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Investigating the Molecular Mechanism of the Self-incompatibility Response in *Brassica*

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The self-incompatibility response has been defined as the inability of a fertile hermaphrodite seed-plant to produce zygotes after self-pollination. Many members of the genus *Brassica* exhibit sporophytic self-incompatibility, rejection of self-pollen occurring on the stigma surface. Over the last 15 years a number of genes have been implicated in the self-incompatibility response in *Brassica*. These include both genes at the *S* locus, which are potentially involved in the recognition of self-pollen, and genes at unlinked loci, which are though to be involved in processes downstream of the recognition event such as signal transduction and self-pollen rejection. Here we review data from recent studies that have focused on determining the function of these genes, and their respective gene products, in the self-incompatibility response.

Key words: *Brassica oleracea*, cell recognition, flower, kale, pollination, receptor protein kinase, self-incompatibility, signal transduction, *S* locus glycoprotein.

INTRODUCTION

The phenomenon of self-incompatibility (SI) in flowering plants has fascinated biologists for over two centuries. Although a great deal has been learnt about SI since Charles Darwin described it as 'one of the most surprising facts I have ever observed' (quoted in Matton *et al.*, 1994), a relatively large number of questions still remain unanswered. The history of the investigation of SI has many of the qualities of a good detective story, with painstaking analysis of clues, clever insights and a few startling twists in the plot. In the last 15 years, the identification of *S* locus genes from a number of different systems has opened up the possibility of understanding these systems at a molecular level (for recent reviews see McCubbin and Kao, 1996; Nasrallah, 1997; McCormick and Gaude, 1999). However, these advances have also added new complexities and raised new questions. If anything, in recent years, work on SI, and particularly on SI in *Brassica*, has come to resemble more and more the complex story of a police thriller with many twists to the plot, some exciting climaxes and, of course, some characters who turn out not to be what they seem. The aim of this paper is to describe recent work investigating the molecular mechanism of SI in *Brassica* and to attempt to clarify the roles of the genes identified by these studies.

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POLLEN REJECTION ON THE STIGMA SURFACE: THE SCENE OF THE CRIME

In compatible cross-pollinations, when a pollen grain arrives on the stigma surface, it adheres, hydrates and emits a pollen tube. The pollen tubes grows through the stigma, carrying the male gametes to the ovary where they will fertilize an ovule. These processes are blocked when a self-pollen grain arrives on the stigma of a self-incompatible *Brassica* plant. The rapidity with which the pollination process is blocked depends both on genetic and environmental factors so that the self-pollen grain may either fail to hydrate completely, may hydrate but fail to germinate or, in some cases, germination may occur but the pollen tube will usually fail to penetrate the stigma surface.

Although the above analogy between research in recent years into the mechanism of self-incompatibility in *Brassica* and the plot of a detective novel is apt in many respects, there is one important difference that adds an additional twist to the plot. This is that, although the arrest of pollen on a self-incompatible stigma may resemble 'murder most foul', all is not what it seems and the arrested pollen grain, in fact, remains viable for at least 24 h and can continue its development if SI breakdown is induced by treatment with cycloheximide (Sarker *et al.*, 1988). Therefore, in *Brassica*, and in contrast to other SI systems that have been studied, SI appears to act by a cytostatic rather than cytotoxic mechanism and models of the molecular mechanism of the response need to take this fact into account.

In *Brassica* recognition of self-pollen by the SI system is controlled by a single, highly polymorphic genetic locus, the

S locus. Alleles of the *S* locus are referred to as haplotypes because of the complex nature of this locus (see below). Dominance interactions occur between *S* haplotypes in a heterozygous state and haplotypes can be ordered in a non-linear dominance hierarchy (Thompson and Taylor, 1966). Two major groups can be distinguished in the dominance hierarchy: class I haplotypes tend to be dominant and confer a strong SI phenotype whereas class II haplotypes tend to confer a weaker phenotype and to be recessive to the class I haplotypes.

