RUPTURED POLLEN GRAIN1, a Member of the MtN3/saliva Gene Family, Is Crucial for Exine Pattern Formation and Cell Integrity of Microspores in Arabidopsis

Yue-Feng Guan, Xue-Yong Huang, Jun Zhu, Ju-Fang Gao, Hong-Xia Zhang, and Zhong-Nan Yang*

National Key Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China (Y.-F.G., H.-X.-Z.); and College of Life and Environment Sciences, Shanghai Normal University, Shanghai 200234, China (X.-Y.H., J.Z., J.-F.G., Z.-N.Y.)

During microsporogenesis, the microsporocyte (or microspore) plasma membrane plays multiple roles in pollen wall development, including callose secretion, primexine deposition, and exine pattern determination. However, plasma membrane proteins that participate in these processes are still not well known. Here, we report that a new gene, RUPTURED POLLEN GRAIN1 (RPG1), encodes a plasma membrane protein and is required for exine pattern formation of microspores in Arabidopsis (Arabidopsis thaliana). The rpg1 mutant exhibits severely reduced male fertility with an otherwise normal phenotype, which is largely due to the postmeiotic abortion of microspores. Scanning electron microscopy examination showed that exine pattern formation in the mutant is impaired, as sporopollenin is randomly deposited on the pollen surface. Transmission electron microscopy examination further revealed that the primexine formation of mutant microspores is aberrant at the tetrad stage, which leads to defective sporopollenin deposition on microspores and the locule wall. In addition, microspore rupture and cytoplasmic leakage were evident in the rpg1 mutant, which indicates impaired cell integrity of the mutant microspores. RPG1 encodes an MtN3/saliva family protein that is integral to the plasma membrane. In situ hybridization analysis revealed that RPG1 is strongly expressed in microsporocyte (or microspores) and tapetum during male meiosis. The possible role of RPG1 in microsporogenesis is discussed.

In angiosperms, the pollen wall usually consists of an outer exine layer and an inner intine layer (Heslop-Harrison, 1971; Piffanelli et al., 1998). The exine layer includes an outer sculptured sexine and a simpler foot layer, the nexine (Heslop-Harrison, 1971). The sexine can be further divided into the rod-shaped bacula and the tectum. The exine wall plays important roles in protecting the pollen from various environmental stresses and in the species-specific adhesion of pollen grains to the stigma (Zinkl et al., 1999). The intine, secreted by the microspore, is the innermost layer and is located between plasma membrane and nexine (Heslop-Harrison, 1971). In addition, a trypnine layer (or pollen coat), which is derived from the remnant of tapetum, fills the gaps within the exine layer and covers the pollen grain.

Successful pollen wall development, especially exine wall formation, requires precise coordination of the microspore and tapetum (Paxson-Sowders et al., 1997; Piffanelli et al., 1998). During meiosis, microsporocytes secrete β-1,3-glucan (callose) between the plasma membrane and the primary cell wall. Multiple roles of the callose wall have been proposed, including preventing microspores from fusion (Waterkeyn and Bienfait, 1970), serving as a molecular filter, and as a mold for primexine patterning (Heslop-Harrison, 1971). At the tetrad stage, primexine matrix, the footprint of the exine layer, is deposited on microspores based on the regular undulation of the plasma membrane (Fitzgerald and Knox, 1995; Paxson-Sowders et al., 1997). After the release of microspores, the main component of exine, sporopollenin, is secreted by the tapetum and deposited on the microspores following the patterning of the primexine (Paxson-Sowders et al., 1997; Piffanelli et al., 1998).

In Arabidopsis (Arabidopsis thaliana), several male sterile mutants with pollen wall formation defects have been reported. In the male sterility1 (ms1) mutant, the microspore cytoplasm and tapetum become abnormally granular and vacuolated with microspore degeneration (Wilson et al., 2001). Primexine formation is aberrant on the microspore at the tetrad stage, which results in random deposition of sporopollenin (Viczay-Barrena and Wilson, 2006). Further studies showed that Ms1 is a PHD-finger family transcription...
the cell wall (Aarts et al., 1997). Callose synthase 5 (CalS5), one of the Arabidopsis inner envelope membrane protein that may play a role in maintaining the integrity of the plastid envelope (Ariizumi et al., 2004). MS2 encodes a putative fatty acid reductase that is involved in sporopollenin synthesis. In the ms2 mutant, microspores are not able to form a thick exine wall (Aarts et al., 1997). Callose synthase 5 (CalS5), one of the Arabidopsis β-1,3-glucan synthases, is expressed specifically in pollen mother cells (Dong et al., 2005; Nishikawa et al., 2005). Ablation of CalS5 results in less callose deposition in the tetrad and alters exine formation in microspores (Dong et al., 2005; Nishikawa et al., 2005). Moreover, microspores are degenerated in strong alleles of calS5 (Dong et al., 2005).

In the defective in exine formation 1 (dex1) mutant, the plasma membrane of the meiocyte is irregular with flattened protuberances, which results in abnormal primexine deposition and exine pattern formation (Paxson-Sowders et al., 1997, 2001). DEX1 encodes a predicted membrane-associated protein that contains several potential calcium-binding domains (Paxson-Sowders et al., 2001). In another conditional sterile mutant, faceless pollen 1 (flp1/cer3-7), the inviable pollen has a smooth surface due to the coverage of the exine bacule of the pollen grain by an excess of trypine (Ariizumi et al., 2003). The FLP1 (recently designated CER3/WAX2/NRE/FLP1) gene encodes a lipid transfer protein that is important for wax biosynthesis (Ariizumi et al., 2003; Chen et al., 2003; Kurata et al., 2003; Rowland et al., 2007).

As indicated by histological and molecular analysis, the microspore plasma membrane plays multiple roles in pollen wall development. The microspore plasma membrane is essential for membrane trafficking and for the secretion and deposition of enzymes, callose, and primexine matrix. In exine pattern determination, the timely undulation of the microspore plasma membrane is thought to determine the deposition site of primexine, which is the scaffold of the exine layer (Paxson-Sowders et al., 1997). Therefore, the integrity and function of the microspore plasma membrane is crucial for microsporogenesis and pollen wall development. Nevertheless, the plasma membrane proteins that may participate in these processes are still not clear. Here, we report a new mutant, ruptured pollen grain 1 (rpg1), which is defective in exine pattern formation and in the cellular integrity of the microspores. RPG1 is an MtN3/saliva family protein that is localized at the plasma membrane. We provide evidence that RPG1 plays an important role in primexine pattern determination and the cellular integrity of microspores.

