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Commentary

Pollen–pistil interactions in compatible pollination

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Plant sexual reproduction depends on highly specific interactions between pollen and pistil, the male gametophyte and the female reproductive organ, respectively (1, 2). The pistil is composed of three major structural parts: the stigma, the style, and the ovary. The stigma has a pollen-receptive surface and several underlying secretory cell layers. The style connects the stigma to the basally located ovary containing the ovules. Within each ovule, an egg cell develops inside the embryo sac. In compatible pollination, the pollen grain germinates and extrudes a pollen tube upon landing on the stigmatic surface. Each pollen tube penetrates the stigmatic cell layers and elongates within a specialized tissue in the style called the transmitting tissue, eventually reaching the ovary, where it enters an ovule and penetrates the embryo sac. The pollen tube tip bursts in the embryo sac to release the male germinal cells for fertilization. Incompatible pollen may be arrested at the stigma or anywhere along the pathway of pollen tube elongation. Pollen has a high capacity to support its activity during germination and tube growth (3, 4). Pistil tissues are believed to provide physical and chemical supports and directional guidance to the pollen tube growth process (1, 2, 4–6). The arrest of pollen germination and tube growth in transgenic plants in which the stigmatic or the transmitting tissues are ablated by cytotoxins (7, 8) and the loss of directional pollen tube growth in the ovary of embryo sac-defective mutants (9) indicate that important functions are contributed by the pistil to pollen germination and tube growth. Cytological and biochemical observations (1, 2) suggest that the pollen and pistil extracellular matrix (6) and the pollen cytoskeleton (10) are important for the pollination process. Recent experiments have identified some of these components and many candidate molecules which may participate in compatible pollen–pistil interactions. One of these molecules, a pollen-specific extracellular matrix protein, Pex-1, from maize is described by Rubinstein *et al.* (11) in this issue of the *Proceedings*. These findings will be discussed with a view toward exploring how pollen and pistil extracellular components may interact and how these extracellular interactions may be transduced and translated into the activities needed for pollen tube growth.

Extracellular Components of Pollen and Pistil

Until the pollen tube enters the embryo sac, its entire journey is within the extracellular matrix of pistil tissues. This matrix is enriched in secretory materials, including sugars, amino acids, and fatty acids, and many of these are found in more complex molecules such as polysaccharides, glycoproteins, and glycolipids. They are believed to serve variously as recognition molecules, nutrients, adhesives, and attractants for pollen germination and tube growth (12–15). Arabinogalactan proteins (AGPs) constitute a major class of proteins in the stigmatic and transmitting tissue extracellular matrix (12–14, 16–18). Several pistil AGPs or AGP-like proteins have been purified (13, 19, 20). A tobacco stylar transmitting tissue-specific AGP, TTS protein, has been shown to promote pollen tube growth and to attract pollen tubes *in vitro* (H. M. Wu, H. Wang, and A.Y.C., unpublished data). When the level of TTS protein is suppressed in transgenic tobacco plants, the pollen tube growth rate is also reduced, leading to reduced female fertility. TTS protein adheres to the surface and tip of pollen tubes and is incorporated into pollen tube walls. Moreover, pollen tubes deglycosylate TTS molecules *in vitro*, causing a substantial reduction in their molecular weights while their polypeptide backbones remain intact. *In vivo*, pollination leads to the accumulation of underglycosylated TTS protein (21). These results provide biochemical evidence for the involvement of a stylar transmitting tissue protein in pollen tube growth and suggest that the sugar moieties on TTS protein may be a source of nutrients for this process. The role of other stylar extracellular matrix proteins (19, 20, 22–25) in pollen tube growth remains to be determined.

It has been shown that the stylar transmitting tissue of three angiosperms can translocate inert latex beads, implicating an extracellular matrix with properties adequate to support a process similar to pollen tube growth (26). Lily pollen tubes have been observed to adhere to each other and to the outer wall of the epidermal cells in the transmitting canal (27), suggesting the presence of surface adhesive molecules on the pollen tube surface and along the transmitting tract. Lord and Sanders (6) suggested that the transmitting-tract extracellular matrix may support pollen tube growth based on surface adhesive molecules. The observation that the tobacco stylar AGP, TTS protein, adheres efficiently to pollen tubes suggests that it may function as a surface adhesive for pollen tube growth (unpublished data).

