The *S* locus glycoprotein and the *S* receptor kinase are sufficient for self-pollen rejection in *Brassica*

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Self-incompatibility (SI) is one of several mechanisms that have evolved to prevent inbreeding in plants. SI in *Brassica* is controlled by the polymorphic *S* locus complex. Two *S* locus-encoded proteins are coordinately expressed in the stigma epidermis: the cell wall-localized *S* locus glycoprotein (SLG) and the plasma membrane-anchored *S* receptor kinase (SRK). These proteins are thought to recognize a pollen factor that leads to the rejection of self-pollen. Evidence has accumulated that indicates that both proteins are necessary for the ability of the stigma to inhibit self-pollen. However, it has not been possible to prove this necessity definitively or to demonstrate that these genes are sufficient for this phenotype, because previous attempts to transfer this phenotype via transformation have not been successful. In this study, two overlapping *S* locus genomic clones, which cover ≈55 kilobases of DNA and contain the *SLG*, *SRK*, and an anther-expressed gene in the region common to the two, were introduced into a self-compatible *Brassica napus* line. The resulting transgenic plants were shown to carry the female part of the SI phenotype, rejecting pollen in a haplotype-specific manner. However, the pollen SI phenotype was not found in any of the transgenic plants. These data show that the SLG and SRK are sufficient for the female side but not the male side of the SI phenotype in *Brassica* and that there must be an independent pollen *S* factor encoded outside the cloned region.

Self-incompatibility (SI) is one of the mechanisms evolved by higher plants to promote outbreeding and is also a good genetic model to study cell-to-cell communication. In *Brassica* species, SI is controlled by haplotypes of the *S* locus complex (1, 2). The control is of the sporophytic type in which the pollen parent genotype determines the phenotype of the pollen (1). When a haplotype in the pollen parent matches that of the pistil, pollen germination is arrested at the stigma surface. Thus, sporophytic SI not only prevents self-fertilization but also prevents crossing between genetically related individuals. The *S* haplotypes are generally codominant with each other, and SI is dominant over self-compatibility (3, 4).

Two genes have been found to be associated with the SI phenotype. The first encodes a secreted *S* locus glycoprotein (SLG; ref. 5), and the second encodes an *S* receptor kinase (SRK; ref. 6). The latter protein has a receptor-like domain very similar to SLG, a transmembrane domain, and a kinase domain. Both genes are expressed primarily in stigma papilla cells (5–7). Although some transcripts have been detected in anther tissue, both proteins could be detected only in stigma (8–10). Consistent with this finding, accumulated genetic evidence indicates that expression of both genes in papillar cells is required for the operation of SI (11–17), though recently the necessity of SLG for SI has been questioned (18). Several self-compatible mutant strains of *Brassica* have been identified in which self-compatibility is associated with spontaneous mutations at the *S* locus causing disruption of the *SRK* gene (13, 14). In addition, spontaneous mutations at loci unlinked to the *S* locus that down-regulate the *SLG* gene (12) and transgene-induced mutations that down-regulate the *SLG* (11, 17) and *SRK* (15, 16)

genes are associated with loss of the pistil's ability to reject self-pollen.

A model for the *Brassica* SI response has been proposed (6, 7) based on the above observations and on what is known about receptor kinase interactions with their specific ligands in mammalian cells (19). The SRK protein would form a receptor complex in the stigma papilla cells that recognizes an allele-specific ligand present on the pollen surface causing a cascade of reactions leading to rejection of self-pollen. The SLG protein either would be involved in bringing the ligand into contact with the SRK protein or would serve as part of the receptor complex. The pollen ligand is thought to be a protein encoded by a separately linked gene and recognized by the receptor complex in a haplotype-specific manner. Although this hypothesis is reasonable, a number of aspects of this model need to be tested experimentally. For example, although the data mentioned above indicate that both SLG and SRK are necessary for SI, it is not clear whether these are sufficient for operation of the SI response in the pistil. Attempts to modify SI specificity by transformation experiments with the *SLG* and/or *SRK* genes have thus far been unsuccessful, largely as a result of transgene-induced cosuppression (15–17).

