An S Receptor Kinase Gene in Self-Compatible Brassica napus Has a 1-bp Deletion

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S locus glycoprotein (SLG) and S locus receptor kinase (SRK) cDNAs were isolated from an S allele present in a number of self-compatible Brassica napus lines. This A10 allele did not segregate with self-incompatibility in crosses involving other self-incompatible B. napus lines. The SLG-A10 cDNA was found to contain an intact open reading frame and was predicted to encode an SLG protein with sequence similarities to those previously associated with phenotypically strong self-incompatibility reactions. SLG-A10 transcripts were detected in the developing stigma at steady state levels even higher than those detected for SLG alleles linked with self-incompatibility. Analysis of the corresponding SRK-A10 cDNA showed that it was very similar to other S locus receptor kinase genes and was expressed predominantly in the stigma. However, a 1-bp deletion was detected in the SRK gene toward the 3' end of the SLG homology domain. This deletion would lead to premature termination of translation and the production of a truncated SRK protein. The A10 allele was determined to represent a B. oleracea S allele based on its segregation pattern with the B. oleracea S24 allele when both these alleles were present in the same B. napus background. These results suggest that a functional SRK gene is required for Brassica self-incompatibility.

INTRODUCTION

In the Brassica family, fertilization can be controlled by a self-incompatibility system that is inherited as a dominant genetic locus called the S locus (for reviews, see Nasrallah et al., 1991; Dzelzkalns et al., 1992). Although the diploid species Brassica oleracea and B. campestris are typically self-incompatible, B. napus, an allotetraploid composed of both of these genomes, generally occurs as a self-compatible plant (Downey and Rakow, 1987). Some naturally occurring self-incompatible B. napus lines have been isolated (Olsson, 1960; Gowers, 1974), and in addition, B. napus can be made self-incompatible by the introgression of S alleles from one of its progenitor species (Mackay, 1977). The presence of a functional S allele results in a barrier to fertilization when the pollen grain originates from a plant carrying the same S allele as the pistil. The pollen phenotype, which is determined by the diploid parental genotype and not by the haploid pollen, is thought to be propagated by putative S allele gene products deposited on the outside wall of the pollen grain by the surrounding tapetum during pollen development (de Nettancourt, 1977). Thus, during pollen–pistil interactions, there is a recognition of self versus nonself, leading to either a block in pollen germination when both parents carry the same S allele or successful fertilization when different S alleles are present.

There are multiple alleles at the S locus, and in B. oleracea, up to 50 different alleles have been identified (Ockendon, 1974, 1982). In addition, two different genes cosegregate with the self-incompatibility phenotype: the S locus glycoprotein (SLG) gene and the S locus receptor kinase (SRK) gene. At a single locus, there is considerable DNA sequence homology between the SLG and SRK genes, with the S' end of the SRK gene showing ~80% DNA homology with the SLG gene (Stein et al., 1991; Goring and Rothstein, 1992). By analogy to other receptor kinases, the protein domain coded for by this part of the gene would be the receptor that recognizes self-pollen (Hanks et al., 1988). The remainder of the SRK gene encodes a putative transmembrane domain and a cytoplasmic kinase domain that has been shown to encode a functional serine/threonine kinase (Goring and Rothstein, 1992).

The DNA sequences of SLG and SRK genes from different S alleles are somewhat variable and have been associated with different self-incompatibility phenotypes. The majority of characterized SLG genes fall into one group with DNA homologies greater than 80% (Dwyer et al., 1991; Goring et al., 1992a, 1992b). Some of these alleles were isolated from lines with phenotypically strong self-incompatibility reactions and showed, in the presence of a second allele from this group, a nonhierarchic pattern of dominance in the pollen (Thompson and Taylor, 1966). A second group of alleles was found to be always recessive to the first group in the pollen and tended to display a weaker self-incompatibility reaction (Thompson and Taylor, 1966). The two characterized alleles from this group, S2 and S5, share high levels of homology, but only show ~70% DNA homology with the first group (Chen and Nasrallah, 1990; Scutt and Croy, 1992).
Transformation experiments have shown that the SLG gene is not sufficient to confer self-incompatibility to a self-compatible B. napus (Nishio et al., 1992). However, there is evidence that SLG genes are required for self-incompatibility. A naturally occurring variant of B. campestris with normal SRK expression, but low levels of SLG expression, has been associated with self-compatibility (Nasrallah et al., 1992). In this study, we have examined an S locus present in a number of self-compatible B. napus lines to determine possible causes for self-incompatibility in these lines. Whereas the SLG gene appears to be normal, the SRK gene has acquired a mutation that is predicted to lead to a truncated gene product. This suggests that the SRK gene is also required for self-incompatibility.

