Supporting Information

Kitashiba et al. 10.1073/pnas.1115283108

SI Materials and Methods

***A. thaliana* Accessions and *SRK-SCR* Transgenes.** The experiments described here used *A. thaliana* Col-0 or C24 plants transformed with the *SRKb-SCRb* genes isolated from the *A. lyrata Sb* haplotype (1). As described previously, Col-0[*SRKb-SCRb*] transformants express transient SI (i.e., a strong SI response in stigmas of stage 13 and early stage 14 floral buds, with subsequent breakdown of SI in more mature stigmas) and these plants set seed (2). In contrast, C24[*SRKb-SCRb*] transformants express a strong SI response at all stages of stigma development, and consequently these plants set very little, if any, seed (3, 4). Because T-DNA insertional mutants are typically available in the Col-0 accession, we used Col-0[*SRKb-SCRb*] transformants to assess the effect of T-DNA insertional mutations on SI. However, for experiments involving transgenic down-regulation or overexpression of candidate genes, we used *SRKb-SCRb* transformants of the C24 accession, which allowed us to monitor the strength of SI by both microscopic examination of pollen tube growth and the amount of seed set.

In addition to the previously described *SRKb-SCRb* transformation construct (4), which contains the *A. lyrata SRKb* and *SCRb* transcriptional units under the control of their native promoters, we also used a transformation plasmid that allowed direct monitoring of SRK protein expression. This plasmid contained an intact *SCRb* gene and a chimeric *SRKb* gene designed to express SRKb protein tagged at its N terminus with YFP under control of a 360-bp *Eco*R1-*Kpn*I fragment corresponding to the promoter of the *AtS1* gene (At3g12000). The *AtS1* promoter was previously shown to confer strong and specific expression in epidermal cells of the stigma (5). The *AtS1pr::YFP-SRKb*/*SCRb* construct was transferred into *Agrobacterium tumefaciens* strain GV1301 and introduced into *A. thaliana* C24 plants by the floral dip method (6). DNA gel blot analysis was used to identify transformed lines carrying single integrations of the transgene pair as described by Boggs et al. (3), and transgenic strains homozygous for the *AtS1pr::YFP:SRKb*/*SCRb* transgene were established.

T-DNA Mutant Strains and Transgenic Manipulation of Candidate Gene Expression. ***AtAPK1b and AtAPK1a.*** The Salk_055314 line, which carries a T-DNA insertion in the *AtAPK1b* (At2g28930) locus, was obtained from the *Arabidopsis* Biological Resource Center (Ohio State University, Columbus, OH) and crossed with a transgenic *A. thaliana* Col-0[*SRKb-SCRb*] homozygote. The genotype of F_2 plants was determined by PCR using a leaf-punch method (7). The T-DNA insertional allele was detected using primers 1842R and LBb1-ROK2 (Table S4), and the WT allele was detected using primers 1842R and 1045F (Table S4). The presence of the *SRKb-SCRb* transgenes was detected using primers SRKbHVRssF and SRKbHVRssR (Table S4).

An artificial microRNA (amiRNA) strategy (8) was used to generate transgenic plants in which *AtAPK1a* (At1g07570) expression was suppressed specifically in the stigma epidermis. The amiRNA target sequence in *AtAPK1a*, 5′-AAGCATGTAAAGTGGCTACTC-3′, was selected using the MicroRNA Designer program (available at http://wmd3.weigelworld.org/cgi-bin/webapp.cgi). This amiRNA target sequence was cloned using four primers designed according to a protocol reported at http://wmd3.weigelworld.org/cgi-bin/webapp.cgi: 5′-GATAGTAGCTACTTTACACGCTTTCTCTCTTTTGTATTCC-3′ (primer I), 5′-GAAAGCGTGTAAAGTAGCTACTATCAAAGAGAATCAATGA-3′ (primer II), 5′-GAAAACGTGTAAAGTTGCTACTTTCACAGGTCGTGATATG-3′ (primer III), and 5′-GAAAGTAGCAACTTTACACGTTTTCTACATATATATTCCT-3′ (primer IV). A pCAMBIA 1300 (GenBank accession no. AF234296) derivative containing the resulting amplicon placed downstream of the *AtS1* promoter was constructed and introduced into *A. thaliana* C24 plants. Transformants carrying a single integration of the transgene pair were identified by DNA gel blot analysis as described above. Plants exhibiting reduced expression of *AtAPK1a* in stigmas were identified by real-time RT-PCR as described below, and a plant showing the greatest reduction in *AtAPK1a* transcripts was crossed with a C24 plant homozygous for the *SRKb-SCRb* transgenes. F_2 plants were screened by PCR using primers AtS1profp1 and OCSrp (Table S4) to amplify a fragment that includes the amiRNA target. SI phenotype was determined by pollination assays as described below.