Brassica napus is an amphidiploid plant that is normally self-compatible, whereas its presumptive progenitors, *Brassica oleracea* and *Brassica rapa*, are diploid SI species (20). SI haplotypes can be transferred into *B. napus* from the progenitor species via interspecific crosses, with the resulting individuals being repeatedly backcrossed to the *B. napus* parent, thus generating SI lines (21, 22). The SI *B. napus* line W1 was produced by introducing a functional *S* haplotype called *910* into the self-compatible Westar line (21). Other than the region involved in SI, these lines are virtually isogenic. We have recently isolated and characterized a ≈65-kilobase (kb) fosmid contig spanning the *SLG-910* and *SRK-910* genes (23). Herein, we present the results of the transgenic analysis of the fosmid clones in the Westar background to determine whether the *SLG* and *SRK* genes are sufficient for the stigma side of the SI phenotype. Further, we wished to see which, if any, of the genes encoded in this region would confer the male part of SI.

Materials and Methods

Transgene Constructs, Plant Transformation, and Cross-Pollination Analysis. The inserts of the Fos20 and Fos22 genomic clones that overlap with *S* locus linked 1 (*SLL1*), *SLG*, and *SRK* in the overlapping region (23) were released by *Not*I digestion, re-

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cloned as *Not*I fragments into the plant transformation vector binary bacterial artificial chromosome 2 (BIBAC2; refs. 24 and 25), and introduced into *Agrobacterium tumefaciens* strain COR338 (24) via triparental mating (26). The self-compatible *B. napus* Westar line was used as recipient for the two transgene constructs. Plant transformation was performed by using *Agrobacterium*-mediated methods (27). The *NPTII*- and *SLG-910*-specific primers used for testing the presence of the transgenes have been described (21, 24). SI was tested by measuring seed set. Pollination bags were applied 2 days before anthesis for both self- and cross-pollination. Cross-pollinations were performed on fully open flowers 2 days after emasculation.

Genomic DNA and RNA Gel Blot Analysis. For pulsed field gel electrophoresis analysis, high molecular mass genomic DNA was isolated from nuclei of young leaf tissue and embedded into agarose plugs as described by Zhang *et al.* (28). For regular genomic DNA blot analysis, DNA was extracted from floral buds as described (29). Approximately 5 μg of DNA was digested, size fractionated through agarose gel, and transferred to nylon membrane in 20× SSC (1× SSC is 150 mM NaCl/15 mM sodium citrate).

Total RNA was extracted from stigmas isolated from 5- to 6-mm-long floral buds by using a hot phenol method (30). Approximately 6 μg of each RNA sample was fractionated through a 1.2% agarose-formaldehyde gel and transferred to nylon membrane in 20× SSC. Equal loading of RNA samples was indicated by rehybridization of the membrane by using the cDNA to 18S rRNA from *B. rapa* (31) as probe. Prehybridization and hybridization steps were performed as described (29). Membranes were washed for 50 min at a final stringency of 0.1× SSC and 0.1% SDS at 65°C. Gel-purified DNA fragments labeled by random priming (32) were used as probes.

Results

BIBAC–Fosmid Constructs and Transformation of Self-Compatible *Brassica*. Previously, the entire *SLG-SRK* region was not cloned on a single genomic fragment (29, 33, 34). Transformation with single genes driven by heterologous promoters, thus potentially generating gain-of-function plants, has been previously unsuccessful (15). We have recently isolated a ≈65-kb fosmid contig covering the *SLG-910* and *SRK-910* genes from the SI *B. napus* line W1 (23). The distance between the two *S* genes is only ≈6 kb of DNA, which is the shortest among the *S* haplotypes characterized to date (23, 34, 35). A vector for the transformation of large pieces of DNA into plants called the BIBAC vector has been constructed (24, 25), which makes the transformation of the entire fosmid genomic clones (≈40 kb) possible. A method for routine introduction of DNA fragments larger than ≈25 kb into plant nuclear genome had not yet been demonstrated (24). Two fosmid clones, Fos20 and Fos22, were chosen for cloning into the BIBAC vector to transform the self-compatible Westar line. As shown in Fig. 1, Fos22 contains the *SRK*, *SLG*, the anther-specifically expressed gene *SLL1*, and several upstream genes, whereas Fos20 contains *SLL1*, *SLG*, *SRK*, and the gene *SLL3*, which encodes a putative small secreted protein, as well as a transposon and a nonfunctional kinase gene. The two genomic clones overlap in the region containing the *SLL1*, *SLG*, and *SRK* genes. *SLL1* is an anther-specific gene (29) and should not play a role in the stigma. Thus, these clones provide us an opportunity for gain-of-function transgenic studies to address the issue of whether the *SLG* and *SRK* genes are sufficient for conferring the SI phenotype on the stigma side and whether any other genes from this region are functionally relevant.

