also be considered that PTs can rapidly release diffusible repellents, without no concrete evidence yet (Palanivelu and Preuss, 2006). The specific *Zea mays EGG APPARATUS1* gene (*ZmEA1*) of maize encodes a small protein present not only in the synergids, but also in the egg cell, filiform apparatus and micropylar nucellus, and is necessary for micropylar orientation (Márton *et al.*, 2005;

Márton *et al.*, 2012). No homologous candidate was found in *A. thaliana*, but there is no doubt about the importance of embryo sac cells for a successful PT guidance.

GAMETE-EXPRESSED3 (GEX3) encodes a membrane protein and is expressed, both in male and female tissues. Micropylar PT guidance defects were caused by aberrant expression of *GEX3* in the egg cell (Kanaoka and Higashiyama, 2015).

In turn, AGPs are also in ovule and synergid tissues as attractive and/or signalling molecules for the PT guidance, considering their immunolocalization evidences in ovules and in the synergids filiform apparatus of different species (Coimbra and Salema, 1997; Coimbra and Duarte, 2003; Coimbra *et al.*, 2007; Lopes *et al.*, 2016). AGP23 is intensively present upon fertilization, inside the embryo sac, where the PT bursts (Pereira *et al.*, 2016a).

The JAGGER protein, AGP4, is essential to prevent the polytubey occurrence. The *jagger* persistent synergid does not undergo PCD and more than one PT is attracted to the ovule, in a polytubey effect. JAGGER controls the persistent synergid death and ends the multiple PTs attraction (Pereira *et al.*, 2016b).

Arabidopsis has LURE1 receptors such as leucine-rich repeat (LRR) kinase type receptors (RLKs): MALE DISCOVERER 1 (MDIS1), MDIS1-INTERACTING LIKE KINASE RECEPTOR (MIK1, MIK2) (Wang *et al.* 2016) and POLLEN RECEPTOR-LIKE KINASE 6 (PRK6) (Takeuchi and Higashiyama, 2016). The recombinant AtLURE1 may induce heterodimerization of MDIS, MIK1 and MIK2 (Wang *et al.*, 2016). These three receptors, at the PT plasma membrane showed their knockout mutants with micropylar guidance defects. The other PT-specific receptors found by Takeuchi and Higashiyama (2016), the PRK (PRK6) receptors, at the PT tip, enable it to detect AtLURE1, controlling its elongation

and attraction. The detection of AtLURE1 by PRK6 is achieved in cooperation with other PRK family receptors like PRK1, PRK3 and PRK8, but PRK6 is the key receptor. PRK6 also interacts with pollen-expressed ROPGEFs (Rho of plant guanine nucleotide-exchange factors), which are important for PT growth through activation of the signalling switch Rho GTPase ROP1 (Kaothien *et al.*, 2005; Zhang and McCormick, 2007; Takeuchi and Higashiyama, 2017).

PT guidance is also affected by nitric oxide (NO) and D-serine. NO has diverse effects on plant development as a growth regulator, and acts as a negative chemotropic cue during *in vitro* PT growth and re-orientation, which was mediated by a cGMP signalling pathway (Prado *et al.*, 2004). Mutations in *A. thaliana NITRIC OXIDE SYNTHASE1 (AtNOS1*) generates low fertility because NO production is affected (Guo *et al.*, 2003), indicating some possible fuctions of NO during PT guidance. Further experiments with *semi-in vivo* isolated ovules and PTs confirmed that NO is necessary for ovular PT guidance in *Lilium longiflorum*, depending this signalling pathway on Ca^{2+} signalling (Prado *et al.*, 2008). Still it is unclear how the PT surface perceives NO.

The Ca^{2+} signalling network can be puzzling enough due to the existence of multiple Ca^{2+} channels, distinct regulation levels and plenty of Ca^{2+} PTs sensors. The Cyclic nucleotide-gated ion channel 18 (CNGC18) appears to be, among other Ca^{2+} channels expressed in pollen, an essential required Ca^{2+} channel in PT growth and guidance (Frietsch *et al.,* 2007). The two point-mutated *cngc18* mutants obtained showed that the cyclic nucleotide (cNMP) activation of CNGC18 is likely involved in ovular guidance, but not PT growth (Gao *et al.,* 2016). Thus, Ca^{2+} influx may regulate the PT growth and guidance in a double manner: basal influx for PT growth and the cNMP-activated influx for PT reorientation (Li *et al.*, 2018).

LOST IN POLLEN TUBE GUIDANCE 1 (LIP1) and LIP2 are also involved in the AtLURE1-dependent orientation mechanism (Liu *et al.*, 2013; Pereira *et al.*, 2016a; Higashiyama, 2018). LIPs do not bind directly to the attractive molecules, including the AtLURE1 peptides, due to the lack of an extracellular region, but they may form complexes with direct receptors of the LUREs peptides, acting in the signal transduction of the PT cytoplasm (Takeuchi and Higashiyama, 2016).

It is also important to map distinct proteins from the endoplasmic reticulum (ER), since the folding of these proteins may be important for the PT competence, enabling it to respond to attraction or orientation signals (Braakman and Herbert, 2013). POLLEN DEFECTIVE IN GUIDANCE 1 (POD1) is a ER protein that interacts with the chaperone CALRETICULIN 3 (CRT3) controlling the PT membrane proteins folding and regulating their signalling (Li *et al.*, 2011). Several molecules are acting in an intrinsic signalling pathway, during the micropylar guidance stage, however, their composition and entanglement is sparse (Pereira *et al.*, 2016a; Mizuta and Higashiyama, 2018) and are in need of new quests.

The aimed treasure is beyond synergids

Finally, the PT reaches the micropyle of its target ovule, entering the embryo sac through one of the synergids, stops its growth and bursts, releasing the two male gametes (Leshem *et al.*, 2013), that will fuse with the female ones, while the multiple PTs blocking system acts (Figure 6). The receptive synergid undergoes PCD shortly before the entry of the PT or even upon its entry (Faure *et al.*, 2002). In a complex signalling system and communication between the PT and the female tissues, the aimed treasure will be finally reached by generating a new seed, granting the reproductive success of flowering plants (Pereira *et al.*, 2016a).

Synergids gather the utmost point of PT attractive molecules, able to receive and rupture it. The first PT receptor found, from the synergid filiform apparatus, was a serine/threonine-type FERONIA/SIRENE (FER/SRN). *fer/srn* mutation leads to the continuous growth of the PT within the embryo sac, with no release of the male gametes (Huck *et al.*, 2003; Escobar-Restrepo *et al.,* 2007; Rotman *et al.*, 2008).

FER is a receptor like kinase (RLK), from the *Catharanthus roseus* CrRLK1L-1 subfamily, with an extracellular portion similar to a malectin domain, so its binding molecule can be a carbohydrate or a cell wall glycoprotein (Lindner *et al.*, 2012; Pereira *et al.*, 2016a). FER is thus, an essential factor to stop the PT growth, its reception, correct rupture and gametes release and may also be the link between extracellular events and intracellular orientation (Stegmann *et al.*, 2017).

LORELEI (LRE) is a GPI anchor protein predominantly expressed in the synergids and the *lre* mutant has a similar phenotype to the *fer/srn* mutation (Capron *et al.*, 2008). LRE and FER work together in the PT synergid reception with LRE acting as a co-receptor with FER (Hafidh *et al.*, 2016; Liu *et al.*, 2016). LRE and LLG1 (LORELEI-like GPI anchored protein 1) interact with FER in the ER lumen, acting as chaperones to conduct FER to the filiform apparatus and regulate PT rupture (Li *et al.*, 2015).

NORTIA (*NTA*) also has a *fer*-like phenotype (Kessler *et al.*, 2010). NTA acts in a FER dependent way, in the same signalling cascade. Its C-terminal calmodulin domain suggests ability for the Ca^{2+} oscillations perception in the synergids (Iwano *et al.*, 2012). Both *lre* and *nta* mutants have a similar *fer* mutant phenotype, but not fully penetrant as this last one. They play important roles in PT-synergid interaction, but maybe acting redundantly with another yet

unknown factor, still need to be known in order to understand this signalling mechanism (Pereira *et al.*, 2016a).

In *A. thaliana*, in parallel to the FER signaling pathway, vacuolar acidification in synergid cells is impaired in low expression levels of AP1G, the γ-subunit of the tetrameric ADAPTOR PROTEIN1 (AP1). Thus, upon PT arrival, there is a delay in synergid cell degeneration. AP1 regulates protein sorting at the trans-Golgi network/early endosome. In synergids cells with functional loss of V-ATPases, the *ap1g* mutant has a defect in PT reception, indicating that acidification of the vacuole represents an important mechanism for its degeneration (Wang *et al.*, 2017).

