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CellPress REVIEWS

Review

Molecular Basis of Pollen Germination in Cereals

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Understanding the molecular basis of pollen germination in cereals holds great potential to improve yield. Pollen, a highly specialized haploid male gametophyte, transports sperm cells through a pollen tube to the female ovule for fertilization, directly determining grain yield in cereal crops. Although insights into the regulation of pollen germination and gamete interaction have advanced rapidly in the model *Arabidopsis thaliana* (arabidopsis), the molecular mechanisms in monocot cereals remain largely unknown. Recently, pollen-specific genome-wide and mutant analyses in rice and maize have extended our understanding of monocot regulatory components. We highlight conserved and diverse mechanisms underlying pollen hydration, germination, and tube growth in cereals that provide ideas for translating this research from arabidopsis. Recent developments in gene-editing systems may facilitate further functional genetic research.

Germination of Pollen

In flowering plants, the distribution of **pollen** (see Glossary) across land by wind and/or animals drives the spread of genetic variation within a species. Agriculturally, successful fertilization of flowers in an increasingly harsh and unpredictable climate is the key determinant of grain production and yield in cereal crops [1]. Mature pollen usually contains only two cell types – sperm cells and the much larger vegetative (or tube) cell – and desiccates before release from the anther. On adhering to a compatible **stigma**, pollen grains respond to signals from the mother plant and become active, undergoing cytoplasmic reorganization and activation of stored RNA and protein to produce a **pollen tube** originating from the vegetative cell. The pollen tube elongates within the **pistil** until it reaches the egg cell, where the sperm cell divides to enable the **double-fertilization** event typical of plants (Figure 1, Key Figure).

The factors that control successful pollen germination have been the focus of plant reproduction, evolution, and breeding research to increase crop yield and to overcome hybridization barriers [1,2]. Barriers to fertilization include interspecific incompatibility as well as self-incompatibility, which maintains genetic diversity within a local population. However, overcoming some of these constraints in a breeding environment can contribute to the production of novel elite lines for specific end-uses. Since 2003, pollen transcriptomic studies, together with identified mutants, have rapidly expanded our knowledge of the regulatory mechanisms that govern the fertilization process, including signaling pathways and cytoskeleton proteins [3–11]. However, most of this research has occurred in model plant species such as arabidopsis; despite massive agronomic and economic interest, relatively little is known about these molecular processes in cereal crops (the Poaceae). This review focuses on the unique characteristics of pollen grain adhesion, hydration, germination, and pollen tube growth in monocot cereals, and provides an overview of our current understanding of the regulators governed by the male **gametophyte** in the best-studied species, rice (*Oryza sativa*) and maize (*Zea mays*) (Figure 1C).

Key Features of Pollen Germination in Cereals

Pollen Structure and Viability

Mature pollen grains have a specialized cell wall that comprises two protective layers: an inner pectocellulosic **intine** acts as a storage reserve for hydrolytic enzymes required for pollen tube penetration, and a textured outer **exine** composed of sporopollenin, a highly resistant biopolymer of aliphatic and phenolic compounds [12]. A pollen coat (also called tryphine or pollenkit), comprising a sticky, hydrophobic mixture of lipids, waxes, flavonoids, and proteins, fills the cavities of the pollen exine, conferring adhesive properties to the pollen grains and providing a conduit for water to pass from the stigma during pollen germination [13]. Each pollen grain contains at least one **aperture**, a region on the surface where exine deposition is reduced or absent, which regulates the rate of water entry upon pollen hydration, and which serves as the site of exit for pollen tubes during germination [14].

Highlights

Successful fertilization is the key determinant of crop yield, but the molecular mechanisms that regulate pollen germination in cereals remain largely unknown.

Recent transcriptomics and proteomics studies in rice and maize pollen have produced breakthroughs in understanding pollen germination and its regulation.

Fifteen genes crucial for pollen germination and tube growth have been identified in grass species (*OsMLO12*, *RIP1*, *OsSPS1*, *OsPBP1*, *OsBOR4*, *RUPO*, *RMD*, and *OsMATL* from rice; *PEX1*, *Zm908p11*, *STK*, *EXPB1*, *ZmCPK*, *ZMGa1P*, and *MTL/NLD/ZmPLA1* from maize). Homologs of relevant arabidopsis genes expressed in pollen tissues provide novel targets for research in cereals.

To date, the lack of cereal mutants homozygous for male gamete defects has significantly hindered functional characterization of genes controlling cereal pollen germination. Cutting-edge gene-editing technologies, in combination with new genetic information from monocot and model dicot plants, will deliver new male-sterile plant resources to optimize crop yield.

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Compared with the three equatorial furrow-shaped apertures of arabidopsis and most eudicot pollen, the apertures in monocot species comprise a single germinal pore that occupies a small portion of the pollen surface (Figure 1B). Apertures are a crucial factor for pollen tube emergence in cereal crops because inaperturate maize pollen completely loses the ability to germinate [15]. Pollen size, exine patterns, and the position and number of apertures can vary enormously between species [16]. Grass pollen, that is usually distributed by wind, often has a smoother and less-sticky coat than pollen of insect-pollinated or self-pollinated species, which often have a more sculptured surface with reticulate cavities filled with coating [12].

