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Pollen–Stigma Signaling in the Sporophytic Self-Incompatibility Response

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INTRODUCTION

Many flowering plants reproduce sexually and generate variation by the process of recombination. Because the possibility of continued evolution is dependent, at least in part, on the production of new variant combinations of genes derived from different individuals, the potential for adaptation is believed to be better under cross-pollination than under self-pollination. In fact, if self-incompatible plants are manipulated to undergo forced self-pollination, they invariably produce progeny with marked inbreeding depression. It is therefore not surprising to find that plants have evolved a variety of mechanisms that favor outcrossing. For example, the crucifer family (Brassicaceae) has an elaborate genetic self-incompatibility (SI) system that controls mating in natural populations of nearly half of the species belonging to this family. This system acts early in pollination to arrest pollen derived from "self." Such plants require cross-pollination to ensure maximal seed production. Radishes, kales, and cabbages are examples of crucifers that rely on outcrossing via cross-pollination by insect vectors to complete their life cycle in the wild. Genetic SI in crucifers is controlled by a complex and highly polymorphic *S* locus with many specificities or mating reactions. In this review, we discuss the role of the gene products encoded at the *S* locus in light of recent molecular genetic data derived from the analysis of three *Brassica* species, *B. oleracea*, *B. campestris*, and *B. napus*.

GENETIC AND CYTOLOGICAL FEATURES OF THE SPOROPHYTIC SELF-INCOMPATIBILITY SYSTEM OF *BRASSICA*

SI is based on the ability of the pistil to discern the presence of self-pollen and to inhibit the germination or subsequent development of self-related, but not genetically unrelated, pollen. In the Brassicaceae, the genetic control of SI was deciphered in the early 1950s with Bateman's analysis of *Iberis amara* (Bateman, 1955). His genetic model, which has since been generalized to other Brassicaceae and is widely accepted (for review, see Nasrallah and Nasrallah, 1989) includes three basic features: (1) SI is genetically controlled by a single, multiallelic locus, the *S* locus; (2) pollen phenotype is determined not gametophytically by the haploid genotype of the pollen grain but sporophytically by the diploid genotype of the parent plant; and (3) codominant and/or dominant allelic interactions occur that determine the ultimate phenotype of stigma and pollen. The essence of this model is that an incompatible response occurs when the same *S* allele is active in stigma and pollen. The number of alleles that occur at the *S* locus is usually large, estimated at 22 in *Iberis* (Bateman, 1955), 34 in *Raphanus* (Sampson, 1957), and 60 in *B. oleracea* (Ockendon, 1974).

Sporophytically controlled SI systems have been described in members of the Compositae and Convolvulaceae as well as in the Brassicaceae. A common feature of plants with sporophytic SI is that the inhibition of self-pollen is very rapid and occurs at the pollen–stigma interface. This site of inhibition, together with the genetic control of pollen SI phenotype, distinguishes sporophytic SI from other SI systems. For example, in the Solanaceae, SI is controlled gametophytically, and the inhibition of self-pollen is typically delayed until the developing pollen tubes extend into the style (see Newbigin et al., 1993, this issue).

The best-characterized sporophytic SI system is that which operates in members of the Brassicaceae. A schematic of the reproductive organs of crucifers and a scanning electron micrograph of a pollinated stigma are shown in Figure 1. The early events of compatible as well as incompatible pollen–pistil interactions in the Brassicaceae can be viewed as specific cell–cell interactions between a stigmatic papillar cell and a pollen grain or pollen tube. Successful pollinations with compatible, or non-self, pollen result in a rapid succession of events that include hydration and germination of pollen grains and the subsequent invasion of the papillar cell wall by the emerging pollen tube (Heslop-Harrison, 1975; Elleman et al., 1992). In contrast, incompatible pollinations with self-pollen are manifested by the inability of the pollen to germinate and/or the inability of the emerging pollen tube to invade the papillar cell wall (Sears, 1937; Kanno and Hinata, 1969; Dickinson and Lewis, 1973; Ockendon and Gates, 1975; Stead et al., 1980). The capacity of the stigma to discriminate between self-pollen and cross-pollen is a developmentally regulated phenomenon: the stigmas of immature buds are self-compatible and only become self-incompatible 1 day before flower opening. This developmental regulation allows the generation and maintenance of *S* locus homozygotes by bud pollination.

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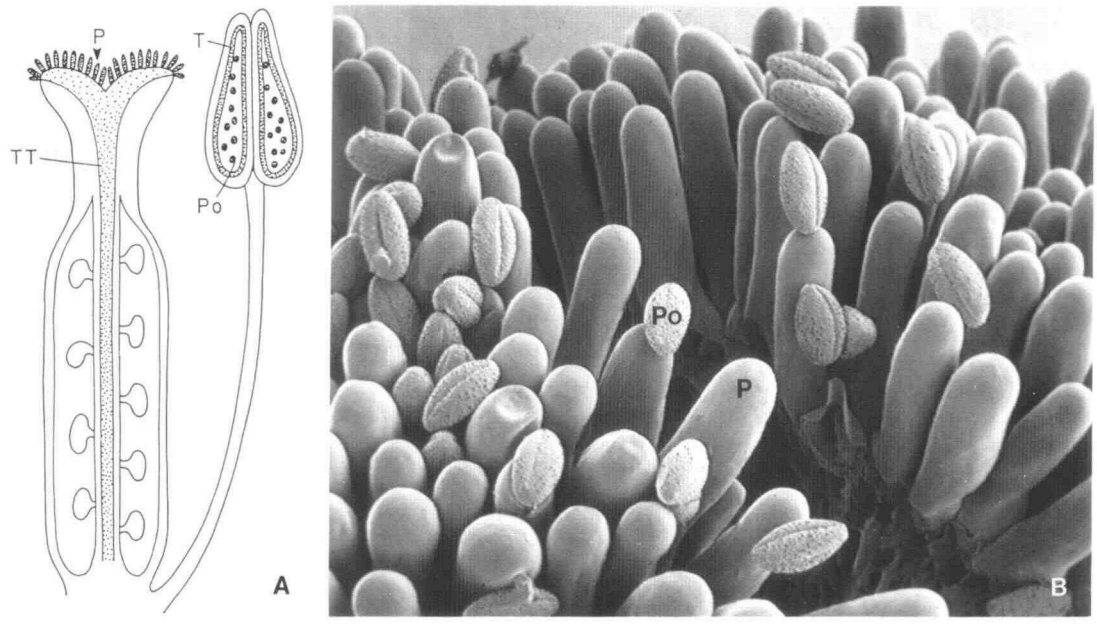