Evidences were found on others female gametophyte CrRLK1L receptors in the PT perception (Galindo-Trigo *et al.*, 2018). HERCULES RECEPTOR KINASE1 (HERK1) and ANJEA (ANJ) are strongly localised at the filiform apparatus of the synergid cells, with a double mutant PT overgrowth. A yeast two hybrid assay showed that HERK1 and ANJ extracellular membrane domains interacted with LRE, leading the assumption that HERK1-LRE and ANJ-LRE may belong to a filiform apparatus receptor complex, responsible for mediating PT reception.

TURAN (TUN), encoding a UDP-glycosyltransferase protein, may also be involved in the PT reception, with a mutation revealing again a PT overgrowth phenotype (Lindner *et al.*, 2015). The TF VERDANDI (VDD) belongs to the Reproductive Meristem (REM) family, and *vdd* revealed defects in the cellular identity of the antipodals and synergids, with no PT explosion, and thus the continuation of PT growth after reaching the synergids (Matias-Hernandez *et al.*, 2010). Recently, VDD and VALKYRIE (VAL), also a REM family member, were shown to act as a complex regulating the receptive synergid death, and PT death, by bursting (Mendes *et al.*, 2016).

A small group of chimeric AGPs, the EARLY NODULIN-LIKE (ENODLs), EN11-15, are essential for the PT reception in Arabidopsis, with high expression levels found in the funiculus and in the ovules. EN14 and EN15, are present in the synergids filiform apparatus. A five-fold mutant revealed a similar phenotype to the *fer* mutant, with, at least EN15 interacting with FER, and thus, maybe being involved in the signalling pathway activation, essential for the coordination of the male-female communication needed in the double fertilization process (Hou *et al.*, 2016).

There are also male players involved in the synergids PT reception and rupture, notoriously interacting in a coordinated way with the female players. The first identified set of pollen-specific MYB transcription factors MYB97, MYB101 and MYB120 act controlling the PT reception and regulating its gene expression, including genes encoding the secretion of small peptides (Liang *et al.*, 2013).

The Ca^{2+} pump ACA9 expressed in the PT was also shown to regulate PT growth and rupture in the synergid (Schiott *et al*. 2004).

ANXUR 1 (ANX1) and ANX2 are receptor like kinases from the PT and FER homologues that induce PT burst (Boisson-Dernier *et al.*, 2009). When the receptive synergid goes into PCD, responses of Ca^{2+} are activated (Boisson-Dernier *et al.*, 2013, Ngo *et al.*, 2014). The overexpression of ANX1/ANX2 receptors, at the plasma membrane of the PT tip, lead to the blockage of its growth by excessive activation of exocytosis and accumulation of secreted material by the cell wall. The *anx1anx2* double mutant stops the PT development, by premature tip rupture (Boisson-Dernier *et al.*, 2013). It is accepted that ANX blocks the PT rupture and premature gametes release, so it continues to grow in the female tissues, granting the cell wall integrity, until it reaches the FG (Boisson-Dernier *et al.* 2009; Miyazaki *et al.*, 2009; Pereira *et al.*, 2016a). The results from Muro *et al.* (2018) have shown that the PT tip

localization of ANX receptors is strictly mediated by two ANTH domain-containing proteins via the clathrin-mediated endocytosis pathway. *phosphatidylinositol binding clathrin assembly protein 5a* (*picalm5a*) *picalm5b* double mutant exhibited an altered distribution of ANX receptors in the PT and an identical phenotype to *anx1anx2*: a premature burst of PTs during their growth.

It has to be an extremely coordinated mechanism of signalling between the female and male cells during these interactions. Once in the FG, the FER-dependent signalling cascade located in the synergid is activated to mediate the reception and fertilization, while the ANX-dependent signalling present in the PT is blocked, enabling the PT rupture and gametes release (Boisson-Dernier *et al.*, 2009; Miyazaki *et al.*, 2009). This is an interesting example, where duplicate sibling genes of an ancestral gene work on cells of the opposite sex during male-female interactions (Higashiyama, 2018).

RAPID ALKALINIZATION FACTORs (RALFs) are secreted peptides, working as extracellular signals and translated into the cell. These peptides are spread along the plant kingdom and Arabidopsis has 37 members (Murphy and De Smet, 2014), regulating various physiological processes (Stegmann *et al.*, 2017).

RALF peptides expression were searched in the PTs by Mecchia *et al.* (2017), and RALF4 and RALF19, the closest homologues, were chosen. The *ralf4ralf19* double mutant showed a similar phenotype to *anx1anx2*: no PT growth maintenance. So RALF4/19 are crucial for the PT growth, possibly acting upstream of ANX1/2.

Searching for more CrRLK1Ls involved in PT reception, Ge *et al.* (2017) found BUPS1 and BUPS2, new receptors predominantly expressed in PT, also with

bups1bups2 mutant having a similar phenotype to *anx1anx2*. Settling the map, it is possible to find RALF4/19 interacting with BUPS1/2 and these two peptides interacting with ANX1/2. Besides, BUPS1/2 and ANX1/2 interact with each other. Considering the same phenotype showed by *anx1anx2* and *bups1bups2* double mutants of premature PT rupture, it may be suggested a model in which ANX1/2 and BUPS/2 form a heterodimer. Quests are needed to find whether RALF4/19 binding induces heterodimerization of ANX and BUPS. RALF34 expressed only in the female tissues, is the closest homolog of RALF4/19 and induces PT rupture at a 2 nM concentration, when compared with RALF4/19 that show no activity even at a 20 µM concentration. RALF34 competes with RALF4/19 *in vitro*, in the BUPS1 and ANX interaction; so RALF34 can control PT rupture through female tissues, still to understand how this happens, and further studies will be needed (Liang and Zhou, 2018).

The autocrine signalling mediated by RALF4/19 and ANX-BUPS is critical to maintain a long distance PT growth and the tip cell wall integrity. Quests remaining to answer are how external autocrine and paracrine signals for the PT tip change during the long journey and how PTs respond to them (Higashiyama, 2018).

The improved understanding of the PT guidance and fertilization process includes a limited number of molecular players. Considering the FER and ANXUR1/2 description and their role in Ca^{2+} mediated fertilization, members of the CrRLK1L family had stepped into the regulation of polarized growth. ERULUS (ERU), also a CrRLK1L protein, has been described as a core root hair (RH) regulator, involved in the establishment of a functional apical $[Ca^{2+}]_{cyt}$ gradient (Bai *et al.*, 2014).

In vivo eru pollen are less competitive than WT pollen in terms of plant fertilization efficiency (Schoenaers *et al.*, 2017) and also with aberrant ovule

targeting of PTs, more mutant PTs growing around the funiculus with multiple turns before targeting the micropyle. This is a similar phenotype to the *lip1lip2* double mutant, which is defective in two PT plasma membrane localized RLKs (Liu *et al.*, 2013). LIP1 and LIP2 are crucial players in the receptor complex regulating PT guidance in response to the micropyle-secreted AtLURE1 signalling peptide. One could, thus, suggest that ERU could function as LURE receptor, and as such could regulate PT targeting in a complex with LIP1 and LIP2 (Schoenaers *et al.*, 2017).

After all the PT-synergid interactions, PT ruptures and releases its content, occurring the male-female gamete fusion (Figure 7). For the successful gametes fusion, three crucial players are interacting: GAMETE EXPRESSED 2 (GEX2), GENERATIVE CELL SPECIFIC 1/HAPLESS 2 (GSC1/HAP2) and EGG CELL1 (EC1) (Mori *et al.*, 2006; Besser *et al.*, 2006; Sprunck *et al.*, 2012). GEX2 encodes a membrane-expressed protein of the male gametes, containing extracellular immunoglobulin-like domains and required for gametes fusion (Mori *et al*., 2014). The male gametes with the *gcs1hap2* mutation when delivered to the ovules cannot initiate fertilization, and the released gametes remain in the fusion site with the female gametes, leading eventually to the attraction of multiple PTs (Mori *et al.*, 2006; von Besser *et al.*, 2006). EC1 is a cysteine-rich protein, a possible activating molecule of the male gametes, to permit gametes fusion and *ec1* quintuple mutants show that successful male-female gamete interactions prevent multiple-sperm cell delivery (Sprunck *et al.*, 2012).