Pollen secretes a variety of molecules, including polysaccharides and proteins (1, 28). Some of these are deposited in the pollen tube walls, presumably as structural components or as recognition molecules for interactions with the pistil. In this issue, Rubinstein *et al.* (11) describe a maize gene, *Pex-1*, which encodes a pollen-specific extensin-like protein (18). *Pex-1* is expressed most actively in mature pollen. Preliminary immunodetection data indicate that the Pex-1 protein accumulates in the pollen tube walls. Pex-1 protein has an N-terminal globular domain and a C-terminal extensin-like domain, similar to the sexual agglutinins of *Chlamydomonas* which mediate the initial recognition of opposite mating types (29). Rubinstein *et al.* propose that the Pex-1 protein may mediate recognition between pollen or pollen tubes and the pistil or play a structural role in the pollen tube walls. Based on its deduced amino acid composition, Rubinstein *et al.* suggest that the Pex-1 protein may be an AGP. It has been shown that arabinogalactan epitopes and pectins are deposited along the inner and outer pollen tube wall layers, respectively (30, 31). Pectins may interact with AGPs in the extracellular matrix (32). It will be interesting to see whether the pollen tube surface pectins act as linker molecules for AGPs in the transmitting tissue extracellular matrix and those associated with the pollen tubes.

Cytoskeletal Components of the Pollen Tube

Characterization of *in vitro* pollen tubes has provided much of the understanding of the cellular and biochemical basis of pollen tube growth. Pollen tube elongation is a tip-growth process. The pollen cytoplasm, its vegetative nucleus, and two sperm cells are restricted to the tip of the

tube (2–4, 10). Cytoskeletal components—including microfilaments, myosins, profilins, microtubules, and kinesins—have been detected in pollen tubes. An actomyosin-based motor is believed to be the primary force for the cytoplasmic streaming which transports secretory vesicles from distally located Golgi apparatus and endoplasmic reticulum to the tube apex to deposit new membrane and wall materials at the tip to support its extension. The microtubule system may play a more subtle and yet unknown role in pollen tube growth.

Ca^{2+} is essential for pollen tube growth (33) and is believed to control this process by regulating the activity of the pollen cytoskeleton (3, 4, 10). An intracellular gradient of Ca^{2+} exists in the pollen tube, and an influx of Ca^{2+} occurs across the pollen tube tip. Using ratiometric ion imaging and a Ca^{2+}-specific vibrating electrode, Pierson *et al.* (34) have shown convincingly that *in vitro* grown lily pollen tubes have a steep, tip-focused intracellular Ca^{2+} gradient (from about ≈ 3 μM at the apex to 0.2 μM at about 20 μm from the tip) and that a tip-directed influx of extracellular Ca^{2+} occurs across the tube tip. The intracellular Ca^{2+} gradient is dissipated by ionophoretic application of Ca^{2+}-chelating buffers. This is accompanied by the abolition of Ca^{2+} influx and the arrest of pollen tube growth. The normal cytoplasmic streaming pattern is perturbed and organelles in the subapical zone of the pollen tube invade the apex. These results suggest that the Ca^{2+} gradient and Ca^{2+} influx are closely coupled and important to pollen tube growth and to the proper cytoplasmic streaming pattern in the pollen. Furthermore, the Ca^{2+} influx is stimulated by tip stretching and dissipated by a loss in turgor pressure at the tip, suggesting that a stretch-activated channel is involved in its regulation, as has been shown in other tip-growth cells (35). Several Ca^{2+}-binding proteins are associated with pollen tube tips (10), and they may participate in regulating the pollen cytoskeletal activities.

In their model for pollen tube growth, Lord and Sanders (6) invoked the participation of pollen tube plasma membrane-associated linker molecules to integrate the extracellular interactions between pistil and pollen to the pollen cytoskeleton to elicit cellular activities needed for pollen tube growth. Star-shaped clusters of microfilaments have been observed in the cytoplasm of *in vivo* lily pollen tubes (27, 36). It has been speculated (27) that these structures may be analogous to focal adhesions in migrating animal cells which link the extracellular matrix to the cortical cytoskeleton via transmembranous receptors (37). More structural, biochemical, and molecular definitions are necessary to substantiate this model, which incorporates cell surface molecules from both pollen and pistil, as well as plasmalemma and cytoskeletal components from the pollen, all of which are needed to fully interpret pollen tube growth *in vivo*.

