Two Mitogen-Activated Protein Kinases, MPK3 and MPK6, Are Required for Funicular Guidance of Pollen Tubes in Arabidopsis[1[W][OPEN]]

Yuefeng Guan, Jianping Lu, Juan Xu, Bruce McClure, and Shuqun Zhang*

Division of Biochemistry, University of Missouri, Columbia, Missouri 65211 (Y.G., B.M., S.Z.); College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China (J.L., J.X.); and Shanghai Center for Plant Stress Biology, Shanghai 201602, China (Y.G.)

Double fertilization in flowering plants requires the delivery of two immotile sperm cells to the female gametes by a pollen tube, which perceives guidance cues, modifies its tip growth direction, and eventually enters the micropyle of the ovule. In spite of the recent progress, so far, little is known about the signaling events in pollen tubes in response to the guidance cues. Here, we show that MPK3 and MPK6, two Arabidopsis (*Arabidopsis thaliana*) mitogen-activated protein kinases, mediate the guidance response in pollen tubes. Genetic analysis revealed that *mpk3 mpk6* double mutant pollen has reduced transmission. However, direct observation of *mpk3 mpk6* mutant pollen phenotype was hampered by the embryo lethality of double homozygous $mpk3^{-/-}$ $mpk6^{-/-}$ plants. Utilizing a fluorescent reporter-tagged complementation method, we showed that the *mpk3 mpk6* mutant pollen had normal pollen tube growth but impaired pollen tube guidance. In vivo pollination assays revealed that the *mpk3 mpk6* mutant pollen tubes were defective in the funicular guidance phase. By contrast, semi-in vitro guidance assay showed that the micropylar guidance of the double mutant pollen tube was normal. Our results provide direct evidence to support that the funicular guidance phase of the pollen tube requires an in vivo signaling mechanism distinct from the micropyle guidance. Moreover, our finding opened up the possibility that the MPK3/MPK6 signaling pathway may link common signaling networks in plant stress response and pollen-pistil interaction.

In flowering plants, successful fertilization is dependent on extensive cell-cell communication between male and female gametophytes. After landing on a compatible stigma surface, a mature pollen grain germinates to form a pollen tube, which penetrates the stigma, perceives guidance cues along the growth path, and modifies its tip growth direction toward the ovule (Hülskamp et al., 1995). In Arabidopsis (*Arabidopsis thaliana*), the pollen tube guidance can be divided into two phases: funicular guidance, in which the pollen tube emerges from the septum and proceeds to a funiculus, and micropylar guidance, in which the pollen tube grows toward and enters the micropyle of an ovule (Hülskamp et al., 1995).

In pollen tube, it is believed that receptors on the tube tip perceive various guidance cues and regulate downstream signaling pathways to modify tip reorientation toward the ovule (Higashiyama, 2010; Takeuchi and Higashiyama, 2011). Two receptor-like kinase genes, *Lost In Pollen tube guidance1* (*LIP1*) and *LIP2*, are involved in guidance control of pollen tubes. *LIP1* and *LIP2* were anchored to the membrane in the pollen tube tip region via palmitoylation, which was essential for their guidance control (Liu et al., 2013). Therefore, *LIP1* and *LIP2* are the essential components of the receptor complex in micropylar guidance. The Glu receptor-like channels facilitate Ca^{2+} influx across the plasma membrane and regulate pollen tube growth and morphogenesis (Michard et al., 2011). This interesting work revealed that there is a signaling mechanism between the male gametophyte and pistil tissue that is similar to the amino acid-mediated communication in animal nervous systems (Michard et al., 2011). Recent findings also highlight the importance of the endoplasmic reticulum (ER), ion homeostasis, and protein processing in pollen tube guidance (Li et al., 2011; Lu et al., 2011; Li and Yang, 2012). Two pollen-expressed cation proton exchangers (CHXs), CHX21 and CHX23, were reported to mediate K^+ transport in ER and are essential for the pollen tube to respond to directional signals from the ovule in Arabidopsis (Lu et al., 2011). POLLEN DEFECTIVE IN GUIDANCE1 plays an important role in micropylar guidance in pollen tube (Li et al., 2011). It is an ER luminal protein involved in ER protein retention and interacts with a luminal chaperone involved in Ca^{2+} homeostasis and ER quality control (Li et al., 2011). Therefore, the ER quality control is likely an important mechanism in surveillance of signaling factors in pollen tube guidance (Li and Yang, 2012).