Recently, RBOHH and RBOHJ were found to act downstream of ANXs, controlling ROS production during PT growth (Boisson-Dernier *et al*., 2013). Besides, the tube burst phenotype of both *anx1anx2* and *rbohhrbohJ* can be suppressed by a point mutation ($MRIR^{240C}$) in the receptor-like cytoplasmic

kinase MARIS (MRI) (Boisson-Dernier *et al*. 2015), while RALF4 and 19 were evidenced to function upstream of MARIS (Mecchia *et al*., 2017). Thus, RALF-BUPS/ANX complex may be regulating downstream RBOHH, RBOHJ and MRI, to keep PT integrity during its growth. Moreover, RALF4 and 19 bind cell wall extensin LRX8, LRX9 and LRX11 expressed in PT, having a vital role in cell wall materials deposition (Mecchia *et al.,* 2017).

Ca^{2+} plays, once more, a preponderant role in regulating the release of male gametes and fertilization (Iwano *et al.*, 2012). Several molecules initiate their signalling mechanism and interaction after Ca^{2+} oscillations events. Specific signatures of this secondary messenger are essential to control the PT and the receptive synergid PCD, for male-female gametes fusion and double fertilization success (Denninger *et al.*, 2014; Ngo *et al.*, 2014).

Conclusion and outlook

The aimed treasure can finally emerge, after a stunning PT pathway, full of traces and signals of a complex genetic regulatory network, that step by step, establishes a precise and refined crosstalk, since the landing of the pollen on stigma to the generation of the new born seed. This proposal tried to settle the overall map of this aimed treasure hunt, gathering the known clues and, in a clearer and simpler step by step set of frameworks, state the acquired knowledge to a further establishment of links and filling of gaps.

For sure, a broader framework of signalling pathways should include the interaction between the hormonal signalling control of tissues domains positioning with the complex genetic regulatory network interacting along the double fertilization process, if we consider the reciprocal dependency of the PT on the TT.

Besides all the involved players, one could also highlight fundamental keys of this overall PT journey that will surely make it able to reach the aimed treasure, as it is the quiet studied signalling molecule Ca^{2+}, fully present during all the stages of the double fertilization process, and their humble supporters, the AGPs family, responsible for vital interactions in each step.

Nevertheless, new key players are now being discovered to complete the overall perspective on the network of the male female crosstalk during this sexual plant reproduction process.

Conflict of Interest Statement

The authors declare that this review was written in the absence of any potential conflict of interest.

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References

Alvarez J, Smyth DR. 2002. *CRABS CLAW* and *SPATULA* genes regulate growth and pattern formation during gynoecium development in *Arabidopsis thaliana*. International Journal of Plant Sciences 163, 17– 41.

Bai L., Ma X., Zhang G, Song S, Zhou Y, Gao L. 2014. A receptor-like kinase mediates ammonium homeostasis and is important for the polar growth of root hairs in Arabidopsis. The Plant Cell 26, 1497–1511.

Beale KM, Leydon AR, Johnson MA. 2012. Gamete fusion is required to block multiple pollen tubes from entering an Arabidopsis ovule. Current Biology 22, 1090–1094.

Boex-Fontvieille E, Rustgi S, Reinbothe S, Reinbothe C. 2015. A Kunitz-type protease inhibitor regulates programmed cell death during flower development in *Arabidopsis thaliana*. Journal of Experimental Botany 66, 6119–6135.

Boisson-Dernier A, Franck CM, Lituiev DS, Grossniklaus U. 2015. Receptor-like cytoplasmic kinase MARIS functions downstream of CrRLK1L-dependent signaling during tip growth. Proceedings of the National Academy of Sciences of the United States of America 112, 12211-12216.

Boisson-Dernier A, Lituiev DS, Nestorova A, Franck CM, Thirugnanarajah S, Grossniklaus U. 2013. ANXUR receptor-like kinases coordinate cell wall integrity with growth at the pollen tube tip via NADPH oxidases. PLoS Biology 11, e1001719.

Boisson-Dernier A, Roy S, Kritsas K, Grobei MA, Jaciubek M, Schroeder JI, Grossniklaus U. 2009. Disruption of the pollen-expressed FERONIA homologs ANXUR1 and ANXUR2 triggers pollen tube discharge. Development 136, 3279–3288.

Bosch M, Hepler PK. 2005. Pectin methylesterases and pectin dynamics in pollen tubes. The Plant Cell 17, 3219-3226.

Braakman I, Hebert DN. 2013. Protein folding in the endoplasmic reticulum. Cold Spring Harbor Perspectives in Biology 5, 17.

Capron A, Gourgues M, Neiva LS, Faure J-E, Berger F, Pagnussat G, Krishnan A, Alvarez-Mejia C, Vielle-Calzada J-P, Lee Y-R. 2008. Maternal control of male-gamete delivery in Arabidopsis involves a putative GPI-anchored protein encoded by the LORELEI gene. The Plant Cell 20, 3038–3049.

Chae K, Kieslich CA, Morikis D, Kim SC, Lord EM. 2009. A Gain-of-Function Mutation of Arabidopsis Lipid Transfer Protein 5 Disturbs Pollen Tube Tip Growth and Fertilization. The Plant Cell 21, 3902–3914.

Chapman LA, Goring DR. 2010. Pollen–pistil interactions regulating successful fertilization in the Brassicaceae. Journal of Experimental Botany 61, 1987–1999.

Chebli Y, Geitmann A. 2007. Mechanical principles governing pollen tube growth. Functional Plant Science and Biotechnology 1, 232–245.

Chen YH, Li HJ, Shi DQ, Yuan L, Liu J, Sreenivasan R, Baskar R, Grossniklaus U, Yang W-C. 2007. The central cell plays a critical role in pollen tube guidance in Arabidopsis. The Plant Cell 19, 3563–3577.

Cheung AY, Wang H, Wu HM. 1995. A floral transmitting tissue-specific glycoprotein attracts pollen tubes and stimulates their growth. Cell Press 82, 383–393.

Coimbra S, Almeida J, Junqueira V, Costa ML, Pereira LG. 2007. Arabinogalactan proteins as molecular markers in Arabidopsis thaliana sexual reproduction. Journal of Experimental Botany 58, 4027–4035.

Coimbra S, Duarte C. 2003. Arabinogalactan proteins may facilitate the movement of pollen tubes from the stigma to the ovules in *Actinidia deliciosa* and *Amaranthus hypochondriacus*. Euphytica 133, 171–178.

Coimbra S, Salema R. 1997. Immunolocalization of arabinogalactan proteins in *Amaranthus hypochondriacus L*. ovules. Protoplasma 199, 75–82.

Costa M, Nobre MS, Becker JD, Masiero S, Amorim MI, Pereira LG, Coimbra S. 2013a. Expression-based and co-localization detection of arabinogalactan protein 6 and arabinogalactan protein 11 interactors in Arabidopsis pollen and pollen tubes. BMC Plant Biology 13, 19.

Crawford BC, Ditta G, Yanofsky MF. 2007. The *NTT* gene is required for transmitting-tract development in carpels of *Arabidopsis thaliana*. Current Biology 17, 1101–1108.

Crawford BC, Yanofsky MF. 2008. The formation and function of the female reproductive tract in flowering plants. Current Biology 18, R972–R978.

Crawford BC, Yanofsky MF. 2011. *HALF FILLED* promotes reproductive tract development and fertilization efficiency in Arabidopsis thaliana. Development 138, 2999–3009.

Crepet WL. 2000. Progress in understanding angiosperm history, success, and relationships: Darwin's abominably "perplexing phenomenon". Proceedings of the National Academy of Sciences of the United States of America 97, 12939–12941.

Deb J, Bland HM, Ostergaard L. 2018. Developmental cartography: coordination via hormonal and genetic interactions during gynoecium formation. Current Opinion in Plant Biology 41, 54–60.

Denninger P, Bleckmann A, Lausser A, Vogler F, Ott T, Ehrhardt DW, Frommer WB, Sprunck S, Dresselhaus T, Grossmann G. 2014. Male–female communication triggers calcium signatures during fertilization in Arabidopsis. Nature Communications 5, 4645.

Dresselhaus T. 2006. Cell–cell communication during double fertilization. Current Opinion in Plant Biology 9, 41–47.

Dresselhaus T, Franklin Tong N. 2013. Male Female Crosstalk during Pollen Germination, Tube Growth and Guidance, and Double Fertilization. Molecular Plant 6, 1018–1036.

Erbar C. 2003. Pollen tube transmitting tissue: place of competition of male gametophytes. International Journal of Plant Sciences 164, S265–S277.

Escobar Restrepo J-M, Huck N, Kessler S, Gagliardini V, Gheyselinck J, Yang W-C, Grossniklaus U. 2007. The FERONIA receptor-like kinase mediates male-female interactions during pollen tube reception. Science 317, 656–660.