AtExo70A1. To generate transgenic plants that overexpress transcripts of the *AtExo70A1* (At5g03540) gene specifically in stigma epidermal cells, the *AtExo70A1* transcriptional unit was amplified from Col-0 genomic DNA in two fragments: a 5′ fragment that extended 3,348 bp from the initiating methionine codon and was generated using primer ExoKpnF, which contained a KpnI site (5′-CGAAAGGTACCGAGAAAAAGTAT-3′) and primer Exo3348R (TTAAAGCGTGGAAAGGGACAAT-3′), and a 3′ 2,096-bp fragment that included 3′ untranslated sequences and was generated using primer Exo2938F (5′-AGTGGAAGTTTGCTATCTAGGACGACAT-3′) and primer ExoPstR, which contained a PstI site (5′-TTAAAACTGCAGAACCATCTCATCACTCTT-5′). After digestion of the 5′ fragment with *Kpn*I and *Xba*I and of the 3′ fragment with *Xba*I and *Pst*I, the transcriptional unit was reassembled by ligation downstream of the *AtS1* promoter in the pCAMBIA 1300 plasmid. Transgenic plants carrying a single integration of the *AtS1pr::AtExo70A1* transgene and overexpressing *AtExo70A1* transcripts were identified as described above. A transgenic plant demonstrating a high level of *AtExo70A1* transcripts in stigmas was crossed with a C24 plant homozygous for the *SRKb-SCRb* transgenes and a C24 plant homozygous for the *AtS1pr::YFP-SRKb*/*SCRb* transgenes. The genotypes of F_2 plants generated from each cross were determined by DNA gel blot analysis.

Pollination Assays. Developing floral buds were staged as described by Smyth et al. (9). Pollination assays were carried out using stage-13 or stage-14 stigmas and pollen grains from mature anthers. Pollinations were typically allowed to proceed for 2 h before processing for epifluorescence microcopy. Overnight pollinations were also scored for a few *AtExo70A1* overexpressing plants, with the same results as obtained in 2-h pollinations. Each pollination assay was performed in triplicate and on at least two different dates to ensure reproducibility of the results. A pollination was scored as highly incompatible (0–5 pollen tubes per pollinated stigma), partially incompatible (6–29 pollen tubes per pollinated stigma), or compatible (>30 pollen tubes per pollinated stigma).

Expression Analysis. Twenty-five stigmas were dissected from floral buds at the stage-13 of development. Total RNA was extracted using Triazol reagent (Invitrogen), and aliquots of 100 ng were treated with RNase-free DNaseI (Invitrogen) and reverse-transcribed using a first strand cDNA synthesis kit for real-time PCR (USB Corp). The single-stranded cDNA was subjected to semiquantitative RT-PCR or quantitative real-time PCR using primer sets specific for each target gene and for the *UBC* gene (At5g25760) as a control (Table S4).