Figure 1. Reproductive Organs Relevant to Pollen–Stigma Interactions in Crucifers.

(A) Schematic representation of pistil and stamen. Pollen grains (Po) develop in anther locules that are lined with sporophytically derived tapetal cells (T). Successful pollinations in crucifers are dependent on cell–cell interactions between pollen and papillar cells (P), ~2000 of which are displayed at the stigma surface. Compatible pollen grains produce tubes that invade the papillar cell wall, grow within the wall, and emerge into the middle lamellae of cells in the transmitting tract (TT).

(B) A scanning electron micrograph of a pollinated stigma showing the two interacting cell types, papillar cells (P) and pollen grains (Po).

THE *BRASSICA S* LOCUS COMPLEX

Molecular genetic analyses have demonstrated that two transcribed genes map to the Mendelian *S* locus. The analysis of restriction fragment length polymorphisms in F_2 populations segregating for different SI genotypes has demonstrated that these two genes are genetically inseparable from each other and from the SI phenotype (Stein et al., 1991) and thus behave as components of a single locus. Furthermore, genomic analysis by pulsed-field gel electrophoresis (Boyes and Nasrallah, 1993) has demonstrated that the two genes are physically linked and are separated by ~200 kb of DNA. The molecular genetic data have prompted us to adopt the term "*S* haplotype" instead of the classic "*S* allele" to designate genetic constitution at the complex *S* locus. The concept of haplotype, originally applied to the major histocompatibility complex, a region of several genes involved in the immune response of mammals, has been extended to describe "the particular combination of alleles or restriction sites (or any other genetic marker) present in some defined area of the genome" (Lewin, 1990).

The two *S* locus–linked genes are related members of the *S* family of genes (Nasrallah et al., 1988; Dwyer et al., 1989; and see below). Figure 2A shows the structures of these two genes. One of the two genes is the *S Locus Glycoprotein* (*SLG*) gene, which encodes a primary protein of 431 amino acids that includes a signal peptide and a 400–amino acid sequence corresponding to the mature secreted glycoprotein. The other gene is the *S* locus *R*eceptor *K*inase (*SRK*) gene, which is predicted to encode a membrane-associated protein (Stein et al., 1991). The predicted SRK protein consists of a potentially glycosylated extracellular domain that shares extensive sequence similarity with SLG, including the placement of the 12 cysteine residues found in all members of the *S* gene family in the Brassicaceae. This "S" domain is joined via a single-pass transmembrane domain to a cytoplasmic region that has sequence similarity to protein kinases. The sequence of the cytoplasmic domain of SRK is more similar to the serine/threonine kinase consensus than to the tyrosine kinase consensus. Indeed, bacterially expressed SRK kinase domains show serine/threonine autophosphorylating activity (Goring and Rothstein, 1992; Stein and Nasrallah, 1993). It should be noted that the term "receptor" is used in connection with SRK because of the similarity of its overall structure to the growth factor receptor tyrosine kinases in animals (Ullrich and Schlessinger, 1990). However, ligand-induced activation of the kinase and kinase activity toward specific substrates have yet to be demonstrated.

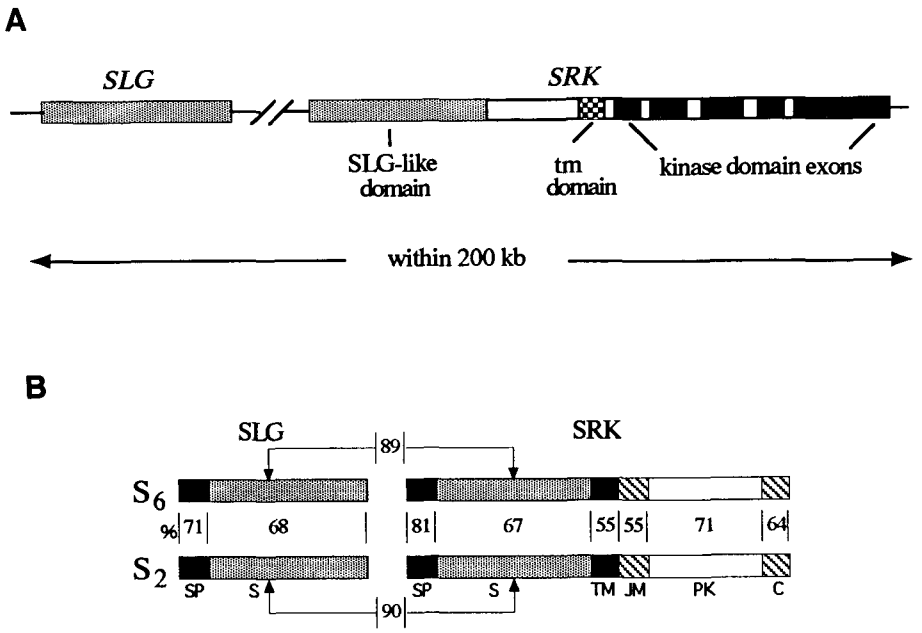

Figure 2. The *Brassica S* Locus and the *SLG/SRK* Gene Pair.