RESULTS

Phenotypical Identification of the rpg1 Mutant

To identify new genes essential for male fertility, T-DNA-tagged lines from a pool of Arabidopsis were screened for mutants that showed a reduced seed set (Qin et al., 2003). One of the tagged lines exhibited very few seed sets upon self-pollination. Based on the observation of postmeiotic rupture of microspores in this mutant (see below), it was designated rpg1. During the vegetative growth phase, rpg1 was indistinguishable from wild-type plants. However, the fertility of rpg1 was dramatically reduced, as indicated by their short siliques (Fig. 1A).

We further compared the seed yield of wild-type and rpg1 plants by examining the number of seeds per siliques. Wild-type plants produced about 25 siliques on the inflorescence axis and approximately 50 seeds per siliques during reproductive development (n = 3), whereas the average seed yield per siliques was reduced by 90% in rpg1 plants (n = 15). Moreover, the fertility of rpg1 varied depending on the reproductive development stage. In the early formed siliques, only one or no seed was produced per siliques. Seed yield of the mutant gradually recovered as the inflorescence development progressed. In the siliques generated from the 25th to 30th flowers on the inflorescence of rpg1, an average of 11 seeds were produced in each siliques (n = 15). Thus, the rpg1 mutation could be propagated in a homozygous state, with all progeny exhibiting a mutant phenotype. To investigate the possible mechanism of this phenomenon, we examined the fertility of the mutant plants in different environments, including under drought, high humidity, and long-day or short-day illumination conditions. The variation of fertility in rpg1 appeared to be consistent under different conditions (data not shown).

Siliques with normal seed set were produced by cross-pollination of the mutant stigmas with wild-type pollen (data not shown), indicating that female reproductive development is not affected. Progeny of heterozygous mutant plants segregate fertile and mutant plants with an approximate 3:1 ratio (320:104), indicating that rpg1 is caused by a single, recessive, nuclear mutation.

Microsporogenesis of the rpg1 Mutant Is Aberrant after Meiosis

Detailed histological observations were performed to analyze the male reproductive developmental defects of rpg1. In rpg1 mutant plants, stamen development was impaired and few pollen grains were observed on the stigmas (Fig. 1, B and C). To examine the vitality of rpg1 pollen grains, we used Alexander’s stain, which distinguishes aborted pollen grains from...
mature pollen grains (Alexander, 1969). Wild-type pollen grains were stained with purple color (Fig. 1D), suggesting that they had successfully completed early development (well-developed protoplasm of pollen grains). In contrast, we observed that green-stained pollen remnants filled the anther in \textit{rpg1} (Fig. 1E), which indicated that most of the pollen grains were aborted during early development (did not develop protoplasm). Consistent with the restoration of fertility, pollen grains from early initiated flowers were mostly stained green, whereas purple-stained pollen grains appeared to be increased in the anthers of later flowers along the axis (data not shown). To examine the male meiosis of \textit{rpg1}, we performed chromosome spreading with 4'/6-diamidino-2-phenylindole (DAPI) staining. The tetrads of \textit{rpg1} plants are indistinguishable from those of the wild type (Fig. 1, F–I), which suggested that nuclear division of male meiosis is not affected in the mutant.

We also generated anther cross sections to compare anther development of wild-type and \textit{rpg1} plants (Fig. 2). In Arabidopsis, anther development can be divided
into 14 well-ordered stages by morphological characteristics (Sanders et al., 1999). Until anther development stage 7, when tetrads of haploid microspores are formed, the mutant appeared to be comparable with wild-type plants in tetrad formation and callose secretion (Fig. 2, A and B). However, when the callose wall of tetrads degenerated, released microspores of rpg1 were distinguishable from those of the wild type. At stage 8, microspores were angular in shape in wild-type plants (Fig. 2C), whereas microspores of rpg1 plants appeared to be turgid and bumpy (Fig. 2D). At stage 9, microspores of wild-type plants generated the basic exine wall and became vacuolated (Fig. 2E). In contrast, in rpg1 plants, the cytoplasm of microspores was shrunken and disintegrated (Fig. 2F). Furthermore, granules were observed in the locule of the mutant (Fig. 2F). During stages 10 and 11 in the wild type, anther microspores underwent asymmetric mitotic divisions and generated a significant pollen wall (Fig. 2, G and I). In contrast, in rpg1, most of the microspores were degenerated and the granules became more evident (Fig. 2, H and J). Eventually, pollen grains of the wild type were released following anther dehiscence (Fig. 2K), whereas most of the mutant microspores were aborted (Fig. 2L). Some of the microspores in the mutant managed to develop further; nevertheless, they were usually irregular in shape (Fig. 2L). The cell fate of the tapetum in RPG1 plants appeared to be unaffected, as tapetum differentiation and degeneration occurred in the same manner as in wild-type plants.

**rpg1 Microspores Are Defective in Exine Pattern Formation and Cell Integrity**

To further elucidate the mechanism of pollen degeneration of rpg1, we compared the ultrastructure of microspore development in both wild-type and rpg1 plants by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) examinations. Unlike the wild-type pollen grains (Fig. 3A), significant aberration of pollen grain and exine pattern formation was observed in rpg1 by SEM examination. Consistent with the description above, most of the pollen grains were aborted and collapsed in rpg1 plants (Fig. 3B). Moreover, in contrast to the reticulate exine pattern that was observed in wild-type pollen grains (Fig. 3C), those aborted pollen grains exhibited a spotted exine pattern (Fig. 3D). This indicated that the sporopollenin deposition and exine patterning were defective in the rpg1 mutant. Although a small portion of pollen grains appeared to be olive-shaped, as observed for wild-type plants, their exine patterning was often incomplete or flawed (Fig. 3, E and F). In addition, fragments of membrane-like material, which usually encases the pollen grains, were observed to surround the anther locule (Fig. 3B). We also examined the pollen grains of heterozygous plants by SEM. The anther development and pollen grains appeared to be indistinguishable from those of the wild type (data not shown), which suggested that the abortion of pollen grains in rpg1 was caused by the defects in somatic development but not in male gametophytic development.