S LOCUS GENES EXPRESSED IN STIGMAS: RINGLEADERS AND OTHER SUSPICIOUS CHARACTERS

A major first step in the molecular analysis of the *S* locus was the identification of the *S* locus glycoprotein (*SLG*) gene (Nasrallah *et al.*, 1985). Analysis of proteins extracted from the stigmas of plants carrying different *S* alleles had revealed the presence of abundant, polymorphic glycoproteins characteristic of each *S* allele. Using this information, an *SLG* cDNA was identified using a differential screening approach. Subsequent analysis of *SLG* alleles from different haplotypes has shown that *SLG* is highly polymorphic (Nasrallah *et al.*, 1987; Kusaba *et al.*, 1997). Based on sequence data, *SLG* alleles can be grouped into two classes which correspond to the class I and II groups defined on the basis of SI phenotype. Stigmas acquire the ability to reject self-pollen as they mature. *SLG* has been shown to be expressed specifically in pistils and the accumulation of SLG protein coincides with the acquisition of the SI phenotype. The majority of the SLG protein accumulates in the stigma although SLG can also be detected in the transmitting tissue of the style and ovary (Kleman-Mariac *et al.*, 1995). The only other organ in which *SLG* transcripts have been detected is anthers but SLG protein does not accumulate to a detectable level (Delorme *et al.*, 1995).

The identification of *SLG* was important for several reasons. In addition to encoding a potential component of the SI response, it was the first molecular marker to be identified at the *S* locus and it was also the first member of a large gene family in plants, the S gene family. Both of these latter characteristics were important in the identification of a second *S* locus gene, the *S* locus receptor kinase (*SRK*) gene. *SRK* is an *S*-locus-linked gene that shares sequence similarity with *SLG* and which encodes a plasma-membrane anchored glycoprotein that resembles animal receptor kinases (Stein *et al.*, 1991). The domain with similarity to SLG (which has been designated the S domain and is present in all members of the S gene family) is predicted to be extracellular and to be separated from a cytosolic kinase domain by a single, membrane-spanning alpha helix. Analysis of a number of different alleles of *SRK* have shown that, like *SLG*, this gene is highly polymorphic and that *SRK* alleles can be grouped into two classes that correspond to the class I and class II groups defined on the basis of SI phenotype (Stein *et al.*, 1991; Kusaba *et al.*, 1997; Cabrillac *et al.*, 1999). SRK protein is only detected in stigmas. Low levels of *SRK* transcripts can be detected in anthers but no SRK protein has been detected in this organ. *SRK*, therefore, shares many characteristics with *SLG*. When *SLG* and *SRK* were first identified, the many similarities between these two genes (map position, expression pattern, subcellular localization of their gene products) indicated strongly that both of their protein products functioned in the stigma to recognize self-pollen. This hypothesis was supported by the fact that, in some *S* haplotypes, *SLG* and *SRK* were shown to have evolved convergently so that the S domains of the two genes are more similar to each other than they are to those of other *SLG* and *SRK* alleles.

Recent analysis has shown that both *SLG* and *SRK* are complex genes and that certain alleles of both genes encode more than one protein product. For example, in the class I S_3 haplotypes, SRK_3 has been shown to encode at least seven different transcripts including transcripts from both strands of the gene (Delorme *et al.*, 1995; Cock *et al.*, 1997). Several of the sense transcripts retain all or part of the first intron and, because there is a termination codon just after the 5′ end of the intron, are predicted to encode a soluble, truncated form of SRK that corresponds to the predicted extracellular domain (Giranton *et al.*, 1995). This truncated form of SRK, which has been called eSRK for extracellular SRK, resembles SLG. The function of eSRK is unknown but it is interesting that no eSRK protein was detected in stigmas of plants homozygous for the class II haplotype S_{15} (Cabrillac *et al.*, 1999). Analysis of SRK alleles from other haplotypes will be necessary to determine whether eSRK is associated exclusively with class I *S* haplotypes.

Tantikanjana *et al.* (1993) showed that the *SLG* allele of the class II S_2 haplotype possesses two exons and that alternative transcripts of this allele can encode both a secreted form of SLG and a membrane-anchored form designated mSLG. The *SLG* allele of the class I S_6 haplotype, on the other hand, was shown to possess only one exon and to encode only a secreted form of SLG. Based on these observations it was suggested that the presence or absence of mSLG may determine whether a haplotype is dominant or recessive, respectively.