Faure JE, Rotman N, Fortuné P, Dumas C. 2002. Fertilization in *Arabidopsis thaliana* wild type: developmental stages and time course. The Plant Journal 30, 481–488.

Feijo JA, Malho R, Obermeyer G. 1995. Ion dynamics and its possible role during in-vitro pollen germination and tube growth. Protoplasma 187, 155–167.

Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JDG, Davies JM, Dolan L. 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422, 442–446.

Frietsch S, Wang YF, Sladek C, Poulsen LR, Romanowsky SM, Schroeder JI, Harper JF. 2007 A cyclic nucleotide-gated channel is essential for polarized tip growth of pollen. Proceedings of the National Academy of Sciences of the United States of America 104, 14531–14536.

Galindo-Trigo S, Blanco-Tourinan N, DeFalco TA, Zipfel C, Gray JE, Smith LM. 2018. CrRLK1L receptor-like kinases HERK1 and ANJEA are female determinants of pollen tube reception. bioRxiv.

Gao QF, Gu LL, Wang HQ, Fei CF, Fang X, Hussain J, Sun SJ, Dong JY, Liu H, Wang YF. 2016. Cyclic nucleotide-gated channel 18 is an essential Ca2+ channel in pollen tube tips for pollen tube guidance to ovules in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America 113, 3096–3101.

Ge Z, Bergonci T, Zhao Y, Zou Y, Du S, Liu MC, Luo X, Ruan H, García-Valencia LE, Zhong S. 2017. Arabidopsis pollen tube integrity and sperm release are regulated by RALF-mediated signaling. Science 358, 1596–1600.

Goring DR, Glavin TL, Schafer U, Rothstein SJ. 1993. An S receptor kinase gene in self-compatible *Brassica napus* has a 1-bp deletion. The Plant Cell 5, 531–539.

Guan Y, Lu J, Xu J, McClure B, Zhang S. 2014. Two mitogen-activated protein kinases, MPK3 and MPK6, are required for funicular guidance of pollen tubes in Arabidopsis. Plant Physiology 165, 528-533.

Guo, FQ, Okamoto, M, Crawford, NM. 2003. Identification of a plant nitric oxide synthase gene involved in hormonal signaling. Science, 302, 100–103.

Hafidh S, Fíla J, Honys D. 2016. Male gametophyte development and function in angiosperms: a general concept. Plant Reproduction 29, 31–51.

Hao L, Liu J, Zhong S, Gu H, Qu L-J. 2016. AtVPS41-mediated endocytic pathway is essential for pollen tube–stigma interaction in Arabidopsis. Proceedings of the National Academy of Sciences 113, 6307–6312.

Hepler PK, Lovy WA, McKenna ST, Kunkel, JG. 2006. Ions and pollen tube growth. The Plant Cell 3, 23.

Heslop HJ. 1987. Pollen germination and pollen-tube growth. International Review of Cytology. Vol. 107, Elsevier, 1–78.

Heslop HY, Heslop-harrison J, Reger BJ. 1985. The pollen-stigma interaction in the grasses .7. Pollen-tube guidance and the regulation of tube number in Zea-Mays-L. Acta Botanica Neerlandica 34, 193–211.

Higashiyama T. 2010. Peptide signaling in pollen–pistil interactions. Plant and Cell Physiology 51, 177–189.

Higashiyama T. 2018. Plant Reproduction: Autocrine Machinery for the Long Journey of the Pollen Tube. Current Biology 28, R266–R269.

Higashiyama T, Hamamura Y. 2008. Gametophytic pollen tube guidance. Sexual Plant Reproduction 21, 17–26.

Higashiyama T, Kuroiwa H, Kawano S, Kuroiwa T. 1998. Guidance in vitro of the pollen tube to the naked embryo sac of *Torenia fournieri*. The Plant Cell 10, 2019–2031.

Higashiyama T, Takeuchi H. 2015. The mechanism and key molecules involved in pollen tube guidance. Annual Review of Plant Biology 66, 393–413.

Higashiyama T, Yabe S, Sasaki N, Nishimura Y, Miyagishima S-y, Kuroiwa H, Kuroiwa T. 2001. Pollen tube attraction by the synergid cell. Science 293, 1480–1483.

Horade M, Kanaoka MM, Kuzuya M, Higashiyama T, Kaji N. 2013. A microfluidic device for quantitative analysis of chemoattraction in plants. RSC Advances 3, 22301–22307.

Hou Y, Guo X, Cyprys P, Zhang Y, Bleckmann A, Cai L, Huang Q, Luo Y, Gu H, Dresselhaus T. 2016. Maternal ENODLs are required for pollen tube reception in Arabidopsis. Current Biology 26, 2343–2350.

Huang B-Q, Russell SD. 1992. Female germ unit: organization, isolation, and function. International Review of Cytology Vol. 140, Elsevier, 233–293.

Huck N, Moore JM, Federer M, Grossniklaus U. 2003. The Arabidopsis mutant *feronia* disrupts the female gametophytic control of pollen tube reception. Development 130, 2149–2159.

Iwano M, Ngo QA, Entani T, Shiba H, Nagai T, Miyawaki A, Isogai A, Grossniklaus U, Takayama S. 2012. Cytoplasmic Ca^{2+} changes dynamically during the interaction of the pollen tube with synergid cells. Development 139, 4202–4209.

Iwano M, Shiba H, Miwa T, Che F-S, Takayama S, Nagai T, Miyawaki A, Isogai A. 2004. Ca^{2+} dynamics in a pollen grain and papilla cell during pollination of Arabidopsis. Plant Physiology 136, 3562–3571.

Jiang LX, Yang SL, Xie LF, Puah CS, Zhang XQ, Yang WC, Sundaresan V, Ye D. 2005. VANGUARD1 encodes a pectin methylesterase that enhances pollen tube growth in the Arabidopsis style and transmitting tract. The Plant Cell 17, 584-596.

Kanaoka MM, Higashiyama T. 2015. Peptide signaling in pollen tube guidance. Current Opinion in Plant Biology 28, 127–136.

Kanaoka MM, Kawano N, Matsubara Y, Susaki D, Okuda S, Sasaki N, Higashiyama T. 2011. Identification and characterization of TcCRP1, a pollen tube attractant from *Torenia concolor*. Annals of Botany 108, 739–747.

Kasahara RD, Portereiko MF, Sandaklie-Nikolova L, Rabiger DS, Drews GN. 2005. MYB98 is required for pollen tube guidance and synergid cell differentiation in Arabidopsis. The Plant Cell 17, 2981–2992.

Kaothien, P. et al. Kinase partner protein interacts with the LePRK1 and LePRK2 receptor kinases and plays a role in polarized pollen tube growth. 2005. The Plant Journal 42, 492–503.

Kaya H, Nakajima R, Iwano M, Kanaoka MM, Kimura S, Takeda S, Kawarazaki T, Senzaki E, Hamamura Y, Higashiyama T, Takayama S, Abe M, Kuchitsu K. 2014. Ca^{2+}-activated reactive oxygen species production by Arabidopsis RbohH and RbohJ is essential for proper pollen tube tip growth. Plant Cell 26, 1069-1080.

Kessler SA, Shimosato-Asano H, Keinath NF, Wuest SE, Ingram G, Panstruga R, Grossniklaus U. 2010. Conserved molecular components for pollen tube reception and fungal invasion. Science 330, 968–971.

Kim S, Mollet JC, Dong J, Zhang KL, Park SY, Lord EM. 2003. Chemocyanin, a small basic protein from the lily stigma, induces pollen tube chemotropism. Proceedings of the National Academy of Sciences of the United States of America 100, 16125–16130.

Kitashiba H, Nasrallah JB. 2014. Self-incompatibility in Brassicaceae crops: lessons for interspecific incompatibility. Breeding Science 64, 23–37.

Kumar V, Trick M. 1994. Expression of the S-locus receptor kinase multigene family in *Brassica oleracea*. The Plant Journal 6, 807–813.

Kusaba M, Nishio T, Satta Y, Hinata K, Ockendon D. 1997. Striking sequence similarity in inter- and intra-specific comparisons of class I SLG alleles from *Brassica oleracea* and *Brassica campestris*: Implications for the evolution and recognition mechanism. Proceedings of the National Academy of Sciences of the United States of America 94, 7673–7678.

Lamport DTA, Kieliszewski MJ, Showalter AM. 2006. Salt stress upregulates periplasmic arabinogalactan proteins: using salt stress to analyse AGP function. New Phytologist 169, 479–492.

Lamport DTA, Tan L, Held MA, Kieliszewski MJ. 2018. Pollen tube growth and guidance: Occam's razor sharpened on a molecular arabinogalactan glycoprotein Rosetta Stone. New Phytologist 217, 491–500.