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* Address correspondence to zhangsh@missouri.edu.

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In spite of the recent progresses, so far, little is known about the cytoplasmic signaling events in pollen tubes in response to the guidance cues. Mitogen-activated protein kinase (MAPK, or MPK) cascades are conserved signaling pathways that respond to extracellular stimuli and regulate various cellular activities. In Arabidopsis, MPK3 and MPK6 are induced by various biotic and abiotic stresses and collaboratively play important roles in defense response and plant development (Zhang, 2008). Here, we show that MPK3 and MPK6 are also critical to pollen tube guidance. Utilizing a fluorescent reporter-tagged complementation method, we demonstrated that *mpk3 mpk6* pollen was defective in pollen tube guidance at the funicular guidance phase. Intriguingly, the micropylar guidance of *mpk3 mpk6* pollen tube is not affected.

RESULTS AND DISCUSSION

Genetic Analysis Reveals Distorted Transmission of *mpk3 mpk6* Pollen

In Arabidopsis, double mutation of *MPK3* and *MPK6* leads to embryo lethality (Wang et al., 2007). In addition, we noticed that the *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants exhibited distorted male transmission when backcrossed to wild-type pistils (*mpk3 mpk6*:*mpk3 MPK6* = 35:316, $P < 0.0001$, male transmission efficiency = 11.08%; male transmission efficiency: no. of mutant progenies/no. of wild-type progenies × 100%). Back crosses using *mpk3*$^{+/-}$, *mpk6*$^{+/-}$, or *mpk3*$^{+/-}$ *mpk6*$^{-/-}$ as male parents showed that *mpk3* single mutant pollen had a normal transmission rate (*mpk3*:*MPK3* = 89:80, P = 0.4887, male transmission efficiency = 111.25%), while the *mpk6* single mutant pollen transmission was moderately reduced in competition with the wild-type pollen (*mpk6*:*MPK6* = 74:156, $P < 0.0001$, male transmission efficiency = 47.24%). The transmission defect of *mpk3 mpk6* pollen was partially suppressed when a limited number (<20) of pollen grains from *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants were used for pollination (*mpk3 mpk6*:*mpk3 MPK6* = 95:144, P = 0.0019, male transmission efficiency = 65.97%). This result indicated that the *mpk3 mpk6* pollen is less competitive than *mpk3* pollen but is mostly able to fertilize ovules in the absence of competitor pollen. *MPK3* and *MPK6* are both expressed in pollen, with *MPK6* showing a higher expression level than *MPK3* (Supplemental Fig. S1; Supplemental Materials and Methods S1). In pollen tubes, MPK3 and MPK6 protein were enriched in nuclei, as revealed by the yellow fluorescent protein (YFP) fusion (Supplemental Fig. S2; Supplemental Materials and Methods S1).

