Plant Reprod
DOI 10.1007/s00497-015-0272-4

REVIEW

Male gametophyte development and function in angiosperms: a general concept

Said Hafidh[1] · Jan Fíla[1] · David Honys[1,2]

Received: 12 September 2015 / Accepted: 19 December 2015

Key message **Overview of pollen development.**

Abstract Male gametophyte development of angiosperms is a complex process that requires coordinated activity of different cell types and tissues of both gametophytic and sporophytic origin and the appropriate specific gene expression. Pollen ontogeny is also an excellent model for the dissection of cellular networks that control cell growth, polarity, cellular differentiation and cell signaling. This article describes two sequential phases of angiosperm pollen ontogenesis—developmental phase leading to the formation of mature pollen grains, and a functional or progamic phase, beginning with the impact of the grains on the stigma surface and ending at double fertilization. Here we present an overview of important cellular processes in pollen development and explosive pollen tube growth stressing the importance of reserves accumulation and mobilization and also the mutual activation of pollen tube and pistil tissues, pollen tube guidance and the communication between male and female gametophytes. We further describe the recent advances in regulatory mechanisms involved such as posttranscriptional regulation (including mass transcript storage) and posttranslational modifications to modulate protein function, intracellular metabolic signaling, ionic gradients such as Ca^{2+} and H^{+} ions, cell wall synthesis, protein secretion and intercellular signaling within the reproductive tissues.

Keywords Pollen development · Male gametophyte · Pollen tube growth · Flowering plants

Communicated by Enrico Schleiff.

A contribution to the special issue 'Pollen development and stress response'.

Electronic supplementary material The online version of this article (doi:10.1007/s00497-015-0272-4) contains supplementary material, which is available to authorized users.

✉ David Honys
david@ueb.cas.cz

[1] Institute of Experimental Botany ASCR, v.v.i., Rozvojová 263, 165 00 Prague 6, Czech Republic

[2] Department of Experimental Plant Biology, Faculty of Science, Charles University in Prague, Viničná 5, 128 44 Prague 2, Czech Republic

Introduction

Reproduction is a characteristic feature of living organisms ensuring the continuity of life on earth through a series of successive generations. In plants, there is an alternation of sexual and asexual generations, sporophyte and gametophyte. In the sporophyte, the individuals consist of diploid cells and reproduce asexually through haploid spores generated by the meiotic division in sporogenous tissue. The spores germinate into the gametophyte, where specialized reproductive organs, female archegonia and male antheridia are formed in which haploid gametes of separate sexes arise. The fusion of male and female gametes leads to the formation of a diploid zygote, the first cell of the next sporophyte generation. In the evolutionary line of vascular plants (*Tracheophyta*), gametophytic reduction and increased functional dependence on the sporophyte is apparent. Angiosperms, which currently account for more than 280,000 species (reviewed by Scotland and Worltey 2003), constitute the overwhelming majority of plant species with maximum reduction of the gametophyte. This reduction together with protection of the reproductive organs within a flower and stringent selection of the fittest pollen to

Published online: 04 January 2016

reproduce is considered to be the main cause of the evolutionary success of angiosperms (Mulcahy 1979; Mulcahy et al. 1996). Most angiosperms form bisexual flowers containing anthers and pistils, where both male and female gametophytes develop. Both structures—pollen grain and ovule—are microscopic, consisting of only a few cells fully supported by the surrounding sporophytic tissues and organs for their development. The only time, when gametophytes of flowering plants exist independently of the sporophyte, is when mature pollen is shed from the anther and is carried to the stigma to undergo reproduction with female gametes.

In the past, the small size of the male gametophyte made its exploration—and even its discovery—very complicated. Therefore, the external appearance of pollen grains stood at the beginning of pollen research (Malpighi 1675, 1679; Purkyně 1830). Further knowledge of the pollen and pollen tube structure and function was gradually acquired together with the other basic phenomena associated with the sexual reproduction of angiosperms, such as the existence of bi- and tri-cellular pollen (Elfving 1879), differentiation of vegetative and generative cells (Strasburger 1884) and the double fertilization (Nawaschin 1898). Subsequent considerable progress in pollen research has been driven by the rapid development of cytological and molecular methods. Equally important was the introduction of in vitro, semi in vivo and in vivo techniques and in particular the onset of "high-throughput" technologies in the recent 20 years.

Male gametophyte

Male gametophyte development of angiosperms is a complex process that requires coordinated activity of different cell types and tissues of both gametophytic and sporophytic origin underlaid by specific gene expression patterns. Male gametophyte development is comprised of two consecutive phases, developmental and functional.

The developmental phase takes place in anther loculi and leads to the release of mature pollen grains from the anthers. It is characterized by the functional specialization of two cell types—vegetative cells and male gametes, the sperm cells, which together represent the male germline (reviewed by Berger and Twell 2011). This process is underpined by two successive cell divisions accompanied by remarkable morphological and physiological differentiation of both cell types including the synthesis of specialised pollen cell wall and the storage of protective substances and metabolic reserves.

The functional or progamic phase is initiated after the pollen grain lands on the stigma. Pollen is activated by rehydration, germinates and produces a long pollen tube that grows into the pistil tissues. Its growth ends with a double fertilization after reaching the ovule. It is not only the pollen tubes explosive growth that makes the progamic phase interesting but also the mutual activation of pollen tube and pistil tissues, pollen tube guidance and the communication between the two gametophytes (reviewed by Dresselhaus and Franklin-Tong 2013).

Unlike the sporophyte, the male gametophyte represents highly reduced, two- or three-celled model system providing a unique opportunity to study the developmental regulation of cell morphogenesis and differentiation at many levels as well as the functional interactions between different cell types (Berger and Twell 2011; Borg et al. 2009; Dresselhaus and Franklin-Tong 2013; Dresselhaus and Sprunck 2012; Hafidh et al. 2014; Kessler and Grossniklaus 2011; Ma 2005; Rutley and Twell 2015; Twell 2011).

Pollen maturation: get ready for the race!

Diploid microspore mother cells (microsporocytes) are encapsulated in the young anther loculi surrounded by four cell layers—tapetum, middle layer, endothecium and epidermis. The microsporocytes secrete cell wall materials consisting of β-1,3-glucan and callose. After two meiotic divisions, the microsporocytes divide into four haploid microspores forming a tetrad (Fig. 1). In *Avena sativa*, microspore mother cells communicate via cytoplasmic bridges enabling the synchronization of meiotic divisions throughout the loculus (Brett and Waldron 1990). The second meiotic division is followed by the synthesis of callose walls between the individual microspores within a tetrad. However, the timing of callose wall formation differs between different plant species (Chen and Kim 2009; De Storme and Geelen 2013; Lu et al. 2014).

During meiosis, the secretory tapetal cells differentiate into binuclear polar cells lacking the primary cell wall, especially at the loculi-facing side. These cells contain abundant ribosomes, mitochondria, endoplasmic reticulum, Golgi apparatus and specialized lipid-rich organelles, tapetosomes (Bedinger 1992; Hsieh and Huang 2005; Ting et al. 1998), close to the plasma membrane facing the anther loculi. The tapetal cells are interconnected by cytoplasmic bridges allowing the coordination of their activities. Transcriptomic studies of anther tissues in various plant species demonstrated the precise control of the activity and subsequent programmed cell death of tapetal cells and the tight coordination of these processes with pollen development (Huang et al. 2011).

Young microspores in tetrads undergo rapid development accompanied by synthesis of the cell wall consisting of an inner intine and outer exine. After the emergence of partially formed exine, microspores are released from tetrads in a synchronised manner (Fig. 1). The release of the microspores is prompted by the activity of an enzyme

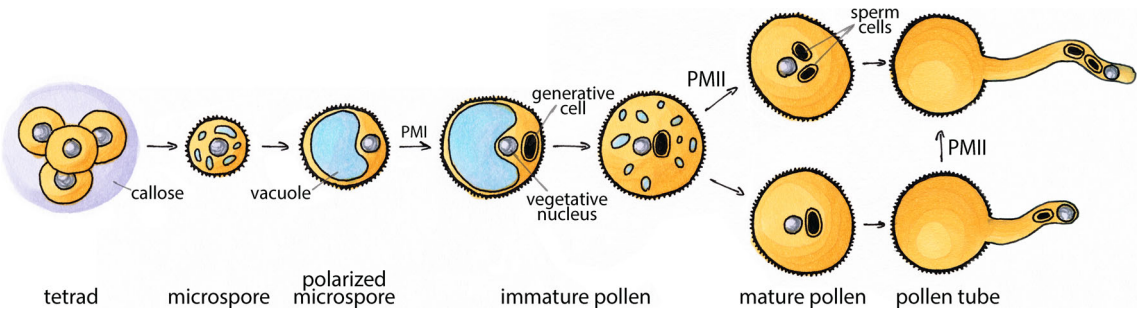

Fig. 1 Schematic diagram describing pollen development (adapted and simplified from Honys et al. 2006)

mixture secreted by tapetal cells, with callase (β-1,3-glucanase) being its essential component responsible for the callose degradation (Lu et al. 2014; Scott et al. 2004). Callase synthesis was shown to be controlled by MYB-family transcription factor AtMYB80 that links pollen maturation with tapetum development resulting in programmed cell death of tapetal cells (Phan et al. 2011; Zhang et al. 2007). Proper timing of callase secretion is one of the critical moments in microsporogenesis and its distortion causes male sterility (Worrall et al. 1992). Free microspores rapidly enlarge and numerous small vacuoles eventually merge into one large vacuole pushing the microspore nucleus from its central position to the cell periphery. The actual microspore polarisation is not just a passive event caused by the growth of vacuoles but a highly dynamic process requiring the active participation of microtubules (Oh et al. 2010; Park et al. 1998; Twell 2011). During microsporogenesis, the tapetal cells remain highly metabolically active. They secrete proteins, lipids, carbohydrates and secondary metabolites to the loculi to be used by developing microspores to synthesize membranes, for exine formation and as a source of energy (Pacini 1990). Despite the obvious importance of tapetum, it is possible to achieve the proper maturation of functional pollen in vitro from uninuclear microspores in the presence of essential nutrients (Tupý et al. 1991).

The highly specialised biological role of angiosperm pollen is reflected by the unique composition of the surrounding cell wall. The sculptured pollen wall not only protects the male gametophyte and its precious cargo but facilitates the broad communication with the stigma surface (Scott et al. 2004). Its inner layer, intine, is of gametophytic origin whereas the outer layer, exine, is mostly sporophytic. The synthesis of pollen wall begins already in tetrads, immediately after the completion of meiosis. Microspores in tetrads first synthesize the pectin-cellulose primexine functioning as a matrix for the deposition of sporopollenin precursors preceding their subsequent polymerization. As a complex compound of fatty acids and phenylpropanoids, sporopollenin belongs among the toughest known biopolymers and its role is the protection of the pollen internal environment (including genetic information) after pollen shedding from the anthers. Although the biochemical pathway of sporopollenin synthesis remains elusive, its synthesis requires close cooperation between microspores and tapetum (Ariizumi and Toriyama 2011; Dobritsa et al. 2009, 2010; Quilichini et al. 2015). The recalcitrant exine is not distributed evenly around pollen grains; it is not deposited or it is reduced in the apertures, where pollen tubes emerge (Furness and Rudall 2004). The number, size and distribution of apertures are an important classification factor that is under strict sporophytic control. In Arabidopsis, aperture marking depends on the prior localisation of INAPERTURATE POLLEN1 protein (Dobritsa and Coerper 2012; Dobritsa et al. 2011).

Formation of the pollen coat is completed in the later stages of microgametogenesis when the residues of the degenerating tapetum are deposited on the surface of pollen grains (Quilichini et al. 2015). Pollen coat determines the pollen adhesiveness, colour, taste and aroma. These properties as well as the often highly elaborated structure of the pollen wall are species-specific. This is of key importance not only for pollen interaction with papillary cells on the stigma but also to facilitate its recognition by pollinators. Insect- and other animal-pollinated species shed pollen that can be decorated with extremely complex surface structures facilitating its adhesion to the pollinators whereas in wind-pollinated species these structures are often absent (Fellenberg and Vogt 2015).

Polarised microspores undergo an asymmetric division during pollen mitosis I (PMI, Fig. 1) resulting in the formation of two unequal daughter cells with distinct cell fates, a large vegetative cell and a small generative cell. Both cells are present in the space bound by the microspore cell wall. The generative cell soon migrates into the vegetative cell to form a unique "cell-within-a-cell" structure (Russell and Jones 2015; Russell et al. 1996). PMI represents another critical moment in the male gametophyte development; it ensures the fixation of the ongoing male gametophytic developmental program, as demonstrated in

various plant species by transcriptomic (Bokvaj et al. 2015; Honys and Twell 2004; Wei et al. 2010) and proteomic studies (Chaturvedi et al. 2013; Grobei et al. 2009; Holmes-Davis et al. 2005; Ischebeck et al. 2014; Noir et al. 2005; Sheoran et al. 2006). Therefore the reversal from the gametophytic to the sporophytic development is achievable only with unicellular microspores but not with bicellular pollen grains (Gaillard et al. 1991). PMI also results in the initiation of the male germline; the generative cell retains its proliferative activity and divides once more during pollen mitosis II (PMII) to produce two male gametes, the sperm cells (Berger and Twell 2011; Twell 2011). The disruption of the asymmetry of PMI either by centrifugation (Terasaka and Niitsu 1987), by the application of microtubule-destabilizing agents, e.g. colchicine (Eady et al. 1995; Zaki and Dickinson 1991), or in particular pollen mutants, e.g. MOR1/GEM1 (Park et al. 1998), leads to the formation of two similar cells with the vegetative cell fate. Arabidopsis *MOR1/GEM1* (Park et al. 1998; Twell et al. 2002) and its tobacco ortholog *TMBP200* (Oh et al. 2010) encode microtubule-associated proteins, plant orthologs of the MAP215/Dis1 protein family. Phenotype defects resulting from the knock-out of the above genes clearly demonstrated the importance of microtubules in the establishment of cellular polarity preceding PMI, asymmetry of which is the key factor in the initiation of male germline (Twell 2011).

The generative cell undergoes yet another mitotic division—PMII—resulting in the formation of two sperm cells. The vegetative nucleus remains in a close physical connection with the two sperm cells after PMII forming the male germ unit (MGU). It plays the vital role not only in the delivery of sperm cells to the female gametophyte, but especially in direct communication between the cells and their nuclei of somatic origin and the germline (McCue et al. 2011) perhaps including the modulation of their gene expression (Slotkin et al. 2009).