RESULTS

Occurrence of the SLG-A10 Allele

The SLG-A10 cDNA was isolated during a search for an SLG allele associated with self-incompatibility in the self-incompatible B. napus ssp oleifera (canola) lines R2 and T2 (Goring et al., 1992a). While the SLG-A10 cDNA was one of the more predominant SLG cDNAs isolated from the stigma cDNA library, it did not segregate with self-incompatibility in these lines. A survey of different canola cultivars revealed that the A10 allele was present in several of these lines. As shown in Figure 1, the SLG-A10 cDNA hybridizes to two HindIII fragments at 8.4 and 9.2 kb, and further analysis has revealed that the SLG-A10 gene is represented by the lower 8.4-kb HindIII band, while the upper 9.2-kb band results from cross-hybridization of the SLG-A10 cDNA to the SRK-A10 gene (see below).

In the self-compatible canola cultivars Ceres (Figure 1, lane 1), Regent (Figure 1, lane 2), and Westar (Figure 1, lane 5), the A10 allele is present in a homozygous form as determined by the segregation of this allele into progeny. This locus is also occasionally found in the self-compatible Topas line, as is shown for the two Topas plants tested, where one is heterozygous for the A10 allele because not all self-progeny carry this allele (Figure 1, lane 4) and the other does not carry the A10 allele (Figure 1, lane 3). In the self-incompatible line W1, which was developed from a cross between a self-incompatible B. campestris and the self-compatible canola cultivar Westar (Goring et al., 1992b), the A10 allele is again present in a homozygous state. A cross between W1 and a self-compatible winter canola line, which does not carry the A10 allele, shows the segregation pattern of the A10 allele relative to self-incompatibility. Of the five genomic DNA samples from self-incompatible plants, only two (Figure 1, lanes 9 and 11) contain the A10 allele in their genomes, while genomic DNA from four self-compatible plants (Figure 1, lanes 12 to 15) carry this allele. The allele responsible for self-incompatibility in this line has been determined to be the 910 allele (Goring et al., 1992b), which is only present in the W1 plant (Figure 1, lane 6) and in the resulting self-incompatible plants from the cross involving W1 (Figure 1, lanes 6 to 11). Thus, in the W1 line, the A10 allele does not segregate with self-incompatibility.

Sequence Analysis of the SLG-A10 cDNA

The sequence of the SLG-A10 cDNA was analyzed to determine if some alteration was responsible for the inability of this allele to elicit a self-incompatibility reaction. It was found that the SLG-A10 sequence could be translated into a full-length SLG protein, as shown in Figure 2. The predicted amino acid sequence shows that the SLG-A10 protein contains several characteristic features of SLG proteins, such as the signal peptide at the 5’ end, several potential sites for N-glycosylation, and the 12 conserved cysteine residues at the carboxyl end of the sequence (Figure 2) (Nasrallah et al., 1987; Dwyer et al., 1991). The SLG-A10 sequence shows high levels of DNA homology to a majority of characterized SLG genes (Trick and Flavell, 1989; Dwyer et al., 1991; Goring et al., 1992a, 1992b), ranging from 84 to 91% for DNA and 79 to 86% similarity for the predicted amino acid sequences. Some of these alleles have been associated with phenotypically strong self-incompatibility reactions (Thompson and Taylor, 1986; Goring
et al., 1992a, 1992b). The amino acid homology is much lower (67%) for the pollen recessive S2 allele (Chen and Nasrallah, 1990). Thus, the sequence predictions suggest that the SLG-A10 allele should be able to promote a strong self-incompatibility reaction.