A Fluorescent Reporter-Tagging Complementation Method for Isolating *mpk3 mpk6* Pollen Grains

Our previous study using *mpk3*$^{+/-}$ *mpk6*$^{-/-}$ plants in the *quartet1* background demonstrated that *mpk3 mpk6*

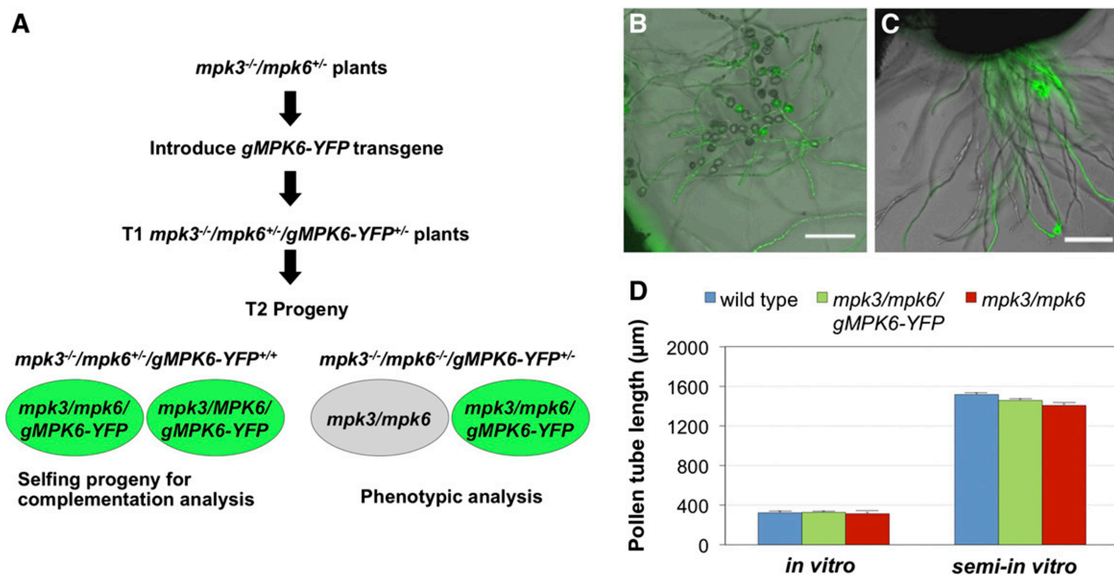

Figure 1. Reduced *mpk3 mpk6* pollen transmission is not associated with defective pollen germination or pollen tube growth. A, Generation of fluorescent reporter-tagged *MPK6* complemented *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ mutant lines. *gMPK6-YFP* construct was introduced into *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants. In T2 generation, *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ *gMPK6-YFP*$^{+/+}$ plants were identified to determine the complementation of *mpk3 mpk6* pollen transmission by *gMPK6-YFP*. Plants of *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/-}$ genotype, which produce the nonfluorescent *mpk3/mpk6* double mutant pollen and the YFP-tagged complemented pollen (*mpk3 mpk6 gMPK6-YFP*), were identified for phenotypic analysis in vivo. B and C, Double *mpk3 mpk6* mutant (nonfluorescent) and complemented *mpk3 mpk6 gMPK6-YFP* (fluorescent) pollen tubes grew at similar rates in both in vitro pollen germination (B) and semi-in vitro pollen germination assays (C). Bar = 100 μm. D, Quantitative comparison of pollen tube length of wild-type, *mpk3 mpk6* double mutant, and *mpk3 mpk6 gMPK6-YFP* complemented pollen. Error bar indicates SE of the mean.

pollen has no developmental defect (Wang et al., 2008). To test if *mpk3 mpk6* pollen tube growth is impaired, we performed backcrosses using pollen from *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants and wild-type pistils and collected each individual seed along the siliques. We found that *mpk3*$^{+/-}$ *mpk6*$^{+/-}$ seeds were randomly distributed, suggesting no pollen tube growth defect of the double mutant in vivo (Supplemental Table S1).