On being released from anthers, most pollen grains have dehydrated to 15–35% water content and are metabolically dormant [17]. Pollen viability is highly correlated with the degree of dehydration and the structure of carbohydrate and lipid reserves. Grass pollen grains are well hydrated and contain high levels of starch, and are therefore relatively short-lived compared with drier dicot pollen, which tends to accumulate soluble sugars in the cytoplasm [17]. This rapid loss of germinative ability of monocot pollen, even in controlled *in vitro* conditions, increases the difficulty of studying pollen germination defects in grasses [18,19].

Pollen Germination

Stigmata provide adhesion, hydration, and germination media for pollen grains [20], and may be classified as wet if coated with sticky secretion (Solanaceae, Leguminosae, and Orchidaceae) or dry if covered with a proteinaceous pellicle (Brassicaceae, Poaceae, and Gramineae). A matrix of carbohydrates and lipids on the surface of wet stigmata promotes the adhesion of most pollen, whereas dry stigmata provide a barrier to **pollination** by selectively promoting pollen adhesion and invasion. *Brassica* pollen carries factors that determine compatibility and mediates recognition by stigmata [10], but species-specific recognition in grasses is regulated later by gametic genes because incompatible pollen can adhere to and germinate on grass stigmata [2].

The initial interaction between pollen and stigma appears to be relatively unspecific in grasses, but generally occurs very quickly. The time between pollen capture and germination is <5 minutes in many grass species [21]. A growing rice pollen tube can reach the embryo sac within 40 minutes, representing peak growth rates of 5400 μm/h [19,22]. Maize pollen tubes can penetrate through over 30 cm of maize silk (stigmata) and reach ovules within 24 h, indicating growth speeds in excess of 1 cm/h [23]. By contrast, dicot pollen can take over 1 h to hydrate, and arabidopsis pollen tubes grow at less than 100 μm/h [19]. Rapid pollen tube growth in grasses is a highly energy-consuming process that is dependent on storage materials within pollen grains [24], and this may explain the presence of starch reserves within grass pollen that would otherwise compromise viability.

Genetic and Proteomic Screening for Key Factors in Pollen Germination

Transcriptomic and proteomic studies have found that transcripts implicated in cell-wall metabolism, signaling, and cytoskeletal dynamics are synthesized during late pollen development and are stored until pollen germination [3,25–27]. Protein synthesis begins rapidly once pollen germination is initiated. *In vitro*, single ribosomes, mRNAs, ribosomes, and tRNAs aggregate into polysomes within 2 minutes [28].

Genome-wide investigations have been performed with the male gametophytic transcriptomes and proteomes in 10 plant species including two cereal crops, rice, and maize [25–27,29–36]. Commonly, pollen transcriptomes contain fewer expressed genes than other plant tissues, but comprise large numbers of genes that are pollen-specific or upregulated in pollen compared with **sporophytic tissues** [3,29,35,37]. About 30% of annotated genes (5939 and 5945 genes) were highly expressed at mature and germinated pollen compared with vegetative tissues [29]. This is a little less than the number of genes highly expressed in mature pollen of arabidopsis (~50%, 6044 genes) [3]. In maize, 43% of genes (14 591 of 39 365) were highly expressed in mature pollen, of which 734 were pollen-specific, and 52–70% of genes were expressed in other reproductive and vegetative tissues [35]. Especially in rice, gene expression profiles of mature and germinated pollen were highly correlated [29],

Glossary

Aperture: thin or modified region of pollen exine through which the pollen tube emerges.
Arabinoxylan: cell wall polysaccharide highly abundant in monocot plants, consisting of a β-1,4-linked xylopyranosyl backbone substituted with arabinose residues.
Double fertilization: fertilization process in the plant female gametophyte. One haploid sperm and a haploid egg combine to form a diploid zygote, which matures into the new plant; the other sperm cell fuses with two female haploid polar nuclei to produce the triploid endosperm that sustains the growing seedling until it can photosynthesize for itself.
Exine: the outer pollen wall. It generally contains the outer reticulate layer, the sexine (baculum and tectum), and a flat layer, termed the nexine or foot layer. The major constituent is sporopollenin, a biopolymer of polyhydroxylated aliphatic compounds and phenolics. The structure and amount vary between species.
Gametophyte: a haploid cell, generated by meiosis, which will fuse with another haploid cell during fertilization.
Intine: innermost layer of the pollen wall underlying the exine. It consists of pectin, cellulose, hemicellulose, hydrolytic enzymes, and hydrophobic proteins, and helps to maintain the structural integrity of the pollen grain and germinated pollen tube.
Pistil: female reproductive organ, consisting of stigma, style, and ovary.
Pollen: haploid microspores produced from the anther of angiosperm flowers develop into pollen grains, containing a vegetative cell and a generative cell that divides into two sperm cells. The size of pollen is highly variable, ranging from 5 to 200 μm.
Pollen tube: long cylindrical extension of the vegetative cell in pollen grain.
Pollination: landing and subsequent germination of the pollen on the stigma.
Sporophytic tissue: a diploid tissue which contains a double set of chromosomes, one set from each parent.