The two BIBAC–fosmid constructs were transformed into the self-compatible cultivar Westar line, which carries the nonfunctional *A10 S* haplotype, likely because of a 1-bp deletion in the *SRK-A10* gene (13). Six putative transgenic plants (T_0) that regenerated shoots and roots on selective medium were analyzed
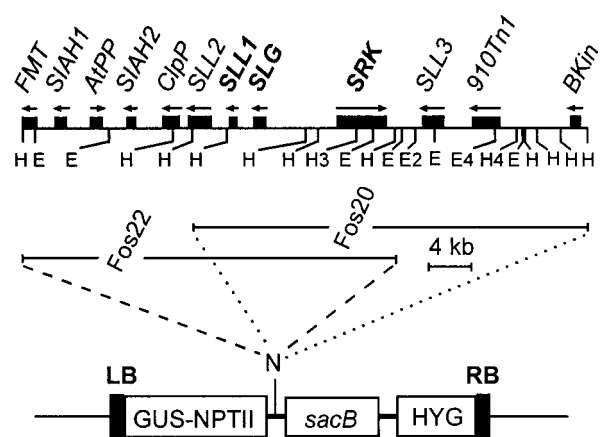
Fig. 1. Schematic representation of the transgene constructs. (*Upper*) The *910 S* haplotype genomic region covered by the Fos20 and Fos22 genomic clones (23). *FMT*, methionyl-tRNA transformylase; *SIAH*, *Drosophila* seven-in-absentia; *AtPP*, *Arabidopsis* putative protein; *ClpP*, Clp protease; *910Tn1*, a Spm-like transposon; *Bkin*, a nonfunctional kinase. (*Lower*) Part of the BIBAC2 vector (24, 25). *GUS*, β-glucuronidase; *NPTII*, neomycin phosphotransferase II; *sacB*, levansucrase; *HYG*, hygromycin phosphotransferase. RB and LB denote right and left borders of T-DNA. E, *Eco*RI; H, *Hin*dIII; N, *Not*I. The number after the letters indicates multiple sites.

subsequently by genomic DNA gel blot analysis by using the selectable marker gene *NPTII* as a probe. Further, the PCR with primers specific to the *NPTII* and *SLG-910* genes was used to ascertain presence of the transgenes (data not shown). Consequently, one of the six was found to be a false positive. Among the five positive transgenic lines, four were from the Fos20 construct, and one was from Fos22. One of the four Fos20 transgenic lines was male sterile (no pollen) and was eliminated from further analysis. The other four lines set self-seeds, suggesting that neither of the two fosmid clones contains the complete functional *S* haplotype. Self-seeds (T_1) were collected for further analysis.

Phenotype of the Transgenic Lines. T_1 progeny were grown from each of the four primary transgenic lines for analysis of pollination phenotypes. Presence of the transgenes was indicated by PCR with primers specific to the *SLG-910* and *NPTII* genes. All progeny plants that inherited the transgenes set roughly the same amount of self-seeds as plants that did not inherit the transgenes (data not shown). To determine the pollination phenotype of these plants in the stigma and pollen, large numbers of reciprocal crosses between transgenic plants and the SI W1 as well as the self-compatible Westar lines were performed. As negative controls, the same crosses were also performed for the progeny plants that did not inherit the transgenes. The pollination results are summarized in Table 1.

Among the progeny of all three Fos20-transformed lines (3A, 5B, and 6A), all 19 plants that inherited the transgene were SI on the stigma side as indicated by the cross-pollination results of transgenic stigmas with W1 and Westar pollen. When transgenic stigmas were fertilized with Westar pollen, the resulting pods were fully developed, and normal numbers of seeds were set (average of ≈20.5 per pod). These results were similar to those obtained when W1 was fertilized with Westar pollen (average of ≈18.3 per pod). In contrast, those fertilized with W1 pollen set very few seeds (average of ≈1.6 per pod), which is typical of the strong SI response shown by W1 when self-pollinated (average of ≈0.2 per pod). All 10 plants that did not inherit the transgene because of segregation were compatible (average of ≈18.7 and 20.1 per pod when pollinated with pollen from W1 and Westar, respectively), confirming that the change in phenotype was indeed caused by the transgene. The T2