TEM demonstrated abnormal primexine deposition and rupture of microspores in rpg1 plants (Fig. 4). At the tetrad stage in wild-type plants, following the regular undulation of microspore plasma membrane, primexine matrix was deposited directly outside the microspore plasma membrane (Fig. 4A). In rpg1, primexine was also deposited within the callose wall (Fig. 4B). However, the microspore was irregular in primexine deposition (Fig. 4B). At the released microspore stage, microspores of the wild type presented basic exine wall formation with bacula and tectum (Fig. 4C). In contrast, the tectum was absent and the bacula was malformed in rpg1 microspores (Fig. 4D).

![Figure 3. SEM examination of dehiscent anthers and pollen grains of wild-type (A and C) and rpg1 (B, D, E, and F) plants. A and B, Dehiscent anthers. Wild-type anther contained numerous pollen grains, whereas most rpg1 pollen grains degenerated. The white arrow indicates membrane-like material that wraps the rpg1 pollens. Bars = 50 μm. C, Wild-type pollen grains with a regular reticulate exine pattern. D, Ruptured rpg1 pollen grains with a spotted exine pattern. The white arrowhead indicates breach of pollen. E and F, Less impaired rpg1 pollen grains. The exine pattern formation was impaired or flawed. In C to F, bars = 5 μm.](https://academic.oup.com/plphys/article/147/2/852/6107522)
Moreover, the microspore plasma membrane and pollen wall were bumpy and wavy in \textit{rpg1} (Fig. 4D), which led to breakage of microspores at a later stage (Fig. 4F). Following the rupture of microspores, cytoplasmic leakage of microspores was observed, and the intine layer was not formed (Fig. 4F). When pollen wall development was complete, a wild-type pollen grain was surrounded by an intine layer, an exine layer, and a pollen coat (Fig. 4G). However, pollen grains of the mutant were mostly collapsed and emptied, with no intine layer and an aberrant exine layer. While a small portion of microspores appeared to complete microsporogenesis and microgametogenesis, the exine pattern formation was usually abnormal, with irregular bacula and absence of tectum (Fig. 4H). Tapetum development of the wild type and mutant was comparable (data not shown), whereas lipid accumulation was aberrant in \textit{rpg1}. Unlike the wild type (Fig. 4I), electron-dense granules that aggregated onto the locule wall were also observed in \textit{rpg1} (Fig. 4J), which may be responsible for the membrane-like material in SEM examination (Fig. 3B).

\textbf{RPG1 Encodes an MtN3/saliva Family Protein That Localizes to the Plasma Membrane}

To identify the corresponding \textit{RPG1} gene, a genomic DNA fragment that flanked the left border of T-DNA was recovered by thermal asymmetric interlaced (TAIL)-PCR (Liu et al., 1995). Sequencing of the TAIL-PCR products suggested that the T-DNA was inserted in the last intron of a predicted open reading frame (At5g40260; Fig. 5A). PCR analysis with T-DNA and genome-specific primers indicated that all mutant plants analyzed were homozygous for the insertion (data not shown). Because the T-DNA insertion appeared to only affect the last intron and exon of \textit{RPG1}, we performed reverse transcription (RT)-PCR with different primer sets to examine the expression of At5g40260 in \textit{rpg1} plants. The result showed that the expression of the 5’ genomic region of the gene was not affected, while no expression of the 3’ region was detected (Fig. 5C). Genetic complementation was then performed to validate the results. A 3.6-kb DNA fragment, which included the genomic sequences of At5g40260 and 1.6-kb sequences upstream from the initiation codon, was cloned from wild-type Arabidopsis and introduced into the homozygous mutant plants. Twenty of 21 transgenic plants showed normal fertility. PCR analysis verified that these plants were homozygous for the \textit{rpg1} mutation with the transformed At5g40260 DNA fragment (data not shown). These results verified that At5g40260 is \textit{RPG1} and that the 3.6-kb genomic region is sufficient for \textit{RPG1} function.

In addition, we obtained three individual \textit{rpg1} alleles from the SIGnal collection at the Arabidopsis Institute of Biological Sciences.
Biological Resource Center: *rpg1-1* (SALK-142803), *rpg1-2* (SALK-092239), and *rpg1-3* (SALK-062567; Fig. 5A). The three T-DNA-tagged lines were verified by PCR. Both *rpg1-1* and *rpg1-2* showed a similar phenotype with that of *rpg1* (Supplemental Fig. S1). In *rpg1-3*, the fertility of mutant plants was moderately affected. SEM analysis showed that pollen grains of *rpg1-3* were shriveled, but exine formation of the pollen grains resembled that of the wild-type pollen grains (Supplemental Fig. S1H). RT-PCR analysis revealed that the *RPG1* gene was moderately knocked down in *rpg1-3*, which may have resulted in the partial function of *RPG1* (data not shown).

To verify the primary structure of the *RPG1* gene, its full-length genomic DNA and cDNA were cloned and sequenced. Genomic sequences of the *RPG1* gene are 1,686 bp in length and comprise six exons and five introns, which is consistent with the gene predictions in the National Center for Biotechnology Information (NCBI) database (Gi: 22328163). There are two putative gene models of At5g40260 in the NCBI database that differ in the predicted splice site of the last intron (At5g40260.1, Gi: 26451731; At5g40260.2, Gi: 79329352). The cDNA we cloned from inflorescences is identical to At5g40260.1. Furthermore, RT-PCR with At5g40260.1- and At5g40260.2-specific primers showed that only At5g40260.1 cDNA could be amplified from inflorescence cDNA (data not shown). Therefore, we chose At5g40260.1 mRNA and protein sequences for further analyses.

The *RPG1* mRNA encodes an unknown protein of 209 amino acids with a putative molecular mass of 27 kD. Domain analysis showed that *RPG1* protein is an integral membrane protein with seven putative transmembrane helices (Fig. 5B) and contains two copies of the MtN3/saliva domain (Fig. 6C). This domain was originally identified in root nodulin-related proteins of the legume *Medicago truncatula* (Gamasa et al., 1996).
and in the saliva protein of Drosophila melanogaster (Artero et al., 1998). MnN3/saliva family proteins are prevalent in eukaryotes, including yeast, mammals, and plants (data from the Pfam database, http://pfam.sanger.ac.uk/).