The PMII can occur before or after pollen maturation and therefore the mature pollen grain can be shed as bicellular (in which the vegetative cell engulfs a single undivided generative cell) or as tricellular (where the vegetative cell is associated with two sperm cells) (Brewbaker 1967). From an evolutionary point of view, the bicellular pollen is a plesiomorphy trait whereas the tricellular pollen represents an advanced trait. However, it was postulated recently that a reverse transition from tricellular to bicellular pollen has also occurred (Williams et al. 2014). Thus, some species sheding bicellular pollen underwent two evolutionary changes rather than keeping the original trait permanently (Williams et al. 2014). The mature pollen grain has a dehydrated cytoplasm; bicellular pollen was usually shown to be in a more dehydrated and quiescent state than tricellular pollen. This assumption was based on the observation that tricellular pollen was usually quicker in the onset of pollen germination and subsequent tube growth (Brewbaker 1967). Furthermore, the basal angiosperm *Annona cherimola* was shown to shed both bicellular and tricellular pollen in different ratios according to the surrounding air temperature (Lora et al. 2009). In a higher temperature (30 °C), almost half of the pollen grains were tricellular whereas at lower temperature (20 °C), the bicellular pollen grains strongly prevailed. It was speculated that in warmer weather, a higher proportion of tricellular pollen was biologically relevant since it allowed faster germination and fertilization that was beneficial since stigma tissues were shorter-lived in a warmer climate. On the other hand, at lower temperature a higher proportion of bicellular pollen can successfully be more widely dispersed due to its higher dehydration and longer life span. This effect is further supported by a longer life span of pistils under milder temperatures around 20 °C (Lora et al. 2009).

Progamic phase: catch me if you can

As pollen grain reaches the papillary cells of the stigma, it is rehydrated and activated (Firon et al. 2012). Under in vitro conditions, Arabidopsis pollen does not increase in volume during the first 21 min of activation (Vogler et al. 2015). The period of no apparent pollen grain enlargement is even longer in more dehydrated bicellular pollen (Barnabas and Fridvalszky 1984). Then, the pollen tube emerges and starts growing with gradually increasing speed (Vogler et al. 2015). The pollen tubes growth rate reaches up to 1 cm per hour, placing them among the fastest-growing plant cells (Lim and Gumpil 1984). The pollen tube growth dynamics and its dependence on stored nutrition reserves differs between studied bicellular and tricellular pollen species (Mulcahy and Mulcahy 1988). Bicellular pollen tube growth consists of two phases—the first growth period is relatively slow with no formation of callose plugs. The second growth period showed more rapid growth characteristics accompanied by callose plugs formation (Fig. 2c). Growth during the first period consumed the reserves carried by the pollen grain itself whereas the second phase mostly relied on nutrition from the style. On the other hand, tricellular pollen started to grow quickly together with callose plug formation that relied on stylar nutrition from the beginning (Mulcahy and Mulcahy 1988).

Pollen tube growth is not isodiametric but together with root hairs, fungal hyphae and vertebrate axons represents an example of tip growth (Palanivelu and Preuss 2000; Šamaj et al. 2006). It is characterized by continuous elongation at one tip of the cell without any further

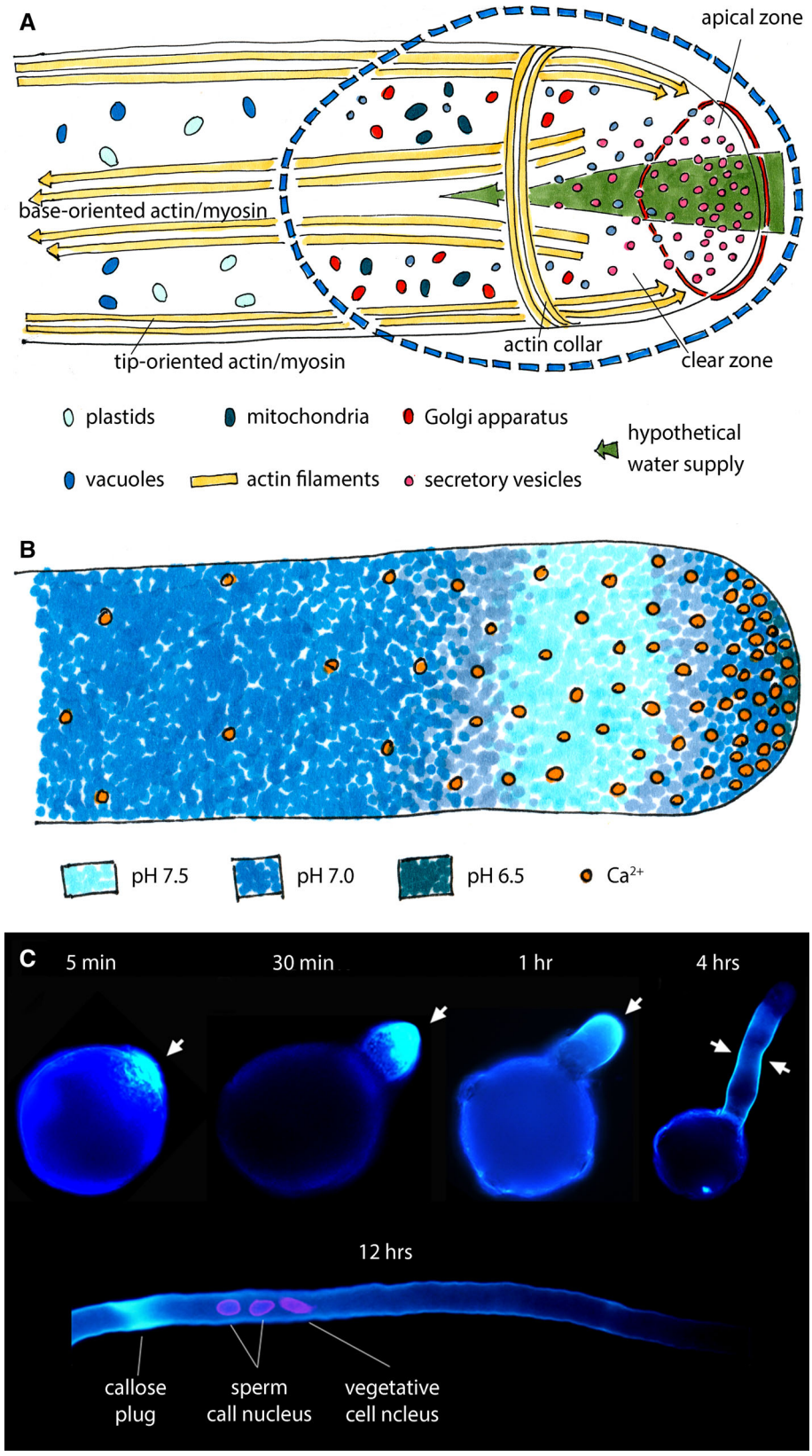

Fig. 2 Pollen tube apical region. **a** Scheme of the lily pollen tube tip showing actin cytoskeleton dynamics and pollen tube zonation (adapted from Hepler and Winship 2015); individual structures are not drawn to scale). **b** Ca^{2+} and H^{+} gradients in lily pollen tube apex (adapted from Hepler and Winship 2015); individual structures are not drawn to scale). **c** Distribution of callose (*arrow*) in activated tobacco pollen grains and during pollen tube germination observed by aniline blue staining. Callose plugs are only visible >4 h post germination (Hafidh et al. unpublished data)

divisions of the vegetative cell. Pollen tubes can reach a maximum of 50 cm (Mascarenhas 1993) in plant species with long styles, for example maize. Tip growth requires several mechanisms that are evolutionary conserved in various tip-growing tissues of different organisms: cytoskeleton organization, vesicular trafficking, small GTPases signalling and ion gradient formation (Palanivelu and Preuss 2000; Šamaj et al. 2006). Moreover, novel formation of cell wall is required in the growing pollen tubes since they usually outgrow the diameter of the pollen grain several-fold. This is achieved by the formation of callose plugs in regular distances to maintain constant amount of cytoplasm required for filling the inside space of the pollen tube (Ferguson et al. 1998; Mogami et al. 2006).

From this point of view, pollen tube contains several zones. The tip-most zone is called clear zone as it looks "clear" under the microscope because the organelles present there have quite low refractivity—in particular, the starch-containing amyloplasts are missing from this part of pollen tube (Hepler and Winship 2015; Vidali et al. 2001). This clear zone comprises two distinct regions, apical and subapical (Fig. 2a). The apical region is typical for its inverted cone-shape, in which endoplasmic reticulum elements and vesicles are present. On the other hand, the subapical region of the clear zone contains also Golgi apparatus and mitochondria. Behind the clear zone, there are also larger organelles, such as amyloplasts and vacuoles (Lancelle and Hepler 1992). The refractivity of this region is also higher than that of the clear zone so its appearance is quite different in both light and electron microscopes (Hepler and Winship 2015).

Fast pollen tube growth and overall morphology is underlaid by the organization and function of the cytoskeleton. Starting behind the subapical zone of lily pollen, actin filaments are organized in parallel bundles (Staiger et al. 2010; Wilsen et al. 2006). The cortical filaments transport vesicles towards the tip whereas the central filaments are dedicated for the basipetal vesicular transport (Fig. 2a). Thus, the vesicular flow resembles a reversed fountain. Myosins, the motor molecules along the actin filaments, move towards the barbed ends of microfilaments. In order to allow the transport in the desired direction, the cortical and central filaments are oriented the opposite way: the central ones have their barbed ends facing the tube tip whereas the cortical filaments face the tip by their pointed ends (Lenartowska and Michalska 2008). Near the border between pollen tube apical and subapical regions an actin collar is formed and its position is likely to be regulated by a higher pH 7.5 and Ca^{2+} gradient. In comparison, pH at the very tip of the pollen tube reaches 6.5 (Feijó et al. 2004). These two factors (pH and Ca^{2+} concentration) control actin polymerization together with actin-binding proteins activity (see below). In the apical region, actin filaments are randomly oriented so they are organized into a net structure. This tip-most region is shaped as a reversed cone and beside the net structure of actin filaments; it typically contains vesicles (Hepler and Winship 2015). The vital importance of this actin net structure in the apical zone was demonstrated by the application of latrunculin B that strongly impaired pollen tube growth and morphology (Vidali et al. 2001). Latrunculin B binds to the actin monomers and blocks them from polymerization whilst the stable actin filaments remain unimpaired by latrunculin B treatment. The role of microtubules in the growing pollen tubes was thought to be less important and originally it seemed that the organelles and vesicles were mainly transported along actin filaments since the colchicine application did not stop pollen tube growth (Heslop-Harrison et al. 1988). However, later, mitochondria were reported to be transported along microtubules in pollen tubes grown in vitro (Romagnoli et al. 2007) and tubulin was shown to be part of RNA storage particles (Honys et al. 2009). Recently, a more prominent role of microtubules in pollen tubes (for instance in vesicular transport) was reported (Onelli et al. 2015). Microtubules are therefore likely to attract more attention in the future pollen tube studies.

The distribution of pollen tube organelles as well as the organization of pollen tube growth rely also on ion fluxes and gradients. Among them, calcium ions (Ca^{2+}) and protons (H^{+}) are of a key importance (Michard et al. 2009). Ca^{2+} ions show a gradually increasing concentration towards the pollen tube tip, a distribution usually called "tip-focused gradient" (Fig. 2b). In the pollen tube apex, the Ca^{2+} concentration reaches 1–10 μM concentration, ten- to hundredfold higher than in the pollen tube shank with Ca^{2+} concentration ranging between 0.1 and 0.3 μM (Holdaway-Clarke et al. 1997). An increased Ca^{2+} concentration in the pollen tube apex can serve as a signal for Ca^{2+}/calmodulin protein kinases, some of which were identified in pollen trancriptome and/or proteome (Supplementary Table 1 and the section below). The Ca^{2+} gradient is also crucial for the regulation of actin cytoskeleton dynamics since Ca^{2+} ions activate villin/gelsolin, which promotes the destabilization of actin filaments (Ren and Xiang 2007). Moreover, the activity of profilin in monomer actin sequestration is promoted by Ca^{2+} (Kovar et al. 2000). Thus, these two proteins collectively destabilize actin filaments and prevent its re-polymerization in the tip-most region of the pollen tube. It should be stressed once more that the increased Ca^{2+} concentration is only at the pollen tube tip since further from the tip, the excessive Ca^{2+} ions are sequestered by endoplasmic reticulum and mitochondria and the Ca^{2+} concentration is kept at the normal physiological values (reviewed in Hepler and Winship 2015). Another process that is promoted by an

elevated Ca^{2+} concentration is exocytosis (Camacho and Malho 2003).

The proton gradient is formed in a similar way—protons enter the pollen tube cytoplasm via proton pumps at the tip where there is a slightly acidic pH (pH 6.5). The distal band in the clear zone remains, on the other hand, slightly alkaline (pH 7.5; Feijó et al. 2004) (Fig. 2b). Interestingly, this apical acidic pH area resembles the reverse-cone shape discussed above (Michard et al. 2008). Similar to calcium ions gradient, the pH variation contributes to the actin cytoskeleton dynamics via actin-binding proteins. The slightly alkaline pH in the subapical region promotes ADF/cofilin complex that cuts the actin filaments to more parts (Allwood et al. 2002; Chen et al. 2002; Lovy-Wheeler et al. 2006; Staiger et al. 2010) and thus promotes actin dynamics in the cortical region of pollen tube. In the shank and at the pollen tube tip, there is neutral pH 7.0 or slightly acidic pH 6.5, respectively, under which ADF/cofilin is inactive.

Taken together, the ion fluxes and gradients are not static in a growing pollen tube but exhibit regular oscillations (Feijó et al. 1999; Holdaway-Clarke et al. 1997; Shi et al. 2009). These gradient oscillations together with cytoskeleton dynamics are reflected in pollen tube growth. For example, growth rate of in vitro cultivated lily pollen tubes oscillates between 0.1 and 0.4 $\mu m\ s^{-1}$ within 15–50 s (Pierson et al. 1996). All of the above mentioned factors seem to be coordinated but a general pacemaker is still not known (Michard et al. 2009).

Both cytoskeleton dynamics and ion gradient oscillations tightly co-operate with the activity of small GTPases from the Rab, Arf and Rop/Rac families that play a key role during vesicular transport and other processes in the growing pollen tube (Šamaj et al. 2006). These small G-proteins have two states, the active form binding GTP and the inactive form bound to GDP (Bishop and Hall 2000). Moreover, the activity of the small GTPases is regulated by three main classes of protein factors—guanine nucleotide exchange factors (GEFs), GAFs and GDIs. GEFs promote the activity of small GTPases by exchanging GDP for GTP. The GTPases inactivate themselves by their GTPase activity, and this inactivation can be accelerated by GTPase accelerating factors (GAFs) that promote the GTPase activity of G-proteins. Finally, guanine nucleotide dissociation inhibitors (GDIs) conserve the G-protein in the GDP-bound form and thus block GTPase reactivation (Feher and Lajko 2015).

Arf GTPases are involved in the vesicular transport and localize to both endosomes and Golgi apparatus. Mutations directly affecting Arf GTPases themselves were not described in pollen tube functional studies. However, several experiments showed their roles during pollen tube growth by analysing *gnom* (Arf GEF) mutants or by application of brefeldin A that acts as a GNOM inhibitor (Zhang and McCormick 2010). Rab proteins are associated with endomembranes and show usual specificity to particular parts of the endomembrane system (Woollard and Moore 2008). RAB11B in tobacco and RABA4D in Arabidopsis were shown to be required for a correct pollen tube growth (de Graaf et al. 2005; Szumlanski and Nielsen 2009). Not surprisingly, these small GTPases were important for pollen tube growth since they are vital for exocytosis of various compounds such as cell wall precursors, membrane components and signalling molecules which promote pollen tube growth (Qin and Yang 2011). Rop GTPases with bound GTP are usually localized in the apical plasma membrane. They execute several important functions such as organization of the actin cytoskeleton. They generate reactive oxygen species, and mediate calcium-dependent signaling (Zheng and Yang 2000; Šamaj et al. 2006). ROP1 was discovered in apical membranes of tobacco and lily pollen tubes (Fu et al. 2001; Zhao and Ren 2006). Not only the GTPases themselves but also their regulating proteins such as RhoGDI were shown to play important roles in pollen tube growth (Klahre et al. 2006); they are integrated into the cellular signalling network of kinases acting both upstream and downstream the Rop GTPases (Feher and Lajko 2015).