For semiquantitative RT-PCR, single-stranded cDNA was added to a reaction mix containing 1 × ExTaq buffer, 0.2 mM of each dNTP, 0.5 μM of each primer, and 0.025 U/μL of ExTaq DNA polymerase (TaKaRa), in accordance with the manufacturer's instructions. Amplification was performed under the following conditions: 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s. For quantitative real-time PCR, single-stranded cDNA was mixed with SYBR green fluorescence regent (iQ SYBR Green Super Mix; Bio-Rad), and the reaction was processed in an ABI Prism 7900HT sequence detection system (Applied Biosystems). The relative amount of transcripts from triplicate experiments was calculated using the comparative CT (threshold cycle) method and normalized to the endogenous *UBC* reference. The mean CT values were calculated from three replicates of each sample.

For protein gel blot analysis, proteins were extracted from 25 stigmas and subjected to SDS/PAGE analysis on a 7.5% (wt/vol) acrylamide gel, then transferred electrophoretically to PVDF membranes, which were processed for immunologic detection as described previously (10). The membranes were treated with a 1:1,000 dilution of a monoclonal anti-GFP primary antibody (Covance), followed by a 1:5,000 dilution of a monoclonal anti-mouse secondary antibody (Sigma-Aldrich). Immunoreactive bands were visualized using the ECL Plus Western Blotting Detection System (GE Healthcare Biosciences) and exposure to X-ray film. The protein blots were subsequently probed with a 1:3,000 dilution of a monoclonal anti-actin antibody (courtesy of M. Parthasarathy, Cornell University, Ithaca, NY) as a loading control, followed by a 1:5,000 dilution of the monoclonal anti-mouse secondary antibody.

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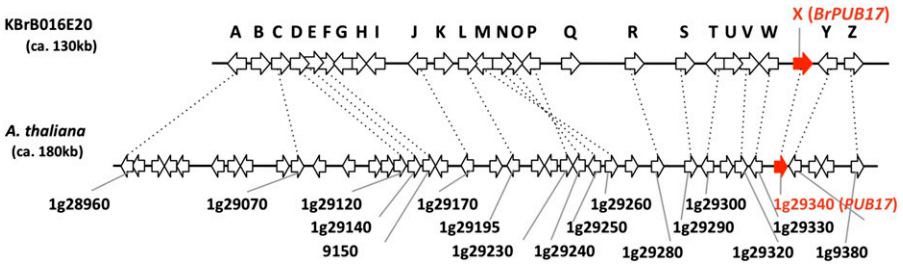

Fig. S1. Synteny of the *PUB17*-containing regions of the *B. rapa* and *A. thaliana* genomes. E-values are listed in Table S5.

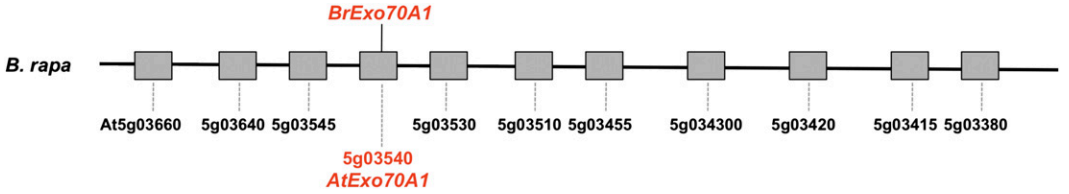

Fig. S2. Synteny of the *Exo70A1*-containing regions of the *B. rapa* and *A. thaliana* genomes. *B. rapa Exo70A1* is part of scaffold AENI01009342 (*ca*. 50 kb), which is located on A10 (chromosome 10 of *B. rapa*).

Table S1. Comparison of *Brassica MLPK*, *ARC1*, and *Exo70A1* to members of the *A. thaliana RLCK VII*, *PUB*, and *Exo70* gene families