A more recent study has shown that the information obtained from the S_2 haplotype cannot be extrapolated to all class II haplotypes (Cabrillac *et al.*, 1999). The class II S_{15} haplotype was shown to include two different *SLG* genes, *SLGA* and *SLGB* (Fig. 1A). Both of these genes possess two exons interrupted by a single intron but only *SLGA* possesses a second exon that encodes a membrane-spanning domain. *SLGA*, therefore, is predicted to encode a secreted SLGA protein and a membrane-anchored mSLGA protein whereas *SLGB* only encodes a secreted SLGB protein. This prediction was confirmed by an analysis of the proteins present in S_{15} stigmas. Comparison of S_{15} with two other class II haplotypes, S_2 and S_5, indicated that these two haplotypes lacked the *SLGB* and *SLGA* genes, respectively (Cabrillac *et al.*, 1999; Fig. 1A). This was consistent with the absence of an mSLG protein in stigmas of the S_5 homozygous line. These observations indicate that mSLG need not necessarily be present for a haplotype to show a class II phenotype. The role of the mSLG protein, therefore, remains unclear at present.

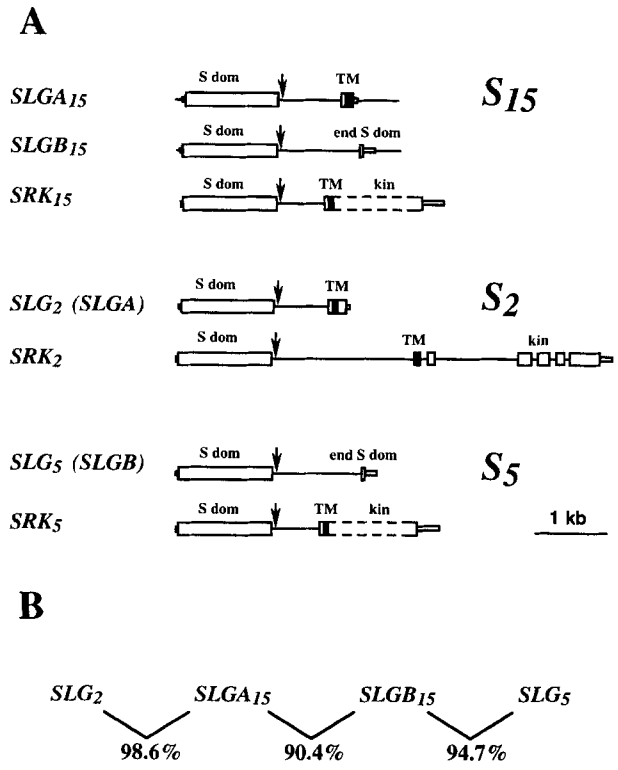

FIG. 1. Comparison of the structures and sequences of *SLG* and *SRK* alleles of three class II haplotypes. A, Schematic representation of *SLG* and *SRK* alleles of three class II *S* haplotypes. Two different *SLG* genes, *SLGA* and *SLGB*, have been identified in the class II S_{15} haplotype (Cabrillac *et al.*, 1999). The S_2 haplotype includes an allele of *SLGA* but not *SLGB* whereas the S_5 haplotype includes an allele of *SLGB* but not *SLGA*. *SLGA* encodes both secreted and membrane-anchored proteins (SLGA and mSLGA, respectively) whereas *SLGB* encodes only secreted SLG proteins (SLGB). Transcribed regions and coding regions are indicated by narrow boxes and thick boxes, respectively. The exon/intron structure of the kinase domains of SRK_{15} and SRK_5 has not been determined and these regions are therefore represented by dotted lines. Membrane-spanning domains (TM) are indicated by black boxes. Arrows indicate stop codons near the 5′ ends of introns that potentially allow the production of truncated proteins from alternatively spliced transcripts that retain all or part of the intron sequences. S dom, S domain; kin, kinase domain. B, Percentage amino acid similarity between alleles of *SLGA* and *SLGB*.

Another conclusion from this study stems from the fact that the S_2 and S_5 haplotypes lack *SLGB* and *SLGA* respectively (Fig. 1A). This suggests that either (1) these genes are redundant or (2) neither is required for the SI response. Moreover, the fact that the *SLGA* and *SLGB* alleles of the S_{15} haplotype are more similar to SLG_2 and SLG_5, respectively, than they are to each other argues against redundancy (Fig. 1B). The conclusions from this study conflict with those of previous studies that have proposed a key role for SLG in the SI response. In the following paragraphs we will review the data available concerning the function of SLG in an attempt to resolve this contradiction.