Because the *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ double mutant is embryo lethal, we cannot obtain homogeneous *mpk3 mpk6* pollen population. To observe directly the phenotype of *mpk3 mpk6* pollen, we developed a fluorescent reporter-tagged complementation method (Fig. 1A). A construct with a genomic *MPK6* fragment (*gMPK6*) tandemly linked to a pollen-specific *Late Anther Tomato53* (*LAT52*) promoter-driven *YFP* reporter cassette (Twell et al., 1990), designated *gMPK6-YFP*, was introduced into *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants. Selfing progenies of *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ *gMPK6-YFP*$^{+/+}$ plants revealed normal pollen transmission (*mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/+}$:*mpk3*$^{-/-}$ *mpk6*$^{+/-}$ *gMPK6-YFP*$^{+/+}$: *mpk3*$^{-/-}$ *MPK6 gMPK6-YFP*$^{+/+}$ = 47:98:44, $P = 0.89$, expected 1:2:1), indicating successful complementation of the double *mpk3 mpk6* mutant pollen function by the *gMPK6-YFP* transgene. Furthermore, plants in *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/-}$ genotype were identified, in which the nonfluorescent *mpk3 mpk6* double mutant pollen was distinguishable from the YFP-tagged-*gMPK6* complemented pollen (genotype: *mpk3 mpk6 gMPK6-YFP*; Fig. 1A). In vitro and semi-in vitro germination of pollen of the *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/-}$ plants verified that the double mutant pollen is indistinguishable from complemented pollen in germination and pollen tube growth (Fig. 1, B–D).

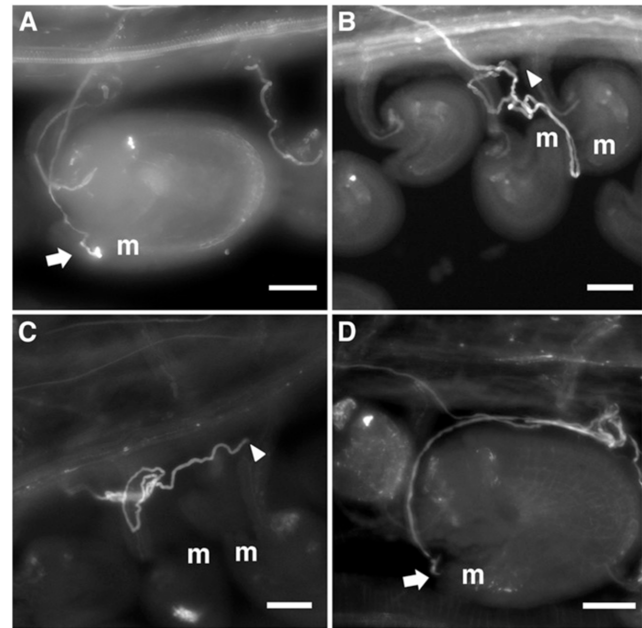

Figure 2. Defective navigation of *mpk3 mpk6* mutant pollen tubes in limited pollination. Pollen tube growth in wild-type pistils was visualized by aniline blue staining. A, An ovule fertilized by a wild-type pollen tube in limited pollination. B to D, Limited pollination of wild-type pistils with the nonfluorescent *mpk3 mpk6* pollen grains isolated from those produced by *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/-}$ plants. B and C, *mpk3 mpk6* pollen tubes exhibited wandering phenotype without targeting any ovule. D, A *mpk3 mpk6* pollen tube eventually targeted an ovule after wandering growth. Arrows indicate pollen tube tips that entered the micropyle. Arrowheads indicate the position of pollen tube tips that did not grow onto the ovule. The letter m indicates position of the micropyle. Bar = 100 μm.

mpk3 mpk6 Pollen Is Defective in Pollen Tube Guidance

To observe the *mpk3 mpk6* pollen tube growth in vivo, we isolated nonfluorescent *mpk3 mp6* pollen grains from *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/-}$ plants under a fluorescent dissecting microscope and performed limited pollination. In limited pollination, all wild-type pollen tubes could target and fertilize the ovules (Fig. 2A). After emerging from the transmitting tract onto the septum, wild-type pollen tubes proceeded directly onto funiculi toward micropyles for fertilization. By contrast, the majority (58%, $n = 117$) of the *mpk3 mpk6* pollen tubes showed defects in navigation. Some of the pollen tubes were lost in the septum and elongated in random directions without growing onto the funiculus (20%, $n = 117$; Fig. 2, B and C). Others exhibited a wandering phenotype before eventually targeting the ovules (38%, $n = 117$; Fig. 2D). However, the response of the *mpk3 mpk6* pollen tubes to the guidance signals was not completely blocked, because most mutant pollen tubes could eventually approach micropyle (80%, $n = 117$) in the absence of competing wild-type pollen. This is consistent with the genotyping result of recovered mutant transmission in limited pollination.