Expression of the SLG-A10 Gene

To determine if the lack of a self-incompatibility reaction was due to an altered expression pattern of the SLG-A10 allele, RNA levels in different tissues from the W1 line were examined. The SLG-A10 steady state mRNA levels were then compared to that of the SLG-910 allele, which is associated with W1 self-incompatibility. As shown in Figure 3, both genes were found to be predominantly expressed in the stigma tissue, with mRNA transcripts detected in samples before and after anthesis (Figures 3A and 3B). When the levels were compared to plasmid controls, the steady state levels of SLG-A10 mRNA were found to be approximately four to eight times higher than the SLG-910 transcripts (Figures 3A and 3B). Similar results were also detected in the R2 line when the SLG-A10 steady state mRNA levels were compared to that of the SLG-A14 gene, the allele associated with self-incompatibility in the R2 line (data not shown). When the developmental profile of the SLG-A10 mRNA levels was examined in stigma samples from developing W1 buds (Figure 3C), it was determined to be very similar to that observed for the SLG-910 allele (Goring et al., 1992b). Finally, loss of self-incompatibility was not associated with an absence of SLG-A10 expression because high levels of SLG-AW transcripts were also detected in stigma RNA samples from developing buds in the self-compatible Westar line (data not shown). Thus, there are no indications that the absence of self-incompatibility is due to an altered expression profile, unless the higher steady state levels of SLG-A10 RNA somehow exert a negative effect.

Figure 2. The Predicted Amino Acid Sequence of the SLG-A10 cDNA.

The underlined section represents a putative signal peptide. Conserved cysteine residues are marked by stars above the amino acid residues. Potential N-glycosylation sites are represented by double underlines.

Isolation and Expression of the SRK-10 cDNA

Because the sequence and expression pattern of SLG-A10 was similar to a functional SLG gene, the SRK gene associated with the A10 allele was then analyzed. Using the polymerase chain reaction (PCR) with primers to conserved regions in SLG genes, an 800-bp internal fragment from the SRK-A10 gene (Figure
1, upper band) was first isolated and sequenced. The corresponding 2.7-kb cDNA was then amplified using the rapid amplification of cDNA ends (RACE) technique with specific primers derived from the genomic fragment (Frohman et al., 1988). To determine in which tissues the SRK-A10 gene was expressed, W1 and Westar RNA samples were analyzed by RNA PCR. As shown in Figure 4, the SRK-A10 gene is predominantly expressed in the pistils throughout bud development in both W1 and Westar RNA samples. This is the primary site of expression seen for other SRK genes in Brassica (Stein et al., 1991; Goring and Rothstein, 1992). However, while very weak expression of other SRK genes has also been found in the anthers, SRK-A10 transcripts were not detected in this tissue.

Sequence Analysis of the SRK-A10 Gene

The SRK genes from different S alleles encode proteins with similar features, namely a region at the N-terminal end very similar in sequence to SLGs, a transmembrane domain, and a C-terminal kinase domain. Alignment of the SRK-A10 cDNA sequence to other SRK genes revealed that it was very similar to the SRK-910 (Goring and Rothstein, 1992) and SRK6 (Stein et al., 1991) genes showing 86 and 87% DNA homology, respectively. However, as determined for other SLG–SRK pairs, the SRK-A10 sequence was most similar to the SLG-A10 sequence in the SLG domain (92%). An unusual feature of this homology is the presence of a 590-bp region with 100% sequence identity between the two genes, suggesting that a gene conversion event has occurred, as shown in Figure 5A. Whereas SLG–SRK pairs at a locus are very similar to each other, the three S loci characterized to date do not have an analogous region of identical sequence. As shown in Figure 5B, a comparison of the SLG-A10 and SRK-A10 sequences revealed a few deletions or insertions occurring in multiples of 3 bp, which would result in the removal or addition of amino acids in the SLG domain. This also has been detected in the SLG–SRK pairs at the S2 and S6 loci. However, just downstream of the region of 100% homology at position 948 in the SRK-A10 sequence, there is a 1-bp deletion (Figure 5, arrow) leading to a shift in the reading frame. Translation of the DNA sequence revealed that premature termination would occur at nucleotide 978 and a truncated protein would be produced (Figure 5B, double underlined). Except for this 1-bp deletion, this gene codes for the predicted structures of a receptor kinase, including the transmembrane and kinase domains. The predicted kinase domain contains all of the conserved amino acids found in kinases, and similar to the other S receptor kinase genes, it shows greatest sequence similarity to serine/threonine kinases (Hanks et al., 1988). Thus, due to the frameshift mutation, this gene no longer codes for a functional S receptor kinase.