Several pieces of circumstantial evidence imply a role for *SLG* in the SI response. These include its location at the *S* locus, the fact that SLG protein accumulates specifically in pistils (and particularly in the stigma), the highly polymorphic nature of *SLG* and evidence for convergent evolution of *SLG* and *SRK* in some *S* haplotypes. However, although several groups have attempted to demonstrate directly a role for *SLG* in the SI response by transgenic or genetic approaches, the results of these studies have been ambiguous (Toriyama *et al.*, 1991; Nasrallah *et al.*, 1992; Nishio *et al.*, 1992; Shiba *et al.*, 1995; Conner *et al.*, 1997). A correlation has been reported between loss of *SLG* expression and the acquisition of a self-compatible phenotype using both approaches, but the interpretation of these results is often complicated by the fact that *SRK* expression may also have been affected. Simultaneous effects on the two genes are highly likely because of their shared sequence similarity, and cosuppression of *SLG* and *SRK* has been described (Conner *et al.*, 1997; Stahl *et al.*, 1998). In addition, recent work described by June Nasrallah (Cornell University, NY, USA) at the Pollen–Stigma Interactions Meeting indicates that SLG is required for correct post-transcriptional expression of SRK, at least in some genetic backgrounds. As a result of these phenomena it may be difficult to distinguish between direct effects of SLG and indirect effects via SRK. For example, in one study using self-compatible plants homozygous for a mutant allele of the suppresser gene *SCF1* the abundance of *SLG* transcripts in stigmas was shown to be significantly reduced and no SLG protein was detectable whilst *SRK* transcripts accumulated to a normal level (Nasrallah *et al.*, 1992). However, the abundance of SRK protein in stigmas of this line was not determined and the new data presented by the Cornell University group suggests that the self-compatible phenotype may have been due to disruption of post-transcriptional expression of SRK, rather than a direct result of the absence of SLG.

Arguments against a role for SLG in the SI response include evidence from the analysis of class II lines described above and a study by Gaude *et al.* (1995) which showed that the level of expression of *SLG* is not correlated with the strength of the SI response. SLG was abundant in stigmas of a self-compatible line homozygous for the S_{15} haplotype but weakly expressed in self-incompatible S_2 plants. This study indicated that, if SLG does play a role in the SI response, it must be capable of functioning at low abundance (or that there is redundancy and other stigma proteins can substitute for SLG). Finally, at the Pollen–Stigma Interactions Meeting, Takeshi Nishio's group (Tohoku University, Sendai) reported the characterization of two self-incompatible *Brassica* lines that carry *S* haplotypes with defective *SLG* genes. These data provide the most convincing argument yet against a role for SLG in the haplotype-specific recognition of self-pollen.

Recently, another clue to the role of SLG has come, unexpectedly, from work on the *S* locus related 1 (*SLR1*) gene. *SLR1* is not genetically linked to the *S* locus but encodes a protein which resembles SLG. Analysis of transgenic plants expressing antisense *SLR1* transcripts and experiments involving treatment of stigmas with an anti-SLR1 antibody indicated that SLR1 has a role in

pollen adhesion (Luu *et al.*, 1999). Treatment of stigmas with an anti-SLG antibody also reduced the strength of pollen adhesion (Luu *et al.*, 1999). In both cases the force of pollen adhesion was only partially reduced indicating that several factors, including the proteins SLR1 and SLG, contribute in an additive manner to pollen adhesion. Luu *et al.* (1997) have shown that the strength of pollen adhesion is not influenced by the self-incompatibility response. Therefore, a function for SLG in pollen adhesion would be independent of the SI system.

If SLG does not play a direct role in the recognition of self-pollen it becomes necessary to explain why the *SLG* and *SRK* alleles of some haplotypes exhibit convergent evolution. One possible explanation for this phenomenon may be that it is unrelated to gene function and is simply due to a combination of reduced recombination between different haplotypes of the *S* locus and the tendency of linked genes to exchange sequence information via gene conversion (see Cabrillac *et al.*, 1999).