Reserves storage and mobilization: shop till you drop

The vegetative cell of the immature pollen grain contains a dense cytoplasm with numerous organelles. The maturing pollen grain shows considerable metabolic activity and the vegetative cell accumulates a considerable amount of various metabolic reserves including carbohydrates, proteins and lipids necessary for the rapid growth of the pollen tube (Pacini 1996; Pacini et al. 2006). A specific portion of reserves comprises osmoprotectants, e.g. disaccharides, proline and glycinebetaine, protecting cellular membranes and proteins from damage caused by dehydration (Schwacke et al. 1999). The generative cell inherits from the microspore a very small portion of the cytoplasm and organelles. Whereas the generative nucleus contains highly condensed chromatin, the larger vegetative nucleus with numerous pores exits the cell cycle in G1 phase and contains decondensed chromatin. Therefore higher transcriptional activity of the vegetative nucleus in comparison to the generative cell can be assumed; however, that is not negligible either (Borges et al. 2008).

From PMI to the maturity, pollen accumulates both mRNA and proteins (Hafidh et al. 2011; Honys et al. 2009). In this period, pollen volume doubles, the amount of total RNA increases seven times and mRNA content increases

thirteen to twenty times (Schrauwen et al. 1990; Tupý 1982). Pioneering experiments using transcription and translation inhibitors in tobacco pollen tubes showed that transcription was detected only during the first several hours of the pollen tube growth, which led to the conclusion that pollen tube growth was mainly dependent on translation but virtually independent of transcription (Čapková et al. 1988). However, the introduction of high-throughput technologies led to the identification of numerous genes transcribed specifically after pollen germination in vitro in all species studied—Arabidopsis (Qin et al. 2009; Wang et al. 2008), rice (Wei et al. 2010) and tobacco (Hafidh et al. 2012a, b). Moreover, many transcripts were *de novo* synthesized even in the tobacco pollen tubes cultivated as long as 24 h (Hafidh et al. 2012a). Interestingly, the interaction of the pollen tube with the pistil tissues, during which pollen tubes gained the competence for fertilization (Higashiyama et al. 1998; Palanivelu and Preuss 2006), activated a specific set of 1254 genes that were not detected in in vitro cultivated pollen tubes (Qin et al. 2009). Moreover, 383 of these genes were pollen-enriched. De novo expression of genes involved predominantly in signal transduction, transcription and pollen tube growth in pistil-activated pollen tubes suggested the possibility of a female-responsive regulatory network orchestrating pollen tube gene expression upon growth through the pistil (Qin et al. 2009). A set of pistil-activated genes required for pollen tube differentiation and sperm cells release was later found to be controlled by three SIV pollen-tube expressed related MYB transcription factors—MYB97, MYB101 and MYB120 (Leydon et al. 2013).

In tobacco, stored transcripts were shown to be associated with large translationally silent ribonucleoprotein particles (EPP complexes; Honys et al. 2000). EPP complexes are associated with the cytoskeleton and contain small and large ribosomal subunits (Honys et al. 2009). Upon pollen activation and during subsequent pollen tube growth, the transcripts stored in EPPs are de-repressed and translated (Honys et al. 2009). Furthermore, EPP particles are likely to be transported towards the tip of the growing pollen tube and could represent a transport form of the transcripts originating from mature pollen. The translation itself leads to production of native polypeptides that undergo a plethora of possible post-translational modifications (PTMs) to form a functional mature protein product. These include phosphorylation, methylation, glycosylation, myristoylation, acetylation etc. (Knorre et al. 2009) that are usually essential for the proper protein structure and function. Here we will focus in more detail on protein glycosylation and phosphorylation since these modifications have been studied most intensely in the male gametophyte.

Protein glycosylation is a co-translational or post-translational covalent attachment of carbohydrate chains (glycans) to the polypeptide backbone. According to the atom, by which the carbohydrate residues are bound to the peptide, three types of glycosylation are recognized. *N*-glycosylated proteins bear the glycans on nitrogen atoms of asparagin in the Asn-X-Ser/Thr consensus sequence, where X is any amino acid except proline, serine, and threonine (Lerouge et al. 1998). *O*-glycosylation is executed on the oxygen atom of serine, threonine or hydroxyproline (Hanisch 2001). Finally, rare *S*-glycosylated proteins carry their glycan moiety on the sulphur atom of cysteine (Stepper et al. 2011).

Protein glycosylation usually increases protein stability and plays a role in protein–protein interactions (Ueda et al. 1996). Likewise, many pollen allergens are glycosylated (reviewed by Puc 2003). Glycoproteins or more heavily glycosylated proteoglycans are important components of cell walls and they are often found in association with membranes or as secreted proteins. In all these compartments, numerous members of a large family of the proline/hydroxyproline-rich glycoproteins are prominent. This family was originally classified into three separate classes: none or subtly glycosylated proline-rich proteins (PRPs), moderately glycosylated extensins and heavily glycosylated arabinogalactan-proteins (AGPs) (for review see Ellis et al. 2010; Wu et al. 2001). The latter two classes are defined by the presence of *O*-glycosylated hydroxyproline as hydroxyproline-rich glycoproteins (HRGPs) and their heterogeneity and abundance attributed them to multiple functions in plant growth and development, plant defence and signalling (Ellis et al. 2010). Recently, HRGPs were identified as a component of calcium signalling pathway (Lamport and Varnai 2013).

In Arabidopsis, a specific subset of AGPs was shown to be expressed in reproductive tissues with the majority of them being present in female tissues along the path taken by the growing pollen tube (Pereira et al. 2014). However, four AGPs, namely two classical AGPs (AGP6, AGP11) and two AG-peptides (AGP23 and AGP40) were specifically expressed in pollen and pollen tubes (Nguema-Ona et al. 2012). A subset of AGPs, namely those expressed in the male gametophyte, are attached to the plasma membrane by glycosylphosphatidylinositol (GPI) anchor (Lalanne et al. 2004). One function of these proteoglycans is the control of nexine formation (Jia et al. 2014) and subsequent pollen germination. Higher number of early germinating pollen tubes within the anthers was observed in *agl6/agl11* double and *agl6/agl11/agl40* triple mutants (Nguema-Ona et al. 2012). Accordingly, the knockout of pollen-expressed AGPs resulted in a reduced seed set. The expression pattern of male gametophytic AGPs is supposed to be balanced since the up-regulation of AGP40 and

AGP23 was observed in *agl6/agl11* double null mutant pollen tubes (Nguema-Ona et al. 2012). Recently it was reported that the expression of all four pollen-specific AGPs is directly controlled by the AT-hook nuclear localized (AHL) family DNA-binding TEK protein (Jia et al. 2014), which therefore indirectly controls nexine formation in the pollen wall (Lou et al. 2014).

Extensins (EXTs), the second large class of HRGPs, are also present in the cell wall where they are involved in the formation of crosslinking networks. Therefore their activity is predominantly observed in fast growing cells like root hairs and pollen tubes (Cannon et al. 2008; Lamport et al. 2011). Unlike AGPs, extensins do not show strict pollen-specific expression patterns and their co-expression in pollen tubes and root hairs was observed (Dupľáková et al. 2007; Hruz et al. 2008). This is also the case for EXT18, a classical extensin required for vegetative growth, reproductive development, pollen viability and fertility. Among other phenotypic defects, *ext18* mutants show significantly slower pollen tube growth and reduced seed set (Choudhary et al. 2015).

Although prominent, *O*-glycosylated HRGPs are not the only glycoproteins associated with male gametophyte function. In tobacco pollen tubes, two highly abundant cell wall *N*-glycoproteins of 66 and 69 kDa were identified (Čapková et al. 1997). The block of their *N*-glycosylation by tunicamycin caused reduction of the callose deposition into pollen tube cell wall and consequently impaired pollen tube growth. Glycoproteins of similar sizes were found also in several other angiosperm species (Fidlerová et al. 2001). In spite of the abundance and apparent important function of these *N*-glycoproteins, their exact molecular activity is still unknown. Moreover, protein *N*-glycosylation was demonstrated to be of vital importance in pollen tube perception (Lindner et al. 2015). The *EVAN* and *TURAN* genes encode putative uridine diphosphate (UDP)-glycosyltransferase superfamily protein and dolichol kinase, respectively. It is likely that proteins responsible for pollen tube perception in female organs are strongly glycosylated since mutations of these genes taking part in *N*-glycosylation pathway caused premature pollen tube burst (Lindner et al. 2015). Therefore membrane- and cell wall-associated glycoproteins are not only important for pollen tube growth through the pistil but are also good candidates to mediate male–female cross-talk during double fertilization.

Protein phosphorylation is rather fast, dynamic and transient PTM, and its reversible nature predestines it for a regulatory role. The switch from a metabolically quiescent pollen grain to a rapidly growing pollen tube has to be precisely regulated by several mechanisms including protein phosphorylation. In the past decade, plant phosphoproteomics evolved rapidly and nowadays, several protocols are available (Dunn et al. 2010; Fíla and Honys 2012). Some of these protocols deal with the challenges of protein extraction and phosphoprotein/phosphopeptide enrichment from very tough tissues such as mature pollen grain (Fíla et al. 2011, 2012; Mayank et al. 2012; Sheoran et al. 2009). Many phosphoproteomics studies have been performed in plants (Li et al. 2015; Meyer et al. 2012; van Bentem et al. 2008; Wolschin and Weckwerth 2005), however, none of these studies focused on pollen phosphoproteome.

The first male gametophytic phosphoproteomics study identified 962 phosphopeptides corresponding to 598 phosphoproteins in Arabidopsis mature pollen (Mayank et al. 2012). Notably, a high number of phosphoproteins (240 in particular) were newly identified. The most prevalent phosphoprotein categories were regulation of metabolism and protein function, signal transduction and cellular transport. Many kinases were identified, implying that kinases themselves were phosphorylated, for instance AGC protein kinases, calcium-dependent protein kinases, and SNF1-related protein kinases.

In tobacco, the protein phosphorylation dynamics during pollen activation was studied (Fíla et al. 2012). There, dry mature pollen grains and pollen suspension activated in vitro for 30 min were compared. In total, 139 phosphoprotein candidates carrying 52 phosphorylation sites were identified (Fíla et al. 2012). Most phosphoprotein candidates were associated with energy metabolism, a category that has to be precisely regulated after the pollen tube hydration. Other meaningful overrepresented protein categories were protein destination and storage, metabolism, cell structure and protein synthesis. Several phosphopeptides were found to be shared by both Arabidopsis and tobacco pollen (Fíla et al. 2014) pointing to the common nature of pollen activation in angiosperms. Pollen phosphoproteomics studies also significantly contributed to the public PhosPhAt database of *Arabidopsis thaliana* phosphorylation sites (Durek et al. 2010; Heazlewood et al. 2008). Many newly identified phosphorylated proteins were likely to be pollen-specific or -enriched as demonstrated by their transcription profiles (Dupľáková et al. 2007; Hruz et al. 2008; Mayank et al. 2012).

Interestingly, changes of the phosphoproteome in a gymnosperm *Picea wilsonii* pollen and pollen tubes were studied in response to nutrient depletion from pollen tube cultivation media (Chen et al. 2012). 42 phosphoproteins were found to be differentially regulated. Of them, phosphorylation of proteins involved in cytoskeleton dynamics was found to be specifically responsive to Ca^{2+} and sucrose deficiency (Chen et al. 2012). These three studies applied different phosphoproteomic approaches, and thus the data sets between them are not comparable since every protocol biases towards a different segment of phosphoproteome (Bodenmiller et al. 2007). However, together

they provided interesting input in the regulatory processes in male gametophyte.

Protein phosphorylation can be studied directly by phosphoproteomic approaches as mentioned above or alternatively, from the perspective of protein kinases (Supplementary Table 1). Several kinase motifs were reported amongst the phosphorylation sites both in Arabidopsis and tobacco male gametophytes (Fíla et al. 2012; Mayank et al. 2012) and few of them were shared by both species (Fíla et al. 2014). However, this in silico approach only revealed the presence of individual motifs but the actual link between a particular protein kinase and target proteins is still missing. To study the protein kinases activity themselves, several pollen-specific kinases were studied including mitogen-activated protein kinases (MAP kinases). MAP kinases represent a large family of Ser/Thr protein kinases common for all eukaryotes (Kultz 1998). They mediate the prolyl-directed phosphorylation on xxxxxxS*Pxxxxx, and xxxxxxT*Pxxxxx peptide motifs (Lee et al. 2011). However, a subset of these motifs is also recognized by cyclin-dependent protein kinases. Different MAP kinases play their roles in various phases of tobacco male gametophyte development. MAP kinase *NTF4* was activated after pollen hydration but before the actual pollen tube emerged. Its role is likely in the activation of pollen metabolism (Wilson et al. 1997). In Arabidopsis, four MAP kinases were identified in the pollen shotgun proteome dataset—MPK6, MPK8, MPK9, and MPK15 (Grobei et al. 2009). Two of these (MPK8, and MPK15) were shown to be phosphorylated at the TDY motif (Mayank et al. 2012). However, their exact roles during male gametophyte development are not yet clear. The other motif over-represented in the Arabidopsis phosphoproteomic data set was a basophilic motif RxxS*xx, that is recognized by CaM-dependent protein kinase family (Lee et al. 2011). In pollen, three calmodulin protein kinases were identified (Honys and Twell 2003), one of which was present also in the pollen proteome and phosphoproteome (Grobei et al. 2009; Mayank et al. 2012). The kinases can also be functionally studied but this is intricate due to the complexity of protein kinases. However, a double homozygous mutant of two AGC protein kinases showed defects in pollen tube growth and their competitiveness but not in a full penetrance (Zhang et al. 2009); however, the connecting link with the signaling pathways is still missing.

Pollen tube guidance: show me the way

As the pollen tube grows through the pistil tissues, it is guided towards ovules to ensure delivery of two non-motile sperm cells for double fertilization. The guidance process involves both mechanical/physical orientation and chemotropic guidance of the pollen tube by the female reproductive tissues. The physical guidance is attributed to the organization of transmitting tract tissues (TT) as well as its secreted compounds. Some of the identified secreted compounds include sulfinylated azadecalin (S-azadecalin; Qin et al. 2011), γ-aminobutyric acid (GABA; Ling et al. 2013; Palanivelu et al. 2003; Yu et al. 2014), brassinosteroids (Vogler et al. 2014), as well as other hormones and metabolites that direct pollen tube growth towards the ovule. Whereas the chemotropic guidance is associated with secreted signals by the attractant (the ovules) either pre-laid along the pollen tube path towards micropylar entry or intensively secreted as diffusible signals ensuring successful ovule targeting by the pollen tube (Heslop-Harrison 1987; Heslop-Harrison and Heslop-Harrison 1986; Mascarenhas and Machlis 1962). The majority of these signals constitute small secreted peptides predominantly of the defensin-like cysteine rich subfamily (DEFL) secreted from the egg apparatus (reviewed by Bleckmann et al. 2014; Higashiyama 2015). Additionally, fail-safe mechanisms exist whereby undegenerated female synergid cell persist to attract additional pollen tube in a case of failed fertilization by the first pollen tube and ensure double fertilization of the female gametes (Kasahara et al. 2012).