Topology prediction indicated that RPG1 is an integral membrane protein (Fig. 5B), whereas the exact subcellular localization was unclear. Therefore, we constructed a protein fusion in which the GFP was fused to the C terminus of RPG1. The chimeric protein was then introduced into wild-type plants under the control of the cauliflower mosaic virus 35S promoter. GFP fluorescence of transgenic plants was observed under confocal microscopy. The fluorescence of the RPG1::GFP protein was located strictly in the plasma membrane region of epidermal cells (Fig. 5D) and protoplast cells of transgenic plants (Fig. 5E) and was separated from the cell wall when the epidermal cells were plasmolyzed by 0.8 M mannitol (Fig. 5D). These results demonstrated that RPG1 is a plasma membrane-localized protein.

According to the Pfam database (http://pfam.sanger.ac.uk/), there are 18 putative MnN3/saliva family genes in Arabidopsis. Phylogenetic analysis showed that RPG1 solely forms a distinct clade in the phylogenetic tree of the AtMnN3/saliva family, which indicates that RPG1 may play a distinct role in Arabidopsis (Fig. 6A). Furthermore, the homologues of RPG1 protein have also been identified in various plant species by BLASTp or tBLASTn search in the NCBI database and The Institute for Genomic Research functional genome database, including rice (Oryza sativa), grape (Vitis vinifera), poplar (Populus trichocarpa), maize (Ze a mays), alfalfa (Medicago sativa), tomato (Solanum lycopersicum), lily (Lilium longiflorum), petunia (Petunia hybrida), and castor bean (Ricinus communis). For example, the homolog from rice, Os0102726, shared highest identity (50%) and similarity (68%) with RPG1. In petunia, NEC1, an anther dehiscence-associated protein (Ge et al., 2000, 2001), shared identity of 34% and similarity of 58% with RPG1. In lily, LIM7, which was previously reported to be induced in meiotic prophase in microsporocytes (Kobayashi et al., 1994), showed identity of 45% and similarity of 63% with RPG1 over a 159-amino acid region. An alignment with these protein sequences was performed by ClustalW (Fig. 6, B and C). Residues in the predicted MnN3/saliva domains are conserved among these proteins. Moreover, the predicted intracellular residues (TKSVEYMPF) in the second MnN3/saliva domain of RPG1 are highly conserved. Furthermore, when we modeled the putative functional motifs of the RPG1 protein with a motif scan tool (http://scansite.mit.edu/motifscan_seq.plhtml), the two conserved intracellular regions in each MnN3/saliva domain exhibited potential motifs, each of which includes a Ser residue (Fig. 6C). Unexpectedly, the C-terminal intracellular tail, which appeared to be essential for RPG1 function, as T-DNA ablation of this region leads to a mutant phenotype, is highly variable among the proteins.

**Expression Pattern of RPG1**

To determine the expression pattern of RPG1, we performed semiquantitative RT-PCR to analyze its expression levels in the root, stem, rosette leaf, inflorescence, and 7-d-old seedling. RPG1 is preferentially expressed in inflorescence and slightly expressed in stem. In contrast, RPG1 mRNA was barely detectable in other organs (Fig. 7A). This result is consistent with a previous study, in which RPG1 (At5g40260) was reported to be expressed preferentially in male and female gametophytes (Yu et al., 2005). Therefore, we performed RNA in situ hybridization experiments to study the precise expression pattern during anther development. RPG1 transcript was initially detected at anther development stage 4 (Fig. 7B), and the signal was increased significantly in tapetal cells and microsporocytes (or meiocytes) during meiosis (Fig. 7, C and D). After stage 7, the expression of RPG1 was still detectable in tapetum and microspores, but it became less than that at the earlier stages (Fig. 7E). When the tapetum completely degenerated at stage 12, RPG1 transcript was detectable in pollen grains only (Fig. 7F).

**DISCUSSION**

**RPG1 Is Crucial for Exine Pattern Formation and Microspore Cell Integrity in Arabidopsis**

We have characterized an MnN3/saliva family gene, RPG1, that is essential for microspore cell integrity and primexine pattern formation in Arabidopsis. In the rpg1 mutant, the formation of the microspore plasma membrane is affected. As the primexine deposition is abnormal at the tetrad stage, exine pattern formation of the microspores is severely impaired. Moreover, microspores are mostly ruptured and aborted during postmeiotic development. RPG1 exhibits its strongest expression in microsporocytes (or meiocytes) and tapetum during microsporogenesis, which confirms the important role of this gene in the early determination of microspore formation and exine pattern formation.

It has been well documented that nef1, dex1, and cals5 mutants are also defective in primexine patterning during the tetrad stage. Primexine was completely absent in nef1, and sporopollenin failed to deposit on the microspore wall (Ariizumi et al., 2004), whereas the microspore plasma membrane was barely undulated and primexine deposition was affected at the tetrad stage in dex1 (Paxson-Sowders et al., 1997, 2001). Aberrant lipid accumulation on the locule wall, which was observed in rpg1, was also observed in dex1 and nef1 (Paxson-Sowders et al., 1997; Ariizumi et al., 2004). In cals5, primexine pattern defects were caused by the insufficient callose synthesis, and the lipid accumulation defect was not observed in the mutant (Dong et al., 2005; Nishikawa et al., 2005). In rpg1, abnormal primexine development indicates that the mutant is defective in the early determination of exine
pattern formation, which is consistent with the expression pattern of the RPG1 gene.