Pollination Signals and Signal Transduction Molecules

Pollen grains have a bilayered coat: the cellulose, pectin, and protein-containing intine and the pigment-containing exine (1, 28). Lipids and proteins are also embedded in the exine surface in a layer known as the tryphine. A number of surface wax-deficient mutants are male sterile (38), implying a role for long-chain lipids in pollination. The pollen from a male sterile and waxless *Arabidopsis* mutant, *pop-1*, lacks long-chain lipids (C_{29} and C_{30}) and the tryphine coat at maturity (39). They fail to germinate on *pop-1* and wild-type stigmas. However, the *pop-1* pollen can germinate and grow tubes normally *in vitro*. High humidity during pollination by *pop-1* and copollination by *pop-1* and wild-type also restore a normal hydration process for *pop-1* pollen and restore their fertility. These results suggest that pollen hydration cannot occur in the absence of these pollen surface lipids, which are proposed to either directly or indirectly signal the dry stigma in *Arabidopsis* to initiate the pollen hydration process.

Flavonoid mutants have also been observed to be male sterile (40, 41). Pollen from flavonoid-deficient maize and petunia fail to germinate tubes *in vitro* and on the mutant stigma (42). A hydrophobic flavonoid, kaempherol, can restore *in vitro* germination and tube growth from these mutant pollen grains. Coinoculation of flavonoid-deficient mutant stigmas with flavonoid-deficient pollen and exogenous kaempherol can restore germination and normal fertility. These results indicate that kaempferol signals early events in the pollination process. Furthermore, kaempferol is present in wild-type stigma tissues which can support the germination of flavonoid-deficient pollen and restore their male fertility.

Pollination also induces a broad spectrum of physiological, molecular, and developmental responses in various pistil tissues (refs. 43–47 and unpublished data). It is believed that at least some of these changes facilitate pollen germination, tube growth, and pollen tube entrance into the embryo sacs. Pollens from many species contain 1-aminocyclopropane-1-carboxylic acid (ACC), which is released on the stigma upon pollination and converted by stigmatic ACC oxidase to ethylene. Exogenous ACC and ethylene can elicit some of the pollination-induced responses in unpollinated pistils (ref. 44 and unpublished data). Therefore, the pollen-borne ACC is believed to serve as a pollination signal which is converted to ethylene and mediated to downstream events.

It is not clear how pollination signals are transduced to activate subsequent activities in both pollen and pistil. A Ca^{2+}-dependent, calmodulin-independent kinase (CDPK) cDNA and a receptor kinase gene (PRK-1) have been isolated from maize and *Petunia inflata*, respectively (48, 49). Both of these mRNAs accumulate specifically in the late stages of pollen development and during pollen germination, suggesting possible functions in the germination and tube growth process. Addition of oligonucleotides antisense to the CDPK mRNAs impairs pollen germination and tube growth *in vitro*. It has been proposed that the maize pollen-specific CDPK may be associated with pollen microfilaments to regulate pollen tube growth (48). Protein kinases have also been implicated in the transduction of the pollination signal to some of the pistil responses (unpublished data). The identification of the *in vivo* substrates for these pollen and pistil kinases will be important for determining their roles in pollination.

Studies in the *Brassica* self-incompatible pollination system have led to the identification of two classes of proteins related to the *S*-locus glycoprotein, the *S*-locus-related proteins and the *S*-locus kinase-related proteins, which have a kinase domain and an *S*-locus protein domain (50). These *S*-locus-related proteins are expressed in different tissue types in a variety of plants and are speculated to participate in different cell–cell signaling pathways. The stigmatic papilla of the self-compatible *Arabidopsis* expresses an *S*-locus-related protein (51). It will be important to determine whether this protein indeed participates in compatible pollen–pistil interaction.