mpk3 mpk6 Pollen Tube Is Deficient in Funicular Guidance in Vivo

In previously reported Arabidopsis mutants with micropylar guidance defects, multiple pollen tubes were observed on one funiculus (Palanivelu et al., 2003; Kasahara et al., 2005; Shimizu et al., 2008). However, this phenotype was not observed in pistils pollinated with excessive pollen grains from *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants (Supplemental Fig. S3). All the ovules were targeted by a single pollen tube that grew directly into the micropyle, indicating that the *mpk3 mpk6* pollen tubes may be impaired in funicular guidance, i.e. the *mpk3 mpk6* pollen tubes cannot find or take a longer time to find the funiculus. To verify if *mpk3 mpk6* pollen tubes were defective in funicular guidance stage in vivo, we introduced a *LAT52* promoter-driven *tdTomato* fluorescent marker (designated *TdTOM*) into *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/-}$ plants (Fig. 3A). The T2 *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/-}$ *TdTOM*$^{+/+}$ plants produce double mutant pollen tagged by only the TdTOM marker (genotype: *mpk3 mpk6 TdTOM*) and the *gMPK6*-complemented pollen tagged by both TdTOM and YFP (genotype: *mpk3 mpk6 gMPK6-YFP TdTOM*; Fig. 3, B–D). In vivo observation showed that the