(A) Physical linkage and structure of the *SLG* and *SRK* genes. tm, transmembrane.
(B) Sequence relationships between the *SLG* and *SRK* genes in two *S* haplotypes. The numbers refer to percent amino acid sequence identities between the predicted proteins and provide evidence for the concerted evolution of the gene pair within a haplotype and its divergence between haplotypes (after Stein et al., 1991). SP, signal peptide; S, S domain; TM, transmembrane domain; JM, juxtamembrane domain; PK, protein kinase domain; C, carboxy-terminal domain.

THE *S* GENE FAMILY

The cloning of the *SLG* gene has led to the characterization of a group of related sequences that is quickly growing into a superfamily of genes. A major structural hallmark of members of this gene family is a cysteine-rich domain consisting of 10 to 12 cysteines arrayed in a precise order. Based on the structure of the encoded gene product and the expression profiles of the genes, the cloned members of the family can be grouped as shown in Table 1. Two major subfamilies can be recognized, one that encodes transmembrane protein kinases and another that encodes secreted glycoproteins. Further, genes that, like the *Brassica S* locus genes (see below), are

Table 1. *S* Locus Genes and Related Sequences in Plants

Plant	*S* Superfamily Gene	Subfamily	Site of Expression
Brassica	*SRK* (*S* locus)	Protein kinase	Reproductive tissues
	SLG (*S* locus)	Secreted glycoprotein	Reproductive tissues
	SLR1	Secreted glycoprotein	Reproductive tissues
	SLR2	Secreted glycoprotein	Reproductive tissues
Arabidopsis	*ARK1*	Protein kinase	Vegetative tissues
	AtS1	Secreted glycoprotein	Reproductive tissues
	RLK1	Protein kinase	Vegetative tissues
	RLK4	Protein kinase	Vegetative tissues
Maize	*ZmPK1*	Protein kinase	Vegetative tissues
Carrot	*EP1*	Secreted glycoprotein	Vegetative tissues

expressed exclusively in reproductive structures, as well as genes that are primarily expressed in vegetative tissues, have been reported.

In *Brassica*, *S* gene family members that are not linked to the *S* locus have been designated *S Locus-Related* (*SLR*) genes. *SLR1* (Lalonde et al., 1989; Trick and Flavell, 1989; Trick, 1990; Isogai et al., 1991) and *SLR2* (Boyes et al., 1991) belong to the secreted glycoprotein–encoding subfamily and are active in reproductive tissues. The SLR1 gene product was localized to the papillar cell walls of the *Brassica* stigma (Umbach et al., 1990), and its promoter was shown to be active in pistils and pollen of transgenic tobacco (Hackett et al., 1992), a pattern similar to that exhibited by the *SLG* promoter in this transgenic host (Thorsness et al., 1991). In the self-fertile crucifer Arabidopsis, the *AtS1* gene, which has floral-specific expression, is also predicted to encode a secreted glycoprotein (Dwyer et al., 1992).

Among the vegetatively expressed members of the *S* gene family are several putative receptor protein kinase genes. These genes include in maize, *ZmPK1*, a root cDNA isolated following amplification of cDNAs with the polymerase chain reaction (PCR) using primers based on conserved regions of protein kinases (Walker and Zhang, 1990), and in Arabidopsis, the *ARK1* (*A*rabidopsis *R*eceptor *K*inase) gene (Tobias et al., 1992), isolated by hybridization to *SRK*-derived probes, and the *R*eceptor-*L*ike *K*inase *RLK1* and *RLK4* genes, isolated by hybridization to a probe corresponding to the catalytic domain of *ZmPK1* (Walker, 1993). The most recent addition to the family is *EP1*, an epidermis-specific secreted glycoprotein sequence isolated as a cDNA from a carrot embryo and suspension cell library (van Engelen et al., 1993). The functions of these *S* locus–related genes have not been defined. It is likely, however, that the floral-specific *SLR1*, *SLR2*, and *AtS1* genes are involved in some general aspect of pollination responses, perhaps as components of a system that promotes the development of pollen tubes in compatible pollinations.

S LOCUS POLYMORPHISMS

The various naturally occurring, classically defined *S* alleles that have been described in *Brassica* have been arranged in a dominance series based on their genetic behavior relative to other alleles in heterozygous plants (Thompson and Taylor, 1966). Two major classes have been recognized (Nasrallah et al., 1991). High-activity (class I) alleles are placed relatively high on the dominance scale and exhibit a strong incompatibility phenotype in which an average of 0 to 10 pollen tubes develop per self-pollinated stigma. Low-activity (class II) alleles have a weak or leaky incompatibility phenotype in which 10 to 30 pollen tubes develop per self-pollinated stigma, and they exhibit recessive and competitive interactions in pollen.