This chapter will discuss (1) secreted peptides by the female reproductive tissues with role in pollen tube attraction, (2) factors of the pollen tube that could perceive female guidance signals directly or indirectly, (3) a brief discussion and transcriptomic analysis of the Arabidopsis DEFL subfamily, cysteine-rich receptor protein kinases (CRKs) and GPI-anchored proteins in Arabidopsis as potential factors likely to be involved in ovular attraction, signal perception and pollen tube guidance. Recent comprehensive reviews on pollen tube guidance are available, see Bleckmann et al. (2014) and Higashiyama (2015).

Ovular secreted peptides for pollen tube attraction

After successful pollination and penetration through the stigma, a compatible pollen tube grows through the extracellular matrix of the transmitting tract tissues with the aid of female guidance signals to reach and fertilize the female gametes (Maheshwari 1950; Yadegari and Drews 2004). This cell–cell communication has emerged as an important bottleneck for unfavourable fertilization and as a pre-zygotic barrier for interspecies hybridization. Techniques involving the use of single-cell laser ablation, use of genetic mutants and high-throughput genomic approaches such as tissue-specific transcriptomic studies have identified various transmitting tract and ovular secreted peptides

involved in pollen-pistil interactions and as ovular attractants with conserved roles across plant species (Márton et al. 2012; Okuda et al. 2009; Takeuchi and Higashiyama 2011). Among them are Arabinogalactan proteins, cysteine-rich polypeptides (CRP), defensin-like proteins (DEFL), S-RNases, transmitting tissue-specific proteins (TTS), class III pistil extensin-like proteins (PELPIII) and lipid transfer proteins (LTP) (Chae and Lord 2011; Dresselhaus and Franklin-Tong 2013; Hamamura et al. 2011). Characterisation and the continuous search for ovular secreted pollen tube attractants have spearheaded better understanding of the molecular dialogue during pollen tube-ovular attraction and successful fertilization.

To exit the transmitting tract and reorient towards target ovules, pollen tubes are attracted by secreted signals directly derived from the ovules. Genetic evidence have shown that functional female gametophyte plays an essential role in pollen tube attraction towards the ovule (Hulskamp et al. 1995; Ray et al. 1997; Shimizu and Okada 2000) by the secretion of ovular attractants (reviewed in Bleckmann et al. 2014; Higashiyama 2015). The ovular attractants for pollen tube guidance identified so far include LURE proteins from Torenia and Arabidopsis and ZmEA1 from maize. LURE proteins are short antifungal/antimicrobial polypeptides (typically 50–100 amino acids) belonging to the defensin-like subfamily of cysteine rich proteins first identified as secreted in *Torenia fourieri* synergid cells and termed LURE1 and LURE2 (Okuda et al. 2009). Through orthologous protein searches, other LURE proteins were identified in *Torenia concolor*, TcCRP1 (Kanaoka et al. 2011) and in *Arabidopsis thaliana* (AtLURE1.1–1.6) and *Arabidopsis lyrata* (AlLURE1.1–1.10) (Takeuchi and Higashiyama 2012). All LURE proteins were shown to be expressed and secreted by the synergid cells. Using semi in vitro assay and microfluidic device techniques (Agudelo et al. 2013; Arata and Higashiyama 2014; Horade et al. 2013; Sanati Nezhad et al. 2014), TfLURE1/2 and AtLURE1 proteins were confirmed as pollen tube attractants with long-range activities in a species-preferential manner (Horade et al. 2013). Antisense knockdown of TfLURE1 or TfLURE2 abolished pollen tube attraction and fertilization in *Torenia fourieri* (Okuda et al. 2009). Intriguingly, these identified LURE proteins are capable of cross-species activities and are sufficient to attract pollen tubes of distantly related species. This was elegantly demonstrated by the successful attraction and embryo sac entry of Arabidopsis pollen tubes by *Torenia fourieri* ovules expressing AtLURE1.2 peptides (Takeuchi and Higashiyama 2012). The range at which the LURE proteins are perceived by the approaching pollen tube is still unclear (see below).

Similar to LURE proteins, *Zea mays* egg apparatus 1 protein (ZmEA1), plays an essential role in pollen tube ovular attraction (Fig. 3a) (Márton et al. 2005, 2012). ZmEA1 belongs to EAL family and is expressed in the egg apparatus predominantly in the synergid cells. ZmEA1 is specific to monocots. Heterologous expression of ZmEA1 in Arabidopsis ovules is sufficient to attract maize pollen tubes to the micropylar entry (Márton et al. 2005). These findings demonstrate that ovular secreted attractants are likely candidates to impose interspecific prezygotic barriers during pollen tube guidance.

One unresolved aspect of this cell–cell crosstalk event is the range at which the ovular attractants travel and are perceived by the pollen tubes. Clearly, the intercellular growth of the pollen tubes in transmitting tract happens basipetally and predominantly involves mechanical guidance by the female sporophytic tissues (Heslop-Harrison 1987; Heslop-Harrison and Heslop-Harrison 1986). Later, Sanders and Lord proposed the model that the pollen tubes are effectively dragged down the transmitting tract through interaction with the extracellular matrix of the transmitting tract tissues (Hulskamp et al. 1995; Sanders and Lord 1989, 1992). In lily and *Torenia* species, germinating pollen tubes emerge from opposite ends of the cut style (distal or proximal) or at both ends of cut style when germinated from a cut slit at the middle of the style (Higashiyama 2015). All above findings emphasise that mechanical guidance predominate intercellular pollen tube growth within the transmitting tract. Thereafter, deviation of the pollen tubes from the transmitting tract tissues onto the surface of the septum towards the target ovule requires additional independent signals (Schwemmie 1968). Precisely, this turning point marks the end of mechanical guidance and the beginning of ovular chemotropic attraction. Isolated sporophytic genetic mutations specifically affecting female gametogenesis (but not the sporophytic tissues) at various developmental stages are known to significantly reduce pollen tube-ovule targeting success including pollen tube emergence onto the surface of the septum (Hulskamp et al. 1995; Ray et al. 1997; Shimizu and Okada 2000). In support, secreted AtLURE1 peptides are detectable beyond the micropylar, at the surface of funiculus and septum (Higashiyama 2010, 2015), suggesting their likely involvement in pre-ovular guidance. Together, these results support the notion that ovular secreted peptides could have a long attraction/guidance range. Intuitively, it could be predicted that mutants affecting protein secretion in ovules would consequently impact on pollen tube guidance and attraction. Therefore, it is of great importance to further resolve the range through which ovular attraction signals operate and whether the transport of these signals involves diffusion or specific carrier molecules such as nanovesicles, carbohydrates moieties and/or encapsulated lipid molecules to reach their target cells, the pollen tube.

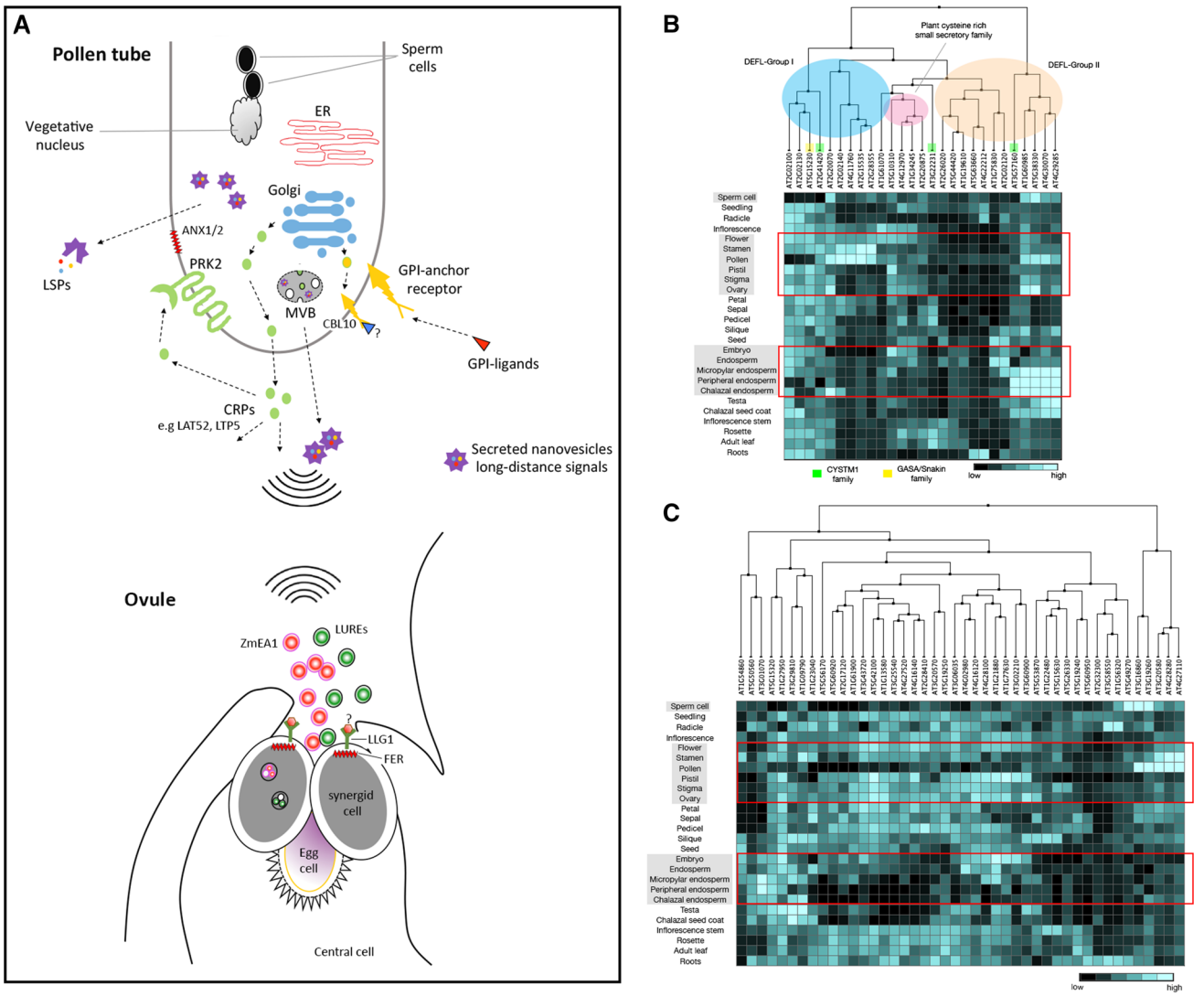

Fig. 3 Empirical model of pollen tube-pistil/ovule crosstalk. **a** A peptidome perspective of cell–cell communication during pollen tube-ovular guidance showing known and predicted secreted molecules involved. **b**, **c** Expression profile of Arabidopsis cysteine-rich polypeptide family proteins of <150 aa's and predicted GPI-anchored proteins outsourced from the UniProtKB and UniRef100 databases. LSP's are pollen tube Leaderless secreted proteins (without N-terminal signal peptide) that could be secreted via nanovesicles exosomes

Perception of female guidance signals by the pollen tube

Although to date there has not been a direct demonstration of female-secreted ligands binding to pollen tube surface receptors, with the exception of AtLURE1 that is indirectly perceived by LIP1/2 receptor kinases (Liu et al. 2013), the continuous efforts (including innovated techniques) are edging closer to reach such resolution of understanding. Several male mutants have been isolated that are defective in pollen tube-ovule targeting (reviewed in Bleckmann et al. 2014; Higashiyama 2015). Intriguingly, they include genes involved in cellular homeostasis, actin dynamics and those encoding membrane- and surface-anchored receptors. Among them is Arabidopsis LOST IN POLLEN TUBE GUIDANCE 1 (LIP1) and LIP2. LIP1 and LIP2 are pollen-expressed membrane-anchored receptor-like kinases without extracellular domains and localize beneath the membrane at the pollen tube tip (Liu et al. 2013). Simultaneous knockdown of LIP1 and LIP2 results in reduced pollen tube ovule targeting ability and reduced attraction towards synthetic AtLURE1.2 peptides. This observation suggests an indirect perception of LURE ovular attractants by LIP1 and LIP2 pollen tube surface receptor proteins (Liu et al. 2013). Another pollen tube receptor identified is COBRA-LIKE 10 (COBL10). COBL10 is a GPI-anchored protein that localizes at the pollen tube tip through its C-terminal GPI-anchor sequence and is involved in the deposition of

apical pectin cap, cellulose microfibrils and functions in pollen tube guidance (Li et al. 2013). Its knock-out results in defects in ovule targeting that mimics those of *abnormal pollen tube guidance1* (*aptg1*), *seth1* and *seth2* knockdown mutants which all are involved in GPI biosynthetic pathway. These results suggest that GPI-mediated membrane anchoring of COBL10 is essential for pollen tube guidance (Li et al. 2013). Perception of ovular attraction signals by the pollen tube also involved proteins with a structural role. MICROTUBULE ASSOCIATE PROTEIN 18 (MAP18) and MICROTUBULE-DESTABILIZING PROTEIN 25 (MDP25), both possess actin filament severing activity and their mutants lack competence in perceiving ovule attraction signals but show normal pollen tube growth (Qin et al. 2014; Zhu et al. 2013). It is surprising that *map18* and *mdp25* mutants do not show defects in pollen tube growth as previously reported for other proteins involved in actin organization (Guan et al. 2013). Here they demonstrate that actin dynamics plays an exclusive role in directing the pollen tube towards the ovules (Higashiyama 2015). Two pollen tube potassium transporters, CHX21 and CHX23, were also identified as essential factors for pollen tube competence in ovule targeting (Lu et al. 2011). They are likely to regulate cytosolic cation dynamics rendering pollen tube competence in response to ovular attractants. Similar to secretion of ovular attractant peptides, pollen tube protein secretion and protein folding are also likely to play an important role in pollen tube competence to perceive ovular attractants (Hafidh, Potěšil, Fíla, Čapková, Zdráhal and Honys, unpublished). This was demonstrated for ER-localized POLLEN DEFECTIVE IN GUIDANCE 1 (POD1) protein, since *pod1* mutant pollen tubes were incompetent in ovular attraction response (Li et al. 2011). These findings suggests that ER-protein folding and likely secretion of membrane-associated and extracellular proteins from the ER are critical for pollen tube responsiveness towards female guidance signals. Moreover, genes involved in regulating secretory pathways are also likely to be essential in pollen tube guidance.