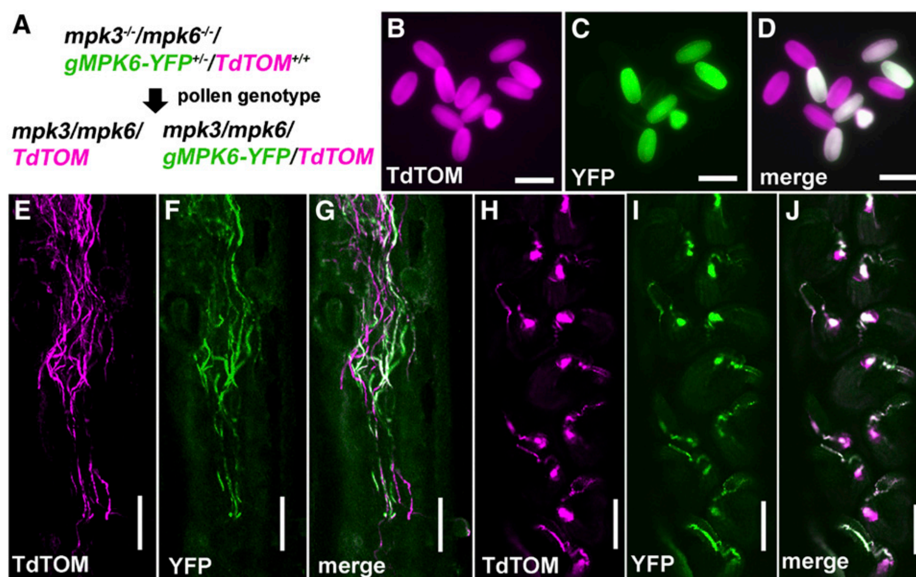

Figure 3. Deficient funicular guidance of *mpk3 mpk6* pollen tubes in vivo. A, Double fluorescent reporter-tagged complementation line. Plants of $mpk3^{-/-}$ $mpk6^{-/-}$ $gMPK6\text{-}YFP^{+/-}$ $TdTOM^{+/+}$ genotype produce double mutant pollen tagged by TdTOM only and complemented pollen tagged by both YFP and TdTOM. B to D, Fluorescent images of TdTOM (B), YFP (C), and merged (D) channels of pollen grains from $mpk3^{-/-}$ $mpk6^{-/-}$ $gMPK6\text{-}YFP^{+/-}$ $TdTOM^{+/+}$ plants. Pollen of *mpk3 mpk6 TdTOM* genotype is indicated by the cyan color in merged channel. Pollen of *mpk3 mpk6 gMPK6-YFP TdTOM* genotype is indicated by the white color in merged channel. Bar = 25 μm. E to G, Pistil with carpel and ovules removed at 7 h after pollination. The double mutant and complemented pollen tubes showed similar growth rates and morphology in the transmission tract. E, TdTOM. F, YFP. G, Merged. Bar = 100 μm. H to J, Pistil with carpel removed at 7 h after pollination to exhibit the ovules. In this part of the pistil, all ovules were fertilized by complemented pollen tubes. Note no double mutant pollen tubes were observed on funiculus. H, TdTOM. I, YFP. J, Merged. Bar = 100 μm.

growth of *mpk3 mpk6* pollen tubes in the transmitting tract is similar to the *gMPK6*-complemented pollen tubes (Fig. 3, E–G). Consistent with the genetic analysis, most of the fertilized ovules were targeted by the *gMPK6*-complemented pollen tubes instead of *mpk3 mpk6* pollen tubes (Fig. 3, H–J; 71 of 82 observed ovules, $P < 0.0001$; targeting efficiency of *gMPK6*-complemented pollen = 86.59%). The absence of *mpk3 mpk6* pollen tubes on most funiculi indicated impaired funicular guidance of the mutant.

Response of *mpk3 mpk6* Pollen Tubes to Micropyle Attractants Is Normal in the Semi-in Vitro Guidance Assay

To examine if *mpk3 mpk6* pollen is also defective in micropylar guidance, we performed a semi-in vitro pollen tube guidance assay, which recapitulates pollen tube navigation by micropylar attractants (Palanivelu and Preuss, 2006; Stewman et al., 2010), using pollen from $mpk3^{-/-}$ $mpk6^{-/-}$ $gMPK6\text{-}YFP^{+/-}$ $TdTOM^{+/+}$ plants. The results showed that double mutant pollen tubes were not defective in navigation to the micropyle (ovules targeted by *mpk3 mpk6 TdTOM*:*mpk3 mpk6 gMPK6-YFP TdTOM* pollen = 43:46, expected 1:1, $P = 0.75$). In addition, when approaching the ovules, the double mutant pollen tubes could make sharp turns toward and enter micropyles, even in competition with *gMPK6*-complemented pollen tubes (Fig. 4, A and B). These results indicated that the pollen tube response to and growth toward the micropylar guidance cues are not affected in *mpk3 mpk6* pollen. Therefore, MPK3/MPK6 signaling is required for in vivo pollen tube guidance response, which is distinct from the micropylar guidance.