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suggesting that genes expressed in late-maturing pollen are transcribed or translated for use during pollen germination and tube growth.

Stigma (plural, stigmata): the sticky or feathery portion of carpel that captures pollen, typically located at the tip of the style.

Recently, genome-wide analyses using meta-anatomical expression identified 627 and 773 late pollen-specific genes in rice and arabidopsis, respectively [34]. Comparative analyses revealed ~20% functional conservancy between them, related to cell-cycle regulation, transcription, the ubiquitin/26S proteasome system, cell-wall synthesis, lipid metabolism, phytohormone signaling, the kinase system, and defense/stress responses. Cell-wall modification and major carbohydrate metabolism genes were found only in rice [34]. Cell wall-related metabolic processes have also been identified in maize pollen, and, in both maize and rice, pectin modification is the most significant response [32,38].

About 43% of rice pollen-preferred genes had no homologs in arabidopsis, suggesting key differences in the molecular regulation of grass pollen germination. Certainly, there are temporal differences in gene transcription: in rice, pollen germination is reliant on translation of mRNA presynthesized during pollen maturation, whereas in arabidopsis a large number of genes are transcribed *de novo* during pollen hydration [29]. Notably, 15–20% of pollen-specific genes in rice and arabidopsis are not annotated or characterized. Limited information even in these model plant species suggests that our knowledge on these processes is far from complete.

Proteomic analysis has validated genetic analyses, revealing a large number of proteins that are specific to germinated pollen [26]. Analysis in rice of the plasma membrane (PM), which forms the pollen tube, found 1121 PM-related proteins, of which 192 showed differential expression in the mature and germinated pollen. These PM proteins were involved in signal transduction, transport, cell-wall remodeling and metabolism, and membrane trafficking [33]. Quantitative proteomic analysis of sterol-rich PM microdomains, which play a key role in directing cell polarity in the pollen aperture and at the tip of germinating pollen tubes, revealed 237 microdomain-associated proteins that regulate the polar growth network, such as Rho-like GTPase from plants (ROP), signaling proteins and kinases, lipid signaling proteins, calcium ATPase, actin-binding proteins, pectin-modifying enzymes, and exocyst complex proteins [27]. Although few functional genes have been studied in grass pollen, conserved pathways are clearly important for polar pollen tip growth (Figure 1A).

Key Pathways Identified for Pollen Germination in Cereals

Pollen Coat Lipids and Proteins Involved in Pollen Hydration

Pollen coat lipids and proteins play a key role in adhesion and hydration at the stigma surface [36,39]. Long-chain fatty acids in the pollen coat may establish a gradient of water potential between the pollen and stigma that is essential for hydration [40]. Arabidopsis *eceriferum* (*cer*) mutants, which lack very long chain fatty acids (≥C20), adhered to the stigma but failed to hydrate [40–42]. In rice, mutation of a conserved triterpene poaceatapetol synthase gene, *OsOSC12/OsPTS1*, compromised pollen coat formation, leaving pollen dehydration dependent on humidity. Mutant pollens were rescued by treatment with a mixture of linolenic (18:3), palmitic (16:0), and stearic (18:0) acids that could prevent overdehydration, suggesting an essential function for poaceatapetol in the grass pollen coat [43].

Maintaining lipids in a proper conformation may require oleosin oil-binding proteins. Extracellular lipase 4 (*EXL4*) and an oleosin-domain-containing glycine-rich protein, *GRP17*, encode major pollen coat proteins (PCPs) in arabidopsis that may alter lipid composition at the pollen–stigma interface [44,45]. The oleosin domain may solubilize lipid droplets during coat mobilization, whereas the repetitive, but highly divergent, C-terminus could act as a species-specific signal to regulate interspecific incompatibility [44]. Indeed, a possible ortholog, *Os05g50110*, is expressed in rice pollen but requires further functional characterization.

Cell Wall-Modifying Enzymes

In rice and maize, PCPs related to cell-wall modification and carbohydrate metabolism are prevalent [25,34,36,46], compared with the lipid metabolism genes that dominate in arabidopsis [44]. Distinct

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Key Figure

Pollination in Cereals versus *Arabidopsis thaliana* (Arabidopsis)