The high degree of *S* locus polymorphism is reflected in the sequence variability of *S* locus genes. In comparisons between different *S* haplotypes, extensive sequence polymorphisms are observed between alleles of the *SLG* gene (Nasrallah et al., 1987; Trick and Flavell, 1989; Chen and Nasrallah, 1990; Dwyer et al., 1991; Goring et al., 1992a, 1992b; Scutt and Croy, 1992; Gaude et al., 1993) and the *SRK* gene (Stein et al., 1991; Goring and Rothstein, 1992). To date, detailed characterizations have been performed on three class I haplotypes—the *B. oleracea* S_6 haplotype (Stein et al., 1991), a *B. oleracea* haplotype introgressed into *B. napus* (Goring and Rothstein, 1992), and the *B. campestris* S_8 haplotype (J.B. Nasrallah, unpublished data)—and one class II haplotype, the *B. oleracea* S_2 haplotype (Chen and Nasrallah, 1990; Stein et al., 1991; Tantikanjana et al., 1993). Alleles of the *SLG* and *SRK* genes isolated from the class I haplotypes share ~80% amino acid sequence identity. By contrast, sequence divergence as high as 30% has been noted between class I and class II haplotypes. For example, the *SLG* and *SRK* genes of the S_2 haplotype share only ~70% sequence identity with class I alleles of these genes (Chen and Nasrallah, 1990; Stein et al., 1991). These relationships are shown in Figure 2B.

Examination of the DNA sequence variability among several class I *SLG* alleles reveals an ancient evolutionary history strongly influenced by intragenic recombination as well as diversifying selection (C.F. Aquadro, J.B. Nasrallah, and M.E. Nasrallah, unpublished observations). The average nucleotide divergence at synonymous (non-amino acid–changing) sites between alleles is 21.4%, ranging from 15.2 to 27.6% for the 433 to 436 codons compared. Assuming a substitution rate of 5×10^{-9}/site/year/lineage, a value calculated for one plant nuclear gene (Wolfe et al., 1987) and close to the average rate of 4.65×10^{-9} calculated for vertebrate nuclear genes (Li et al., 1985), it appears that the alleles diverged more than 21 million years ago. The conclusion that *S* locus polymorphisms are ancient and likely to have predated speciation in the genus *Brassica* is supported by the finding that a class I *B. oleracea* *SLG* allele shares a higher degree of sequence identity with a *B. campestris* allele than with other class I *B. oleracea* alleles (Dwyer et al., 1991).

Interestingly, the sequence variation between alleles is distributed in a highly nonrandom fashion across the gene and with respect to nonsynonymous nucleotide sites. Nonsynonymous sites show remarkable variation across the gene, with one 80-bp segment in the central region of the protein differing by as much as 28%. This observation suggests that natural selection for protein diversity is at least partly responsible for the tremendous allelic variation at this locus. These data, combined with the great age of the alleles found in present populations, are consistent with the expectations of population genetic models of SI, which suggest that new alleles will have a strong selective advantage and be maintained polymorphic in the population for long periods of time (Wright, 1939). In addition, given the age of the *SLG* alleles and the fact that they occur naturally as heterozygotes, one might expect recombination to have played an important role in the evolutionary history of the locus. In fact, analysis of the patterns of substitutions along the genes has suggested that part of the sequence heterogeneity observed among *SLG* alleles

is due to intragenic recombination, leading to alleles composed of segments with vastly different evolutionary histories—some very old and highly divergent, others recently diverged and quite similar at the sequence level.

EVOLUTION OF *S* HAPLOTYPES

The occurrence of a pair of related but not identical genes at the *S* locus presents an interesting problem in gene evolution. Within a haplotype, SLG and the S domain of SRK share ~90% amino acid sequence identity (Stein et al., 1991; Goring and Rothstein, 1992; Figure 2B). This sequence conservation, which contrasts with the high degree of sequence divergence between haplotypes, suggests that selective pressure acts to retain extensive sequence similarity between the two genes, perhaps by a mechanism involving gene conversion. Some clues to the evolutionary history of the *SLG/SRK* gene pair are provided by the structures of these genes and the intrinsic variability of *S* haplotypes.

The *SLG* alleles reported to date, the majority of which are derived from class I haplotypes, have been shown to produce a 1.6-kb transcript (Nasrallah et al., 1985b, 1987, 1988; Trick and Flavell, 1989; Goring et al., 1992a, 1992b). Where genomic clones are available, this transcript is found to be colinear with the genomic sequence, leading to the conclusion that the gene is intronless (Nasrallah et al., 1988; Dwyer et al., 1989). By contrast, the *SRK* gene encodes a 3.0-kb transcript and consists of six exons separated by seven introns (Stein et al., 1991). Like many other eukaryotic genes, the *SRK* gene appears to have originated by the shuffling of exons that represent distinct functional domains. Thus, exon 1 encodes the extracellular S domain, exon 2 encodes the transmembrane and juxtamembrane domain, and exons 3 through 7 encode the cytoplasmic kinase domain. The nature of exon 1 as a distinct structural entity is further underscored by its physical separation from the remaining exons by the longest intron, which varies from 896 bp to over 5.5 kb (Stein et al., 1991; Tantikanjana et al., 1993; J.B. Nasrallah, unpublished data). This gene organization appears to be a common feature of the class of genes within the crucifer *S* gene family that encode transmembrane protein kinases. It has also been described for the Arabidopsis *ARK1* gene, a vegetatively expressed *S* receptor–like protein kinase gene (Tobias et al., 1992).