Similar to ovule-secreted peptides, the range of activities for pollen tube-secreted peptides also needs to be addressed. Cysteine-rich family protein LAT52 and Lipid transfer family protein LTP5 are the only known pollen tube-secreted ligand proteins that are perceived by pollen tube receptor like kinase, PRK2 (Zhao et al. 2013). Once secreted, LAT52 and LTP5 are believed to participate in an autocrine signalling involving RopGEFs to control polar tip growth of the pollen tube (Fig. 3a). It is not known whether LAT52 and LTP5 act as ligands to female receptors during pollen tube-pistil interaction. Furthermore, discovery of other pollen tube secreted proteins/peptides was hampered by the inaccessibility of the pollen tube "secretome" within the transmitting tract, however, current developed techniques offer a compromised access to such molecules and have a potential to speed up the discovery of the pollen tube "peptidome" (Hafidh et al. 2014). The challenge ahead is to demonstrate the range of activities for LAT52, LTP5 and other pollen tube secreted peptides/proteins and how these secreted peptides reach their target receptors. An empirical model would be that short range intercellular signals might reach their targets by diffusion whereas long-range intercellular signals could be encapsulated within "carrier organelles/molecules" such as lipid bilayer capsules or secreted nanovesicles/exosomes (Prado et al. 2014) and either taken up or released by endocytosis upon contact with the target cell (Fig. 3a). With application of techniques such as microfluidic devices with live cell imaging capabilities (Cheung et al. 2010; Horade et al. 2013; Rotman et al. 2003; Uebler and Dresselhaus 2014), the intercellular dialogue between pollen tube ovular signal perception and ovular attraction will gain resolution to the molecular level and increase better understanding of the fertilization process and prezygotic interspecific barriers of flowering plants.

Cysteine-rich polypeptides and GPI-anchored proteins predicted as secreted in Arabidopsis

Ovular attractants that have been identified to date belong to the defensin-like DEFL subfamily of the cysteine-rich polypeptide group of proteins. The CRP proteins are present as isoforms and paralogs across species, whereas others are species-specific defensin-like proteins (Márton et al. 2005, 2012; Takeuchi and Higashiyama 2012). It is likely that other members of this family play an essential role in pollen tube guidance. We have surveyed the Arabidopsis genome using the proteome data from UniProt repository (http://www.uniprot.org/) and selected annotated CRP family peptides of <150 amino acids that were predicted as secreted (Supplementary Table 2). Transcriptomic analysis of publicly deposited dataset (Dupľáková et al. 2007; Hruz et al. 2008) followed by phylogenic classification using Eucledian distance algorithms and optimal leaf ordering based on co-expression and similar vector branching, highlighted two main groups of DEFL-like proteins and a small subset of "plant cysteine-rich small secretory family" of proteins (Fig. 3b). DEFL-like group I consisted of defensin-like peptides that showed consistent expression in male and female reproductive tissues but more strongly in pollen grains than in ovary (Fig. 3b). The DEFL-like group II on the other hand, displayed more variable expression patterns with much stronger expression in the female gametophyte and in dissected endospem (Fig. 3b). The subgroup of plant cysteine-rich small secretory family constituted exclusively of EPIDERMAL patterning factor-like proteins (EPFL).

EPFL's are <50 aa secreted peptides within the mesophyll cells and are known to increase stomatal formation through positive and negative protein–protein interaction and likely through their interaction with receptor-like proteins such as TMM receptor kinases (Lee et al. 2015). Their expression is uniform throughout plant development (Fig. 3b). Whether EPFL plays role also in pollen tube guidance remains to be demonstrated.

Another noteworthy subfamily is CRKs, cysteine-rich receptor-like kinases. CRKs are not secreted to the extracellular matrix but reside on the membrane with a single-pass transmembrane helix (Supplementary Table 2) and are likely to function as receptors for secreted ligands during pollen tube guidance. For majority, their expression in several plant tissues is also constitutive and shows no prominent specificity in particular tissues (Supplementary Fig.S1). However, five CRK genes showed exceptional profile; CRK42 (AT5G40380), CRK17 (AT4G23250), CRK33 (AT4G11490), CRK43 (AT4G28670) and CRK1 (AT1G19090), all appeared to be exclusively enriched in pollen and in sperm cells compared to any other tissues (Fig. S1). Their pattern hint towards possible role in kinase-mediated signalling in pollen tubes and in sperm cells during pollen-pistil interaction and during fertilization. To date, only one example of possible ligand-receptor interaction, LIP1/2–LUREs, has been reported (Li et al. 2013). Embedment of CRKs within the membrane places them as potential receptors that could link and transduce signals from cell surface receptors (such as GPI-anchored proteins, example COBL10) to inner receptors such as LIP1 and LIP2 (Fig. 3a). Therefore, understanding the role of these CRKs will pave way in the understanding the cascades of signal transduction during male–female crosstalk.

Similarly, GPI-anchored proteins are among the gene families identified as regulators of pollen tube guidance and reception. LLG1, a Lorelei-like GPI-anchored protein is expressed exclusively in synergid cells and could perceive signals secreted by the approaching pollen tube (Capron et al. 2008). Knockdown of LLG1 severely perturbs pollen tube reception resulting in pollen tube overgrowth, defective sperm cell release and embryo development (Capron et al. 2008; Tsukamoto et al. 2010). In pollen tubes, COBRA-like 10, is expressed and localized at the pollen tube tip through its C-terminal GPI-anchor (Li et al. 2013). Knockdown of CBL10 results in defects in pollen tube growth and ovule targeting. In mammals, GPI-anchored proteins are commonly deployed as cell sensors during cell–cell communications including during egg-sperm cell recognition. We have analysed expression of annotated secreted Arabidopsis GPI-anchored proteins in several tissues as potential candidate transient receptors during pollen-pistil interaction and pollen tube-ovule targeting (Supplementary Table 3). The majority showed a general widespread expression pattern in all tissues including the gametophytes with the exception of COBL9 (AT5G49270), COBL8 (AT3G16860), COBL10 (AT3G20580), LLG3 (AT4G28280) and COBL11 (AT4G27110), which showed significantly higher expression levels in stamens, pollen grains and in sperm cells (Fig. 3c). Of them, only COBL10 has been reported to play critical role in pollen tube growth and in cell–cell crosstalk during pollen-pistil interaction and fertilization (Li et al. 2013). GPI-anchored proteins are likely to function as primary receptors in pollen tube and in synergid cell and the female gametophyte to perceive secreted signals. The aforementioned genes (as well as others on Table 3) are worth a detailed investigation to establish their role in pollen tube signal perception during male–female signaling and fertilization.

Termination of pollen tube guidance and attraction

Once the pollen tube successfully entered the embryo sac and released the two sperm cells, gamete fusions (both plasmogamy and karyogamy) of sperm cells with the egg cell and the central cells mark the end of pollen tube guidance and an ovule stops attracting any additional pollen tubes. This is preceeded with induced programme cell death of the persistent synergid cell approximately 20 h post pollination (Beale et al. 2012). If plasmogamy or karyogamy fails with the first pollen tube, the persistent synergid cell is reactivated to attract additional pollen tubes and ensure double fertilization of both female gametes. Up to three pollen tubes can be attracted by a single ovule (Kasahara et al. 2012; Williams 2009). This phenomena is termed polytubey and ensures fertilization recovery. Polytubey can also lead to hetero-fertilization where male gametes involved in the fertilization are delivered by different pollen tubes (Maruyama et al. 2013). Ovule mutants defective in micropylar guidance such as *myb98* (Kasahara et al. 2005), *magatama* (Shimizu and Okada 2000), and *central cell guidance* (Chen et al. 2007), or those defective in pollen tube reception, such as *feronia/sirene* (Huck et al. 2003; Rotman et al. 2003) and *lorelei* (Capron et al. 2008; Tsukamoto et al. 2010), all show the polytubey phenotype. Similarly, male components that are required for gamete fusion and fertilization such as *GENERATIVE CELL SPECIFIC 1/HAPLESS 2* (*GCS1/HAP2*) which encodes a sperm cell plasma membrane protein and required for gamete fusion (Mori et al. 2005; von Besser et al. 2006), *cdka;1* (Hamamura et al. 2012), *duo1*, *duo3* (Beale et al. 2012; Kasahara et al. 2012) and *kokopeli* (Hamamura et al. 2012), which are all defective in producing two competent sperm cells, also display polytubey phenotypes suggesting that a cessation of pollen tube attraction is a direct consequence of the double fertilization event. For ovules that

have undergone successful fertilization, ethylene signalling is induced by ER localized ETHYLENE-INSENSITIVE 2 and 3 (EIN2 and EIN3) and perceived by the remaining synergid cell (Völz et al. 2013). Ethylene perception induces programmed cell death of the synergid cell and marks the end of pollen tube attraction and beginning of embryogenesis.

Conclusion

Unlike animals, the specification of specialized cells that give rise to gametes (the germline) happens much later and repeatedly during plant development. In angiosperms, the male gametogenesis takes place through coordinated activities of both gametophytic and sporophytic tissues and involves widespread dynamic changes in gene expression. Shed pollen grains constitute a vegetative cell (that forms a pollen tube and delivers two sperm cells for fertilization) and either undivided germ cell (bicellular species) or with two sperm cells (tricellular species). This process is underpinned by two successive cell divisions accompanied by morphological and physiological differentiation of both cell types (reviewed by Berger and Twell 2011). Tremendous efforts including genetic and transcriptomic approaches has led to the isolation of several mutants whose gene function regulates several steps of the male gametogenesis (reviewed by Borg et al. 2011). This has provided better understanding of male sterility and can be used to manipulate and improve male fitness.

A plethora of processes also regulates pollen tube growth, guidance competence and reception by the target ovule. They include posttranscriptional regulation (including mass transcript storage) and posttranslational modifications such as phosphorylation to modulate protein function, intracellular metabolic signalling, ionic gradients such as Ca^{2+} and H^{+} ions, cell wall synthesis, protein secretion and intercellular signalling with the female reproductive tissues. Mechanisms regulating many of the above mentioned processes are being unravelled (reviewed by Dresselhaus and Franklin-Tong 2013). Current efforts have seen a big leap in the understanding of pollen tube guidance and ovular attraction with potential in understanding interspecies hybridization barriers (reviewed by Higashiyama 2015). Future challenges include the identification of other pollen tube guidance factors secreted from the female reproductive tissues, particularly those involved in ovular guidance. Equally critical, male factors involved in perceiving female guidance signals, including secreted receptors and ligands, will pave the way to better understanding of cell–cell communication between male and female gametophytes during pollen tube guidance and fertilization. Innovative techniques including microfluidic devices and live cell imaging will spearhead the discovery of molecules critical for fertilization, understanding pollen tube response towards female attraction signals as well as establish the range of activities through which female and male signalling molecules can be perceived by their target cell. Not to be underestimated, transcriptional approaches still offer a powerful tool to isolate such molecules and aid in the breakthroughs to understand mechanisms governing gametogenesis, fertilization and seed set.

Author contribution statement S.H., J.F. and D.H. wrote the manuscript. All authors read and approved the manuscript.

Acknowledgments The authors thank Barbora Honysová for drawing the Figs. 1 and 2 and Nina Lindstrøm Friggens for assistance with drawing Fig. 3 and the language editing of the manuscript. The authors acknowledge the financial support from the Czech Science Foundation Grants No. 15-22720S, 14-32292S, P305/12/2611 and 15-16050S and Ministry of Education, Youth and Sport CR project COST LD14109.

References

Agudelo CG, Sanati Nezhad A, Ghanbari M, Naghavi M, Packirisamy M, Geitmann A (2013) TipChip: a modular, MEMS-based platform for experimentation and phenotyping of tip-growing cells. Plant J 73:1057–1068. doi:10.1111/tpj.12093

Allwood EG, Anthony RG, Smertenko AP, Reichelt S, Drobak BK, Doonan JH, Weeds AG, Hussey PJ (2002) Regulation of the pollen-specific actin-depolymerizing factor LIADF1. Plant Cell 14:2915–2927. doi:10.1105/tpc.005363

Arata H, Higashiyama T (2014) Poly(dimethylsiloxane)-based microdevices for studying plant reproduction. Biochem Soc Trans 42:320–324. doi:10.1042/bst20130258

Ariizumi T, Toriyama K (2011) Genetic regulation of sporopollenin synthesis and pollen exine development. In: Merchant SS, Briggs WR, Ort D (eds) Annual Review of Plant Biology, vol 62, pp 437–460

Barnabas B, Fridvalszky L (1984) Adhesion and germination of differently treated maize pollen grains on the stigma. Acta Bot Hung 30:329–332

Beale KM, Leydon AR, Johnson MA (2012) Gamete fusion is required to block multiple pollen tubes from entering an Arabidopsis ovule. Curr Biol 22:1090–1094. doi:10.1016/j.cub.2012.04.041

Bedinger P (1992) The remarkable biology of pollen. Plant Cell 4:879–887

Bendtsen JD, Jensen LJ, Blom N, von Heijne G, Brunak S (2004) Feature-based prediction of non-classical and leaderless protein secretion. Protein Eng Des Sel 17:349–356. doi:10.1093/protein/gzh037

Berger F, Twell D (2011) Germline specification and function in plants. Annu Rev Plant Biol 62:461–484. doi:10.1146/annurev-arplant-042110-103824

Bishop AL, Hall A (2000) Rho GTPases and their effector proteins. Biochem J 348:241–255. doi:10.1042/0264-6021:3480241

Bleckmann A, Alter S, Dresselhaus T (2014) The beginning of a seed: regulatory mechanisms of double fertilization. Front Plant Sci 5:452. doi:10.3389/fpls.2014.00452

Bodenmiller B, Mueller LN, Mueller M, Domon B, Aebersold R (2007) Reproducible isolation of distinct, overlapping segments of the phosphoproteome. Nat Methods 4:231–237. doi:10.1038/nmeth1005

Bokvaj P, Hafidh S, Honys D (2015) Transcriptome profiling of male gametophyte development *Nicotiana tabacum*. Genom Data 3:106–111

Borg M, Brownfield L, Twell D (2009) Male gametophyte development: a molecular perspective. J Exp Bot 60:1465–1478. doi:10.1093/jxb/ern355

Borg M, Brownfield L, Khatab H, Sidorova A, Lingaya M, Twell D (2011) The R2R3 MYB transcription factor DUO1 activates a male germline-specific regulon essential for sperm cell differentiation in Arabidopsis. Plant Cell 23:534–549. doi:10.1105/tpc.110.081059

Borges F, Gomes G, Gardner R, Moreno N, McCormick S, Feijó JA, Becker JD (2008) Comparative transcriptomics of Arabidopsis sperm cells. Plant Physiol 148:1168–1181. doi:10.1104/pp.108.125229

Brett C, Waldron K (1990) Physiology and biochemistry of plant cell walls. Unwin Hyman, London

Brewbaker JL (1967) Distribution and phylogenetic significance of binucleate and trinucleate pollen grains in angiosperms. Am J Bot 54:1069–1083. doi:10.2307/2440530

Camacho L, Malho R (2003) Endo/exocytosis in the pollen tube apex is differentially regulated by Ca^{2+} and GTPases. J Exp Bot 54:83–92. doi:10.1093/jxb/erg043

Cannon MC, Terneus K, Hall Q, Tan L, Wang Y, Wegenhart BL, Chen L, Lamport DT, Chen Y, Kieliszewski MJ (2008) Self-assembly of the plant cell wall requires an extensin scaffold. Proc Natl Acad Sci USA 105:2226–2231. doi:10.1073/pnas.0711980105

Čapková V, Hrabětová E, Tupý J (1988) Protein synthesis in pollen tubes: preferential formation of new species independent of transcription. Sex Plant Reprod 1:150–155