Another common characteristic of \textit{rpg1} and other exine pattern mutants (e.g. \textit{ms1}, \textit{ms2}, \textit{nef1}, \textit{dex1}, and \textit{cals5}) is the postmeiotic degeneration of microspores (Aarts et al., 1997; Paxson-Sowders et al., 1997; Wilson et al., 2001; Ariizumi et al., 2004; Dong et al., 2005). Our study demonstrated that microspore abortion in \textit{rpg1}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Phylogenetic analysis of MtN3/saliva family and RPG1 homologous proteins. A, Unrooted phylogenetic tree of the MtN3/saliva family in Arabidopsis. Amino acid sequences of MtN3/saliva family proteins were analyzed by the neighbor-joining method with genetic distance calculated by MEGA3.1. The numbers at the nodes represent percentage bootstrap values based on 1,000 replications. The length of the branches is proportional to the expected numbers of amino acid substitutions per site, with a scale provided at the bottom of the tree. The arrow indicates RPG1 (At5g40260). B, Unrooted phylogenetic tree of RPG1 and homologous proteins. Protein sequence files are as follows: Pt, \textit{Populus trichocarpa}, LG_12590; Rc, \textit{Ricinus communis}, 29822.m003348; Os, \textit{Oryza sativa}, Os01g0605700; LIM7, \textit{Lilium longiflorum}, BAA04837; Sl, \textit{Solanum lycopersicum}, CAE47557; Vv, \textit{Vitis vinifera}, CAN64755; Zm, \textit{Zea mays}, AZM5_12004; Min3, \textit{Medicago truncatula}, CAA69976; NEC1, \textit{Petunia hybrida}, AAG34696. C, Multiple alignments of RPG1 and homologous proteins. Boxes, Similar to MtN3/saliva domain; black bars, putative transmembrane regions; gray bars, conserved intracellular regions of each domain; black dots, conserved Ser that was predicted to be a phosphorylation site. Sequences were aligned using ClustalW and displayed using BOXSHADE (www.ch.embnet.org/software/BOXform.html).}
\end{figure}
is apparently due to the rupture of microspores. This indicated that RPG1 is required for the cell integrity of microspores. Generally, plant cells are bound by a rigid cell wall that prevents cellular migration and maintains cellular integrity (Martin et al., 2001). Previous studies have shown that reduced cell wall strength leads to altered cell formation and integrity. For example, a cellulose synthase-like gene, AtCSDL3 (KOJAK), is required for root hair formation. Mutations of KOJAK/AtCSDL3 result in root hairs that initiate bulge formation but then rupture at their tips (Favery et al., 2001; Wang et al., 2001). In microsporogenesis, a common cell wall is replaced by callose in microspores during meiosis, and exine and intine layers are subsequently formed as the pollen cell wall to protect pollen grains (Heslop-Harrison, 1971). In the rpg1 mutant, plasma membrane formation and pollen wall development of microspores are severely impaired beginning at the tetrad stage. Therefore, the rupture of microspores may be due to the reduced integrity of the plasma membrane and pollen wall. In the weak mutant allele rpg1-3, pollen grains are usually irregular in formation, whereas exine patterning is not severely affected (Supplemental Fig. S1H). This indicates that microspore cell integrity, rather than pollen wall formation, tends to be affected by RPG1 lesions. Our results indicate that proper development of the plasma membrane and pollen wall may be required for the maintenance of microspore cell integrity.

rpg1 is partially fertile later in reproductive development, and apparently pollen development is gradually rescued. A similar phenotype was also reported in ms2 and atgpat1 mutants, although the precise mechanism was unclear (Aarts et al., 1997; Zheng et al., 2003). To investigate the possible mechanism of this phenomenon, we examined the fertility of the mutant plants in different environments, including under drought, high humidity, and different illumination conditions. Unexpectedly, the recovery of fertility in the mutant plants appeared to be consistent (data not shown), which indicated that the rescued pollen development might be caused by some endogenous factors. We speculate that some genes might be expressed in late development (e.g. senescence-induced genes) and could partially complement the function of RPG1.

A previous report demonstrated that RPG1 (At5g40260) is also highly expressed in the embryo sac of the ovule, but an examination of two T-DNA insertion mutant lines, SALK _137176 and SALK_092239 (rpg1-2), suggested no observable phenotype in the embryo sac (Yu et al., 2005). Our results showed that the female fertility of rpg1 and allele mutant lines is indistinguishable from that of the wild type (data not shown). In addition, RPG1 expression in the SALK _137176 line is not significantly affected (data not shown). Therefore, the functional role of RPG1 in female gametophytes may be unnecessary or redundant with other proteins, as proposed previously (Yu et al., 2005).

**RPG1 Encodes an MtN3/saliva Family Protein**

RPG1 is a member of the MtN3/saliva gene family, which exists in a wide variety of eukaryotes. Members of this family usually contain two copies of an MtN3/saliva domain (Gamas et al., 1996; Artero et al., 1998). The MtN3/saliva domain was originally found to be conserved between the root nodulin-related MtN3 protein of the legume _M. truncatula_ and the saliva protein of _D. melanogaster_ (Gamas et al., 1996; Artero et al., 1998). In humans, a member of the MtN3/saliva family, Rga (for Recombination-activating gene 1 gene activation), was found to facilitate the gene activation of Recombination-activating gene 1 (Tagoh et al., 1996). In _Ciona intestinalis_, knockdown of CiRga, an ortholog of mouse Rga, resulted in abnormal embryos in which the cleavage pattern became atypical and the expression of marker genes was suppressed at the tailbud stage (Hamada et al., 2005). In plants, reported MtN3 family genes are usually related to reproductive development. In petunia, the NEC1 gene is expressed preferentially in nectaries and stamens (Ge et al., 2000).

![Figure 7](https://example.com/fig7.png)

**Figure 7. Expression analysis of RPG1.** A, Semiquantitative RT-PCR of RNA isolated from various tissues with RPG- and TUBULIN-specific primer sets. Inf, Inflorescence; Lf, leaf; Rt, root; Sl, seedling; St, stem. B, In situ hybridization of RPG1 transcript within a stage 4 anther with antisense probe. C, In situ hybridization of RPG1 transcript within a stage 5 to 6 anther with antisense probe. D, In situ hybridization of RPG1 transcript within a stage 7 anther with antisense probe. E, In situ hybridization of RPG1 transcript within a stage 9 anther with antisense probe. F, In situ hybridization of RPG1 transcript within a stage 12 anther with antisense probe. G, In situ hybridization of RPG1 transcript within a meiosis stage anther with sense probe. MMC, Microspore mother cells; Mps, microspores; PG, pollen grains; Sp, sporocytes; T, tapetum; Tds, tetrads. Bars = 10 μm.
Partial silencing of NEC1 resulted in premature dehiscence of anthers and reduced fertility (Ge et al., 2001). In rice, an MtN3/saliva gene, Os8N3, was characterized to be a host susceptibility gene for bacterial blight targeted by the type III effector. Also, Os8N3 appeared to be required by inflorescence development, as loss of function of this gene affected pollen development and other floral tissues (Yang et al., 2006).