Table 1. Average seed set per pod for the transgenic lines (T$_1$ generation) when reciprocally crossed with parental lines

Female × Pollen	Transgene*	Seeds per pod†	No. of pods	No. of plants
W1 ($S_{910}S_{910}$) × (Self)		0.2	85	4
W1 ($S_{910}S_{910}$) × Westar		18.3	48	3
Tg‡ (*Fos20*) × W1 ($S_{910}S_{910}$)	+	1.6	446	19
	−	18.7	196	10
Tg (*Fos20*) × Westar	+	20.5	279	19
	−	20.1	145	10
Tg (*Fos20*) × T2 ($S_{A14}S_{A14}$)	+	19.3	82	7
W1 ($S_{910}S_{910}$) × Tg (*Fos20*)		21.8	49	3
Tg (*Fos22*) × W1 ($S_{910}S_{910}$)	+	0.7§	269	7
	−	22.2	175	8
Tg (*Fos22*) × Westar	+	12.8§	112	7
	−	22.2	100	8
Tg (*Fos22*) × T2 ($S_{A14}S_{A14}$)	+	12.4§	23	2
W1 ($S_{910}S_{910}$) × Tg (*Fos22*)		11.4§	52	3

*"+" indicates presence of the transgene; "−" indicates loss of the transgene caused by segregation.
†For the *Fos20* transgenic lines, the data are the combination of all three lines (3A, 5B, and 6A).
‡Tg, transgenic lines in the self-compatible Westar background.
§The somewhat lower seed set for some of the plants was caused by the conditions in the growth chamber.

line is another SI *B. napus* line that contains the functional *A14 S* haplotype (22). Pollen from this line was used to fertilize the transgenic lines yielding fully developed pods having a normal seed set (averaged ≈19.3 per pod) comparable to that seen for Westar, indicating that the rejection of W1 pollen by the transgenic stigmas is a haplotype-specific phenomenon. The pollen from all of the transgenic plants was able to fertilize the W1 line (average of ≈21.8 per pod), indicating that the male part of the phenotype remains unchanged. This result is consistent with the earlier observations that all of the transgenic lines set self-seeds, even though the female SI phenotype was expressed. We did not notice any significant differences in pollination phenotype among plants that inherited the transgene, suggesting that there was no obvious relationship between stigma SI phenotype and transgene dosage.

Similar pollination results were obtained with progeny of the Fos22-transformed line (389A). Nine progeny plants that inherited the transgene as judged by PCR were analyzed (along with eight plants that did not). Of the plants that inherited the transgene, seven plants showed the strong gain-of-function SI phenotype on the stigma side, similar to that seen for the Fos20-transformed lines (Table 1). Further, the response was again haplotype-specific, and the pollen phenotype was not changed (Table 1). The typical phenotypes of seed pods produced after various cross-pollinations are shown in Fig. 2. Pollination of the transgenic stigmas with W1 pollen yielded very few seeds, whereas pollination of the transgenic stigmas with Westar or T2 pollen produced fully developed pods, very similar to those seen in the self-compatible Westar line. Two progeny plants carrying the transgene (389A-5 and 389A-7) did not show any significant differences when pollinated by W1 or Westar
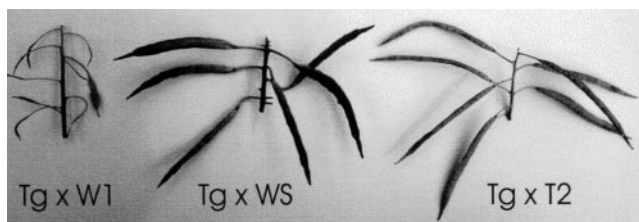
pollens. This result correlated with a lack of expression of the *SLG* and *SRK* genes in these plants (see below).