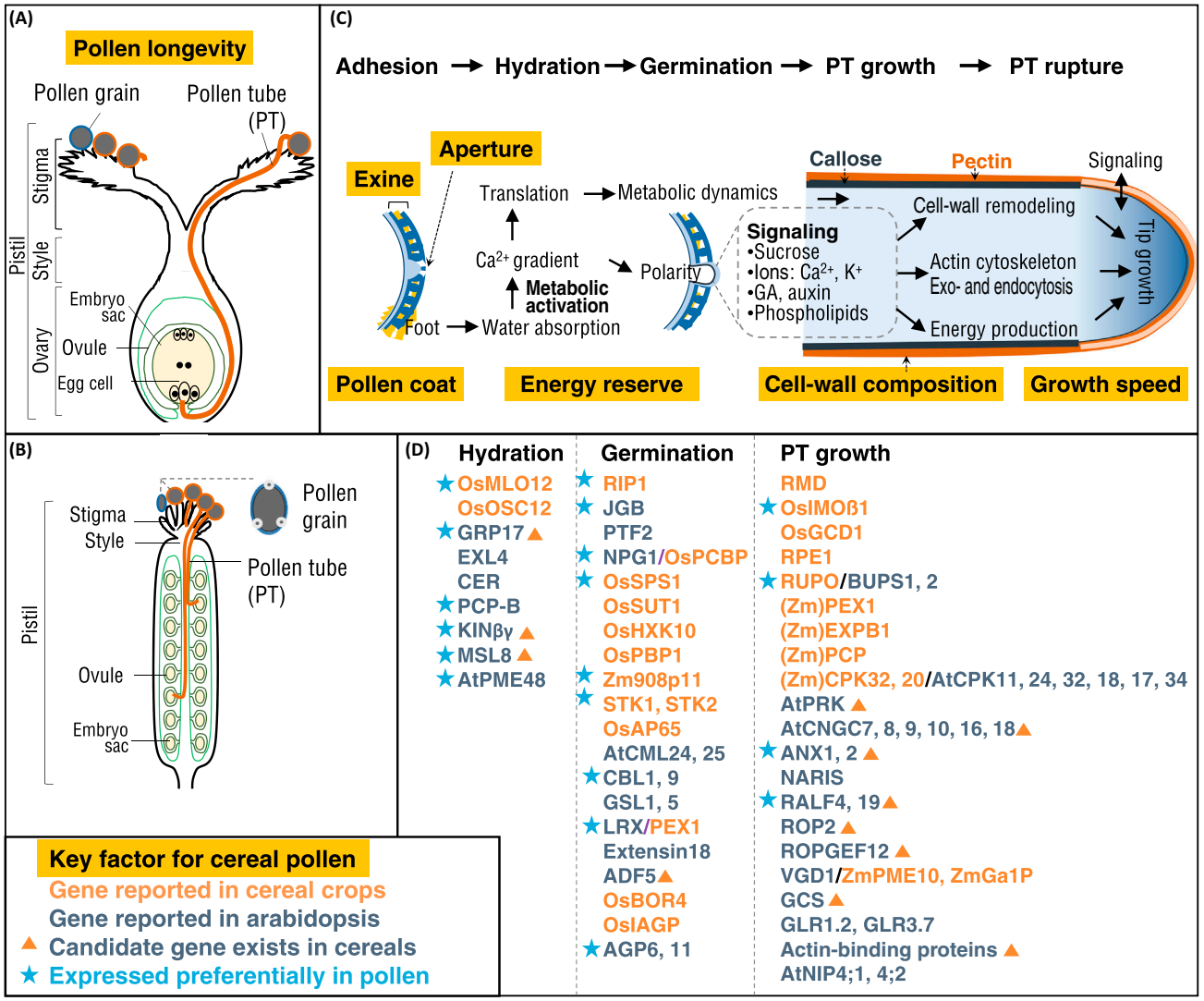

Figure 1. (A) Pistil structure and pollen tube (PT) journey in cereal plants. (B) Pistil structure and pollen tube journey in arabidopsis. Arabidopsis pistils contain many ovules, which enables detection of pollen transmission defect in one ovule, whereas cereal crops only contain one ovule. (C) The conserved pathway of pollen hydration, germination, and tube growth in monocots and dicots. Key features of differences for cereal plants are highlighted in yellow boxes. Once landed on the stigma, the pollen exine mediates adhesion and the pollen coat forms a 'foot', which promotes pollen hydration. After hydration, metabolic activation triggers pollen to germinate a pollen tube from the aperture by multiple coordinated cellular and molecular processes. (D) Conserved/diversified genes involved in pollen germination. See text and Table S1 for full gene/protein names. Abbreviations: GA, gibberellic acid; PT, pollen tube.

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structural differences between grass and dicot cell walls may explain the different requirements for cell wall-modifying enzymes [47]. Pollen allergen β-expansins are unique to grass species. These expansins facilitate pollen tube invasion of the stigma by loosening the cell-wall integrity of maternal tissues, which, in monocots, contain highly substituted **arabinoxylan** [46,48]. In rice, six β-expansin genes are preferentially expressed in pollen and do not have homologs in arabidopsis pollen-preferred genes [34]. Similarly, maize pollen extracts have expansin-like activity [49], and 16 maize expansin genes are expressed specifically in pollen [50]. Maize β-expansin, EXPB1, binds to glucuronoarabinoxylan [51], and *EXPB1* downregulation reduces the competitive ability of pollen *in vivo* as a result of slower pollen tube penetration [52]. The pollen coat of maize also accumulates β-1,3-glucanase, endoxylanase, and exopolygalacturonase in order to specifically hydrolyze cell-wall polysaccharides [36,45,53].