Recently, the Rga protein was revealed to be associated with the ion channel protein TRPV2 in a rat mast cell line (Barnhill et al., 2004; Stokes et al., 2005). TRPV2 is a member of the TRPV (vanilloid receptor-related) subfamily of the TRP (for Transient Receptor Potential) family. Ion channels of the TRP family respond to diverse cellular stimuli, including variations across a physiological and pathophysiological temperature range (Jin et al., 2006). The Rga protein may be localized to a vesicular subcompartment of the endoplasmic reticulum/Golgi apparatus and interact with TRPV2 intracellularly. The interaction depends on a cellular γ glycosylation event, suggesting that Rga may play a chaperone or targeting role for TRPV2 during the maturation of the ion channel protein (Barnhill et al., 2004; Stokes et al., 2005). The function of MtN3/saliva proteins in plants may be different from that in mammals, as the TRP family does not exist in plants. Moreover, the conserved Asn residues in mammalian MtN3/saliva proteins, suggested to be possible N-glycosylation sites, are absent in characterized plant MtN3/saliva proteins (data not shown). Nevertheless, this discovery provides us a clue that MtN3/saliva proteins may function through interaction with other membrane proteins.

**Putative Role of RPG1 in Microsporogenesis**

Our results indicated that the predicted RPG1 intracellular regions are essential for its function. By phylogenetic analysis, we found that the putative intracellular region of the MtN3/saliva domain is highly conserved in RPG1 and other MtN3/saliva proteins (Fig. 6C). In addition, it is worth noting that the highly conserved intracellular region in the MtN3/saliva domain presents putative phosphorylation motifs in RPG1 (Fig. 6C), which indicates a potential role of RPG1 in protein regulatory networks. The C-terminal tail of RPG1, which is also predicted to be intracellular, appears not to be conserved in our analysis. However, ablation effect of T-DNA insertion in the RPG1 gene indicated that this region is essential for RPG1 function. In the two strong mutant alleles, rpg1 and rpg1-2, T-DNA insertion sites are in the last intron and fifth exon, respectively. RT-PCR showed that in the rpg1 mutant the T-DNA insertion mainly affects the transcription of the last exon of RPG1, which corresponds to the C-terminal tail of RPG1 function. There are two possible reasons why the important C-terminal region is not conserved in our phylogenetic analysis. First, this may due to the incomplete genome information for other species. The importance of the C terminus may be specific to RPG1 in Arabidopsis and closely related species (e.g. in *Brassica napus*), and the absence of orthologous proteins in the phylogenetic analysis may lead to false-negative results. Alternatively, the conservation of the C terminus may not be present at the sequence level but rather at the topological level.

In conclusion, our results demonstrate that (1) ablation of RPG1 causes aberrant cell integrity and exine patterning of microspores; (2) RPG1 is localized to the plasma membrane in microspores and tapetum; and (3) the predicted intracellular regions are essential for RPG1 function. Based on these results, we can infer two possible roles of RPG1 in microsporogenesis. First, RPG1 may play a role in maintaining the plasma membrane integrity of microspores by regulating membrane traffic, such as protein maturation or activation. Ablation of RPG1 results in disrupted integrity and abnormal invagination of the microspore plasma membrane, which in turn leads to the rupture of microspores and impaired exine pattern formation. Alternatively, RPG1 may regulate the timely undulation of the microspore plasma membrane, which is required for primexine patterning. Lesions of RPG1 affect the proper undulation of the plasma membrane and result in severely disrupted primexine patterning. Without the protection of a basic exine layer, the developing microspore with a bumpy plasma membrane tends to burst in the stressful condition of the pollen sac.

Although the characterization of the rpg1 mutant provides insights into the role of the plasma membrane protein in pollen wall development, even more questions arise about the RPG1 protein. Which proteins do RPG1 interact with on the plasma membrane? Why is RPG1 expressed highly in both tapetum and microspores? What are the roles of other MtN3/saliva proteins in Arabidopsis? Investigation of these questions will provide further insights into the molecular basis of microsporogenesis and the roles of MtN3/saliva proteins in plant development.

**MATERIALS AND METHODS**

**Plant Growth and Mutant Isolation**

Arabidopsis (*Arabidopsis thaliana*) plants used in this study are in the Columbia-0 background. Seeds were sown on vermiculite and allowed to imbibe for 3 d at 4°C. Plants were grown under long-day conditions (16 h of light/8 h of dark) in an approximately 22°C growth room. The rpg1 mutant was characterized from the p5SK15 activation-tagging T-DNA mutant pools (Qin et al., 2003).

**Phenotype Characterization and Microscopy**

Plants were photographed with a Canon digital camera (Powershot A710IS). Flower images were taken using an Olympus dissection microscope with an Olympus digital camera. Alexander solution and DAPI staining were performed as described (Alexander, 1969; Ross et al., 1996). Cross section and callose staining were performed as described previously (Zhang et al., 2007). Photography was performed with an Olympus BX-51 microscope. For SEM examination, fresh stamens and pollen grains were coated with 8 nm of gold and observed on a JSM-840 microscope (JEOL). For TEM photography was performed with an Olympus BX-51 microscope. For TEM photography was performed with an Olympus BX51 microscope.
examination, Arabidopsis buds from the inflorescence were fixed and embedded as described (Zhang et al., 2007). Ultrathin sections (90–100 nm thick) were observed with a JEM-1230 transmission electron microscope (JEOL).

**TAIL-PCR and Molecular Cloning of the RPG1 Gene**

The presence of T-DNA insertion in the mutant was validated using primers that specifically amplify the BAR gene of T-DNA (Bar-F, 5′-CCACCCATCGTCAACCCATC-3′; Bar-R, 5′-TGTCGAGAAGCCGCAGCTC-3′). For TAIL-PCR, T-DNA left border primers (AtLB1, 5′-ATACCCGCGCGTTAACCTT-3′; AtLB2, 5′-TATAAACGCGGCAACATCAAC-3′; AtLB3, 5′-TTGACCATCATACTCTTGTCG-3′) and genomic DNA of mutant plants were used. The TAIL-PCR procedure and arbitrary degenerate primers were as described (Liu et al., 1995). Cosegregation of the T-DNA insertion site and mutant phenotype were analyzed with AtLB3 and plant-specific primers (LP, 5′-CTCCGAGCGAGAAATCTCAAC-3′; RP, 5′-TTTATCGCCCTCCGCTTGCG-3′). For mutant plants, only PCR with LP and RP primers could amplify a DNA fragment of about 700 bp. For wild-type plants, only PCR with LP or RP primers could amplify DNA fragment of 760 bp. For heterozygous mutant plants, PCR with both primer pairs showed positive results.