Genomic DNA Gel Blot Analysis of the *Brassica* Transgenic Lines. Genomic DNA was isolated from the progeny of each transgenic *Brassica* line, cleaved with various restriction endonucleases, and analyzed by hybridization with several probes. This analysis was used to determine whether the fosmid genomic inserts were transferred intact into the plant chromosome, to estimate the approximate copy number, and to identify unique band patterns for each of the transgenic lines. First, DNA blots of *Not*I-digested high molecular mass genomic DNA were probed with cDNAs for the *SLG-910* and *SRK-910* kinase portion to determine whether the fosmid inserts were transferred intact into the plant chromosomes. As shown in Fig. 3*A*, progeny of all four lines had a ≈40-kb band, indicating that the transgene fragments were intact. In addition to the ≈40-kb fragment, the 3A line had a shorter and weaker band, which might have been caused by the transfer of a partial T-DNA fragment during transformation.

DNA blots of *Hind*III-digested genomic DNA were then probed with the *NPTII* probe to estimate the copy number of the transgenes. As shown in Fig. 3*B*, only the 6A line progeny had two different bands representing two copies at different chromosomal sites, whereas the other three lines had only one copy. The various sizes of the restriction fragments identified by hybridization to the *NPTII*-specific probe indicated that the T-DNA integrated into a unique site in each of the transformed lines. The 3A line contained two copies that hybridized to the *SLG-910* and *SRK-910* cDNAs, and only one fragment hybridized to the *NPTII*-specific left border probe. The shorter copy was likely caused by the partial transfer of the T-DNA, because it is thought that the T-DNA is transferred linearly from the right border to the left border (24).

Finally, DNA blots of *Eco*RI-digested genomic DNA were probed with cDNAs for *SLG-910* and *SRK-910* kinase portion (Fig. 3*C*) to establish the unique band pattern of each of the transgenic lines. *Eco*RI was chosen, because the 25-kb *Eco*RI fragment hybridizing to both *SLG* and *SRK* probes is a hallmark of the *910 S* haplotype (29). In addition, there is a ≈3-kb fragment that hybridizes to the *SRK* kinase portion probe, because there is an *Eco*RI site within the *SRK* gene. All progeny of the Fos20-transformed lines produced the expected ≈3-kb band and various bands larger than ≈17 kb, which represent the ≈17-kb *Eco*RI–*Hind*III transgene fragment plus flanking regions from the host