Lassig R, Gutermuth T, Bey TD, Konrad KR, Romeis T. 2014. Pollen tube NAD(P)H oxidases act as a speed control to dampen growth rate oscillations during polarized cell growth. Plant Journal 78, 94-106.

Lennon KA, Roy S, Hepler PK, Lord E. 1998. The structure of the transmitting tissue of *Arabidopsis thaliana* (L.) and the path of pollen tube growth. Sexual Plant Reproduction 11, 49–59.

Leshem Y, Johnson C, Sundaresan V. 2013. Pollen tube entry into the synergid cell of Arabidopsis is observed at a site distinct from the filiform apparatus. Plant Reproduction 26, 93–99.

Leydon AR, Tsukamoto T, Dunatunga D, Qin Y, Johnson MA, Palanivelu R. 2015. Pollen Tube Discharge Completes the Process of Synergid Degeneration That Is Initiated by Pollen Tube-Synergid Interaction in Arabidopsis. Plant Physiology 169, 485-+.

Li C, Yeh FL, Cheung AY, Duan Q, Kita D, Liu M-C, Maman J, Luu EJ, Wu BW, Gates L. 2015. Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in Arabidopsis. Elife 4.

Li HJ, Yang WC. 2018. Ligands switch model for pollen-tube integrity and burst. Trends in Plant Science 23, 369-372.

Li HJ, Meng JG, Yang W-C. 2018. Multilayered signaling pathways for pollen tube growth and guidance. Plant Reproduction 31, 31–41.

Li HJ, Zhu SS, Zhang MX, Wang T, Liang L, Xue Y, Shi DQ, Liu J, Yang WC. 2015. Arabidopsis CBP1 is a novel regulator of transcription initiation in central cell-mediated pollen tube guidance. The Plant Cell 27, 2880–2893.

Li HJ, Xue Y, Jia D-J, Wang T, Liu J, Cui F, Xie Q, Ye D, Yang W-C. 2011. POD1 regulates pollen tube guidance in response to micropylar female signaling and acts in early embryo patterning in Arabidopsis. The Plant Cell 23, 3288–3302.

Li J, Yu MA, Geng LL, Zhao J. 2010. The fasciclin-like arabinogalactan protein gene, FLA3, is involved in microspore development of Arabidopsis. The Plant Journal 64, 482–497.

Li S, Ge FR, Xu M, Zhao XY, Huang GQ, Zhou LZ, Wang JG, Kombrink A, McCormick S, Zhang XS. 2013. Arabidopsis COBRA-LIKE 10, a GPI-anchored protein, mediates directional growth of pollen tubes. The Plant Journal 74, 486–497.

Li XM, Nield J, Hayman D, Langridge P. 1994. Cloning a putative self-incompatibility gene from the pollen of the grass *Phalaris-Coerulescens*. The Plant Cell 6, 1923–1932.

Liang Y, Tan Z-M, Zhu L, Niu Q-K, Zhou J-J, Li M, Chen L-Q, Zhang X-Q, Ye D. 2013. MYB97, MYB101 and MYB120 function as male factors that control pollen tube-synergid interaction in *Arabidopsis thaliana* fertilization. PLoS Genetics 9, e1003933.

Liang XX, Zhou JM (2018). "The secret of fertilization in flowering plants unveiled." Science Bulletin 63(7), 408-410.

Lindner H, Kessler SA, Müller LM, Shimosato-Asano H, Boisson-Dernier A, Grossniklaus U. 2015. TURAN and EVAN mediate pollen tube reception in Arabidopsis synergids through protein glycosylation. PLoS Biology 13, e1002139.

Liu J, Zhong S, Guo X, Hao L, Wei X, Huang Q, Hou Y, Shi J, Wang C, Gu H. 2013. Membrane-bound RLCKs LIP1 and LIP2 are essential male factors controlling male-female attraction in Arabidopsis. Current Biology 23, 993–998.

Liu X, Castro CA, Wang Y, Noble JA, Ponvert ND, Bundy MG, Hoel CR, Shpak ED, Palanivelu R. 2016. The role of LORELEI in pollen tube reception at the interface of the synergid cell and pollen tube requires the modified eight-cysteine motif and the receptor-like kinase FERONIA. The Plant Cell, tpc, 00703–02015.

Liu LT, Zheng CH, Kuang BJ, Wei LQ, Yan LF, Wang T. 2016. Receptor-like kinase RUPO interacts with potassium transporters to regulate pollen tube growth and integrity in rice. PLoS Genetics 12.

Long JA, Ohno C, Smith ZR, Meyerowitz EM. 2006. TOPLESS regulates apical embryonic fate in Arabidopsis. Science 312, 1520–1523.

Lopes AL, Costa ML, Sobral R, Costa MM, Amorim MI, Coimbra S. 2016. Arabinogalactan proteins and pectin distribution during female gametogenesis in *Quercus suber* L. Annals of Botany 117, 949–961.

Lora J, Laux, T, Hormaza, J. 2018. The role of the integuments in pollen tube guidance in flowering plants. New Phytologist.

Lord EM, Russell SD. 2002. The mechanisms of pollination and fertilization in plants. Annual Review of Cell and Developmental Biology 18, 81–105.

Losada JM, Herrero M. 2012. Arabinogalactan-protein secretion is associated with the acquisition of stigmatic receptivity in the apple flower. Annals of Botany 110, 573–584.

Losdat S, Chang SM, Reid JM. 2014. Inbreeding depression in male gametic performance. Journal of Evolutionary Biology 27, 992–1011.

Lu Y, Chanroj S, Zulkifli L, Johnson MA, Uozumi N, Cheung A, Sze H. 2011. Pollen tubes lacking a pair of K^+ transporters fail to target ovules in Arabidopsis. The Plant Cell 23, 81–93.

Lush WM. 1999. Whither chemotropism and pollen tube guidance? Trends in Plant Science 4, 413-418.

Maheshwari P, Johri BM. 1950. Development of the embryo sac, embryo and endosperm in *Helixanthera-ligustrina* (wall) dans. Nature 165, 978–979.

Márton ML, Cordts S, Broadhvest J, Dresselhaus T. 2005. Micropylar pollen tube guidance by egg apparatus 1 of maize. Science 307, 573–576.

Márton ML, Fastner A, Uebler S, Dresselhaus T. 2012. Overcoming hybridization barriers by the secretion of the maize pollen tube attractant ZmEA1 from Arabidopsis ovules. Current Biology 22, 1194–1198.

Matias-Hernandez L, Battaglia R, Galbiati F, Rubes M, Eichenberger C, Grossniklaus U, Kater MM, Colombo L. 2010. VERDANDI is a direct target of the MADS domain ovule identity complex and affects embryo sac differentiation in Arabidopsis. The Plant Cell 22, 1702–1715.

McCormick S. 1993. Male gametophyte development. The Plant Cell 5, 1265–1275.

McCormick S. 2004. Control of male gametophyte development. The Plant Cell 16, S142–S153.

Mecchia MA, Santos-Fernandez G, Duss NN, Somoza SC, Boisson-Dernier A, Gagliardini V, Martinez-Bernardini A, Fabrice TN, Ringli C, Muschietti JP, Grossniklaus U. 2017. RALF4/19 peptides interact with LRX proteins to control pollen tube growth in Arabidopsis. Science 358, 1600-1603.

Meinke DW. 1994. Seed development in *Arabidopsis thaliana*. Cold Spring Harbor Monograph Series 27, 253–253.

Mendes MA, Guerra RF, Castelnovo B, Silva-Velazquez Y, Morandini P, Manrique S, Baumann N, Groß-Hardt R, Dickinson H, Colombo L. 2016. Live and let die: a REM complex promotes fertilization through synergid cell death in Arabidopsis. Development 143, 2780–2790.

Miyazaki S, Murata T, Sakurai-Ozato N, Kubo M, Demura T, Fukuda H, Hasebe M. 2009. ANXUR1 and 2, sister genes to FERONIA/SIRENE, are male factors for coordinated fertilization. Current Biology 19, 1327–1331.

Mizukami AG, Inatsugi R, Jiao J, Kotake T, Kuwata K, Ootani K, Okuda S, Sankaranarayanan S, Sato Y, Maruyama D, Iwai H, Garenaux E, Sato C, Kitajima K, Tsumuraya Y, Mori H, Yamaguchi J, Itami K, Sasaki N, Higashiyama T. 2016. The AMOR Arabinogalactan Sugar Chain Induces Pollen-Tube Competency to Respond to Ovular Guidance. Current Biology 26, 1091–1097.

Mizuta Y, Higashiyama T. 2018. Chemical signaling for pollen tube guidance at a glance. Journal of Cell Science 131, 8.