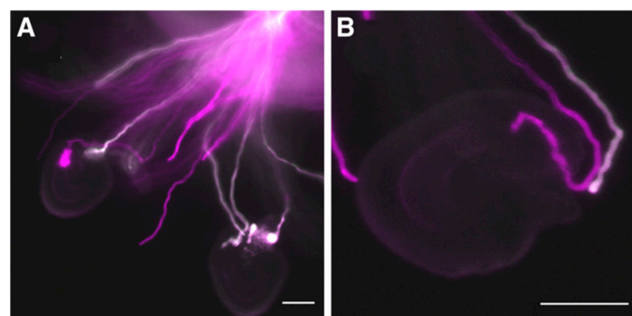

Figure 4. Normal micropylar guidance of the *mpk3/mpk6* double mutant pollen tubes in semi-in vitro guidance assay. A and B, Double mutant pollen tubes can efficiently target micropyles in the semi-in vitro pollen tube guidance assay. Cyan color indicates *mpk3 mpk6* double mutant pollen tubes (genotype: *mpk3 mpk6 TdTOM*), and white color indicates *gMPK6-YFP* complemented pollen tubes (genotype: *mpk3 mpk6 gMPK6-YFP TdTOM*). A, Two ovules targeted by an *mpk3 mpk6* pollen tube and a *gMPK6*-complemented pollen tube, respectively. B, A *mpk3 mpk6* pollen tube made a sharp turn toward an ovule and entered the micropyle ahead of a *gMPK6*-complemented pollen tube. Bar = 50 μm.

In Arabidopsis, MPK3 and MPK6 play multiple roles in signaling plant developmental processes and responses to invading pathogens and abiotic stress stimuli (Zhang and Klessig, 2001; Asai et al., 2002; Liu and Zhang, 2004; Wang et al., 2007, 2008; Beckers et al., 2009). Here, we report that the MPK3/MPK6 signaling is also required for pollen tube guidance, opening up the possibility that the MPK3/MPK6 signaling pathway may link signaling networks between stress response and reproductive development. The micropylar guidance attractants, LUREs, belong to the defensin-like super gene family of secreted Cys-rich proteins, which are an ancient class of small antimicrobial proteins (Aerts et al., 2008; Okuda et al., 2009). Intriguingly, plant antifungal defensins could activate fungal MAPK pathways to change the structural integrity of fungal membranes, which changes the influx/efflux of ion signals (such as Ca^{2+} and K^{+}) and in turn affects fungal growth (Ramamoorthy et al., 2007; Aerts et al., 2008; Dresselhaus and Márton, 2009). The pollen tube response to guidance cues (such as LUREs and funicular guidance signals) might be analogous to the defensin-triggered fungal response and involve the MAPK pathway to regulate the attractant response and reorientation of pollen tube growth (Dresselhaus and Márton, 2009).

The MPK3/MPK6 signaling pathway may act downstream of pollen tube guidance signal receptors (e.g. receptor-like kinases) and is activated when the funicular guidance cues are perceived by these receptors. Previous reports showed that stress-induced activation of MPK6 or its orthologs in other species happens within one to several minutes (Zhang and Klessig, 2001). Thus, it is possible that MPK3 and MPK6 are activated quickly in response to the guidance signals (Supplemental Fig. S4). Alternatively, pollen tube growth through the stigma and pistil tissues is essential for the priming of pollen tubes for guidance response (Qin et al., 2009). We could not exclude the possibility that the MPK3/MPK6 signaling cascade may be activated during pollination to regulate expression of funicular guidance signaling genes. However, the expression of MAPK cascade components including MAPKs, their upstream MAPK kinases (MAPKKs) and MAPKK kinases (MAPKKKs) are not significantly changed in semi-in vitro pollen tubes (Qin et al., 2009). In addition, *mpk3 mpk6* pollen tubes were completely competent in semi-in vitro guidance assay. These results indicated that MPK3/MPK6 downstream pathway may be specifically involved in the in vivo interactions between funiculus and pollen tube, although the underlying mechanism is still unclear. Finally, the MPK3/MPK6 signaling cascade might affect gene expression at earlier stages, such as during pollen development, and the preexisting transcripts or proteins play important roles at a later stage during pollen tube guidance (Supplemental Fig. S4).

In addition, the fluorescent reporter-tagged complementation method we developed is broadly applicable to the male gametophytic mutant study. This method allows one to confidently assign complemented and mutant genotypes to individual pollen grains under a fluorescence microscopy. Homogenous mutant pollen grains can be physically isolated. Furthermore, the method facilitates side-by-side comparison of mutant and complemented pollen in vitro and in vivo.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Arabidopsis (*Arabidopsis thaliana*) ecotype Columbia was used as the wild type. Transfer DNA insertion alleles of *MPK3* (At3g45640), *mpk3-1*, and *MPK6* (At2g43790), *mpk6-2*, were described previously (Wang et al., 2007). Mutant plants with *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ and *mpk3*$^{+/-}$ *mpk6*$^{-/-}$ genotypes were generated by crossing *mpk3-1* and *mpk6-2*. Plant growth and genotyping of mutant alleles was performed as described previously (Wang et al., 2007).