Pollen Tube Guidance

The most intriguing but controversial area in pollen–pistil interactions is that of pollen tube guidance. Although experiments arguing against long-range tropism within the style have been reported (52), pollen tubes universally grow from the stigma to the ovary. Observations suggesting mechanical, electrical, chemical, and pollination-induced signals for pollen tube guidance have all been reported (2, 4, 53, 54). A universal mechanism apparently cannot be invoked to explain this phenomenon. It is possible that a combination of mechanisms, even within one species, may exist to ensure the proper pollen tube growth directions.

The maize silk is probably the best described structure in which cellular architecture and additional factors are involved in pollen tube guidance (4, 54). Pollen grains germinate on silk hair, and pollen tubes elongate following the silk hair orientation. They traverse several

rows of cortical cells to enter the transmitting tissue in the main axis of the silk with their tips pointing toward the ovary and continue their journey in that direction. The maize silk is said to provide mechanical control for pollen tube growth. Additional factors, perhaps chemotropic, must be invoked to explain the targeting of pollen tubes to the transmitting tissue. Similar mechanical guidance for pollen tube directionality has also been attributed to the trichomes on pearl millet stigma (55).

In vitro pollen tubes respond chemotropically to pistil tissues or their extracts from many plant species (2, 53, 56, 57). Ca^{2+} has been shown to attract pollen tubes *in vitro* from a number of species, including *Antirrhinum* (58) and pearl millet (59); the latter also responds chemotropically to glucose. Interestingly, the tobacco stylar TTS proteins are more highly glycosylated at the ovarian end of the style than at the stigmatic end (unpublished data). Furthermore, two-dimensional SDS/PAGE has shown that the more glycosylated TTS proteins have more acidic isoelectric points. Thus, TTS proteins display a gradient of increasing glycosylation and acidity from the stigma to the base of the style, coincident with the direction of pollen tube growth. Since TTS proteins interact intimately with pollen tubes by binding to them, which in turn deglycosylate these proteins, the increasing sugar contents in TTS proteins along the length of the style may contribute to guiding pollen tubes toward the ovary.

The most obvious directional guidance for pollen tubes occurs in the ovary, where they turn from their basally oriented growth direction to enter the ovules. Exudates from the ovules are rich in carbohydrates (60, 61), and the synergid cells have a high concentration of Ca^{2+} (5, 62, 63). Ovary fragments, extracts, and ovules (53, 56, 59) from several species also elicit chemotropic response from *in vitro* grown pollen tubes. Several *Arabidopsis* mutants defective in embryo sac development also show anomalous pollen tube guidance in the ovary (9). Although these mutants do not directly identify what substances are involved in pollen tube guidance; they provide genetic evidence that the embryo sac is important to this phenomenon.

Limitations in studying pollen tube guidance derive partly from the difficulty of going beyond observing pollen tube growth *in vivo* and partly from the reliance on *in vitro* or semi-*in vivo* assay systems which either do not mimic or perturb the environment that pollen tubes normally encounter. A genetic approach to obtain mutants defective in pollen tube growth would be the key to this problem. Extensive mutant screens in *Arabidopsis* have so far not recovered mutants in which pollen tube growth properties are perturbed but all the pistil components are structurally normal (R. Pruitt, Harvard University, personal communication). This may be due to gene redundancy and overlapping functions to safeguard against the obliteration of successful gamete fusions. Alternatively, mutations which affect the processes that produce directional cues for pollen tube growth may also affect essential processes in the vegetative phase, thus precluding the observation of their effects on sexual reproduction. However, further efforts into identifying mutations that directly affect the pollination and fertilization processes in an otherwise normal flower are warranted despite their apparent difficulty.

Concluding Remarks

Current studies on pollen–pistil interactions have the advantage of a voluminous background of cytological research upon which molecular and biochemical experiments can be built. Current research is also burdened, however, by the long history of controversial and seemingly irreconcilable observations, especially in the area of pollen tube guidance. It is apparent that much of our current knowledge awaits integration into a much larger picture of compatible pollen–pistil interactions. The identification of components which are potential participants in the process of pollen tube growth, such as the Pex-1 protein (11), is a key step toward understanding how fertilization is accomplished. This goal will be best achieved by combining molecular, biochemical, and cell biological approaches with genetic and transgenic approaches. With more defined molecular and genetic markers, investigations into the pollination and fertilization processes in the coming years will eventually produce a more lucid understanding of plant sexual reproduction.

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