How did the *SLG/SRK* gene pair originate? A plausible hypothesis has emerged from the analysis of a class II haplotype (Tantikanjana et al., 1993). In this haplotype, the *SLG* gene has an unusual structure and consists of two exons, the first of which encodes the S domain of the gene. Two transcripts that differ at their 3′ ends are produced by this class II *SLG* gene. One transcript contains sequences from the first exon, terminates within the first intron, and encodes a secreted glycoprotein. The second, larger transcript contains sequences from the first and second exons and encodes a membrane-anchored form of SLG. The sequence similarity shared by *SLG* and *SRK* in this haplotype not only includes the S domain but also extends into the intron and second exon of the genes. Based on these similarities, Tantikanjana et al. (1993) suggest that *SLG* is derived from *SRK* by duplication. It is possible that the ancestral duplication event involved the entire *SRK* gene and was followed by the deletion of a varying number of exons to generate the present-day haplotypes. Alternatively, the duplication event that generated the *SLG/SRK* gene pair may have occurred more than once in the evolutionary history of the *S* locus. In any event, the duplication of *SRK*-like genes and the generation of genes that encode secreted glycoproteins appear to have occurred with some frequency during the evolution of the *S* gene family. Independent evidence for such duplications has been recently found in the analysis of a secreted glycoprotein–encoding member of the *S* gene family that also maintains an intron downstream of its *S* region (T. Tantikanjana, M.E. Nasrallah, and J.B. Nasrallah, unpublished observations).

SELF-RECOGNITION VIA A SIGNALING RECEPTOR COMPLEX

It is theoretically possible that the SRK protein acts alone to initiate the SI response. By analogy to the ligand-activated receptor tyrosine kinases described in animal systems (Ullrich and Schlessinger, 1990), the binding of an unidentified ligand to the extracellular domain of SRK could result in receptor oligomerization and the subsequent activation of the kinase domain. Activation of the receptor and inter-receptor transphosphorylation would be followed by the phosphorylation of specific target substrates, resulting in a phosphorylation cascade that ultimately leads to the observed cellular response.

SRK-mediated signaling appears to be more complex, however. For one thing, the apparent coevolution of the *SLG* and *SRK* genes within a haplotype implies a functional interaction between their products. In addition, as described in the following sections, data from expression studies and from the analysis of self-compatible mutations argue in favor of the involvement of a receptor complex in self-recognition.

Expression of the *SLG* and *SRK* Genes

The expression pattern exhibited by the *SLG* and *SRK* genes is consistent with models of SI in which both pollen and stigma bear determinants of recognition derived from the *S* locus. Both genes have been shown to be expressed in anthers and pistils by RNA gel blot analysis and amplification of transcripts by PCR (Nasrallah et al., 1985b; Trick and Flavell, 1989; Stein et al., 1991; Goring and Rothstein, 1992). The *SLG* gene, which has been characterized in detail, is expressed exclusively in reproductive structures, as demonstrated by reporter gene analysis (Sato et al., 1991; Thorsness et al., 1991; Toriyama et al., 1991a) and genetic ablation experiments using transgenic plants that express a chimeric gene consisting of the

SLG promoter fused to a β-glucuronidase reporter gene or to the subunit A protein of diphtheria toxin (DT-A) (Thorsness et al., 1991; Kandasamy et al., 1993; Thorsness et al., 1993).

In the pistil, *SLG* expression is developmentally regulated and correlates with the onset of SI (Nasrallah et al., 1985a, 1985b, 1988). Furthermore, *SLG* is expressed predominantly in the surface papillar cells of the stigma (Nasrallah et al., 1988; Sato et al., 1991), and its glycoprotein product accumulates to high levels in the walls of these cells (Kandasamy et al., 1989). This localization is consistent with cytological observations that demonstrate the arrest of pollen or pollen tube development at the stigma surface. In anthers, the *SLG* promoter is active sporophytically in the tapetum and gametophytically in the developing microspores (Sato et al., 1991). The sporophytic expression of the *SLG* gene is consistent with the sporophytic control of SI phenotype in *Brassica* pollen. The gametophytic expression of *SLG*, on the other hand, is unexpected, and its functional significance can be best assessed when the identity and localization of the SLG protein in pollen are determined.

The high degree of sequence similarity shared by the *SLG* and *SRK* genes extends into the promoter regions of the genes. Recent reporter gene analysis has shown that the *SRK* promoter exhibits a pattern of activity similar to that of the *SLG* promoter, being most highly active in the papillar cells of the stigma (J.C. Stein and J.B. Nasrallah, unpublished observations). Thus, both *S* locus genes are expressed during the development of the two cell types that interact during the SI response. Clearly, and particularly in papillar cells, the opportunity exists for the SLG and SRK proteins to interact.

Analysis of Self-Compatible Strains

Genetic evidence also reveals a requirement for both SLG and SRK in the SI response and suggests that both molecules cooperate to initiate a productive signal between pollen and stigma. Transgenic experiments have shown that the introduction of a cloned *SLG* gene into a self-incompatible strain of *B. oleracea* resulted in a modified pollination response (Toriyama et al., 1991b). In these experiments, a self-compatible transgenic phenotype was generated that was associated with the down regulation of the resident *SLG* gene in a manner similar to the recently observed but poorly understood phenomenon of "sense" suppression (Mol et al., 1990). This down regulation drastically reduced the amount of *SLG* glycoprotein product but did not affect the *SRK* or the *S* locus–related *SLR1* and *SLR2* genes.

Additional genetic evidence for the importance of SLG in SI derives from the analysis of self-compatible variants of the predominantly self-incompatible species *B. oleracea* and *B. campestris*. These self-compatible variants have generally been attributed to mutations in "modifier" genes that are unlinked to the *S* locus (Thompson and Taylor, 1971; Nasrallah, 1974; Hinata and Okasaki, 1986). The molecular basis of these mutations is not known except for a variant *B. oleracea* strain in which SI was associated with a reduction in SLG in the stigma (Nasrallah, 1974).