Pollen tube elongation is supported by Golgi-derived secretory vesicles that deliver new components to the pollen tube apex, which consists primarily of elastic pectins and is easily stretched by turgor pressor [54]. Homogalaturonans, a major component of pectin, are secreted into the wall in a highly methylesterified state and are subsequently de-esterified by the wall-associated pectin methylesterase (PME). It allows the formation of Ca^{2+}-based crosslinks that cause the cell wall to become more rigid. PME activities are post-translationally regulated by PME inhibitors (PMEIs). A PMEI may have selective effects on different PMEs depending on environmental factors such as pH, as shown in arabidopsis root [55]. In rice and arabidopsis, >10 PME and PMEI genes were identified in the group of pollen-preferred genes [34].

Several studies illustrate the importance of PME and PMEI in coordinating the strength and plasticity of the apical cell wall to direct pollen tube growth [56,57]. Mutation of the pollen-specific arabidopsis PME gene, *vgd1*, resulted in unstable pollen tubes that burst more frequently than wild type tubes [58], and lack of AtPPME1 altered the shape of the pollen tube without affecting fertility [59]. *AtPME48* was also shown to be important for the mechanical properties of the intine wall during pollen maturation. Homozygous mutants displayed delayed hydration and germination *in vitro* and *in vivo*, with two tubes frequently emerging, resulting in reduced fertility [60]. In maize, three PME proteins (ZmPME3, ZMGa1P, and ZmPME10-1) have also been implicated in conferring a self-incompatibility phenotype, although the mechanisms have not yet been defined [61,62]. Only one pollen-specific gene, *OsPME1*, has been analyzed among 43 rice PMEs, and was shown to be regulated by various abiotic stresses and hormones [63]. In maize, exogenous application of ZmPMEI1 leads to subapical bursting of the pollen tube and swelling at the tip [64]. Further analysis will be necessary to understand whether the PMEs act redundantly or have discrete functions, and to characterize their interaction with PMEIs for pectin modification.

Controlling Cell-Wall Integrity

The *Catharanthus roseus* receptor-like kinase (*CrRLK1L*) genes have been implicated in pollen tube integrity, timely growth arrest, and rupture, including arabidopsis pollen-specific *ANXUR1* (*ANX1*) and *ANX2*, and buddha's paper seal 1 (*BUPS1*) and *BUPS2* [65,66], which direct downstream production of reactive oxygen species (ROS). In cereals, only one member, ruptured pollen tube (*RUPO*), has been functionally studied. It is expressed specifically in pollen, localizing to the apical PM and vesicle of the pollen tube [67]. Pollen tubes of homozygous CRISPR-knockout lines burst shortly after germination, as in the *anx1*/*anx2* double mutant of arabidopsis. *RUPO* negatively regulates high-affinity potassium transporters (OsHAK1, OsHAK19, OsHAK20) via phosphorylation-dependent interactions, controlling K^+ homeostasis for pollen tube growth and integrity [67]. Recently, BUPS1/2 was shown to form heterodimers with ANX1/2 and to bind to RAPID ALKALINIZATION FACTOR (RALF) 4 and 19 peptides [68]. RALF4/19 bind to CrRLK1L proteins and BUPS/ANX, as well as to the cell-wall protein, leucine-rich repeat extensin (LRX), and regulate pollen tube integrity and burst [68,69]. Thus, monocots and dicots both have CrRLK1L signaling pathways to maintain cell integrity and pollen tube growth; however, it will be interesting to see whether the current known mechanisms that burst the pollen tube via ROS and osmotic pressure in arabidopsis and rice, respectively, are conserved or diversified. At least five *RALF* genes (*Os07g13440*, *Os07g13450*, *Os01g10470*, *Os05g11330*,

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Os12g35690) are highly expressed in rice from genome-wide expression studies [34], but further examination of grass members of the RALF family for their function in pollen tube growth or reception is needed. The ligands and signaling cascade partners of these receptors could be primary targets for studies on grass pollen integrity.

Cytoskeleton Dynamics

An extensive matrix of actin and microtubule bundles accumulates along the length of the pollen tube, reducing in the subapical region, and being largely absent from the apical dome [68]. Actin cables provide tracks for the movement of large organelles and cytoplasmic streaming during pollen tube tip growth, as shown by genetic studies of various actin-binding proteins (ABPs) including fimbrin, formin, actin-depolymerizing factor (ADF), and LIM-domain protein (LIM) [4,70–72]. Mutation of different classes of ADF proteins, including *ADF7* and *ADF5*, result in retarded pollen tube growth [73,74]. Although *ABP* and *ADF* genes are highly expressed in rice pollen [34], there is no information about their function.

A rice type II formin, *RICE MORPHOLOGY DETERMINANT* (*RMD*), has been shown to affect cytoskeleton organization by regulating actin dynamics and influencing pectin deposition at the pollen tube apex [75]. Despite abnormal pollen germination and slower pollen tube growth in *rmd* lines, the seed setting ratio was similar to the wild type, as in arabidopsis ADF mutants, indicating that actin cytoskeleton dynamics are controlled by multiple regulators.