Mori T, Igawa T, Tamiya G, Miyagishima S-y, Berger F. 2014. Gamete attachment requires GEX2 for successful fertilization in Arabidopsis. Current Biology 24, 170–175.

Mori T, Kuroiwa H, Higashiyama T, Kuroiwa T. 2006. GENERATIVE CELL SPECIFIC 1 is essential for angiosperm fertilization. Nature cell Biology 8, 64.

Mouline K, Very AA, Gaymard F, Boucherez J, Pilot G, Devic M, Bouchez D, Thibaud JB, Sentenac H. 2002. Pollen tube development and competitive ability are impaired by disruption of a Shaker K^+ channel in Arabidopsis. Genes & Development 16, 339-350.

Muro K M-TK, Tsukamoto R, Kanaoka M, Ebine K, Higashiyama T, Nakano A, Ueda T. 2018. ANTH domain-containing proteins are required for the pollen tube plasma membrane integrity via recycling ANXUR kinases. Nature Communications Biology,1.

Murphy E, De Smet I. 2014. Understanding the RALF family: a tale of many species. Trends in Plant Science 19, 664–671.

Mutskov V, Gerber D, Angelov D, Ausio J, Workman J, Dimitrov S. 1998. Persistent interactions of core histone tails with nucleosomal DNA following acetylation and transcription factor binding. Molecular and Cellular Biology 18, 6293–6304.

Naito Y, Hino K, Bono H, Ui-Tei K. 2014. CRISPRdirect: software for designing CRISPR/Cas guide RNA with reduced off-target sites. Bioinformatics 31, 1120–1123.

Ngo QA, Vogler H, Lituiev DS, Nestorova A, Grossniklaus U. 2014. A calcium dialog mediated by the FERONIA signal transduction pathway controls plant sperm delivery. Developmental Cell 29, 491–500.

Okuda S, Suzuki T, Kanaoka MM, Mori H, Sasaki N, Higashiyama T. 2013. Acquisition of LURE-binding activity at the pollen tube tip of *Torenia fournieri*. Molecular Plant 6, 1074–1090.

Okuda S, Tsutsui H, Shiina K, Sprunck S, Takeuchi H, Yui R, Kasahara RD, Hamamura Y, Mizukami A, Susaki D. 2009. Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. Nature 458, 357.

Oldknow J, Trick M. 1995. Genomic sequence of an srk-like gene linked to the s-locus of a self-incompatible *Brassica oleracea* line. Sexual Plant Reproduction 8, 247–253.

Palanivelu R, Brass L, Edlund AF, Preuss D. 2003. Pollen tube growth and guidance is regulated by POP2, an Arabidopsis gene that controls GABA levels. Cell 114, 47–59.

Palanivelu R, Preuss D. 2006. Distinct short-range ovule signals attract or repel *Arabidopsis thaliana* pollen tubes in vitro. BMC Plant Biology 6, 9.

Palanivelu R, Tsukamoto T. 2012. Pathfinding in angiosperm reproduction: pollen tube guidance by pistils ensures successful double fertilization. Wiley Interdisciplinary Reviews-Developmental Biology 1, 96113.

Park S-Y, Jauh G-Y, Mollet J-C, Eckard KJ, Nothnagel EA, Walling LL, Lord EM. 2000. A lipid transfer–like protein is necessary for lily pollen tube adhesion to an in vitro stylar matrix. The Plant Cell 12, 151–163.

Pereira AM, Lopes AL, Coimbra S. 2016a. Arabinogalactan proteins as interactors along the crosstalk between the pollen tube and the female tissues. Frontiers in Plant Science 7, 1895.

Pereira AM, Masiero S, Nobre MS, Costa ML, Solis MT, Testillano PS, Sprunck S, Coimbra S. 2014. Differential expression patterns of arabinogalactan proteins in *Arabidopsis thaliana* reproductive tissues. Journal of Experimental Botany 65, 5459–5471.

Pereira AM, Nobre MS, Pinto SC, Lopes AL, Costa ML, Masiero S, Coimbra S. 2016b. "Love Is Strong, and You're so Sweet": JAGGER Is Essential for Persistent Synergid Degeneration and Polytubey Block in *Arabidopsis thaliana*. Molecular Plant 9, 601–614.

Pereira AM, Pereira LG, Coimbra S. 2015. Arabinogalactan proteins: rising attention from plant biologists. Plant Reproduction 28, 1–15.

Prado AM, Colaco R, Moreno N, Silva AC, Feijo JA. 2008. Targeting of pollen tubes to ovules is dependent on nitric oxide (NO) signaling. Molecular Plant 1, 703–714.

Prado, AM, Porterfield, DM, Feijo, JA. 2004. Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. Development, 131, 2707–2714.

Punwani JA, Rabiger DS, Lloyd A, Drews GN. 2008. The MYB98 subcircuit of the synergid gene regulatory network includes genes directly and indirectly regulated by MYB98. The Plant Journal 55, 406–414.

Qu L-J, Li L, Lan Z, Dresselhaus T. 2015. Peptide signalling during the pollen tube journey and double fertilization. Journal of Experimental Botany 66, 5139–5150.

Ray S, Park S-S, Ray A. 1997. Pollen tube guidance by the female gametophyte. Development 124, 2489–2498.

Rotman N, Gourgues M, Guitton A-E, Faure J-E, Berger F. 2008. A dialogue between the SIRENE pathway in synergids and the fertilization independent seed pathway in the central cell controls male gamete release during double fertilization in Arabidopsis. Molecular Plant 1, 659–666.

Samuel MA, Chong YT, Haasen KE, Aldea-Brydges MG, Stone SL, Goring DR. 2009. Cellular pathways regulating responses to compatible and self-incompatible pollen in Brassica and Arabidopsis stigmas intersect at Exo70A1, a putative component of the exocyst complex. The Plant Cell 21, 2655-2671.

Schiott M, Romanowsky SM, Baekgaard L, Jakobsen MK, Palmgren MG, Harper JF. 2004. A plant plasma membrane Ca^{2+} pump is required for normal pollen tube growth and fertilization. Proceedings of the National Academy of Sciences of the United States of America 101, 9502-9507.

Schoenaers, S., D. Balcerowicz, A. Costa and K. Vissenberg. 2017. "The Kinase ERULUS Controls Pollen Tube Targeting and Growth in *Arabidopsis thaliana*." Frontiers in Plant Science 8, 10.

Shimizu KK, Okada K. 2000. Attractive and repulsive interactions between female and male gametophytes in Arabidopsis pollen tube guidance. Development 127, 4511–4518.

Shimizu KK, Ito T, Ishiguro S, Okada K. 2008. MAA3 (MAGATAMA3) helicase gene is required for female gametophyte development and pollen tube guidance in *Arabidopsis thaliana*. Plant and Cell Physiology 49, 1478–1483.

Showalter AM. 2001. Arabinogalactan-proteins: structure, expression and function. Cellular and Molecular Life Sciences 58, 1399–1417.

Showalter AM, Keppler BD, Lichtenberg J, Gu D, Welch LR. 2010. A bioinformatics approach to the identification, classification, and analysis of hydroxyproline-rich glycoproteins. Plant Physiology, pp. 110–156554.

Smith DK, Jones DM, Lau JBR, Cruz ER, Brown E, Harper JF, Wallace IS. 2018. A Putative Protein O-Fucosyltransferase Facilitates Pollen Tube Penetration through the Stigma-Style Interface. Plant Physiology 176, 2804–2818.

Sogo A, Tobe H. 2005. Mode of pollen-tube growth in pistils of *Myrica rubra* (Myricaceae): a comparison with related families. Annals of Botany 97, 71–77.

Sommer-Knudsen J, Lush WM, Bacic A, Clarke AE. 1998. Re-evaluation of the role of a transmitting tract-specific glycoprotein on pollen tube growth. The Plant Journal 13, 529–535.

Sprunck S, Rademacher S, Vogler F, Gheyselinck J, Grossniklaus U, Dresselhaus T. 2012. Egg cell–secreted EC1 triggers sperm cell activation during double fertilization. Science 338, 1093–1097.

Stegmann M, Monaghan J, Smakowska-Luzan E, Rovenich H, Lehner A, Holton N, Belkhadir Y, Zipfel C. 2017. The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. Science 355, 287–289.

Stührwohldt N, Dahlke RI, Kutschmar A, Peng X, Sun MX, Sauter M. 2015. Phytosulfokine peptide signaling controls pollen tube growth and funicular pollen tube guidance in *Arabidopsis thaliana*. Physiologia Plantarum 153, 643–653.