More recently, a mutation in *B. campestris* was identified that also results in drastically reduced levels of stigma SLG and in the loss of the incompatibility response in the stigma but not in pollen (Nasrallah et al., 1992). Genetic analysis of this strain has identified a locus unlinked to the *S* locus, designated *SCF1*, that is required for the normal developmental induction of SI and that regulates *S* locus function in *trans*. The self-compatible strain carries a single recessive mutation at the *SCF1* locus that down regulates the RNA levels of the *SLG*, *SLR1*, and *SLR2* genes but not the *SRK* gene. As described earlier, the *SLR1* and *SLR2* genes are not linked to the *S* locus and are not, therefore, believed to be determinants of specificity in the SI response. They do, however, exhibit an expression pattern similar to *SLG*, with maximal expression in the papillar cells of the stigma. The simultaneous down regulation of *SLG*, *SLR1*, and *SLR2* and the breakdown of SI in *scf1* homozygotes supports the involvement of these *S* gene family members, *SLG* in particular, in the pollen–stigma interaction of SI. It is likely that the mutation disrupts a regulatory gene that encodes a positive *trans*-acting factor that binds to the very similar promoters of the three genes and is required for their high-level stigmatic transcription. In this context, it should be noted that the occurrence of such a pistil-specific *trans*-acting factor is predicted from the recently characterized structure of the *SLG* promoter. Functional promoter studies in transgenic tobacco have shown that this promoter has a modular organization and consists of separable DNA elements that independently specify pistil- and pollen-specific expression: a 196-bp region sufficient to confer stigma and style specificity to reporter gene expression and two distinct but functionally redundant flanking domains, each of which is sufficient for expression in pollen (Dzelzkalns et al., 1993).

A requirement for SRK is similarly suggested by the analysis of self-compatible variants carrying mutations that segregate at the *S* locus. Two such mutations, which represent nonfunctional self-fertile (S_f) haplotypes, have been identified in *B. oleracea* and *B. campestris* (M.E. Nasrallah, S.J. Rundle, and J.B. Nasrallah, unpublished observations). Unlike *scf1* homozygotes, strains homozygous for these S_f haplotypes produce SLG glycoprotein in the stigma at levels comparable to those in self-incompatible strains. These strains exhibit aberrant *SRK* transcription and appear to represent "null" *SRK* mutants. Genetic alterations that impair *SRK* gene function thus constitute an additional means for generating compatible variants from incompatible ancestors. More generally, the disruption of *S* locus genes at the level of transcription, translation, or protein function may explain the self-compatibility of amphidiploid genera such as *B. napus* and of self-compatible diploid crucifers.

Analysis of a Class II Haplotype

The unusual structure of the class II *SLG* gene described above may provide evidence for the interaction of the SLG and SRK

proteins. The anomalous membrane-bound form of SLG encoded by this gene may be responsible for the leakiness and/or recessive nature of this haplotype. Because *SLG* and the extracellular domain of *SRK* from the same *S* haplotype, although not identical, share a high degree of sequence identity, the *SLG* gene may be viewed as corresponding to the extracellular domain of *SRK*. The membrane-anchored form of SLG would simulate a truncated kinase-negative form of SRK. Truncated forms of receptor protein kinases that lack the kinase catalytic domain have been engineered in animal systems, reintroduced into the appropriate cells, and shown to interfere with the function of the wild-type receptor (see, for example, Hemmati-Brivanlou and Melton, 1992). Ligand-induced receptor dimerization with subsequent activation of receptor protein kinases is thought to be mediated in large part by the extracellular domain of the receptor (reviewed in Ullrich and Schlessinger, 1990; Massague, 1992). The truncated receptors that lack the kinase domain apparently interact with the wild-type receptor through their extracellular domains, thus creating unproductive receptor heterodimers and suppressing signal transduction. If SLG and the extracellular domain of SRK interact, the membrane-anchored form of SLG would similarly interfere with SRK receptor function and result in a leaky SI phenotype. Such a partially active receptor system could also account for the pollen-recessive nature of the haplotype in heterozygotes, if competitive interactions between the proteins encoded by different *S* haplotypes are assumed to occur during the pollen–stigma interaction.

S GENE ACTION IN *BRASSICA*

Both genetic and molecular data are consistent with the hypothesis that the SRK protein kinase is activated by contact between a papillar cell and self-pollen. The operation of a signaling receptor is also consistent with the speed at which the inhibition of self-pollen occurs in crucifers and the nature of the cytological response, which is localized to the papillar cell–pollen interface, as Figure 3A illustrates. By phosphorylating intracellular substrates, the SRK protein could couple the initial molecular recognition events at the papillar cell–pollen interface to the signal transduction chain that leads ultimately to pollen rejection (Stein et al., 1991). The biochemical basis of the requirement for SLG in SI signaling is less obvious. Nevertheless, from the requirement for SLG in SI, it appears that interactions between transmembrane receptors and secreted forms of the extracellular "recognition" domain of the receptor are essential for signaling by this class of molecules across the plant cell wall. This conclusion is supported by the analysis of the Arabidopsis *ARK1* gene. Although we have found no evidence that this gene occurs in close physical linkage with a secreted glycoprotein–encoding gene similar to *SLG*, the *ARK1* gene itself produces two alternative transcripts, one of which has the potential for encoding a secreted form of the extracellular domain, the other of which could encode a full-length transmembrane receptor (Tobias et al., 1992; C.M. Tobias and J.B. Nasrallah, manuscript in preparation).

The observation that, among members of the *S* gene family, two different mechanisms have evolved to generate secreted forms of receptor extracellular domains suggests that such secreted forms are important for receptor function. The *SLG/SRK* gene pair would thus represent an extreme case. It is possible that the duplication of the extracellular domain of *SRK* in the form of the *SLG* gene occurred as a result of unique requirements in pollen–stigma signaling. A large excess of the secreted form of the extracellular domain relative to the transmembrane receptor may be required for the efficient functioning of the SI system. Such a requirement would have been met by the evolution of the *SLG* gene with its highly active promoter and the consequent "amplification" of the secreted form of the extracellular domain. A functional requirement for high levels of this molecular form could also provide the selective pressure to maintain the tight linkage of *SLG* to *SRK* and the extensive sequence similarity between the two genes.