Calcium Signaling

The tip-focused Ca^{2+} gradient is necessary for pollen tube growth and guidance [76,77]. Recent discoveries of Ca^{2+} channels in arabidopsis pollen tubes, including six cyclic nucleotide-gated channels (CNGCs) and two glutamate receptor-like channels (GLRs), have uncovered the molecular regulators of the gradient [78–81]. In arabidopsis, *CNGC18* mutants exhibited complete male sterility as a result of short and deformed pollen tube growth, whereas *GRL1.2* and *GRL3.7* mutants exhibited partial male sterility [78–80]. CNGC18 was found in the pollen tube apex PM, whereas GLRs have been found in the tonoplast and sperm cell membranes [81], indicating that multiple intracellular channels control cytosolic Ca^{2+} homeostasis. Rice pollen expresses at least three potential CNGCs [34], but they have not yet been functionally studied. Of particular relevance is *OsCNGC8* (*LOC_Os12g06570*) [82], the ortholog of *AtCNGC18*, and its role in pollen tube growth and guidance will be interesting to ascertain.

The Ca^{2+} signal can be sensed and transduced by calcium-dependent protein kinases (CPKs). Ten of 42 *CPK* genes in maize are specifically expressed in pollen [83], whereas in rice, seven of 31 *CPK* genes are pollen-specific [84]. In maize, *ZmCPK32* negatively regulates pollen tube length [83]; its closest arabidopsis homolog, *AtCPK24*, also negatively affects pollen tube elongation by regulating potassium influx [85]. Conversely, *ZmCPK20* was shown to promote pollen tube germination and growth [86]; its arabidopsis homologs, *AtCPK17* and *AtCPK34*, were shown to be essential for pollen tube tip growth in response to tropism [87], and *AtCPK34* was later found to target two aquaporin channels [88]. In the same CPK subgroup in rice, *OsCPK25/26* was shown to interact with a DNA helicase, which could affect transcriptional control [84], but its molecular function has not been further investigated. Altogether, different CPK members are associated with discrete functions in pollen tube growth.

In arabidopsis, mutations in calmodulin (CaM)-binding *NO POLLEN GERMINATION* (*NPG*) proteins abolish pollen germination [89]. At least one AtNPG protein interacts with a pectate lyase to modify the pollen cell wall and regulate pollen tube emergence and growth [90]. Homologous pollen-specific pollen CaM-binding protein genes have been identified in rice (*OsPCBP* [91]) and maize (*MPCBP* [92]). OsPCBP localized to the amyloplast of mature pollen and to the pollen tube cell wall, and RNAi down-regulation caused aborted pollen formation [91]. Conserved proteins identified in monocots and dicots, but not in nonplant systems, suggest that NPGs represent a plant-specific CaM-binding protein family. In addition, pollen-specific *MLO* (*POWDERY MILDEW RESISTANCE LOCUS O*) encodes a protein with seven transmembrane helices and a CaM-binding domain; mutation causes a defect in

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pollen hydration and male gamete gene transmission [93]. Probably, OsMLO12 may act as bridge for Ca^{2+} signaling and hydration, but this remains to be investigated.

Phospholipid Signaling

In rice, *C2-domain phospholipid-binding protein* (*OsPBP1*) is expressed highly in pollen and pistil immediately before anthesis. The protein binds phospholipids and is translocated from the cytoplasm and nucleus onto the PM in a calcium-dependent manner *in vitro*. Knockdown transgenic lines exhibited a reduced seed set caused by changes in microfilament distribution that compromised pollen germination, suggesting that OsPBP1 connects Ca^{2+} and phospholipid signaling with cytoskeleton dynamics [94].

A pollen-specific patatin-like phospholipase A, *MATRILINEAL* (*MTL*)/*NOT LIKE DAD* (*NLD*)/*ZmPHOSPHOLIPASE A1* (*ZmPLA1*), was identified from a 'haploid inducer' line of maize [95–97]. The MTL protein localized to the sperm cell, and is hypothesized to function in membrane remodeling to promote the fusion of sperm and egg cells. The truncated protein loses its PM anchorage and disturbs sperm cell membrane lipid homeostasis during fertilization. *MTL* is conserved in cereals, and the rice ortholog *OsMATL* also shows a pollen-specific expression pattern. Knockout mutants of *OsMATL* exhibited reduced seed set and a 2–6% haploid induction rate in rice [98], consistent with the