Brassica gene	Gene family members in *A. thaliana*	*A. thaliana* gene	E-value	Amino acid identity	Synteny
BrMLPK	46	*At2g28930* (*APK1b*)	7.0e-143	76%	High
		At1g07570 (*APK1a*)	5.0e-81	73%	None
		At5g02290 (*NAK*)	2.0e-34	61%	None
		At3g55450 (*PBS1-like*)	8.0e-28	62%	None
BnARC1	42	*AtARC1* [*At2g34250-At2g34290 region*]	Pseudogene		High
		At1g29340 (*PUB17*)	1.0e-134	58%	None
		At5g45900 (*ATAPG7*)	0.12	13%	None
BnExo70A1	23*	*At5g03540* (*Exo70A1*)	1.0e-95	95%	High
		At5g52340 (*Exo70A2*)	6.0e-31	72%	None
		At5g52350 (*Exo70A3*)	6.0e-18	59%	None

For each *Brassica* gene, the two or three most similar *A. thaliana* genes are listed.
*Only three genes are found in the "A" clade of the *Exo70* gene family. The remaining 20 genes are grouped into other clades and exhibit <35% amino acid sequence identity with Exo70A1 (11).

Table S2. SI phenotype of F_2 plants derived from crossing an SRKb-SCRb plant with a plant lacking functional AtAPK1b or with a plant exhibiting down-regulation of AtPK1a

Col-0[*SRKb-SCRb*] × *apk1b*		*AtAPK1b**	*SRKb-SCRb**	Intensity of SI[†]
F2 8	*apk1b apk1b*	+	High	
25	*apk1b apk1b*	+	High	
27	*apk1b apk1b*	+	High	
5	*APK1b APK1b*	+	High	
18	*APK1b APK1b*	+	High	
20	*APK1b APK1b*	+	High	
11	APK1b APK1b	–	Low	
WT Col-0	*APK1b APK1b*	–	Low	
C24[*SRKb-SCRb*] × ami*AtAPK1a*		ami*AtAPK1a**	*SRKb-SCRb**	Intensity of SI[†]
F2 45-3	+	+	High	
45-6	+	+	High	
45-9	+	+	High	
45-16	+	+	High	
45-17	+	+	High	
45-2	–	+	High	
45-8	+	–	Low	

*Genotype was determined by amplification of genomic DNA using gene-specific primers (*Materials and Methods*). In the *RKb-SCRb* and *amiAPK1a* columns, (+) and (–) indicate presence and absence of the transgenes. In the *APK1b* column, *apk1b* indicates the T-DNA allele and *APK1b* indicates the WT allele.
[†]The SI response was determined by pollinating stigmas from stage 13 floral buds with SCRb-expressing pollen. Pollinations were allowed to proceed for 2 h. The intensity of SI was scored as "high" if there were <5 pollen tubes per pollinated stigma, and as "low" if there were >30 pollen tubes per stigma.

Table S3. Pollination phenotype of representative plants overexpressing *AtExo70A1* in stigmas

Plant ID	Genotype*		Intensity of SI†
	YFP-SRKb/SCRb	*AtExo70A1*	
AtS1pr::YFP-SRKb/SCRb × *AtS1pr::AtExo70A1*			
F_1	+	+	High
F_2 #4	+	+	High
F_2 #6	+	+	High
F_2 #8	+	+	High
F_2 #13	+	+	High
F_2 #14	+	+	High
F_2 #9	+	–	High
F_2 #5	–	+	Low
C24[*AtS1pr::YFP-SRKb/SCRb*]	+	–	High
SRKb/SCRb × *AtS1pr::AtExo70A1*			
F_1	+	+	High
F_2 #7	+	+	High
F_2 #6	+	–	High
F_2 #8	–	+	Low
C24[*SRKb-SCRb*]	+	–	High

*(+) and (–) indicate presence and absence of the transgenes as determined by DNA gel blot analysis.
†The SI response was determined by pollinating stigmas from stage-13 or stage-14 floral buds with *SCRb*-expressing pollen. The same results were obtained when pollination was allowed to proceed for 2 h or overnight before processing for microscopy. The intensity of SI was scored according to the number of pollen tubes observed per stigma: high, <5; low, >30.