Čapková V, Fidlerová A, van Amstel T, Croes AF, Mata C, Schrauwen JAM, Wullems GJ, Tupý J (1997) Role of *N*-glycosylation of 66 and 69 kDa glycoproteins in wall formation during pollen tube growth in vitro. Eur J Cell Biol 72:282–285

Capron A, Gourgues M, Neiva LS, Faure JE, Berger F, Pagnussat G, Krishnan A, Alvarez-Mejia C, Vielle-Calzada JP, Lee YR, Liu B, Sundaresan V (2008) Maternal control of male-gamete delivery in Arabidopsis involves a putative GPI-anchored protein encoded by the LORELEI gene. Plant Cell 20:3038–3049. doi:10.1105/tpc.108.061713

Chae K, Lord EM (2011) Pollen tube growth and guidance: roles of small, secreted proteins. Ann Bot 108:627–636. doi:10.1093/aob/mcr015

Chaturvedi P, Ischebeck T, Egelhofer V, Lichtscheidl I, Weckwerth W (2013) Cell-specific analysis of the tomato pollen proteome from pollen mother cell to mature pollen provides evidence for developmental priming. J Proteome Res 12:4892–4903. doi:10.1021/pr400197p

Chen XY, Kim JY (2009) Callose synthesis in higher plants. Plant Signal Behav 4:489–492

Chen CY, Wong EI, Vidali L, Estavillo A, Hepler PK, Wu HM, Cheung AY (2002) The regulation of actin organization by actin-depolymerizing factor in elongating pollen tubes. Plant Cell 14:2175–2190. doi:10.1105/tpc.003038

Chen YH, Li HJ, Shi DQ, Yuan L, Liu J, Sreenivasan R, Baskar R, Grossniklaus U, Yang WC (2007) The central cell plays a critical role in pollen tube guidance in Arabidopsis. Plant Cell 19:3563–3577. doi:10.1105/tpc.107.053967

Chen Y, Liu P, Hoehenwarter W, Lin J (2012) Proteomic and phosphoproteomic analysis of *Picea wilsonii* pollen development under nutrient limitation. J Proteome Res 11:4180–4190. doi:10.1021/pr300295m

Cheung AY, Boavida LC, Aggarwal M, Wu HM, Feijó JA (2010) The pollen tube journey in the pistil and imaging the in vivo process by two-photon microscopy. J Exp Bot 61:1907–1915. doi:10.1093/jxb/erq062

Choudhary P, Saha P, Ray T, Tang Y, Yang D, Cannon MC (2015) EXTENSIN18 is required for full male fertility as well as normal vegetative growth in Arabidopsis. Front Plant Sci 6:553. doi:10.3389/fpls.2015.00553

de Graaf BHJ, Cheung AY, Andreyeva T, Levasseur K, Kieliszewski M, Wu HM (2005) Rab11 GTPase-regulated membrane trafficking is crucial for tip-focused pollen tube growth in tobacco. Plant Cell 17:2564–2579. doi:10.1105/tpc.105.033183

De Storme N, Geelen D (2013) Cytokinesis in plant male meiosis. Plant Signal Behav 8:e23394. doi:10.4161/psb.23394

Dobritsa AA, Coerper D (2012) The novel plant protein INAPERTURATE POLLEN1 marks distinct cellular domains and controls formation of apertures in the Arabidopsis pollen exine. Plant Cell 24:4452–4464. doi:10.1105/tpc.112.101220

Dobritsa AA, Shrestha J, Morant M, Pinot F, Matsuno M, Swanson R, Moller BL, Preuss D (2009) CYP704B1 is a long-chain fatty acid omega-hydroxylase essential for sporopollenin synthesis in pollen of Arabidopsis. Plant Physiol 151:574–589. doi:10.1104/pp.109.144469

Dobritsa AA, Lei Z, Nishikawa S, Urbanczyk-Wochniak E, Huhman DV, Preuss D, Sumner LW (2010) LAP5 and LAP6 encode anther-specific proteins with similarity to chalcone synthase essential for pollen exine development in Arabidopsis. Plant Physiol 153:937–955. doi:10.1104/pp.110.157446

Dobritsa AA, Geanconteri A, Shrestha J, Carlson A, Kooyers N, Coerper D, Urbanczyk-Wochniak E, Bench BJ, Sumner LW, Swanson R, Preuss D (2011) A large-scale genetic screen in Arabidopsis to identify genes involved in pollen exine production. Plant Physiol 157:947–970. doi:10.1104/pp.111.179523

Dresselhaus T, Franklin-Tong N (2013) Male–female crosstalk during pollen germination, tube growth and guidance, and double fertilization. Mol Plant 6:1018–1036. doi:10.1093/mp/sst061

Dresselhaus T, Sprunck S (2012) Plant fertilization: maximizing reproductive success. Curr Biol 22:R487–R489. doi:10.1016/j.cub.2012.04.048

Dunn JD, Reid GE, Bruening ML (2010) Techniques for phosphopeptide enrichment prior to analysis by mass spectrometry. Mass Spectrom Rev 29:29–54. doi:10.1002/mas.20219

Dupl'áková N, Reňák D, Hovanec P, Honysová B, Twell D, Honys D (2007) Arabidopsis Gene Family Profiler (aGFP): user-oriented transcriptomic database with easy-to-use graphic interface. BMC Plant Biol 7:39. doi:10.1186/1471-2229-7-39

Durek P, Schmidt R, Heazlewood JL, Jones A, MacLean D, Nagel A, Kersten B, Schulze WX (2010) PhosPhAt: the *Arabidopsis thaliana* phosphorylation site database: an update. Nucleic Acids Res 38:D828–D834. doi:10.1093/nar/gkp810

Eady C, Lindsey K, Twell D (1995) The significance of microspore division and division symmetry for vegetative cell-specific transcription and generative cell differentiation. Plant Cell 7:65–74. doi:10.1105/tpc.7.1.65

Eisenhaber B, Wildpaner M, Schultz CJ, Borner GH, Dupree P, Eisenhaber F (2003) Glycosylphosphatidylinositol lipid anchoring of plant proteins. Sensitive prediction from sequence- and genome-wide studies for Arabidopsis and rice. Plant Physiol 133:1691–1701. doi:10.1104/pp.103.023580

Elfving F (1879) Studien über die Pollenkörner der Angiospermen. Jenaische Zeitschrift für Naturwissenschaft 13:1–28

Ellis M, Egelund J, Schultz CJ, Bacic A (2010) Arabinogalactan-proteins: key regulators at the cell surface? Plant Physiol 153:403–419. doi:10.1104/pp.110.156000

Feher A, Lajko DB (2015) Signals fly when kinases meet Rho-of-plants (ROP) small G-proteins. Plant Sci 237:93–107. doi:10.1016/j.plantsci.2015.05.007

Feijó JA, Sainhas J, Hackett GR, Kunkel JG, Hepler PK (1999) Growing pollen tubes possess a constitutive alkaline band in the clear zone and a growth-dependent acidic tip. J Cell Biol 144:483–496. doi:10.1083/jcb.144.3.483

Feijó JA, Costa SS, Prado AM, Becker JD, Certal AC (2004) Signalling by tips. Curr Opin Plant Biol 7:589–598. doi:10.1016/j.pbi.2004.07.014

Fellenberg C, Vogt T (2015) Evolutionarily conserved phenylpropanoid pattern on angiosperm pollen. Trends Plant Sci 20:212–218. doi:10.1016/j.tplants.2015.01.011

Ferguson C, Teeri TT, Siika-aho M, Read SM, Bacic A (1998) Location of cellulose and callose in pollen tubes and grains of *Nicotiana tabacum*. Planta 206:452–460. doi:10.1007/s004250050421

Fidlerová A, Smýkal P, Tupý J, Čapková V (2001) Glycoproteins 66 and 69 kDa of pollen tube wall: properties and distribution in angiosperms. J Plant Physiol 158:1367–1374. doi:10.1078/0176-1617-00562

Fíla J, Honys D (2012) Enrichment techniques employed in phosphoproteomics. Amino Acids 43:1025–1047. doi:10.1007/s00726-011-1111-z

Fíla J, Čapková V, Feciková J, Honys D (2011) Impact of homogenization and protein extraction conditions on the obtained tobacco pollen proteomic patterns. Biol Plant 55:499–506. doi:10.1007/s10535-011-0116-5

Fíla J, Matros A, Radau S, Zahedi RP, Čapková V, Mock H-P, Honys D (2012) Revealing phosphoproteins playing role in tobacco pollen activated in vitro. Proteomics 12:3229–3250. doi:10.1002/pmic.201100318

Fíla J, Čapková V, Honys D (2014) Phosphoproteomic studies in Arabidopsis and tobacco male gametophytes. Biochem Soc Trans 42:383–387. doi:10.1042/bst20130249

Firon N, Nepi M, Pacini E (2012) Water status and associated processes mark critical stages in pollen development and functioning. Ann Bot 109:1201–1213. doi:10.1093/aob/mcs070

Fu Y, Wu G, Yang ZB (2001) Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. J Cell Biol 152:1019–1032. doi:10.1083/jcb.152.5.1019

Furness CA, Rudall PJ (2004) Pollen aperture evolution: a crucial factor for eudicot success? Trends Plant Sci 9:154–158. doi:10.1016/j.tplants.2004.01.001

Gaillard A, Vergne P, Beckert M (1991) Optimization of maize microspore isolation and culture conditions for reliable plant regeneration. Plant Cell Rep 10:55–58. doi:10.1007/BF00236456

Grobei MA, Qeli E, Brunner E, Rehrauer H, Zhang R, Roschitzki B, Basler K, Ahrens CH, Grossniklaus U (2009) Deterministic protein inference for shotgun proteomics data provides new insights into Arabidopsis pollen development and function. Genome Res 19:1786–1800. doi:10.1101/gr.089060.108

Guan Y, Guo J, Li H, Yang Z (2013) Signaling in pollen tube growth: crosstalk, feedback, and missing links. Mol Plant 6:1053–1064. doi:10.1093/mp/sst070

Hafidh S, Čapková V, Honys D (2011) Safe keeping the message: mRNP complexes tweaking after transcription. Adv Exp Med Biol 722:118–136. doi:10.1007/978-1-4614-0332-6_8

Hafidh S, Breznenová K, Honys D (2012a) De novo post-pollen mitosis II tobacco pollen tube transcriptome. Plant Signal Behav 7:918–921. doi:10.4161/psb.20745

Hafidh S, Breznenová K, Růžička P, Feciková J, Čapková V, Honys D (2012b) Comprehensive analysis of tobacco pollen transcriptome unveils common pathways in polar cell expansion and underlying heterochronic shift during spermatogenesis. BMC Plant Biol 12:24. doi:10.1186/1471-2229-12-24

Hafidh S, Potěšil D, Fíla J, Feciková J, Čapková V, Zdráhal Z, Honys D (2014) In search of ligands and receptors of the pollen tube: the missing link in pollen tube perception. Biochem Soc Trans 42:388–394. doi:10.1042/BST20130204

Hamamura Y, Saito C, Awai C, Kurihara D, Miyawaki A, Nakagawa T, Kanaoka MM, Sasaki N, Nakano A, Berger F, Higashiyama T (2011) Live-cell imaging reveals the dynamics of two sperm cells during double fertilization in *Arabidopsis thaliana*. Curr Biol 21:497–502. doi:10.1016/j.cub.2011.02.013

Hamamura Y, Nagahara S, Higashiyama T (2012) Double fertilization on the move. Curr Opin Plant Biol 15:70–77. doi:10.1016/j.pbi.2011.11.001

Hanisch FA (2001) O-glycosylation of the mucin type. Biol Chem 382:143–149. doi:10.1515/bc.2001.022

Heazlewood JL, Durek P, Hummel J, Selbig J, Weckwerth W, Walther D, Schulze WX (2008) PhosPhAt: a database of phosphorylation sites in *Arabidopsis thaliana* and a plant-specific phosphorylation site predictor. Nucleic Acids Res 36:D1015–D1021. doi:10.1093/nar/gkm812

Hepler PK, Winship LJ (2015) The pollen tube clear zone: clues to the mechanism of polarized growth. J Integr Plant Biol 57:79–92. doi:10.1111/jipb.12315

Heslop-Harrison J (1987) Pollen germination and pollen-tube growth. In: Giles KL, Prakash J (eds) Pollen: cytology and development. Academic Press, London, pp 1–78

Heslop-Harrison J, Heslop-Harrison Y (1986) Pollen-tube chemotropism: fact or delusion? In: Cresti M, Romano D (eds) In biology of reproduction and cell motility in plants and animals. University of Sienna Press, Siena, pp 169–174

Heslop-Harrison J, Heslop-Harrison Y, Cresti M, Tiezzi A, Moscatelli A (1988) Cytoskeletal elements, cell shaping and movement in the angiosperm pollen tube. J Cell Sci 91:49–60

Higashiyama T (2010) Peptide signaling in pollen–pistil interactions. Plant Cell Physiol 51:177–189. doi:10.1093/pcp/pcq008

Higashiyama T (2015) The mechanism and key molecules involved in pollen tube guidance. Annu Rev Plant Biol 2015:393–413

Higashiyama T, Kuroiwa H, Kawano S, Kuroiwa T (1998) Guidance in vitro of the pollen tube to the naked embryo sac of *Torenia fournieri*. Plant Cell 10:2019–2032

Holdaway-Clarke TL, Feijó JA, Hackett GR, Kunkel JG, Hepler PK (1997) Pollen tube growth and the intracellular cytosolic calcium gradient oscillate in phase while extracellular calcium influx is delayed. Plant Cell 9:1999–2010

Holmes-Davis R, Tanaka CK, Vensel WH, Hurkman WJ, McCormick S (2005) Proteome mapping of mature pollen of *Arabidopsis thaliana*. Proteomics 5:4864–4884. doi:10.1002/pmic.200402011

Honys D, Twell D (2003) Comparative analysis of the Arabidopsis pollen transcriptome. Plant Physiol 132:640–652. doi:10.1104/pp.103.020925

Honys D, Twell D (2004) Transcriptome analysis of haploid male gametophyte development in Arabidopsis. Genome Biol. doi:10.1186/gb-2004-5-11-r85

Honys D, Combe JP, Twell D, Čapková V (2000) The translationally repressed pollen-specific ntp303 mRNA is stored in non-polysomal mRNPs during pollen maturation. Sex Plant Reprod 13:135–144

Honys D, Reňák D, Twell D (2006) Male gametyphyte development and function. In: da Silva JT (ed) Floriculture, ornamental and plant biotechnology: advances and topical issues, vol 1. Global Science Books, London, pp 76–87

Honys D, Reňák D, Feciková J, Jedelský PL, Nebesářová J, Dobrev P, Čapková V (2009) Cytoskeleton-associated large RNP complexes in tobacco male gametophyte (EPPs) are associated with ribosomes and are involved in protein synthesis, processing, and

localization. J Proteome Res 8:2015–2031. doi:10.1021/pr8009897

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

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P (2008) Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinform 2008:420747. doi:10.1155/2008/420747

Hsieh K, Huang AH (2005) Lipid-rich tapetosomes in Brassica tapetum are composed of oleosin-coated oil droplets and vesicles, both assembled in and then detached from the endoplasmic reticulum. Plant J 43:889–899. doi:10.1111/j.1365-313X.2005.02502.x