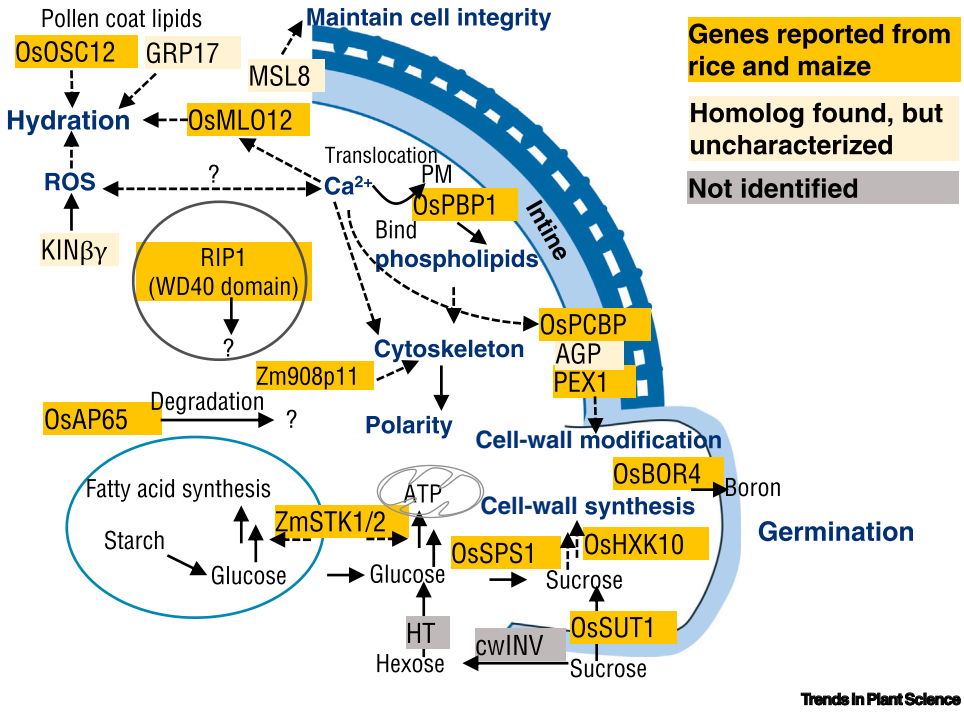

Figure 2. Proposed Pathways of Genes/Proteins Involved in Pollen Germination in Cereals.
Pollen coat lipid-metabolic OsOSC12/OsPTS1 (triterpene poaceatapetol synthase) and OsMLO12 (powdery mildew resistance locus O) function in pollen hydration in rice. Arabidopsis GRP17 (glycine-rich protein), KINβγ (plant-specific subunit of the SNF1-related protein kinase complex), and MscS-like 8 (MSL8) function in pollen hydration. The genes/proteins that generate ROS (reactive oxygen species), a calcium gradient, and phospholipid signaling to trigger cytoskeleton dynamics and polarity for pollen germination are similar to those of dicot plants. Abbreviations: AGP, arabinogalactan glycoprotein; cwINV, cell-wall invertase; HT, hexose transporter; OsAP65, aspartic protease; OsBOR4, boron efflux transporter; OsHXK10, hexokinase 10; OsPBP1, C2-domain phospholipid-binding protein; OsPCBP, calmodulin-binding protein; OsSPS1, sucrose phosphate synthase; OsSUT1, sucrose transporter 1; RIP1, RICE IMMATURE POLLEN 1; PEX1, pollen extensin-like 1; PM, plasma membrane; PME, pectin methylesterase; Zm90811, 97 amino acid short ORF; ZmSTK1/2, serine/threonine protein kinases 1 and 2.

Outstanding Questions

What are the mechanisms controlling pollen germination and rapid pollen tube growth in cereals?

How do the multiple members of one protein family, such as the 31 rice and 42 maize pollen-specific calcium-dependent protein kinases (CPK), coordinate and control pollen germination and tube elongation in cereals?

Is receptor kinase-mediated signaling in cereal pollen germination conserved, as currently understood in arabidopsis?

What are the mechanisms by which cereal pollen germination and pollen tube growth react to abiotic stresses such as high/low temperature and drought?