Su S, Higashiyama T. 2018. Arabinogalactan proteins and their sugar chains: functions in plant reproduction, research methods, and biosynthesis. Plant Reproduction 1–9.

Susaki D, Takeuchi H, Tsutsui H, Kurihara D, Higashiyama T. 2015. Live imaging and laser disruption reveal the dynamics and cell-cell communication during *Torenia fournieri* female gametophyte development. Plant and Cell Physiology 56, 1031–1041.

Takayama S, Isogai A. 2005. Self-incompatibility in plants. Annual Review of Plant Biology, 23.

Takeuchi H, Higashiyama T. 2012. A species-specific cluster of defensin-like genes encodes diffusible pollen tube attractants in Arabidopsis. PLoS Biology 10, e1001449.

Takeuchi H, Higashiyama T. 2016. Tip-localized receptors control pollen tube growth and LURE sensing in Arabidopsis. Nature 531, 245.

Tan HX, Liang WQ, Hu JP, Zhang DB. 2012. MTR1 Encodes a Secretory Fasciclin Glycoprotein Required for Male Reproductive Development in Rice. Developmental Cell 22, 1127–1137.

Tang WH, Kelley D, Ezcurra I, Cotter R, McCormick S. 2004. LeSTIG1, an extracellular binding partner for the pollen receptor kinases LePRK1 and LePRK2, promotes pollen tube growth in vitro. The Plant Journal 39, 343–353.

Vogler F, Schmalzl C, Englhart M, Bircheneder M, Sprunck S. 2014. Brassinosteroids promote Arabidopsis pollen germination and growth. Plant Reproduction 27, 153–167.

von Besser K, Frank AC, Johnson MA, Preuss D. 2006. Arabidopsis *HAP2* (*GCS1*) is a sperm-specific gene required for pollen tube guidance and fertilization. Development 133, 4761–4769.

Wang JG, Feng C, Liu HH, Feng QN, Li S, Zhang Y. 2017. AP1G mediates vacuolar acidification during synergid-controlled pollen tube reception. Proceedings of the National Academy of Sciences of the United States of America 114, E4877-E4883.

Wang T, Liang L, Xue Y, Jia PF, Chen W, Zhang MX, Wang Y-C, Li H-J, Yang WC. 2016. A receptor heteromer mediates the male perception of female attractants in plants. Nature 531, 241.

Woriedh M, Wolf S, Marton ML, Hinze A, Gahrtz M, Becker D, Dresselhaus T. 2013. External application of gametophyte-specific ZmPMEI1 induces pollen tube burst in maize. Plant Reproduction 26, 255-266.

Winship LJ, Obermeyer G, Geitmann A, Hepler PK. 2011. Pollen tubes and the physical world. Trends in Plant Science 16, 353-355.

Wu H, de Graaf B, Mariani C, Cheung AY. 2001. Hydroxyproline-rich glycoproteins in plant reproductive tissues: structure, functions and regulation. Cellular and Molecular Life Sciences 58, 1418–1429.

Yadegari R, Drews GN. 2004. Female gametophyte development. The Plant Cell 16, S133–S141.

Yang J, Sardar HS, McGovern KR, Zhang YZ, Showalter AM. 2007. A lysine-rich arabinogalactan protein in Arabidopsis is essential for plant growth and development, including cell division and expansion. The Plant Journal 49, 629–640.

Yu GH, Zou J, Feng J, Peng XB, Wu JY, Wu YL, Palanivelu R, Sun MX. 2014. Exogenous gamma-aminobutyric acid (GABA) affects pollen tube growth via modulating putative Ca^{2+}-permeable membrane channels and is coupled to negative regulation on glutamate decarboxylase. Journal of Experimental Botany 65, 3235–3248.

Zhang Y, McCormick, S. A distinct mechanism regulating a pollen-specific guanine nucleotide exchange factor for the small GTPase Rop in *Arabidopsis thaliana*. 2007. Proceedings of the National Academy of Sciences of the United States of America 104, 18830–18835.

Figures

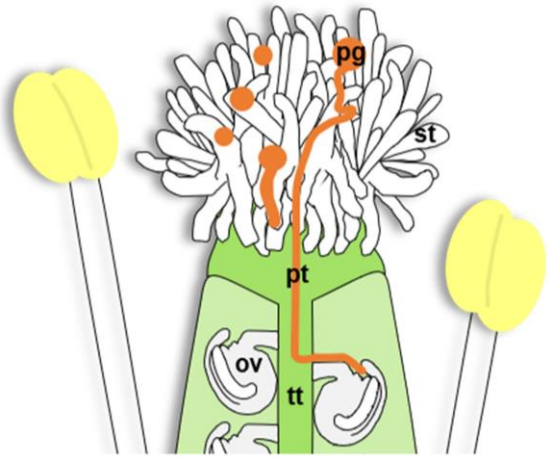
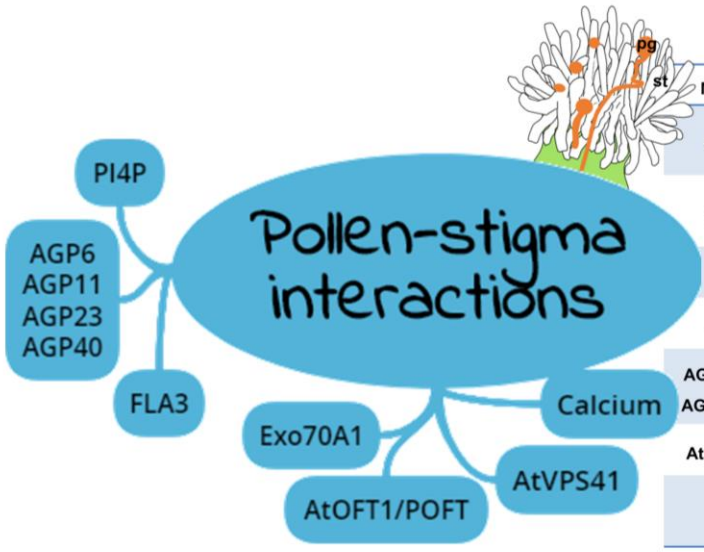


Figure 1. Schematic representation of the treasure hunt scenario - an Arabidopis pistil. st, stigma; ov, ovules; pg, pollen grain; pt, pollen tube.



Molecules	Functions
Exo70A1	Pollen hydration and PT growth
AtvPS41	Recycling molecules via the endocytic pathway - PT penetration in TT
PI4P	Pollen hydration, successful pollination
Calcium	PT formation and polarized growth
AGP6, AGP11, AGP23, AGP40	PT endossome machinery
AtOFT1/POFT	PT penetration and growth
FLA3	Microspores development, cellulose deposition

Figure 2. The players of pollen-stigma interactions and the clues for their roles.

Molecules	Functions
Chemocyanin	Chemotropism of the PT
SCAs	PT growth control and direction
LTP5	PT orientation
STIG1 – LePRK2- PI3P	PT growth promotion
Brs and GABA	PT growth and PT signaling pathways regulation

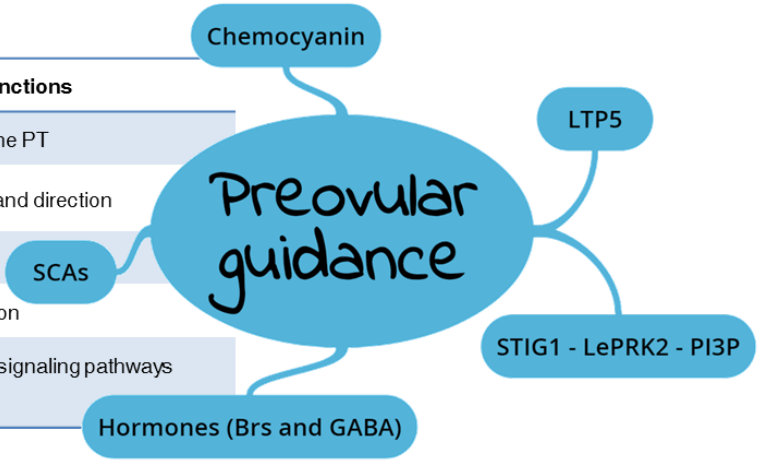


Figure 3. The players involved in the preovular guidance stage and the clues for their roles.