Segregation of the A10 Allele with a Functional S Allele

Because the A10 allele is not associated with self-incompatibility, it was not known whether this allele was genetically linked to the S locus or if it was present in another part of the Brassica genome. There are potentially two S loci in B. napus because it is composed of the genomes of both B. campestris and B. oleracea. The location of the A10 allele can be determined by studying the segregation patterns in crosses between the self-compatible Westar line, which is homozygous for the A10 allele, and a self-incompatible B. napus line that is produced by the introgression of a B. campestris or B. oleracea S allele. In the W1 line, both the B. campestris 910 allele and the A10 allele are homozygous, suggesting that the A10 allele is not at the S locus position in the B. campestris component of the B. napus genome. Furthermore, in lines in which both the 910 and A10 alleles are present as heterozygotes, these alleles segregate randomly in the self-progeny (data not shown).

The segregation pattern of the A10 alleles was next compared to a B. oleracea allele (S24) present in the canola cultivar Karat. The self-incompatible S24 Karat homozygote (negative for the A10 allele) was crossed to the self-compatible Westar (homozygous for the A10 allele), and the resulting F1 progeny were self-pollinated (using salt to break down self-incompatibility) to produce a segregating F2 population. The A10 allele was detected in seedlings both by PCR amplification using primers specific to the SLG-A10 gene and by DNA blot hybridization. Surprisingly, all 30 F2 progeny tested carried the A10 allele. Because a DNA probe for the S24 allele was not available, 19 of these plants were analyzed for the presence of this allele by testing for self-incompatibility, and the S24SLG gene was then identified by cross-hybridization to the SLG-A10 cDNA. Approximately two-thirds of the plants tested (13 of 19) were found to be self-incompatible. Furthermore, all the self-incompatible F2 plants, as shown in Figure 6 (lanes 7 to 10), were found to carry a cross-hybridizing HindIII band.

Figure 4. RNA PCR Analysis of the SRK-A10 Gene.

First strand cDNAs from different W1 and Westar RNA samples were amplified with SRK-A10–specific primers spanning the kinase domain. The anther and pistil samples were extracted from different bud size lengths: (lanes 1) 2 to 3 mm; (lanes 2) 4 to 5 mm; (lanes 3) 6 to 7 mm. Amplification was performed for 30 cycles for the pistil samples and 35 cycles for the remaining samples. PCR products were hybridized to the SRK-A10 cDNA. Only the top hybridizing band, which represents the expected length of 1.1 kb, was visible after staining with ethidium bromide. The smaller bands may have been derived from incorrectly spliced mRNAs, which have been detected during the cloning of another SRK gene, the SRK-910 cDNA (D.R. Goring, unpublished results).
Figure 5. Alignment of the SLG-A10 and SRK-A10 cDNA Sequences.

(A) The coding region of the SLG-A10 cDNA was aligned with the SRK-A10 cDNA sequence. A region of 100% homology between the two genes is marked. The 1-bp deletion in the SRK-A10 gene leading to premature termination is marked by the arrow. Underneath the SRK-A10 cDNA is a representation of the S receptor kinase domains that would be translated if the deletion was not present.