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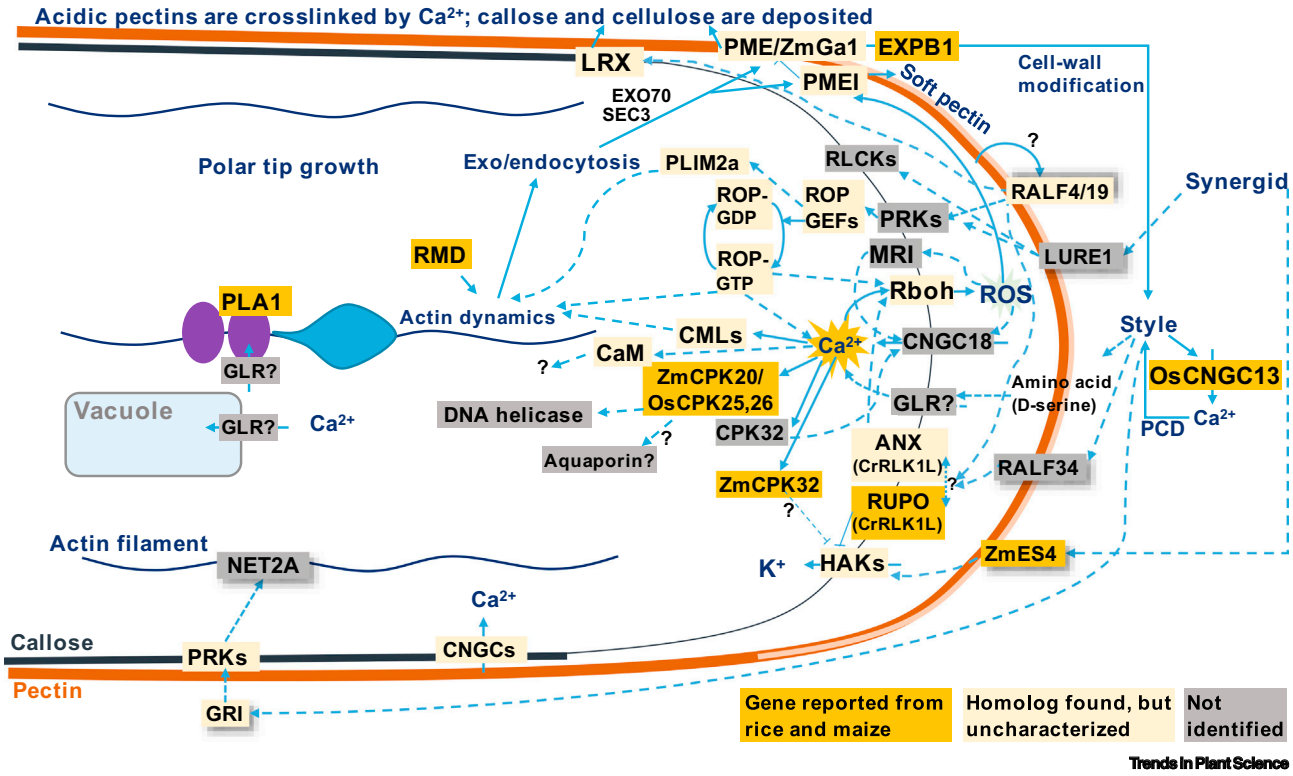

Figure 3. Proposed Pathways in Metabolism and Signaling for Pollen Tube Growth in Cereals.

Only a few reported genes including *RUPO* (*ruptured pollen tube*), *RMD* (*rice morphology determinant*), *PLA1* (*patatin-like phospholipase A*), *CPK* (*calcium-dependent protein kinase*), and the synergid-released peptide *ZmES4* (*Zea mays embryo sac*) and style-expressed *OsCNGC13* (cyclic nucleotide-gated channel) have been functionally studied in pollen tube elongation in cereal plants. Many receptor-like kinases and peptides reported in dicot plants, which transduce Rho-like GTPase from plants (ROP)-mediated calcium and ROS (reactive oxygen species) signaling for cytoskeleton dynamics, vesicular transport, and cell-wall remodeling for polar pollen tube growth, have not yet been identified or characterized in monocot plants. Abbreviations: ANX, ANXUR; CaM, calmodulin; CML, calmodulin-like protein; EXPB1, ß-expansin; GLR, glutamate receptor-like channel; GRI, GRIM REAPER peptide; HAK, potassium transporters; LURE, defensin-like polypeptide; LRX, leucine-rich repeat extensin; NET2A, NETWORKED 2; PCD, plant cell death; PLIM, LIM domain containing protein; PME/ZmGA1, pectin methylesterase; PMEI, pectin methylesterase inhibitor; PRK, pollen receptor kinase; Rboh, NADPH oxidase; ROPGEFs, guanine nucleotide exchange factors for ROPs; RALF, RAPID ALKALINIZATION FACTOR.

maize haploid induction phenotype, suggesting a conserved function for this gene in cereal grasses. These studies highlight the importance of nonnuclear sperm components in male genome transmission, and open new ideas for developing double haploid hybrid seed production [99,100].

Concluding Remarks

Although some genes in the rice and maize mutant lines have been studied at the molecular level, related functional approaches are rare owing to the difficulty of screening mutants in cereals (Table S1 in the supplemental information online). Although sporophyte mutants can be found by screening for male sterility, the isolation of gametophyte mutants affecting pollen function is problematic: heterozygous mutants produce 50% of normal pollen and thus display no easily observable effect on fertility, but it is not possible to obtain homozygous mutant plants because the haploid pollen bearing the mutation is essentially sterile. Although such research has been possible in arabidopsis because the presence of many ovules in the ovary produces a gamete defect phenotype in the silique (Figure 1B), it is now time to generate resources in agriculturally crucial cereal crops. Candidate gamete genes for pollen function can be found using new approaches, such as the targeted creation of homozygous mutants by CRISPR/Cas gene editing. Genome-wide transcriptomic analysis and comparative phylogenetic analysis of gene families will aid the design of reverse genetic approaches

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to identify genes essential for pollen germination (Figure 2) and tube growth (Figure 3), and this will be crucial to anticipate agronomically relevant questions such as environmental stress tolerance (see Outstanding Questions). Further investigation into pollen–pistil interactions will further elucidate how recognition events lead to incompatibility. Better molecular understanding of pollen tube initiation and guidance in grasses will help to overcome hybridization barriers between genotypes, and even between species, to improve the gene pool for breeding.

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Supplemental Information

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