Table S4. Primers used in this study

Name	Sequence (5′ to 3′)	Notes
1842R	TAAACAATACTTCTGAACAAATGA	Genotyping for *APK1b*
LBb1-ROK2	GCGTGGACCGCTTGCTGCAACT	Genotyping for T-DNA insertion allele in *APK1b*
1045F	GGTGGTTGGAGGATAAAATGACTT	Genotyping for *APK1b*
SRKbHVRssF	TGGGTTGGGATGTCAAGAAAG	Genotyping for *SRKb*
SRKbHVRssR	CAACTTCATCTTTCTCAGGCACAA	Genotyping for *SRKb*
AtS1profp1	CATTTGTCTTGTCTGCTA	Genotyping for amiRNA target sequence in *AtAPK1a*
OCSrp	GCGATCATAGGCGTCTCG	Genotyping for amiRNA target sequence in *AtAPK1a*
APK1afp5	AAAGTCATGTCTCTACACGAG	Real-time PCR for *APK1a*
APK1arp6	CCACTCCACAAGGTTCCTC	Real-time PCR for *APK1a*
APK1b-KakiF	CTCAAAAGGGTTTTTGTTTAGTTCTCCTC	Semiquantitive RT-PCR for *APK1b*
APK1b-KakiR	GATTCTCTAAGCTTCCACGAGGCA	Semiquantitive RT-PCR for *APK1b*
APK1bfp5	AAGCCATGTCTCTACGCGG	Real-time PCR for *APK1b*
APK1brp6	CCCATTCCACTAGCTTTTGC	Real-time PCR for *APK1b*
AtExo70A1RTfp2	CTGGGGGAGGAAGTTTAGAG	Real-time PCR for *AtExo70A1*
AtExo70A1RTrp2	CATTGCGATTGTCTCTGATG	Real-time PCR for *AtExo70A1*
UBC-fp1	AGAATGCTTGGAGTCCTGC	Real-time PCR as a control
UBC-rp1	AACCCTCTCACATCACCAGA	Real-time PCR as a control

Table S5. Summary of sequence comparisons

Brassica genomic region	*A. thaliana* locus number	E-value
KBrB068E07 and KBrH083O14 in Fig. 1*A*		
A	At2g28605	2.5e-33
C	At2g28610	2.4e-58
E	At5g59970	1.3e-50
F	At2g28840	8.2e-58
G	At2g28870	2.8e-41
J	At2g28930	4.7e-46
K	At2g29300, 310, 320, 340	6.7e-31
L	At2g29300, 310, 320, 340	2.3e-28
M	At2g29300, 310, 320, 340	6.8e-28
N	At2g29400	1.2e-97
O	At2g29450	7.0e-52
P	At2g29680	4e-67
Q	At2g29690	1.0e-112
R	At2g29700	2e-68
S	At2g29730	0.0
U	At2g29940	0.0
KBrH010M08 in Fig. 2*A*		
A	At1g07390	4.0e-184
B	At1g07410	2.3e-76
C	At1g07420	2.7e-36
D	At1g07430	1.4e-60
E	At1g07450	1.0e-27
F	At1g07460	1.8e-71
G	At1g07470, 480	2.1e-25
H	At1g07490	1.3e-34
K	At1g07510	5.4e-132
L	At1g07520	3.0e-138
O	At1g07560	1.1e-80
P	At1g07570	1.6e-70
R	At1g07590	5.2e-198
S	At1g07620	1.5e-152
KBrB016E20 in Fig. S1		
A	At1g28960	1.1e-42
C	At1g29070	3.9e-67
D	At1g29120	1.8e-28
E	At1g29140	3.9e-51
F	At1g29150	1.6e-210
J	At1g29170	1.4e-112
L	At1g29195	3.5e-66
M	At1g29260	7.0e-171
O	At1g29240	8.3e-104
P	At1g29230	7.1e-249
R	At1g29280	4.0e-104
S	At1g29290	4.8e-37
T	At1g29300	1.1e-183
V	At1g29320	1.1e-35
W	At1g29330	2.0e-40
X	At1g29340	0.0
Y	At1g29350	6.2e-43
Z	At1g29380	1.6e-89