(B) Sequence alignment of the SLG-A10 and SRK-A10 cDNAs. Identical nucleotides are marked by dashes, and gaps are represented by blank spaces. The predicted start and stop codons for both genes are underlined. The 1-bp deletion in the SRK-A10 gene is marked by an arrow. The nucleotide sequences for SLG-A10 and SRK-A10 have been submitted to GenBank as accession numbers L08608 and L08607, respectively.
Self-incompatibility is one of several mechanisms that promote outbreeding. In self-incompatible species like *B. campestris* and *B. oleracea*, progeny of self-fertilized plants show severe inbreeding depression and are less capable of competing with their cross-bred cousins. It is generally thought that self-compatible species have arisen via mutation of the genes important for self-incompatibility (Stebbins, 1957). There are a variety of circumstances where self-compatibility and the resultant capability for inbreeding might prove advantageous. These include cross-pollination difficulties due to the low density of a species under some environment conditions, selfing as an isolating mechanism due to polyploidy, and fixing of a chance variant having local adaptive advantage (for a more complete discussion, see Jain, 1976). *R. napus* is an amphidiploid species derived from a combination of the *B. campestris* and *B. oleracea* genomes (Downey and Rakow, 1987). Presumably the selection for self-compatibility in the original *R. napus* lines was very strong for a variety of reasons, including an initially low population density and a lack of severe inbreeding depression due to polyploidy. It is tempting to conclude that the mutation in the A10 allele occurred during the process of *B. napus* speciation. However, this species has been domesticated for centuries (Downey and Rakow, 1987), and it should be recognized that the cultivars that are presently utilized have a complex breeding history. This makes it difficult to determine when a particular mutation in the self-incompatibility genes occurred.

The A10 allele is present in a number of *B. napus* cultivars and yet all of these are self-compatible lines. The SLGA10 gene is expressed at high levels in the stigma, and sequence analysis suggested that it should encode a functional SLG protein. It has recently been shown that a self-compatible *B. oleracea* line also carries a normal SLG gene and that its gene product is expressed in a manner similar to that for SLGs from self-incompatible lines (Gaufet al., 1993). Analysis of the SRK-A10 gene showed that it also is expressed in stigma tissues; however, the coding region contains a 1-bp deletion in the receptor domain when compared to other SLG and SRK genes. This deletion creates a shift in the reading frame that leads to premature translation termination toward the end of the receptor domain and to the production of a truncated receptor. Receptor kinases have been implicated in a variety of systems to play important roles in signal transduction during cell–cell communication (Cantley et al., 1991; Karin, 1992). Thus, the absence of a functional receptor kinase would presumably lead to a block in the signal pathway that would normally be activated during the self-incompatible response.

Close to the frameshift mutation in the SRK-A10 gene is a region of identical sequence between the SLGA10 and SRK-A10 genes, which is a characteristic product of gene conversion. One explanation for these results is that the SRK-A10 gene suffered DNA damage followed by recombinational repair. During the repair process, if the SLGA10 gene was used to repair the SRK-A10 gene, the result would be gene conversion. This type of homologous recombination between duplicated sequences present on the same chromosome has been shown to be induced by mutagens in mammalian cells (Wang et al., 1988). The SLG gene and the SLG domain of the SRK gene from a single allele are more similar in sequence to each other than they are to these genes isolated from other alleles (Stein et al., 1991; Goring and Rothstein, 1992). Occasional gene

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**DISCUSSION**

Self-incompatibility is one of several mechanisms that promote outbreeding. In self-incompatible species like *B. campestris* and *B. oleracea*, progeny of self-fertilized plants show severe inbreeding depression and are less capable of competing with their cross-bred cousins. It is generally thought that self-compatible species have arisen via mutation of the genes important for self-incompatibility (Stebbins, 1957). There are a variety of circumstances where self-compatibility and the resultant capability for inbreeding might prove advantageous. These include cross-pollination difficulties due to the low density of a species under some environment conditions, selfing as an isolating mechanism due to polyploidy, and fixing of a chance variant having local adaptive advantage (for a more complete discussion, see Jain, 1976). *R. napus* is an amphidiploid species derived from a combination of the *B. campestris* and *B. oleracea* genomes (Downey and Rakow, 1987). Presumably the selection for self-compatibility in the original *R. napus* lines was very strong for a variety of reasons, including an initially low population density and a lack of severe inbreeding depression due to polyploidy. It is tempting to conclude that the mutation in the A10 allele occurred during the process of *B. napus* speciation. However, this species has been domesticated for centuries (Downey and Rakow, 1987), and it should be recognized that the cultivars that are presently utilized have a complex breeding history. This makes it difficult to determine when a particular mutation in the self-incompatibility genes occurred.