Huang MD, Hsing YI, Huang AH (2011) Transcriptomes of the anther sporophyte: availability and uses. Plant Cell Physiol 52:1459–1466. doi:10.1093/pcp/pcr088

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

Hulskamp M, Schneitz K, Pruitt RE (1995) Genetic evidence for a long-range activity that directs pollen tube guidance in Arabidopsis. Plant Cell 7:57–64. doi:10.1105/tpc.7.1.57

Ischebeck T, Valledor L, Lyon D, Gingl S, Nagler M, Meijon M, Egelhofer V, Weckwerth W (2014) Comprehensive cell-specific protein analysis in early and late pollen development from diploid microsporocytes to pollen tube growth. Mol Cell Proteomics 13:295–310. doi:10.1074/mcp.M113.028100

Jia Q-S, Zhu J, Xu XF, Lou Y, Zhang ZL, Zhang ZP, Yang ZN (2014) Arabidopsis AT-hook protein TEK positively regulates the expression of arabinogalactan proteins for Nexine formation. Mol Plant 8:251–260

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*. Ann Bot 108:739–747. doi:10.1093/aob/mcr111

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. Plant Cell 17:2981–2992. doi:10.1105/tpc.105.034603

Kasahara RD, Maruyama D, Hamamura Y, Sakakibara T, Twell D, Higashiyama T (2012) Fertilization recovery after defective sperm cell release in Arabidopsis. Curr Biol 22:1084–1089. doi:10.1016/j.cub.2012.03.069

Kessler SA, Grossniklaus U (2011) She's the boss: signaling in pollen tube reception. Curr Opin Plant Biol 14:622–627. doi:10.1016/j.pbi.2011.07.012

Klahre U, Becker C, Schmitt AC, Kost B (2006) Nt-RhoGDI2 regulates Rac/Rop signaling and polar cell growth in tobacco pollen tubes. Plant J 46:1018–1031. doi:10.1111/j.1365-313X.2006.02757.x

Knorre DG, Kudryashova NV, Godovikova TS (2009) Chemical and functional aspects of posttranslational modification of proteins. Acta Nat 1:29–51

Kovar DR, Drobak BK, Staiger CJ (2000) Maize profilin isoforms are functionally distinct. Plant Cell 12:583–598

Kultz D (1998) Phylogenetic and functional classification of mitogen- and stress-activated protein kinases. J Mol Evol 46:571–588. doi:10.1007/pl00006338

Lalanne E, Honys D, Johnson A, Borner GH, Lilley KS, Dupree P, Grossniklaus U, Twell D (2004) SETH1 and SETH2, two components of the glycosylphosphatidylinositol anchor biosynthetic pathway, are required for pollen germination and tube growth in Arabidopsis. Plant Cell 16:229–240. doi:10.1105/tpc.014407

Lamport DT, Varnai P (2013) Periplasmic arabinogalactan glycoproteins act as a calcium capacitor that regulates plant growth and development. N Phytol 197:58–64. doi:10.1111/nph.12005

Lamport DT, Kieliszewski MJ, Chen Y, Cannon MC (2011) Role of the extensin superfamily in primary cell wall architecture. Plant Physiol 156:11–19. doi:10.1104/pp.110.169011

Lancelle SA, Hepler PK (1992) Ultrastructure of freeze-substituted pollen tubes of *Lilium longiferum*. Protoplasma 167:215–230. doi:10.1007/bf01403385

Lee TY, Bretana NA, Lu CT (2011) PlantPhos: using maximal dependence decomposition to identify plant phosphorylation sites with substrate site specificity. BMC Bioinform 12:13. doi:10.1186/1471-2105-12-261

Lee JS, Hnilova M, Maes M, Lin YC, Putarjunan A, Han SK, Avila J, Torii KU (2015) Competitive binding of antagonistic peptides fine-tunes stomatal patterning. Nature 522:439–443. doi:10.1038/nature14561

Lenartowska M, Michalska A (2008) Actin filament organization and polarity in pollen tubes revealed by myosin II subfragment 1 decoration. Planta 228:891–896. doi:10.1007/s00425-008-0802-5

Lerouge P, Cabanes-Macheteau M, Rayon C, Fischette-Laine AC, Gomord V, Faye L (1998) N-glycoprotein biosynthesis in plants: recent developments and future trends. Plant Mol Biol 38:31–48. doi:10.1023/a:1006012005654

Leydon AR, Beale KM, Woroniecka K, Castner E, Chen J, Horgan C, Palanivelu R, Johnson MA (2013) Three MYB transcription factors control pollen tube differentiation required for sperm release. Curr Biol 23:1209–1214. doi:10.1016/j.cub.2013.05.021

Li HJ, Xue Y, Jia DJ, Wang T, Hi DQ, Liu J, Cui F, Xie Q, Ye D, Yang WC (2011) POD1 regulates pollen tube guidance in response to micropylar female signaling and acts in early embryo patterning in Arabidopsis. Plant Cell 23:3288–3302. doi:10.1105/tpc.111.088914

Li S, Ge FR, Xu M, Zhao XY, Huang GQ, Zhou LZ, Wang JG, Kombrink A, McCormick S, Zhang XS, Zhang Y (2013) Arabidopsis COBRA-LIKE 10, a GPI-anchored protein, mediates directional growth of pollen tubes. Plant J 74:486–497. doi:10.1111/tpj.12139

Li Y, Ye Z, Nie Y, Zhang J, Wang G-L, Wang Z (2015) Comparative phosphoproteome analysis of *Magnaporthe oryzae*-responsive proteins in susceptible and resistant rice cultivars. J Proteomics 115:66–80. doi:10.1016/j.jprot.2014.12.007

Lim ES, Gumpil JS (1984) The flowering, pollination and hybridization of groundnuts (*Arachis hypogaea* L.). Pertanika 7:61–66

Lindner H, Kessler SA, Muller LM, Shimosato-Asano H, Boisson-Dernier A, Grossniklaus U (2015) TURAN and EVAN mediate pollen tube reception in Arabidopsis synergids through protein glycosylation. PLoS Biol. doi:10.1371/journal.pbio.1002139

Ling Y, Chen T, Jing Y, Fan L, Wan Y, Lin J (2013) γ-Aminobutyric acid (GABA) homeostasis regulates pollen germination and polarized growth in *Picea wilsonii*. Planta 238:831–843. doi:10.1007/s00425-013-1938-5

Liu J, Zhong S, Guo X, Hao L, Wei X, Huang Q, Hou Y, Shi J, Wang C, Gu H, Qu LJ (2013) Membrane-bound RLCKs LIP1 and LIP2 are essential male factors controlling male–female attraction in Arabidopsis. Curr Biol 23:993–998. doi:10.1016/j.cub.2013.04.043

Lora J, Herrero M, Hormaza JI (2009) The coexistence of bicellular and tricellular pollen in *Annona cherimola* (*Annonaceae*): implications for pollen evolution. Am J Bot 96:802–808. doi:10.3732/ajb.0800167

Lou Y, Xu XF, Zhu J, Gu JN, Blackmore S, Yang ZN (2014) The tapetal AHL family protein TEK determines nexine formation in the pollen wall. Nat Commun 5:3855. doi:10.1038/ncomms4855

Lovy-Wheeler A, Kunkel JG, Allwood EG, Hussey PJ, Hepler PK (2006) Oscillatory increases in alkalinity anticipate growth and may regulate actin dynamics in pollen tubes of lily. Plant Cell 18:2182–2193. doi:10.1105/tpc.106.044867

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. Plant Cell 23:81–93. doi:10.1105/tpc.110.080499

Lu P, Chai M, Yang J, Ning G, Wang G, Ma H (2014) The Arabidopsis CALLOSE DEFECTIVE MICROSPORE1 gene is required for male fertility through regulating callose metabolism during microsporogenesis. Plant Physiol 164:1893–1904. doi:10.1104/pp.113.233387

Ma H (2005) Molecular genetic analyses of microsporogenesis and micro gametogenesis in flowering plants. Annu Rev Plant Biol 2005:393–434

Maheshwari P (1950) An introduction to embryology of angiosperms. McGraw-Hill, New York

Malpighi M (1675, 1679) Die Anatomie der Pflanzen. I und II Theil. London 1675 und 1679. Engelmann, Leipzig, 1901

Márton ML, Cordts S, Broadhvest J, Dresselhaus T (2005) Micropylar pollen tube guidance by egg apparatus 1 of maize. Science 307:573–576. doi:10.1126/science.1104954

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. Curr Biol 22:1194–1198. doi:10.1016/j.cub.2012.04.061

Maruyama D, Hamamura Y, Takeuchi H, Susaki D, Nishimaki M, Kurihara D, Kasahara RD, Higashiyama T (2013) Independent control by each female gamete prevents the attraction of multiple pollen tubes. Dev Cell 25:317–323. doi:10.1016/j.devcel.2013.03.013

Mascarenhas JP (1993) Molecular mechanisms of pollen tube growth and differentiation. Plant Cell 5:1303–1314. doi:10.1105/tpc.5.10.1303

Mascarenhas JP, Machlis L (1962) The hormonal control of the directional growth of pollen tubes. Vitam Horm 20:347–372

Mayank P, Grossman J, Wuest S, Boisson-Dernier A, Roschitzki B, Nanni P, Nühse T, Grossniklaus U (2012) Characterization of the phosphoproteome of mature Arabidopsis pollen. Plant J 72:89–101. doi:10.1111/j.1365-313X.2012.05061.x

McCue AD, Cresti M, Feijó JA, Slotkin RK (2011) Cytoplasmic connection of sperm cells to the pollen vegetative cell nucleus: potential roles of the male germ unit revisited. J Exp Bot 62:1621–1631. doi:10.1093/jxb/err032

Meyer LJ, Gao J, Xu D, Thelen JJ (2012) Phosphoproteomic analysis of seed maturation in Arabidopsis, rapeseed, and soybean. Plant Physiol 159:517–528. doi:10.1104/pp.111.191700

Michard E, Dias P, Feijó JA (2008) Tobacco pollen tubes as cellular models for ion dynamics: improved spatial and temporal resolution of extracellular flux and free cytosolic concentration of calcium and protons using pHluorin and YC3.1 CaMeleon. Sex Plant Reprod 21:169–181. doi:10.1007/s00497-008-0076-x

Michard E, Alves F, Feijó JA (2009) The role of ion fluxes in polarized cell growth and morphogenesis: the pollen tube as an experimental paradigm. Intern J Dev Biol 53:1609–1622. doi:10.1387/ijdb.072296em

Mogami N, Miyamoto M, Onozuka M, Nakamura N (2006) Comparison of callose plug structure between dicotyledon and monocotyledon pollen germinated in vitro. Grana 45:249–256. doi:10.1080/00173130600726687

Mori T, Kuroiwa H, Higashiyama T, Kuroiwa T (2005) GENERATIVE CELL SPECIFIC 1 is essential for angiosperm fertilization. Nat cell biol 8:64–71

Mulcahy DL (1979) The rise of the angiosperms: a genecological factor. Science 206:20–23. doi:10.1126/science.206.4414.20

Mulcahy GB, Mulcahy DL (1988) The effect of supplemented media on the growth in vitro of binucleate and trinucleate pollen. Plant Sci 55:213–216

Mulcahy DL, Sari Gorla M, Mulcahy GB (1996) Pollen selection: past, present and future. Sex Plant Reprod 9:353–356

Nawaschin S (1898) Resultate einer Revision der Befruchtungsvorgänge bei *Lilium martagon* und *Fritillaria tenella*. Bulletin de l'Académie Impériale des Sciences 9:377–382

Nguema-Ona E, Coimbra S, Vicre-Gibouin M, Mollet J-C, Driouich A (2012) Arabinogalactan proteins in root and pollen-tube cells: distribution and functional aspects. Ann Bot 110:383–404. doi:10.1093/aob/mcs143

Noir S, Bräutigam A, Colby T, Schmidt J, Panstruga R (2005) A reference map of the *Arabidopsis thaliana* mature pollen proteome. Biochem Biophys Res Commun 337:1257–1266. doi:10.1016/j.bbrc.2005.09.185

Oh SA, Pal MD, Park SK, Johnson JA, Twell D (2010) The tobacco MAP215/Dis1-family protein TMBP200 is required for the functional organization of microtubule arrays during male germline establishment. J Exp Bot 61:969–981. doi:10.1093/jxb/erp367

Okuda S, Tsutsui H, Shiina K, Sprunck S, Takeuchi H, Yui R, Kasahara RD, Hamamura Y, Mizukami A, Susaki D, Kawano N, Sakakibara T, Namiki S, Itoh K, Otsuka K, Matsuzaki M, Nozaki H, Kuroiwa T, Nakano A, Kanaoka MM, Dresselhaus T, Sasaki N, Higashiyama T (2009) Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. Nature 458:357–361. doi:10.1038/nature07882

Onelli E, Idilli AI, Moscatelli A (2015) Emerging roles for microtubules in angiosperm pollen tube growth highlight new research cues. Front Plant Sci. doi:10.3389/fpls.2015.00051

Pacini E (1990) Tapetum and microspore function. In: Blackmore S, Knox RB (eds) Microspores: evolution and ontogeny. Academic Press, London, pp 213–237

Pacini E (1996) Types and meaning of pollen carbohydrate reserves. Sex Plant Reprod 9:362–366

Pacini E, Guarnieri M, Nepi M (2006) Pollen carbohydrates and water content during development, presentation, and dispersal: a short review. Protoplasma 228:73–77. doi:10.1007/s00709-006-0169-z

Palanivelu R, Preuss D (2000) Pollen tube targeting and axon guidance: parallels in tip growth mechanisms. Trends Cell Biol 10:517–524. doi:10.1016/s0962-8924(00)01849-3

Palanivelu R, Preuss D (2006) Distinct short-range ovule signals attract or repel *Arabidopsis thaliana* pollen tubes in vitro. BMC Plant Biol 6:7. doi:10.1186/1471-2229-6-7

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

Park SK, Howden R, Twell D (1998) The *Arabidopsis thaliana* gametophytic mutation *gemini pollen1* disrupts microspore polarity, division asymmetry and pollen cell fate. Development 125:3789–3799

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. J Exp Bot 65:5459–5471

Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 8:785–786. doi:10.1038/nmeth.1701

Phan HA, Iacuone S, Li SF, Parish RW (2011) The MYB80 transcription factor is required for pollen development and the regulation of tapetal programmed cell death in *Arabidopsis thaliana*. Plant Cell 23:2209–2224. doi:10.1105/tpc.110.082651

Pierloni A, Martelli PL, Casadio R (2008) PredGPI: a GPI-anchor predictor. BMC Bioinform 9:392. doi:10.1186/1471-2105-9-392

Pierson ES, Miller DD, Callaham DA, van Aken J, Hackett G, Hepler PK (1996) Tip-localized calcium entry fluctuates during pollen tube growth. Dev Biol 174:160–173. doi:10.1006/dbio.1996.0060

Prado N, de Dios Alche J, Casado-Vela J, Mas S, Villalba M, Rodriguez R, Batanero E (2014) Nanovesicles are secreted during pollen germination and pollen tube growth: a possible role in fertilization. Mol Plant 7:573–577. doi:10.1093/mp/sst153