Molecular Cloning and Transformation

For complementation of *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ mutant, the *LAT52:YFP* cassette was PCR amplified from *pZY90-LAT52:YFP* construct (a gift from Dr. Sheila McCormick, Plant Gene Expression Center) and cloned into *pGreenII-gMPK6* to generate the *pGreenII-gMPK6-LAT52:YFP* construct. The *LAT52:TdTOM* construct was prepared using a synthetic sequence (GenBank: KJ081243) encoding *TdTOM* (Shaner et al., 2004) that was codon optimized based on pollen-expressed genes *NTP303* from tobacco (*Nicotiana tabacum*; Weterings et al., 1992) and *LAT52* from tomato (*Solanum lycopersicum*; Twell et al., 1989). The *LAT52* gene promoter was used for pollen-specific expression, and the sequence was cloned upstream of the nopaline synthase terminator from pBIN19 (Bevan, 1984).

Microscopy and Pollen Germination, Growth, and Guidance Assays

Pollen germination assays were performed as described (Boavida and McCormick, 2007). For pollen growth assay, the lengths of more than 100 pollen tubes per sample were measured 4 h after germination using ImageJ software (Abràmoff et al., 2004).

For in vivo fertilization assay, pistils of emasculated flowers were hand pollinated with pollen from *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/-}$ *TdTOM*$^{+/+}$ plants. At 7 h after pollination, the pistils were carefully dissected to remove carpel along one valve and to expose two rows of ovules. The dissected pistil was then placed in the pollen germination medium with exposed ovules facing up, and a cover slip was put on to mount the slide. Confocal images were taken with a Zeiss LSM 510 META NLO confocal microscope. Semi-in vitro pollination and pollen tube guidance assay was performed according to Palanivelu and Preuss (2006). Fluorescence microscopy was performed with an Olympus IX70 inverted microscope with an ORCA digital camera.

For *mpk3 mpk6* mutant pollen isolation, pollen grains from newly opened flowers of *mpk3*$^{-/-}$ *mpk6*$^{-/-}$ *gMPK6-YFP*$^{+/-}$ plants were collected loosely on a slide and counted under a Leica MZFLIII dissecting microscope. Nonfluorescent *mpk3 mpk6* mutant pollen grains were carefully picked up using one hair of a paintbrush and then dabbed onto the stigma surface of emasculated pistils. Each stigma was pollinated with eight pollen grains. To minimize UV damage, the pollen grains on a slide were changed when the total exposure time under UV reached 30 s. For control, the wild-type pollen was exposed under UV light for 30 s and used for pollination. Aniline blue staining of pollen tubes was performed as described previously (Boavida et al., 2009).

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers MPK3 (At3g45640) and MPK6 (At2g43790).

Supplemental Data

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

Supplemental Figure S1. *MPK6* expression is higher than *MPK3* in pollen.

Supplemental Figure S2. Subcellular localization of MPK3 and MPK6 fusion protein.

Supplemental Figure S3. In vivo tube growth of pollen grains from *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ plants in excessive pollination.

Supplemental Figure S4. Proposed model for MPK3/MPK6 signaling in pollen tube guidance.

Supplemental Table S1. Distribution of *mpk3 mpk6* fertilized seeds in *mpk3*$^{-/-}$ *mpk6*$^{+/-}$ pollinated pistils.

Supplemental Materials and Methods S1. Real-time reverse transcription-PCR quantification and subcellular localization of MPK3 and MPK6 in pollen.

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