If the evidence is now against a direct involvement of *SLG* in haplotype-specific recognition of self-pollen, what about *SRK*? The circumstantial evidence implicating *SLG* in the SI response is also valid for *SRK*. Like *SLG*, *SRK* is located at the *S* locus, is expressed specifically (at the protein level) in stigmas and exhibits a high level of DNA polymorphism. Moreover, the resemblance between SRK and animal receptor kinases is consistent with a role in a cell–cell recognition system. More direct evidence for an involvement of SRK in SI has come from the analysis of self-compatible *Brassica* lines. Two examples have been reported of non-functional *SRK* alleles in such lines indicating a correlation between loss of SRK function and acquisition of a self-compatible phenotype (Goring *et al.*, 1993; Nasrallah *et al.*, 1994). Importantly, however, in neither case was the self-compatible phenotype shown to be a direct result of the *SRK* mutation and it is possible that other genes are mutated in these lines. Efforts to express novel SRK alleles or dominant negative forms of SRK in transgenic plants have run into problems due to insufficient expression and cosuppression (Stein *et al.*, 1991; Conner *et al.*, 1997; Gaude and Cock, unpubl. res.). The most convincing evidence that SRK is involved in the SI response has been obtained by Stahl *et al.* (1998) who observed haplotype-specific breakdown of SI in a *B. napus* plant transformed with a kinase-defective *SRK* gene. However, the breakdown of SI in these experiments was partial and the observations were made on a single transgenic line. It will be important to demonstrate a similar phenotype in additional independent transformants.

In summary, the evidence 'convicting' *SRK* of involvement in the recognition of self-pollen is now very convincing. On the other hand, and despite a considerable amount of circumstantial evidence implicating *SLG* in the same process, new evidence suggests that this gene is innocent of the accusations that were originally made against it and is unlikely to be involved directly in the recognition of self-pollen. SLG may, however, be an accessory to the 'crime', acting in a supporting role to SRK.

THE ELUSIVE MALE COMPONENT: RED HERRINGS AND INNOCENT BYSTANDERS

One of the mysteries surrounding the *Brassica* SI system that has been most difficult to crack has been the nature of the male component of the SI interaction. Based on genetic evidence, the *S* locus is predicted to encode a factor that is carried by the pollen and recognized when the pollen interacts with the stigma. SI in *Brassica* is sporophytic so that the pollen carries factors corresponding to both *S* haplotypes present in the diploid genome of the male parent. Several mechanisms have been proposed to explain the sporophytic nature of SI in *Brassica*. Pandey (1959) suggested that the male component of the SI response is synthesized in the diploid progenitors of the microspores before meiosis and is then inherited by the maturing pollen grains. An alternative hypothesis, proposed by Heslop-Harrison (1975), is that the male component accumulates in the diploid cells of the tapetum which line the locule in which the microspores develop. According to this hypothesis, the male component is released when the tapetum breaks down at around the binucleate stage of microspore development, and is deposited onto the exterior of the pollen grain along with the other tapetum-derived components of the pollen coat. A third model, proposed recently by Doughty *et al.* (1998), suggests that the gene that encodes the male component may actually be expressed during the gametophytic stage of the life cycle, in the developing microspores, but that the male component is shared between microspores in the same locule so that each mature pollen grain carries components encoded by the two segregating *S* haplotypes.

Whichever of these models is correct, the rapidity of the SI response following the arrival of a self-pollen grain on the stigma indicates that the male component is on the exterior of the pollen grain and immediately accessible to the papillar cell. Experimental evidence to support this has recently been obtained by Stephenson *et al.* (1997) who showed, using a bioassay, that isolated pollen coats can modulate the SI response *in vivo*. Fractionation experiments indicated that the male component is a small molecule with a molecular mass of probably less than 10 kDa. The fraction that was active in the bioassay also contained PCP-A1 (previously named PCP7), a highly basic, 7 kDa protein that has been shown to interact with SLG (Doughty *et al.*, 1993). PCP-A1 does not bind to SLG in a haplotype-specific manner and, moreover, the gene encoding PCP-A1 is not linked to the *S* locus. PCP-A1 is, therefore, unlikely to be the male component. However, *PCP-A1* is a member of a large gene family in *Brassica* (Stanchev *et al.*, 1996) and it is possible that another member of this family encodes a male component of the SI recognition system.