What is the basis of haplotype specificity in the SI response? Specificity may derive from the haplotype-specific homophilic binding of the SLG and SRK proteins. This possibility is currently being addressed by transformation experiments in which *SLG/SRK* gene pairs derived from the same haplotype are introduced into *Brassica*. However, because of the observation that the *SLG* and *SRK* genes have overlapping temporal and spatial domains of expression and the expectation that their products occur in the same cell types, one or more additional pollen-borne components must be invoked to account for the self-pollen–induced activation of SRK. Such a component would presumably be encoded sporophytically and displayed at the pollen surface.

At least two scenarios, diagrammed in Figure 3B, can be envisaged. One possibility is that SLG may become competent for binding SRK only after it is modified by a pollen-borne molecule with haplotype-specific activity. Alternatively, and by analogy to the ligand-activated receptor tyrosine kinases of animal systems, "self" recognition may result from the allele-specific binding of an as-yet-unidentified extracellular ligand that is delivered to the papillar cell surface by pollen. SLG, which is freely diffusible within the cell wall, could bind this ligand at the papillar cell wall–pollen interface for presentation at the membrane and would thus act as an extracellular regulator of ligand access to the signaling receptor. Such a ligand would presumably bind to amino acid residues within the extracellular domain of SRK and/or SLG. However, a role for the carbohydrate moiety of these glycosylated molecules in receptor–ligand interaction cannot be discounted at present, especially because the various SLG molecules sequenced to date appear to exhibit different patterns of N-glycosylation (Nasrallah et al., 1987; Takayama et al., 1987; Trick and Flavell, 1989; Chen and Nasrallah, 1990), if not structurally different glycan chains (Takayama et al., 1986).

The answer to the question of whether a pollen-borne SRK ligand exists is important for deciphering the mechanism of

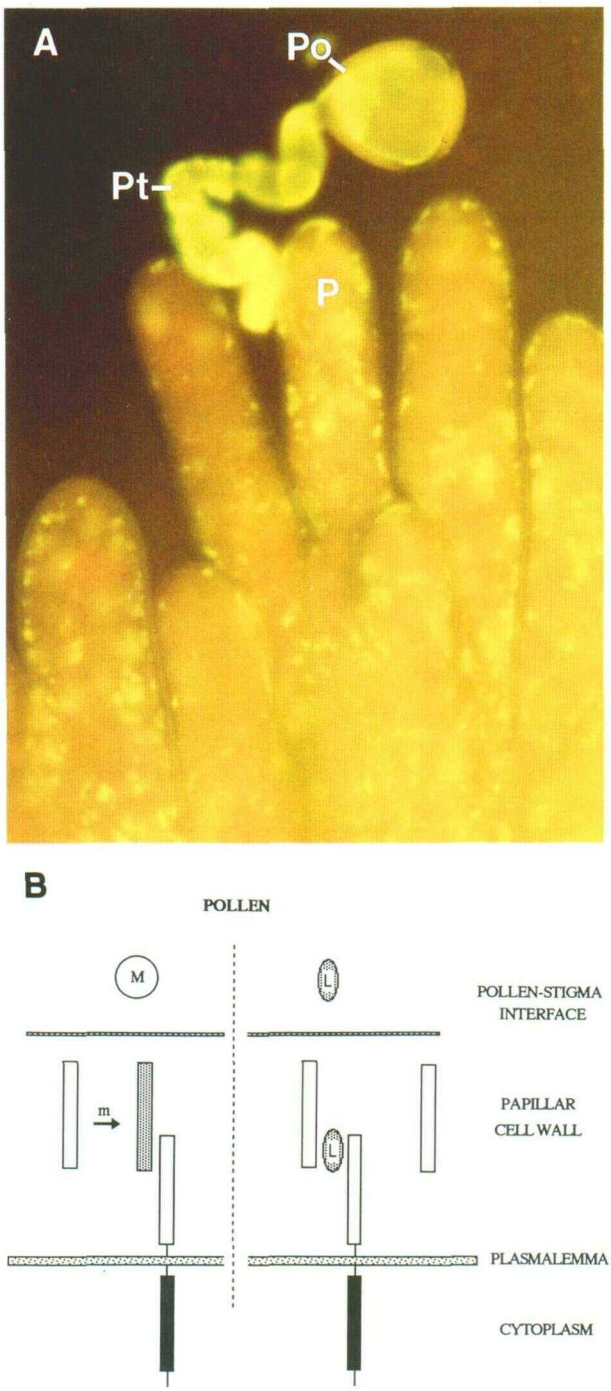

Figure 3. Specificity of the SI Response.

(A) A micrograph showing the inhibition of self-pollen (Po) and the inability of the emerging pollen tube (Pt) to invade the papillar cell (P) wall.