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**Figure 6. Segregation of the A10 Locus with a B. oleracea S Allele in the B. napus Genome.**

Genomic DNA samples were digested with HindIII and hybridized to the SLGA10 cDNA under cross-hybridizing conditions (washes at 50°C in 0.1 × SSC, 0.1% SDS). Sources of genomic DNA are the following: self-incompatible *S*, Karat line (lanes 1 and 2); F₂ progeny from a cross between *S*, Karat and Westar (lanes 3 and 4); self-compatible Westar canola line (lanes 5 and 6); and F₂ progeny from the self-pollination of a *S* Karat/Westar F₁ plant (lanes 7 to 13). The plants from lanes 1 to 4 and 7 to 10 are self-incompatible (SI); and from lanes 5 and 6, and 11 to 13 are self-compatible (SC).

at ~12 kb; this band was present in the *S* Karat parental line (Figure 6, lanes 1 and 2) and in the *S* Karat/Westar F₁ plants (Figure 6, lanes 3 and 4), but absent in the Westar parental line (Figure 6, lanes 5 and 6) and in the self-compatible F₂ plants (Figure 6, lanes 11 to 13).

The A10 allele (represented by the two HindIII bands at 8.4 and 9.2 kb) was present in the *S* Karat/Westar F₁ plants (Figure 6, lanes 3 and 4), the Westar parental line (Figure 6, lanes 5 and 6), and all of the F₂ plants (Figure 6, lanes 7 to 13). The signal intensity of the A10 allele in the F₁ and F₂ populations suggested that there is one copy of the A10 allele in the self-incompatible F₁ and F₂ plants and two copies in the self-compatible F₂ plants and the parental line. Therefore, all 30 progeny plants tested had the A10 allele. Of the 19 tested further, 13 of 19 were self-incompatible, and all appeared to be heterozygous for the A10 allele, with the six self-compatible plants being homozygous for the A10 allele. Although the *S* and A10 alleles appear to be segregating in a 0:2:1 ratio into the progeny with no *S* homozygotes, there is a statistically significant probability that the actual ratio is 0:1:1.
conversion between the SLG and SRK genes at a single allele would explain why these sequences have remained so similar.

Through crosses to self-incompatible *B. napus* lines carrying known *B. campestris* and *B. oleracea* S alleles, the A10 allele was shown to be closely linked to the *B. oleracea* S allele. The evidence of linkage between the A10 allele and the *B. oleracea* S allele is twofold. First, when the S24 allele is present, the A10 allele does not segregate randomly and is in fact present in all progeny plants. This is in contrast to what occurs when the A10 allele is present in the same plant as a *B. campestris* S allele or in self-compatible lines. Second, the A10 allele appears to be heterozygous to the S24 allele in all self-compatible progeny, while being homozygous in the self-compatible *Fp* plants. At this point, it is not possible to determine whether the A10 allele is present right at the *S* locus, because not enough progeny were analyzed to detect rare recombination events.

The segregation of A10 and *B. oleracea* S24 alleles into the *F₂* progeny was not as expected. For a sporophytic self-incompatibility system such as that found in the Brassica species, the parental genotypes determine whether a particular pollination will be rejected. Therefore, if one breaks down the self-incompatibility system by treating the stigmas with salt prior to pollination, the mechanisms preventing pollen germination on the stigma surface would be overcome for all pollen. Therefore, self-pollination of the A10/S24 heterozygotes should yield progeny at a normal Mendelian ratio of 1:2:1. There is evidence that there also may be a gametophytic component in Brassica self-incompatibility (Zuberi and Lewis, 1988). Furthermore, Sato et al. (1991) have demonstrated that an SLG-6 promoter—β-glucuronidase (GUS) fusion gene introduced into Brassica is expressed not only in the stigma papillae cells (sporophytic) but also in the transmitting tissue of the stigma, style, and ovary (gametophytic). This also may indicate a gametophytic component to Brassica self-incompatibility.