Several groups have been using an alternative approach to look for the gene encoding the male component by walking along the chromosome at the *S* locus using *SLG* and *SRK* as starting points. These analyses have revealed several interesting features of the *S* locus region of the chromosome. Comparison of different *S* haplotypes has revealed extensive rearrangements and sequence divergence in the region surrounding *SLG* and *SRK* (Boyes *et al.*,

1997) and there is evidence that polymorphism between certain haplotypes may extend to a region of over 500 kbp (Boyes and Nasrallah, 1993). The region surrounding *SLG* and *SRK* has been found to include several expressed genes although the majority of these genes do not appear to be polymorphic and/or are expressed in organs other than anthers (Yu *et al.*, 1996; Boyes *et al.*, 1997; Suzuki *et al.*, 1997). It is therefore unlikely that they encode the male component of the SI system. In contrast, the *S* locus anther gene, *SLA*, seemed to be a much better candidate when it was first identified for several reasons (Boyes and Nasrallah, 1995). Firstly, it was shown to be located at the *S* locus; *SLA* was first identified 500 bp downstream of *SLG* in the S_2 haplotype. Secondly it was shown to be expressed specifically in anthers; two transcripts that are antisense with respect to each other accumulate specifically in anthers at different stages of development. Thirdly, one of the *SLA* transcripts was shown to include two short open reading frames that could encode short peptides. Fourthly, *SLA* seemed to be highly polymorphic in as far as an *SLA* probe only hybridized to DNA from a small number of haplotypes. Finally, and probably most convincingly, a self-compatible *B. napus* line was shown to carry an *SLA* allele interrupted by a large insertion.

A more recent study carried out in our laboratory, however, indicates that *SLA* is, in fact, only present in a limited number of *S* haplotypes and that it exhibits a very low level of polymorphism (Pastuglia *et al.*, 1997). Moreover, a number of self-incompatible cauliflower lines were identified that carried an allele of *SLA* which was interrupted by a retrotransposon (similar to the allele found in *B. napus*). These data indicate that *SLA* is not required for the SI response and, unfortunately, it is hence unlikely that it encodes the male component.

THE *MODUS OPERANDI*

The previous sections described the genes that have been identified at the *S* locus and the experiments that have been carried out to determine whether they are implicated in the SI response. However, to use the detective story analogy again, these investigations may have identified some interesting suspects but do not tell us how the 'crime' was actually committed. To answer this we need not only to identify the molecules involved in the recognition process but to have a deeper understanding of how these molecules function. A relatively crude model for the molecular mechanism of self-pollen recognition can be proposed based on the structural resemblance of SRK to animal receptor kinases. In this model, the extracellular (S) domain of SRK would act as a receptor and recognize a pollen-borne ligand. Ligand binding would activate the cytosolic kinase domain leading to initiation of the SI response via a signalling cascade.

The data that are currently available for SRK are consistent with this model in as much as the kinase domain of SRK has been shown to possess a serine/threonine protein kinase activity when it is expressed in *Escherichia coli* (Goring and Rothstein, 1992) and SRK has been shown to encode a glycoprotein that is localized in the plasma-membrane in stigmas (Delorme *et al.*, 1995; Stein *et al.*, 1996; Cabrillac *et al.*, 1999). Recently, work carried out in our laboratory, involving expression of kinase-active and kinase-inactive recombinant forms of SRK fused to different epitope tags in a baculovirus/insect cell system, has shown that one molecule of SRK can phosphorylate another SRK molecule in a membrane environment (Giranton, Cock, Dumas and Gaude, unpubl. res.). In addition, evidence has also been obtained, using both cross-linking and velocity sedimentation methodology, showing that SRK is part of a complex *in planta* (Giranton *et al.*, unpubl. res.). These are important observations because the role of oligomerization and trans-phosphorylation in the activation of animal receptor kinases is well established (Heldin, 1995). These experiments, therefore, provide the first biochemical support for models in which SRK functions in a manner analogous to animal receptor kinases.