For complementation, a DNA fragment of 3.6 kb including 1- and 3-kb downstream sequences was amplified using LA-Taq polymerase (Takara, Japan). The complementation fragment and genome-specific primers were as follows: RPG1N-F (5′-AGATCTGAACCCTCTCCGTAGCAGAAATCTCAAC-3′) and genome-specific primers as follows: SALK-142803-RP (5′-TTTTACGGTCTCCCTGTGG-3′). After verification by sequencing, the fragment was cloned into pCAMBIA1300 binary vector (CAMBIA; www.cambia.org.au) and then introduced into homozygous mutant plants using the infiltration method with Agrobacterium tumefaciens introduced into homozygous mutant plants using the infiltration method with Agrobacterium tumefaciens. The TAIL-PCR procedure and arbitrary degenerate primers were as described (Liu et al., 1995). Cosegregation of the T-DNA insertion site and mutant phenotype were analyzed with AtLB3 and plant-specific primers (LP, 5′-CTCCGAGCGAGAAATCTCAAC-3′; RP, 5′-TTTATCGCCCTCCGCTTGCG-3′). For mutant plants, only PCR with LP and RP primers could amplify a DNA fragment of 760 bp. For heterozygous mutant plants, PCR with both primer pairs showed positive results.

**Phylogenetic Analysis**

The multiple sequence alignment of full-length protein sequences was performed using the ClustalW tool online (http://www.ch.embnet.org/software/clustalW.html) and displayed using BOXSHADE (http://www.ch.embnet.org/software/BOX_form.html). Phylogenetic trees were constructed and tested by MEGA3.1 based on the neighbor-joining method.

**Expression Analysis**

For expression analysis of RPG1 in mutant, RNA was extracted from inflorescences of mutant and wild-type plants using TRizol (Invitrogen). Semiquantitative RT-PCR for 30 cycles was used to assess the levels of expression of RPG1 and alleles, using primer sets as follows: RPG1N-F (5′-ATGTTGTGATGCAAAAACAGTTCC-3′) and RPG1N-R (5′-AACACAG-GAGACCGTTAA-3′) were used to examine 5′ region expression (exons 1–3), and LP and RP (see above) were used for 3′ region expression analysis (exons 3–5).

For RT-PCR, RNA was extracted from root, rosette leaves, 14-d-old seedlings, and inflorescences. PCR was performed by LP/RP primer set. In situ hybridization was performed with the DIG (digoxigenin) RNA Labeling Kit (Roche) and the DIG Probe Synthesis Kit (Roche). An RPG1-specific cDNA fragment of 458 bp was amplified and cloned into the pSK vector. Antisense and sense digoxigenin-labeled probes were prepared with EcoRI or BamHI digestion and in vitro transcription using T3 or T7 RNA polymerase, respectively.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers NP_568579, BAA05436, CAE47557, CAN64755, AZM5_12004, CAAB9976, and AAC54696.

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Phenotype of rpg1 mutant alleles.

**ACKNOWLEDGMENTS**

We thank the Salk Institute Genomic Analysis Laboratory for providing the sequence-indexed Arabidopsis T-DNA insertion mutants. We thank Xiao-yan Gao from Shanghai Institute of Plant Physiology and Ecology and Hui-qi Zhang from Shanghai Normal University for their help with SEM and TEM. We are grateful to Dr. Gareth H Jones from the University of Birmingham for kindly suggestions on DAPI staining.

Received February 21, 2008; accepted April 16, 2008; published April 23, 2008.

**LITERATURE CITED**

Aarts MG, Hodge R, Kalantidis K, Florack D, Wilson ZA, Mulligan BJ, Stiekema WJ, Scott R, Pereira A (1997) The Arabidopsis MALE STERILITY 2 protein shares similarity with reductases in elongation/condensation complexes. Plant Cell 12: 615–623

Alexander MP (1969) Differential staining of aborted and nonaborted pollen. Stain Technol 44: 117–122

Ariizumi T, Hatakeyama K, Hinata K, Inatsugi R, Nishida I, Sato S, Kato T, Tabata S, Toriyama K (2005) Disruption of the novel plant protein NFI F affects lipid accumulation in the plastids of the tapetum and exine formation of pollen, resulting in male sterility in Arabidopsis thaliana. Plant J 39: 170–181

Ariizumi T, Hatakeyama K, Hinata K, Sato S, Kato T, Tabata S, Toriyama K (2003) A novel male-sterile mutant of Arabidopsis thaliana, faceless pollen-1, produces pollen with a smooth surface and an acetolysis-sensitive exine. Plant Mol Biol 53: 107–116

Artero RD, Terol-Alcaide Y, Paricio N, Ring J, Bargues M, Torres A, Perez-Alonso M (1998) salto, a new Drosophila gene expressed in the embryonic salivary glands with homologues in plants and vertebrates. Mech Dev 75: 159–162

Barthill JC, Stokes AJ, Koblan-Huberson M, Shimoda LM, Muraguchi A, Adra CN, Turner H (2004) RGA protein associates with a TRPV ion channel during biosynthesis and trafficking. J Cell Biochem 91: 808–820

Chen X, Goodwin SM, Boroff VL, Liu X, Jenks MA (2003) Cloning and characterization of the WAX2 gene of Arabidopsis involved in cuticle membrane and wax production. Plant Cell 15: 1170–1185

Donal L (2001) KO/AR encodes a cellulose synthase-like protein required for root hair cell morphogenesis in Arabidopsis. Genes Dev 15: 79–89

Favery B, Ryan E, Foreman J, Linstead P, Boudonck K, Steer M, Shaw P, Keegstra K (2003) Characterization of cDNAs from Arabidopsis thaliana that encode a potential glucan 1,3-beta-glucosyltransferase. Plant Cell 15: 1161–1171

Fitzgerald MA, Knox RB (1995) Initiation of primexine in freeze-substituted microspores of Brassica campestris. Sex Plant Reprod 8: 99–104

Guan et al.
Paxson-Sowders DM, Owen HA, Makaroff CA, Nishikawa S, Zinkl GM, Swanson RJ, Maruyama D, Preuss D (2005) Partial silencing of the NEC1 gene results in early opening of anthers in Petunia hybrida. Mol Genet Genomics 265: 414–423

Ge YY, Angenent GC, Dahlhaus E, Franken J, Peters J, Wullems GJ, Creemers-Molenaar J (2001) Partial silencing of the NEC1 gene results in early opening of anthers in Petunia hybrida. Mol Genet Genomics 265: 414–423