If a gametophytic component does exist and is not affected by salt treatment, then one would expect the growth of the pollen tubes from gametes carrying the S24 allele to be hindered, allowing the pollen tubes carrying the A10 allele to successfully fertilize the ovules. In this case, one would expect a progeny ratio in the *F₂* generation of 1 A10/S24 heterozygote to 1 A10 homozygote. The segregation ratio of the A10 and S24 alleles into progeny was aberrant, with all progeny tested having the A10 allele and concomitantly none being S24 homozygotes. Although the ratio of 13 heterozygotes to 6 homozygotes fits a segregation ratio of 2:1, not enough samples were tested to statistically eliminate the possibility that the real ratio is 0:1:1. Therefore, it is not possible to demonstrate that there is a gametophytic self-incompatibility component in this case or whether some other mechanism is at work. It should be noted that one does not always fail to detect progeny homozygous for self-incompatibility at the expected frequency when heterozygous plants having one functional *S* allele are self-pollinated. The actual ratio varies considerably depending on which *S* allele is present (D.R. Goring, unpublished results). It is unclear whether this is due to the strength of a certain allele or the result of another closely linked locus (Zuberi and Lewis, 1988).

Because having a single functional *S* allele is sufficient to make a plant self-incompatible, both the *B. campestris* and *B. oleracea* S loci must be mutated to produce self-compatible *B. napus*. Whereas the A10 allele represents a *B. oleracea* S allele in several *B. napus* lines, it is unclear what has happened to the *B. campestris* S locus in these lines. Under cross-hybridizing conditions with SLG probes, bands that may represent a nonfunctional *B. campestris* S allele were not detected (for example, see Figure 6). Therefore, these genes may be absent due to a deletion removing these genes. However, the *B. campestris* locus would not be detected if the SLG and SRK genes have been masked by other cross-hybridizing bands or have diverged sufficiently not to be detected by hybridization.

Whereas direct transformation of self-compatible plants with the SLG and SRK genes would be the most conclusive evidence demonstrating that these genes are involved in the self-incompatibility reaction, the results in this study add to the growing evidence that these genes are responsible for self-incompatibility. We have shown that the A10 locus does segregate with an *S* allele linked to self-incompatibility and that the A10 locus itself is not associated with self-incompatibility. The presence of a frameshift mutation in the SRK-A10 gene suggests that loss of receptor kinase activity is responsible for the loss of self-incompatibility at this locus, although we cannot rule out the possibility that there are other unidentified genes at the *S* locus that are also affected. Nasrallah et al. (1992) have shown that decreased SLG expression is associated with loss of self-incompatibility. Thus, from these studies, it appears that both the SLG and SRK genes are required for the self-incompatibility response. However, it is certainly possible that an additional gene product, such as a ligand recognized by the receptor kinase, is required to confer self-incompatibility.

**METHODS**

**Plant Material and Crosses**

The *S* allele in the W1 line originated from a self-incompatible *Brassica campestris* plant and was introgressed into the *B. napus* ssp *oleifera* cv Westar line as described by Goring et al., (1992b). The *S* allele in a *B. oleracea* broccoli *F₁* hybrid was identified as the *S* allele based on crosses to a *B. oleracea* tester set (supplied by Dr. D.J. Ockendon, Horticultural Research Institute, Woburn, U.K.) and then introgressed into the *B. napus* ssp *oleifera* cv Karat line (V. Ripley and W. Beversdorf, manuscript in preparation). Plants were grown either in a greenhouse or in a growth room under 16-hr–daylight and 8-hr–dark conditions. Following anthesis under pollination bags (to enforce self-pollination), plants were tested for self-incompatibility by measuring seed set or by staining pistils for pollen tube growth with aniline blue and examining them under a fluorescence microscope (Kho and Baer, 1998). Forced self-pollination of the self-incompatible plants involved...
the use of 5% NaCl to block expression of self-incompatibility temporarily and permit self-fertilization.