Puc M (2003) Characterisation of pollen allergens. Ann Agric Environ Med 10:143–149

Purkyně JE (1830) De cellulis antherarum fibrosis nec non de granorum pollinarium formis: commentatio phytomica. Sumtibus J. D. Gruesonii, Breslau, Vratislaviae

Qin Y, Yang ZBA (2011) Rapid tip growth: insights from pollen tubes. Semin Cell Dev Biol 22:816–824. doi:10.1016/j.semcdb.2011.06.004

Qin Y, Leydon AR, Manziello A, Pandey R, Mount D, Denic S, Vasic B, Johnson MA, Palanivelu R (2009) Penetration of the stigma and style elicits a novel transcriptome in pollen tubes, pointing to genes critical for growth in a pistil. PLoS Genet. doi:10.1371/journal.pgen.1000621

Qin Y, Wysocki RJ, Somogyi A, Feinstein Y, Franco JY, Tsukamoto T, Dunatunga D, Levy C, Smith S, Simpson R, Gang D, Johnson MA, Palanivelu R (2011) Sulfinylated azadecalins act as functional mimics of a pollen germination stimulant in Arabidopsis pistils. Plant J 68:800–815. doi:10.1111/j.1365-313X.2011.04729.x

Qin T, Liu X, Li J, Sun J, Song L, Mao T (2014) Arabidopsis microtubule-destabilizing protein 25 functions in pollen tube growth by severing actin filaments. Plant Cell 26:325–339. doi:10.1105/tpc.113.119768

Quilichini TD, Grienenberger E, Douglas CJ (2015) The biosynthesis, composition and assembly of the outer pollen wall: a tough case to crack. Phytochemistry 113:170–182. doi:10.1016/j.phytochem.2014.05.002

Ray SM, Park SS, Ray A (1997) Pollen tube guidance by the female gametophyte. Development 124:2489–2498

Ren HY, Xiang Y (2007) The function of actin-binding proteins in pollen tube growth. Protoplasma 230:171–182. doi:10.1007/s00709-006-0231-x

Romagnoli S, Cai G, Faleri C, Yokota E, Shimmen T, Cresti M (2007) Microtubule- and actin filament-dependent motors are distributed on pollen tube mitochondria and contribute differently to their movement. Plant Cell Physiol 48:345–361. doi:10.1093/pcp/pcm001

Rotman N, Rozier F, Boavida L, Dumas C, Berger F, Faure JE (2003) Female control of male gamete delivery during fertilization in *Arabidopsis thaliana*. Curr Biol 13:432–436

Russell SD, Jones DS (2015) The male germline of angiosperms: repertoire of an inconspicuous but important cell lineage. Front Plant Sci 6:173. doi:10.3389/fpls.2015.00173

Russell SD, Strout GW, Stramski AK, Mislan TW, Thompson RA, Schoemann LM (1996) Development polarization and morphogenesis of the generative and sperm cells of *Plumbago zeylanica*. 1. Descriptive cytology and three-dimensional organization. Am J Bot 83:1435–1453

Rutley N, Twell D (2015) A decade of pollen transcriptomics. Plant Reprod 28:73–89. doi:10.1007/s00497-015-0261-7

Šamaj J, Muller J, Beck M, Böhm N, Menzel D (2006) Vesicular trafficking, cytoskeleton and signalling in root hairs and pollen tubes. Trends Plant Sci 11:594–600. doi:10.1016/j.tplants.2006.10.002

Sanati Nezhad A, Packirisamy M, Geitmann A (2014) Dynamic, high precision targeting of growth modulating agents is able to trigger pollen tube growth reorientation. Plant J 80:185–195. doi:10.1111/tpj.12613

Sanders LC, Lord EM (1989) Directed movement of latex particles in the gynoecia of three species of flowering plants. Science 243:1606–1608

Sanders LC, Lord EM (1992) A dynamic role for the stylar matrix during pollen tube extension. Int Rev Cytol 140:297–318

Schrauwen JA, de Groot PF, van Herpen MM, van der Lee T, Reynen WH, Weterings KA, Wullems GJ (1990) Stage-related expression of mRNAs during pollen development in lily and tobacco. Planta 182:298–304. doi:10.1007/BF00197125

Schwacke R, Grallath S, Breitkreuz KE, Stransky E, Stransky H, Frommer WB, Rentsch D (1999) LeProT1, a transporter for proline, glycine betaine, and gamma-amino butyric acid in tomato pollen. Plant Cell 11:377–392

Schwemmie J (1968) Selective fertilization in Oenothera. Adv Genet 14:225–324

Scotland RW, Worltey AH (2003) How many species of seed plants are there? Taxon 52:101–104

Scott RJ, Spielman M, Dickinson HG (2004) Stamen structure and function. Plant Cell 16(Suppl):S46–S60. doi:10.1105/tpc.017012

Sheoran IS, Sproule KA, Olson DJH, Ross ARS, Sawhney VK (2006) Proteome profile and functional classification of proteins in *Arabidopsis thaliana* (Landsberg erecta) mature pollen. Sex Plant Reprod 19:185–196. doi:10.1007/s00497-006-0035-3

Sheoran IS, Ross ARS, Olson DJH, Sawhney VK (2009) Compatibility of plant protein extraction methods with mass spectrometry for proteome analysis. Plant Sci 176:99–104. doi:10.1016/j.plantsci.2008.09.015

Shi YY, Tao WJ, Liang SP, Lu YT, Zhang L (2009) Analysis of the tip-to-base gradient of CaM in pollen tube pulsant growth using in vivo CaM-GFP system. Plant Cell Rep 28:1253–1264. doi:10.1007/s00299-009-0725-z

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

Slotkin RK, Vaughn M, Borges F, Tanurdzic M, Becker JD, Feijo JA, Martienssen RA (2009) Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. Cell 136:461–472. doi:10.1016/j.cell.2008.12.038

Staiger CJ, Poulter NS, Henty JL, Franklin-Tong VE, Blanchoin L (2010) Regulation of actin dynamics by actin-binding proteins in pollen. J Exp Bot 61:1969–1986. doi:10.1093/jxb/erq012

Stepper J, Shastri S, Loo TS, Preston JC, Novak P, Man P, Moore CH, Havlicek V, Patchett ML, Norris GE (2011) Cysteine S-glycosylation, a new post-translational modification found in glycopeptide bacteriocins. FEBS Lett 585:645–650. doi:10.1016/j.febslet.2011.01.023

Strasburger E (1884) Neue Untersuchungen uber den Befruchtungsvorgang bei den Phanerogamen als Grundlage fur eine Theorie der Zeugung. Gustav Fischer, Jena

Szumlanski AL, Nielsen E (2009) The Rab GTPase RabA4d regulates pollen tube tip growth in *Arabidopsis thaliana*. Plant Cell 21:526–544. doi:10.1105/tpc.108.060277

Takeuchi H, Higashiyama T (2011) Attraction of tip-growing pollen tubes by the female gametophyte. Curr Opin Plant Biol 14:614–621. doi:10.1016/j.pbi.2011.07.010

Takeuchi H, Higashiyama T (2012) A species-specific cluster of defensin-like genes encodes diffusible pollen tube attractants in Arabidopsis. PLoS Biol 10:e1001449. doi:10.1371/journal.pbio.1001449

Terasaka O, Niitsu T (1987) Unequal cell division and chromatin differentiation in pollen grain cells I. Centrifugal, cold and caffeine treatment. Bot Mag (Tokyo) 100:205–216

Ting JT, Wu SS, Ratnayake C, Huang AH (1998) Constituents of the tapetosomes and elaioplasts in *Brassica campestris* tapetum and their degradation and retention during microsporogenesis. Plant J 16:541–551

Tsukamoto T, Qin Y, Huang Y, Dunatunga D, Palanivelu R (2010) A role for LORELEI, a putative glycosylphosphatidylinositol-anchored protein, in *Arabidopsis thaliana* double fertilization and early seed development. Plant J 62:571–588. doi:10.1111/j.1365-313X.2010.04177.x

Tupý J (1982) Alterations in polyadenylated RNA during pollen maturation and germination. Biol Plant 24:331–340

Tupý J, Říhová L, Žárský V (1991) Production of fertile tobacco pollen from microspores in suspension culture and its storage for in situ pollination. Sex Plant Reprod 4:284–287

Twell D (2011) Male gametogenesis and germline specification in flowering plants. Sex Plant Reprod 24:149–160. doi:10.1007/s00497-010-0157-5

Twell D, Park SK, Hawkins TJ, Schubert D, Schmidt R, Smertenko A, Hussey PJ (2002) MOR1/GEM1 has an essential role in the plant-specific cytokinetic phragmoplast. Nat Cell Biol 4:711–714. doi:10.1038/ncb844

Uebler S, Dresselhaus T (2014) Identifying plant cell-surface receptors: combining 'classical' techniques with novel methods. Biochem Soc Trans 42:395–400. doi:10.1042/bst20130251

Ueda T, Iwashita H, Hashimoto Y, Imoto T (1996) Stabilization of lysozyme by introducing *N*-glycosylation signal sequence. J Biochem 119:157–161

van Bentem SD, Anrather D, Dohnal I, Roitinger E, Csaszar E, Joore J, Buijnink J, Carreri A, Forzani C, Lorkovic ZJ, Barta A, Lecourieux D, Verhounig A, Jonak C, Hirt H (2008) Site-specific phosphorylation profiling of Arabidopsis proteins by mass spectrometry and peptide chip analysis. J Proteome Res 7:2458–2470. doi:10.1021/pr8000173

Vidali L, McKenna ST, Hepler PK (2001) Actin polymerization is essential for pollen tube growth. Mol Biol Cell 12:2534–2545

Vogler F, Schmalzl C, Englhart M, Bircheneder M, Sprunck S (2014) Brassinosteroids promote Arabidopsis pollen germination and growth. Plant Reprod 27:153–167. doi:10.1007/s00497-014-0247-x

Vogler F, Konrad SSA, Sprunck S (2015) Knockin' on pollen's door: live cell imaging of early polarization events in germinating Arabidopsis pollen. Front Plant Sci. doi:10.3389/fpls.2015.00246

Völz R, Heydlauff J, Ripper D, von Lyncker L, Groß-Hardt R (2013) Ethylene signaling is required for synergid degeneration and the establishment of a pollen tube block. Dev Cell 25:310–316. doi:10.1016/j.devcel.2013.04.001

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. doi:10.1242/dev.02683

Wang Y, Zhang W-Z, Song L-F, Zou J-J, Su Z, Wu W-H (2008) Transcriptome analyses show changes in gene expression to accompany pollen germination and tube growth in Arabidopsis. Plant Physiol 148:1201–1211. doi:10.1104/pp.108.126375

Wei LQ, Xu WY, Deng ZY, Su Z, Xue Y, Wang T (2010) Genome-scale analysis and comparison of gene expression profiles in developing and germinated pollen in *Oryza sativa*. BMC Genomics 11:338. doi:10.1186/1471-2164-11-338

Williams JH (2009) *Amborella trichopoda* (Amborellaceae) and the evolutionary developmental origins of the angiosperm progamic phase. Am J Bot 96:144–165. doi:10.3732/ajb.0800070

Williams JH, Taylor ML, O'Meara BC (2014) Repeated evolution of tricellular (and bicellular) pollen. Am J Bot 101:559–571. doi:10.3732/ajb.1300423

Wilsen KL, Lovy-Wheeler A, Voigt B, Menzel D, Kunkel JG, Hepler PK (2006) Imaging the actin cytoskeleton in growing pollen tubes. Sex Plant Reprod 19:51–62. doi:10.1007/s00497-006-0021-9

Wilson C, Voronin V, Touraev A, Vicente O, Heberle Bors E (1997) A developmentally regulated MAP kinase activated by hydration in tobacco pollen. Plant Cell 9:2093–2100

Wolschin F, Weckwerth W (2005) Combining metal oxide affinity chromatography (MOAC) and selective mass spectrometry for robust identification of in vivo protein phosphorylation sites. Plant Methods 1(1):9

Woollard AAD, Moore I (2008) The functions of Rab GTPases in plant membrane traffic. Curr Opin Plant Biol 11:610–619. doi:10.1016/j.pbi.2008.09.010

Worrall D, Hird DL, Hodge R, Paul W, Draper J, Scott R (1992) Premature dissolution of the microsporocyte callose wall causes male sterility in transgenic tobacco. Plant Cell 4:759–771. doi:10.1105/tpc.4.7.759

Wu H, de Graaf BHJ, Mariani C, Cheung AY (2001) Hydroxyproline-rich glycoproteins in plant reproductive tissues: structure, functions and regulation. Cell Mol Life Sci 58:1418–1429

Yadegari R, Drews GN (2004) Female gametophyte development. Plant Cell 16:S133–S141. doi:10.1105/tpc.018192

Yu GH, Zou J, Feng J, Peng XB, Wu JY, Wu YL, Palanivelu R, Sun MX (2014) Exogenous γ-aminobutyric acid (GABA) affects pollen tube growth via modulating putative Ca^{2+}-permeable membrane channels and is coupled to negative regulation on glutamate decarboxylase. J Exp Bot 65:3235–3248. doi:10.1093/jxb/eru171

Zaki MAM, Dickinson H (1991) Microspore-derived embryos in Brassica: the significance of division asymmetry in pollen mitosis I to embryogenic development. Sex Plant Reprod 4:48–55

Zhang Y, McCormick S (2010) The regulation of vesicle trafficking by small GTPases and phospholipids during pollen tube growth. Sex Plant Reprod 23:87–93. doi:10.1007/s00497-009-0118-z

Zhang ZB, Zhu J, Gao JF, Wang C, Li H, Li H, Zhang HQ, Zhang S, Wang DM, Wang QX, Huang H, Xia HJ, Yang ZN (2007) Transcription factor AtMYB103 is required for anther development by regulating tapetum development, callose dissolution and exine formation in Arabidopsis. Plant J 52:528–538. doi:10.1111/j.1365-313X.2007.03254.x

Zhang Y, He J, McCormick S (2009) Two Arabidopsis AGC kinases are critical for the polarized growth of pollen tubes. Plant J 58:474–484. doi:10.1111/j.1365-313X.2009.03792.x

Zhao HP, Ren HY (2006) Rop1Ps promote actin cytoskeleton dynamics and control the tip growth of lily pollen tube. Sex Plant Reprod 19:83–91. doi:10.1007/s00497-006-0024-6

Zhao XY, Wang Q, Li S, Ge FR, Zhou LZ, McCormick S, Zhang Y (2013) The juxtamembrane and carboxy-terminal domains of Arabidopsis PRK2 are critical for ROP-induced growth in pollen tubes. J Exp Bot 64(18):5599–5610. doi:10.1093/jxb/ert323

Zheng ZL, Yang ZB (2000) The Rop GTPase: an emerging signaling switch in plants. Plant Mol Biol 44:1–9. doi:10.1023/a:1006402628948

Zhu L, Zhang Y, Kang E, Xu Q, Wang M, Rui Y, Liu B, Yuan M, Fu Y (2013) MAP18 regulates the direction of pollen tube growth in Arabidopsis by modulating F-actin organization. Plant Cell 25:851–867. doi:10.1105/tpc.113.110528