Fig. 2. Comparison of mature *B. napus* pods produced when transgenic stigmas were pollinated with pollen from W1 (Tg × W1), Westar (Tg × WS), and T2 (Tg × T2).
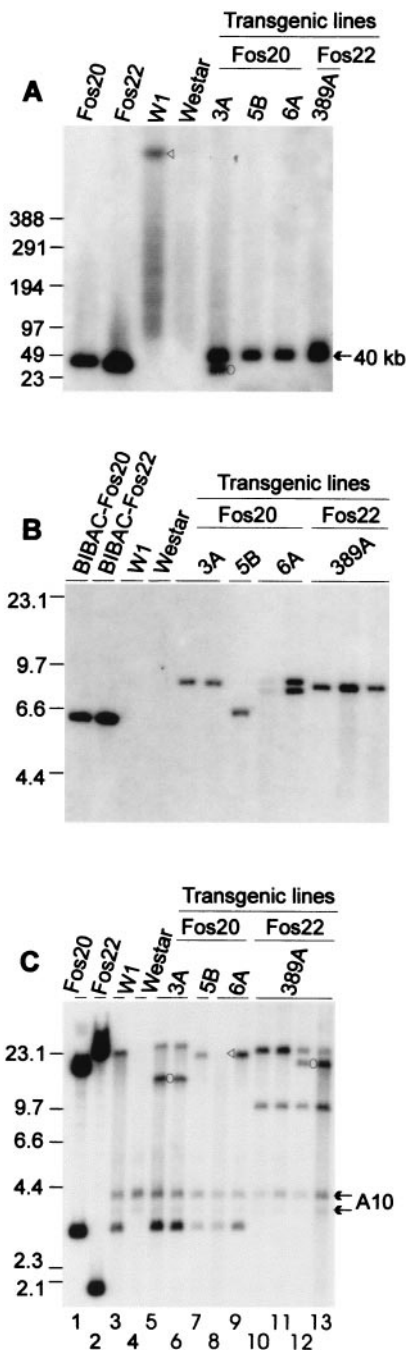
Fig. 3. Genomic DNA gel blot analysis of the T₁ progeny plants. Molecular size markers are indicated at left in kilobases. Sources of DNA are labeled above each lane. Each lane represents DNA from one progeny plant. The transgene constructs, W1, and Westar DNAs were all included as controls. (*A*) Gel-blot hybridization of *Not*I-digested high molecular mass DNA with *SLG-910* and *SRK-910* cDNAs. Restriction fragments were separated by pulsed field gel electrophoresis in a CHEF-DRIII apparatus (Bio-RAD) in 1× Tris-acetate-EDTA at 3 V/cm with a pulse-switching interval ramped from 5 to 120 s over 42 h. The triangle indicates the ≈1,000-kb *Not*I fragment encompassing both the *SLG-910* and *SRK-910* genes, which was identified previously by pulsed field gel electrophoresis analysis (29). The circle indicates a truncated copy in 3A line. (*B*) Gel-blot hybridization of *Hin*dIII-digested DNA with *NPTII*-specific DNA. (*C*) Gel-blot hybridization of *Eco*RI-digested DNA with *SLG-910* and *SRK-910* cDNAs. The two fosmids were cut with *Not*I and *Eco*RI. The ≈12-kb band in lanes 5 and 6 and the ≈16-kb band in lanes 12 and 13 (indicated by circles) represent truncated copies of the transgenes. The triangle in lane 8 indicates a band visible after longer exposure. The arrows indicate the bands likely representing the endogenous *A10* haplotype present in Westar.
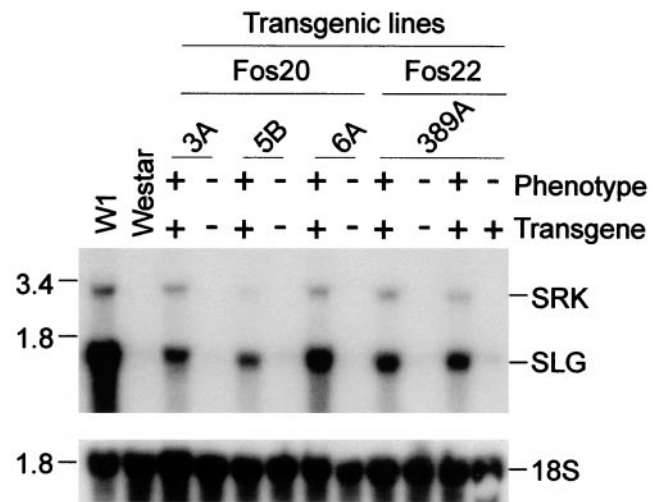
Fig. 4. Expression of the *SLG* and *SRK* transgenes in the stigmas of the T₁ progeny plants. Sources of stigma tissues are indicated above each lane (the last lane is 389A-5). "+" indicates presence of the transgene; "−" indicates loss of the transgene caused by segregation. The blot was probed with *SLG-910* and *SRK-910* cDNAs. "Phenotype" indicates the gain-of-function SI phenotype in the stigmas. Molecular size markers are indicated at left in kilobases. (*Lower*) Rehybridization of the same blot to 18S rDNA.

genome. The intermediate bands (≈12 kb) very likely resulted from the truncated copy shown on the *Not*I-digested blot (Fig. 3*A*). All the 389A line progeny yielded the expected ≈25-kb band as well as a ≈10-kb band, which represents the 1.8-kb *Eco*RI–*Hin*dIII transgene fragment plus the flanking region from the host genome. Surprisingly, some of the 389A progeny yielded an unexpected ≈16-kb band, which might have been created by a postintegration rearrangement event.

Expression of the *SLG-910* and *SRK-910* Transgenes in the Self-Compatible Westar Line. The fact that both Fos20 and Fos22 constructs gave rise to the same phenotype indicates that only the overlapping region between the two is responsible for the SI function. From our recent transcriptional mapping studies of the cloned *910 S* haplotype region (23), it is known that only three genes are encoded in this overlapping region: *SLL1*, *SLG*, and *SRK*. *SLL1* is specifically expressed in anthers (29) and thus should not function in stigmas. To determine whether the phenotype is indeed conferred by expression of the *SLG-910* and *SRK-910* genes, RNA gel blot analysis was performed with the stigma RNA to examine expression of the two genes. As shown in Fig. 4, all the transgenic progeny plants that gained the SI phenotype also expressed both genes at significant levels. The only exception was 389A-5, which inherited the transgenes but showed barely detectable expression and did not gain the SI phenotype. As expected, progeny plants lacking the transgenes through segregation had no expression as did the negative control Westar (faint signals could be observed after a longer exposure because of the presence of the nonfunctional *A10 S* haplotype in Westar that cross-hybridizes with the *SLG-910* and *SRK-910* probes; data not shown). Thus, the expression studies provide a direct correlation between gain of the stigma SI phenotype and expression of the *SLG-910* and *SRK-910* genes in the same tissue. *S* gene expression levels vary among the transgenic lines and are all somewhat lower than the expression level of W1 but were sufficient to confer the phenotype.