Isolation of the SLG-A10 and SRK-A10 cDNAs

The S locus glycoprotein (SLG)-A10 cDNA was isolated from a cDNA library constructed from stigmas collected 1 to 2 days before anthesis and hybridized with the BS29-2 SLG probe (Thick and Flavell, 1989) under cross-hybridizing conditions (Goring et al., 1992a).

The S receptor kinase (SRK)-A10 cDNA was isolated using polymerase chain reaction (PCR) techniques described by Goring et al. (1992b). W1 genomic DNA, digested with HindIII and fractionated on an agarose gel, was used to amplify an 800-bp region using PCR primers to conserved regions in published SLG sequences. The PCR products were cloned into pBluescript KS+ (Stratagene) and identified by sequencing and genomic blot hybridization patterns. Specific primers derived from the genomic PCR clone were then used to amplify the 5' and 3' cDNA ends using the RACE (rapid amplification of cDNA ends) procedure (Frohman et al., 1988) with modifications described by Goring et al. (1992b).

DNA Sequencing

PCR clones were partially sequenced using dideoxy sequencing and the sequence enzyme (U.S. Biochemical Corp.) to confirm that they were derived from the SRK-A10 gene. To completely sequence the full-length SLG-A10 cDNA clone and the SRK-A10 PCR clones, deletions were made using exonuclease III and mung bean nuclease according to the procedure in the Stratagene kit. Overlapping deletions were sequenced for both strands. To avoid errors that may have been introduced during PCR, several cDNA ends derived from separate PCR amplifications were sequenced. The 1-bp deletion in the SRK-A10 gene was detected in both the genomic PCR clones and in the 5' RACE products. All DNA and protein sequence analyses were performed on the DNASIS and PROSIS software (Hitachi America Ltd., San Bruno, CA).

Genomic DNA Blots

Genomic DNA was extracted from leaves as described previously (Goring et al., 1992b). Approximately 5 to 10 μg of genomic DNA was digested with HindIII, fractionated through a 0.7% agarose gel, and transferred to a Zetabind membrane (CUNO, Meriden, CT). The membranes were prehybridized and hybridized as described previously (Goring et al., 1992b). Washing conditions involved two 30-min washes in 0.1 x SSC (1 x SSC is 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS at 50 to 53°C for cross-hybridization, and at 65 to 69°C for specific hybridization. For the probes, the full-length cDNAs were gel purified from vector sequences and labeled by random priming (Feinberg and Vogelstein, 1983).

RNA Analysis

Total RNA was extracted from W1 tissues using the method of Jones et al. (1985). Poly(A)⁺ RNA samples were extracted from Westar tissue using the Micro-FastTrack mRNA isolation kits (Invitrogen, San Diego, CA). For the RNA blots, 10 μg of W1 total RNA was fractionated through a 1.2% formaldehyde gel (Sambrook et al., 1989) and transferred to Zetabind membranes. Hybridization conditions were the same as used for the genomic blots, and washing conditions were followed for specific hybridization.

To examine the expression of the SRK-A10 gene using PCR, W1 total RNA samples and Westar poly(A)⁺ RNA samples were amplified with specific SRK-A10 primers that span the kinase domain, which contains several introns. Twenty micrograms of total RNA and ~1 μg of poly(A)⁺ RNA were first treated with DNase I to remove any contaminating genomic DNA; these samples were then used to synthesize first strand cDNA according to the method of Harvey and Darlison (1991). Controls without reverse transcriptase were also set up for each sample. One-quarter of each cDNA sample was amplified for 30 cycles for the pistil samples and 35 cycles for the remaining samples and then subjected to gel electrophoresis and DNA gel blot analysis.

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