FROM SELF-POLLEN RECOGNITION TO SELF-POLLEN REJECTION: MIDDLEMEN AND TRAFFICKERS

To date, the majority of the work on molecular aspects of SI in *Brassica* has concentrated on the molecules involved in pollen–pistil recognition; very little is known about how the rejection of self-pollen is mediated. In one approach to identify components acting downstream of SRK in the stigma, Daphne Goring's group (York University, Ontario, Canada) have been using the two hybrid system to identify proteins that interact with the cytosolic kinase domain of SRK (Bower *et al.*, 1996; Gu *et al.*, 1998). Three interacting proteins have been identified: two thioredoxin-h-like proteins (THL-1 and THL-2) and arm repeat containing protein 1 (ARC 1) which contains arm repeats similar to those found in the *Drosophila* armadillo protein and β-catenin. ARC1 is perhaps the most interesting of these proteins for a number of reasons: (1) it is expressed specifically in stigmas; (2) it only associates with the phosphorylated form of the SRK kinase domain; and (3) it is specifically phosphorylated by the kinase domain of SRK *in vitro*. Recent work, involving the analysis of transgenic plants carrying antisense ARC1 constructs, was described by the York University group at the Pollen–Stigma Interactions Meeting. A partial breakdown of the SI response was observed in these plants indicating that ARC1 is indeed a component of the SI response.

Based on genetic analysis, the genes directly involved in the recognition step of the SI response are predicted to be located at the *S* locus but genes involved in other steps of the response such as self-pollen rejection need not necessarily be genetically linked to the *S* locus. Indeed, the self-incompatibility response is known to be influenced by a number of unlinked modifier and suppresser loci in addition to the *S* locus (Nasrallah, 1989; Nasrallah *et al.*, 1992). Analysis of these loci provides an alternative approach to identify genes involved in other stages of the SI response.

Using differential display reverse transcription-polymerase chain reaction (DDRT-PCR) analysis, Ikeda *et al.*

(1997) identified an aquaporin-like gene which is expressed in stigmas of wild-type plants but not in self-compatible plants homozygous for a mutant allele of the modifier gene *MOD* (Hinata *et al.*, 1983). The gene encoding the aquaporin-like protein was shown to be very closely linked to the *MOD* locus. Ikeda *et al.* (1997) proposed that the aquaporin-like gene is *MOD* and suggested that its gene product may be involved in controlling the hydration of self-pollen.

This is a very exciting result in that it potentially identifies a novel component of the SI system. However, the implications of the involvement of this protein in the SI response are complex because it is necessary to account for the fact that loss of the protein results in self-compatibility. If a lesson has been learnt from the analyses of the *SLG* and *SLA* genes it is that one should be prudent in assigning a role in the SI response. For this reason, and because of the complexity of the models that explain how the aquaporin-like gene could be implicated in the SI response, it will be important to test these models experimentally and to confirm that the aquaporin-like gene is identical to *MOD*.

FUTURE DIRECTIONS: THE PLOT THICKENS

Despite a number of setbacks and encounters with unexpected complexities a picture is starting to emerge of how rejection of self-pollen is mediated at the molecular level, at least on the female side. However, several questions remain to be answered, the most important being the nature of the male component of the SI response. At the Pollen–Stigma Interactions Meeting, several groups described their ongoing efforts to identify the male component using approaches ranging from differential screening, to mutagenic approaches and chromosome walking at the *S* locus, to biochemical approaches and bioassays. Considering the effort that is going into this work, it is likely that the male component will be identified in the near future.

The nature of the male component is not the only question that is currently being addressed. One important aspect of the SI response, about which very little is known, is the mechanism of self-pollen rejection following recognition by the stigma. Some recent advances in this area have been described above. Work in this area may help explain why some *S* haplotypes are early acting, causing rapid arrest of pollination, whilst others are late acting. Are these differences due to the SI system creating several 'barriers' to pollination at different stages of the pollination process (hydration, germination, pollen tube penetration, etc.) or do the differences in the stage at which arrest occurs reflect a delayed effect of the SI system acting more or less 'strongly', early in the pollination process?

Many other questions will become easier to address as we learn more about how SI works at the molecular level. An obvious example is the molecular basis of dominance between *S* haplotypes. The evidence that SRK molecules are able to associate with each other in an oligomeric complex suggests a possible mechanism for dominance in stigmas. Other questions of this type concern the mechanism of the breakdown of SI under certain environmental conditions, the molecular basis of *S* haplotype specificity and the evolutionary origin of the SI system.

Current research aimed at understanding the molecular mechanism of SI builds on genetic and physiological studies that have been carried out over the past few decades. It is fitting that the Pollen–Stigma Interactions Meeting, which provided such an exciting forum to discuss the recent developments in this area, should have been dedicated to the memory of Jack Heslop-Harrison who made such an important contribution to the laying down of this foundation.

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