Discussion

The results reported herein, when combined with information on sequencing and transcriptional mapping of the cloned *910 S* hap-

lotype region (23), show that SLG and SRK are sufficient to confer the stigma SI phenotype in *Brassica*. The common region shared by the Fos20 and Fos22 genomic clones does not contain any additional genes or significant ORFs except for *SLG*, *SRK*, and the anther-specific *SLL1*. Nevertheless, our results do not mean that both genes are required to determined haplotype specificity on the pistil side. Further, these results show that the *SLG*, *SRK*, *SLL1*, *SLL3*, as well as other genes encoded in this region do not function as the pollen determinant of SI or at the very least are not sufficient for determining the pollen phenotype. This finding is consistent with results from previous biochemical (8), genetic (11–14), and transgenic (15–17) studies and supports the current model for the *Brassica* SI response that proposes SRK as a key signal transmitter and an independent pollen component.

A gain-of-function phenotype by gene transformation provides the most direct proof that the transgene or transgenes are not only necessary but are also sufficient for function, and therefore this phenotype is an important means to study gene function. Unfortunately, previous attempts at introducing *S* genes into *Brassica* have been unsuccessful, perhaps because of homology-dependent cosuppression of the *S* gene family members (15, 16). The experiments reported herein may have been successful in comparison to those previously published, as well as our own previous unsuccessful efforts, for several reasons. First, native promoters may be important to drive the *S* gene expression to appropriate levels. In the work by Conner *et al.* (15), the *SRK* gene was driven by an *SLG* promoter, which may drive *SRK* expression to an abnormally high level and thus trigger the cosuppression response. Second, it may be necessary for the *S* genes to be linked physically to ensure the coordinated spatial and temporal expression as well as their relative level. This physical linkage is a major advantage of large insert transformation via the BIBAC system, because it allows expression of plant genes or gene clusters in their native genomic context and might eliminate site-dependent gene expression. This lack of consistent transgene expression might be an explanation for the previous transformation studies conducted in our laboratory (K.-R. J. Stahl and S.J.R., unpublished data). The two *S* genes with their native promoters were transformed individually into Westar and then crossed into one line. A significant number of transgenic lines were analyzed, but no phenotypic changes were observed. Our success in obtaining the gain-of-function SI phenotype by introducing the cloned *SLG-SRK* genomic region into *Brassica* opens the way for additional functional studies of the *Brassica S* locus. For example, with the transgenic approach, we can now address the issue whether SRK alone is sufficient and/or whether SLG is indeed required for haplotype-specific rejection of self-pollen.

Our results also suggest that there must be a pollen *S* gene outside the ≈55-kb genomic region covered by the Fos20 and Fos22 constructs. This gene has been confirmed by recent findings. After years of intensive search conducted by several laboratories employing various approaches (23, 29, 35–37), the male determinant of SI specificity in *Brassica* has just been identified (38). The recently identified pollen *S* gene, *SCR* (for *S* locus cysteine-rich protein), is predicted to encode a small secreted cysteine-rich protein. Putative alleles of *SCR* have been identified in our sequenced *A14* and *910 S* haplotypes (ref. 23 and N.B., Y.C., Y.-M.B., and S.J.R., unpublished data). The putative *SCR-910* is located just outside the Fos20 clone, ≈2 kb downstream of the *Bkin* gene, and is located in Fos16, a clone that was not used in this transformation study (for a map of the *910 S* haplotype, see ref. 23). Our data, together with those of Schopfer *et al.* (38), strongly suggest a direct interaction between SCR and the SLG/SRK receptors during the initial contact/recognition reaction between self-pollen and stigmatic papillar cells. The interaction is thought to activate the SRK kinase and, subsequently, a signal transduction pathway that leads to self-pollen rejection.

In addition, it should be noted with regards to the evolution of SI that genes for pollen and stigma self-recognition are different and that they are separated by at least three intervening genes.

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