Molecules	Functions
TTS, NOS-NO, SPIK, VGD1, MRI, MSL8	PT growth and orientation
NTT, HEC, HAF, SPT, AtWSCP	TT development and PT growth
AGP- Calcium	PT growth and orientation
AGP1, AGP4, AGP19, AGP12, AGP15	PT growth, being highly expressed on the TT
AMOR	PT competence induction

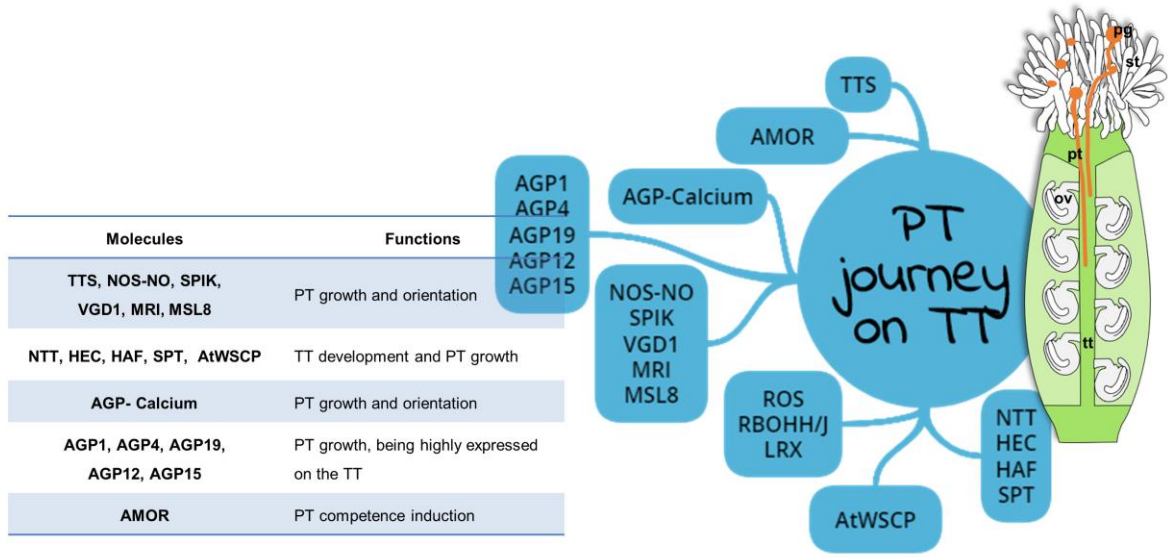
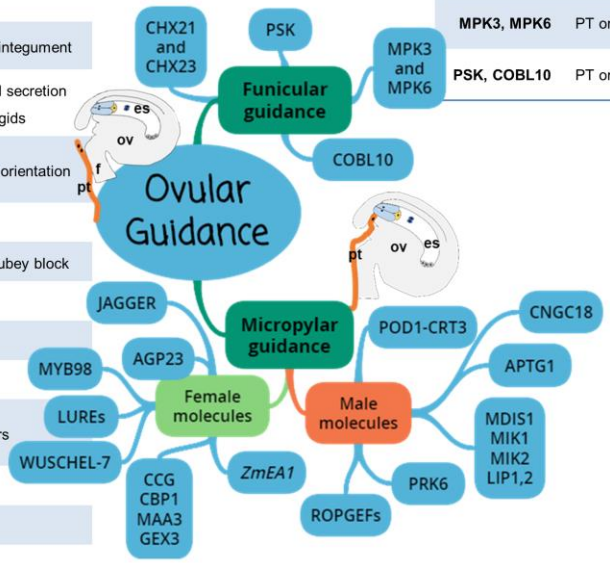


Figure 4. The players of PT navigation along the TT and the clues for their roles.

Micropylar Guidance	
Female	
Molecules	**Functions**
WUSCHEL-7	PT guidance influence by the inner integument
MYB98	Filiform apparatus development and secretion of attraction molecules by the synergids
LUREs , MAA3, GEX3	PT attractive molecules, mycropilar orientation
ZmEA1	Micropylar orientation in maize
AGPs (AGP4)	Persistent synergid death and polytubey block
CCG, CBP1	Micropylar guidance
Male	
Molecules	**Functions**
MDIS1, MIK1, MIK2, LIP1, LIP2	LUREs direct and indirectly receptors
PRK (PRK6)	PT elongation and attraction
ROPGEFs	PT growth
CNGC18	PT growth and guidance
POD1 – CRT3	PT membrane proteins folding and regulation of their signaling
APTG1	PT orientation and growth



Funicular guidance	
Molecules	**Functions**
CHX21, CHX23	Ovule's signals perception
MPK3, MPK6	PT orientation
PSK, COBL10	PT orientation through the TT

Figure 5. The players involved in the ovular guidance stage, divided in funicular and micropylar guidance steps, and the clues for their roles.

PT-synergids	
Female	
Molecules	**Functions**
FER/SRN	PT synergid reception, PT growth arrestment, correct rupture and gametes release
LRE-LLG1	FER interactor and PT rupture regulation
NTA	Ca^{2+} oscillations perception in the synergids
HERK1 and ANJEA	LRE interactor, mediating PT reception
AP1G	Synergid degeneration delay
VDD and VAL	PT and receptive synergid death
ENODLs, TUN	PT reception
RALF34	PT rupture
Male	
Molecules	**Functions**
MYB97, MYB101, MYB120	PT reception and gene expression regulation
ANX1, 2	PT breakdown and gametes release inhibitor, during PT growth
ANTH	PT tip localization of ANX receptors
RALF4, 19	PT growth
BUPS1, 2	ANX interactor and early PT rupture inhibition
ERU	Functional apical $[Ca^{2+}]_{cyt}$ gradient establishment and PT targeting regulation
ACA9	PT growth and synergid rupture

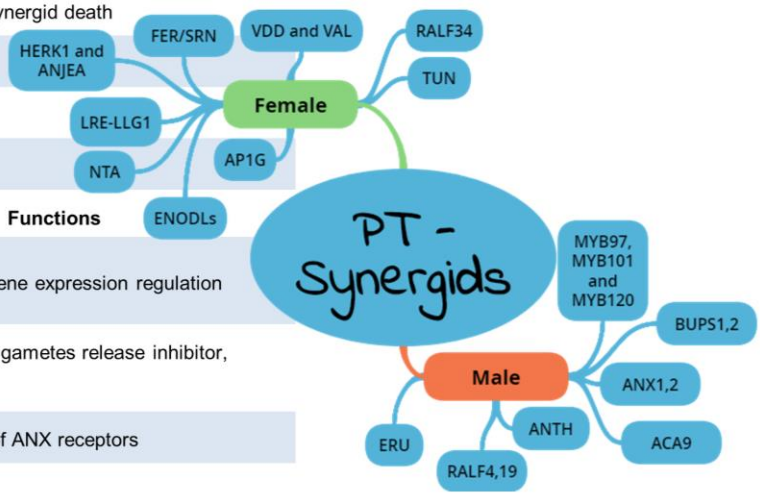
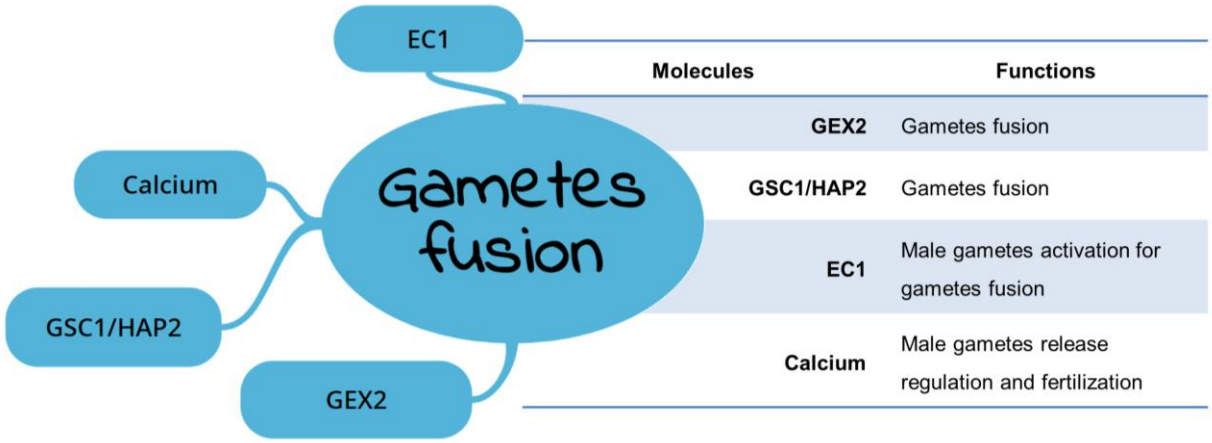


Figure 6. The players involved during the PT- synergids interaction, and the clues for their roles.



Molecules	Functions
GEX2	Gametes fusion
GSC1/HAP2	Gametes fusion
EC1	Male gametes activation for gametes fusion
Calcium	Male gametes release regulation and fertilization

Figure 7. The players involved during gametes fusion interaction, and the clues for their roles.