(B) Two models of *S* gene action. SRK and SLG cooperate in the papillar cell to transduce a signal emanating from pollen. Activation of receptor kinase activity at the papillar cell–pollen interface is postulated to occur by either of two mechanisms. To the left of the dotted line, activation of SRK is depicted as resulting from the action (m) of a pollen-derived modifying activity (M) that acts on SLG to allow its interaction with SRK. To the right, activation of SRK is depicted as resulting from the interaction of a pollen-derived ligand (L) with SLG and SRK. White box, SLG; stippled box, modified SLG; white box connected to black box, SRK.

action of the receptor. In the absence of hard data, we can only speculate on the nature of such a molecule. The putative ligand could belong to a class of nonproteinaceous signaling molecules such as those involved in plant–*Rhizobium* interactions (Lerouge et al., 1990). However, because of the similarity of SRK to animal receptor tyrosine kinases, which, as a rule, are activated by protein ligands, its ligand could turn out to be a polypeptide. Furthermore, by virtue of the specificity and single-locus control of the SI response, the ligand may be polymorphic and encoded in the *S* locus complex. Such a finding would reinforce the analogy of the *Brassica S* locus to the mammalian major histocompatibility complex and would constitute a plant model system for ". . . the concept of multiple polymorphic loci, encoded in a complex, giving rise to one concerted function of recognition and triggering . . ." (Dangl, 1992).

COMPATIBLE POLLEN-STIGMA INTERACTIONS

SI is a signaling system that overrides pollen germination and pollen tube growth through pistil tissues. But what of compatible pollinations? Little is known of the genes required for successful pollen tube development in general. As described earlier, genes related to the *S* locus genes are expressed in reproductive tissues of *Brassica* (Lalonde et al., 1989; Trick and Flavell, 1989; Boyes et al., 1991; Guilluy et al., 1991; Isogai et al., 1991) and Arabidopsis (Dwyer et al., 1992). In addition, proteins related to SLG have been detected in *Brassica* pistils (Isogai et al., 1988; Umbach et al., 1990) and anthers (Watanabe et al., 1992), and a nonpolymorphic pollen peptide that appears to interact with the pistil molecules in vitro has been described (Doughty et al., 1993). The roles of these genes and their products in pollination are not understood. Their expression in reproductive tissues makes it conceivable that the *SLR* genes are involved in the interaction of the pollen tube with cells of the pistil that line its path through the transmitting tissue of the style and ovary and into the funiculus as it grows toward the ovules.

Some questions relating to the basis of compatible pollen tube development have been addressed in genetic ablation experiments aimed at investigating the role of papillar cells in the early pollination events of crucifers. A chimeric gene consisting of the *SLG* promoter fused to the cytotoxic DT-A subunit was introduced into two self-fertile host plants, the Westar cultivar of *B. napus* (Kandasamy et al., 1993) and Arabidopsis (Thorsness et al., 1993). As expected from the pattern of *SLG* promoter activity, transgenic plants of both genera

exhibited specific abnormalities in pistils and anthers. In particular, the pistils of these plants exhibited stunted and biosynthetically deficient papillar cells. Significantly, along with their biochemical dysfunction in transgenic *Brassica*, the ablated papillar cells had lost the capacity to support pollen tube growth. By contrast, the ablated papillar cells of transgenic Arabidopsis were still receptive and allowed pollen tube development in appropriate crosses. Similar differences between the two genera were observed by treatment of flower pistils with protein phosphatase inhibitors, which inhibited pollen tube development in *Brassica* but not Arabidopsis.

Thus, different mechanisms of pollen perception operate in *Brassica* and Arabidopsis despite the similarities between the two plants in the early events of pollination and the path followed by the pollen tube through the tissues of the pistil (Elleman et al., 1992). Metabolically active papillar cells are required for successful pollen tube growth in *Brassica* but are not of critical importance to pollen tube growth in Arabidopsis. The importance of the phosphorylation state of stigmatic proteins to the success of compatible pollinations in *Brassica* indicates further that pollen acceptance in self-fertile strains of this genus is dependent on the operation of a signal transduction system that is distinct from that which operates in SI. Such a signaling system apparently does not operate in Arabidopsis.

FUTURE DIRECTIONS

In this review, we have shown that a definition of the molecular events operating in the signaling between pollen and papillar cells in crucifer species has begun to emerge. The isolation of genes derived from the *Brassica S* locus has been crucial to progress in this area. Future advances in our understanding of the pollen–stigma interaction of SI and its mediation by the large array of naturally occurring *S* haplotypes will depend on a thorough characterization of the *S* locus complex. It will be important to determine whether the *SLG* and *SRK* genes are the only genes required to express a haplotype specificity or whether, as seems more likely, additional genes are encoded in the complex. Recently, the successful transfer of yeast artificial chromosomes (YACs) carrying 250 kb of genomic DNA into mice by pronuclear injection of gel-purified YAC DNA has been reported (Schedl et al., 1993). The application of this technology to plants should significantly increase our ability to define the chromosomal region that encodes an *S* haplotype and, ultimately, to manipulate pollination phenotype.

The discovery of the *S* locus–linked *SRK* gene has suggested a testable model for *S* gene action and has indicated that the rigid wall of plant cells does not preclude signaling via membrane-anchored receptors that are structurally similar to those operating in other biological systems. The involvement of a receptor protein kinase in SI also raises several questions. Does SRK activation require a ligand? If so, what is the nature of this ligand? Is it haplotype-specific and encoded at the *S* locus? What are the downstream events that result in the inhibition of self-pollen? Does SRK-mediated signaling involve changes in calcium ions, the concentrations of which are critical for pollen tube growth? Or does it involve the establishment at the papillar cell surface of a localized physical barrier to pollen tube invasion by inducing the cross-linking of a cell wall component or the activation of callose synthase and the deposition of callose? Is there a role for an SLG/SRK signaling complex in pollen, or is the activity of these molecules in papillar cells sufficient for pollen inhibition? If the signaling pathway is active in pollen, does it target the organization of cytoskeletal components known to be important in tip growth? The continued application of molecular, genetic, and biochemical methodologies, the availability of a rich array of haplotypes, and the ability to manipulate the interacting cells experimentally will no doubt reveal much about how the signals are processed in papillar cells and pollen.

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