Ge YY, Angenent GC, Wittich PE, Peters J, Franken J, Russcher M, Zhang LM, Dahlhaus E, Kater MM, Wullems GJ, et al (2000) NEC1, a novel gene, highly expressed in nectary tissue of Petunia hybrida. Plant J 24: 725–734

Hamada M, Wada S, Kobayashi K, Satoh N (2005) C–Rgl, a gene encoding an MiN3/saliva family transmembrane protein, is essential for tissue differentiation during embryogenesis of the ascidian Ciona intestinalis. Differentiation 73: 364–376

Heslop-Harrison J (1971) Wall pattern formation in angiosperm microsporogenesis. Symp Soc Exp Biol 25: 277–300

Ito T, Nagata N, Yoshida Y, Ohme-Takagi M, Ma H, Shinozaki K (2002) Shaping in plant cells. Curr Opin Plant Biol 5: 265–275

Ito T, Shinozaki K (2000) Two GRAM domain proteins are expressed in tapetal cells and is required for pollen maturation. Plant Cell Physiol 41: 1523–1532

Jin X, Touhey J, Gaudet R (2006) Structure of the N-terminal ankyrin repeat domain of the TRPV2 ion channel. J Biol Chem 281: 25006–25010

Kobayashi T, Kobayashi E, Sato S, Hotta Y, Miyajima N, Tanaka A, Tabata S (1994) Characterization of CDNA induced in meiotic prophase in lily pollen development in Arabidopsis. Plant Physiol 105: 539–549

Li YG, Mitsukawa N, Oosumi T, Whittier RF (1995) Efficient isolation of T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J 8: 457–463

Liu YG, Mitsuwaka N, Osoumi T, Whittier RF (1995) Efficient isolation and mapping of Arabidopsis thaliana T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J 8: 457–463

Martin C, Bhatt K, Baumann K (2001) Shaping in plant cells. Curr Opin Plant Biol 4: 540–549

Nishikawa S, Zinkl GM, Swanson RJ, Maruyama D, Preuss D (2005) Callose (beta-1,3 glucan) is essential for Arabidopsis pollen wall pat- terning, but not tube growth. BMC Plant Biol 5: 22

Paxson-Sowders DM, Dodrill CH, Owen HA, Makaroff CA (2001) DEX1, a novel plant protein, is required for exine pattern formation during pollen development in Arabidopsis. Plant Physiol 127: 1739–1749

Paxson-Sowders DM, Owen HA, Makaroff CA (1997) A comparative ultrastructural analysis of exine pattern development in wild-type Arabidopsis and a mutant defective in pattern formation. Protoplasma 198: 53–65

Piffanelli P, Ross JHE, Murphy DJ (1998) Biogenesis and function of the lipidic structures of pollen grains. Sex Plant Reprod 11: 65–80

Qin G, Kang D, Dong Y, Shen Y, Zhang L, Deng X, Zhang Y, Li S, Chen N, Niu W, et al (2003) Obtaining and analysis of flanking sequences from T-DNA transformants in Arabidopsis. Plant Sci 165: 941–949

Ross KJ, Franz P, Jones GH (1996) A light microscopic atlas of meiosis in Arabidopsis thaliana. Chromosome Res 4: 507–516

Rowland O, Lee R, Franke R, Schreiber L, Kunst L (2007) The CER3 wax biosynthetic gene from Arabidopsis thaliana is allelic to WAX2/YRE/FLPL. FEBS Lett 581: 3538–3544

Sanders PM, Bui AQ, Weterings K, McIntire KN, Hsu Y-C, Lee PY, Truong MT, Beals TP, Goldberg RB (1999) Anther developmental defects in Arabidopsis thaliana male-sterile mutants. Sex Plant Reprod 11: 297–322

Stokes AJ, Wakano C, Del Carmen KA, Koblan-Huberson M, Turner H (2003) Formation of a physiological complex between TRPV2 and RGA protein promotes cell surface expression of TRPV2. J Cell Biochem 94: 649–683

Tagoh H, Kishi H, Muraguchi A (1996) Molecular cloning and characterization of a novel stromal cell-derived cDNA encoding a protein that facilitates gene activation of recombination activating gene (RAG)-1 in human lymphoid progenitors. Biochem Biophys Res Commun 221: 744–749

Vizcay-Barrena G, Wilson ZA (2006) Altered tapetal PCD and pollen wall development in the Arabidopsis ms1 mutant. J Exp Bot 57: 2709–2717

Wang X, Cnops G, Vanderhaeghen R, De Block S, Van Montagu M, Van Lijsebettens M (2001) AICS/LD2, a cellulose synthase-like gene important for root hair growth in Arabidopsis. Plant Physiol 126: 575–586

Waterkeyn L, Bienfait A (1970) On a possible function of the callosic special wall in Ipomoea purpurea (L.) Roth. Grana 10: 13–20

Wilson ZA, Morroll SM, Dawson J, Swarup R, Tighe PJ (2001) The Arabidopsis MALE STERILITY1 (MST1) gene is a transcriptional regula- tor of male gametogenesis, with homology to the PHD-finger family of transcription factors. Plant J 28: 27–39

Yang B, Sugio A, White FF (2006) OsSN3 is a host disease-susceptibility gene for bacterial blight of rice. Proc Natl Acad Sci USA 103: 10503–10508

Yang C, Vizcay-Barrena G, Conner K, Wilson ZA (2007) MALE STERILITY1 is required for tapetal development and pollen wall biosynthesis. Plant Cell 19: 3530–3548

Yoo SD, Cho YH, Sheen J (2007) Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nat Protocols 2: 1565–1572

Yu HJ, Hogan F, Sundaresan V (2005) Analysis of the female gametophyte transcriptome of Arabidopsis by comparative expression profiling. Plant Physiol 139: 1853–1869

Zhang ZB, Zhu J, Gao JF, Wang C, Li H, Li H, Zhang HQ, Zhang S, Wang DM, Wang QX, et al (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

Zheng Z, Xie Q, Dauk M, Shen W, Selvaraj G, Zou JT (2003) Arabidopsis AGL11, a member of the membrane-bound glycerol-3-phosphate acyltransferase gene family, is essential for tapetum differentiation and male fertility. Plant Cell 15: 1872–1887

Zinkl GM, Zweibel BL, Grier DG, Preuss D (1999) Pollen-stigma adhesio in Arabidopsis: a species-specific interaction mediated by lipophilic molecules in the pollen exine. Development 126: 5431–5440

RPGL Is Required for Pollen Wall Development in Arabidopsis