Self-incompatibility and other pollen–pistil interactions

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Self-incompatibility allows plants to recognize and reject pollen from the same plant, thereby reducing inbreeding. Although in most cases self-incompatibility is controlled by a single genetic locus, recent results show that surprisingly complex signal transduction pathways and many players are involved in pollen recognition and rejection.

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Abbreviations
SLA S-locus anther
SLG S-like glycoprotein
SRK S-receptor kinase

Introduction

Pollen–pistil interactions provide an excellent system for studying cell–cell interactions. Although most work has emphasized the pollen rejection events in self-incompatibility, understanding the interactions during compatible (successful) pollinations is of equal importance and will increase our understanding of self-incompatibility.

Self-incompatibility is usually encoded by a single, multiallelic *S* locus that is composed of one or more male and/or female expressed genes. Allelic differences in the proteins encoded by these genes are believed to be the basis for the recognition of self or non-self pollen. The goal in all studies of self-incompatibility is to identify the protein components on each side (male and female) of the interaction. Surprisingly, this has not yet been achieved for any self-incompatibility system. In this review, I summarize recent work in the fields of self-incompatibility and pollen tube growth, and point out promising directions for future work.

Self-incompatibility

Self-incompatibility has been extensively reviewed; most reviews have emphasized the sporophytic self-incompatibility system [1,2] exemplified by *Brassica* species, and the gametophytic self-incompatibility system [3] exemplified by Solanaceous species such as tobacco and petunia (Figure 1). Other self-incompatibility systems, however, have also been studied and have provided useful advances or insights [4]. For example, in the gametophytic self-incompatibility system in poppy, where the gene encoding the style glycoprotein has been cloned, some information is available about the presumed signal transduction pathway that occurs on the pollen side [5,6]. In the grasses, self-incompatibility is gametophytic but two unlinked loci (*S* and *Z*) are required; one gene from the *S* locus has been cloned [7].

Gametophytic self-incompatibility and RNases

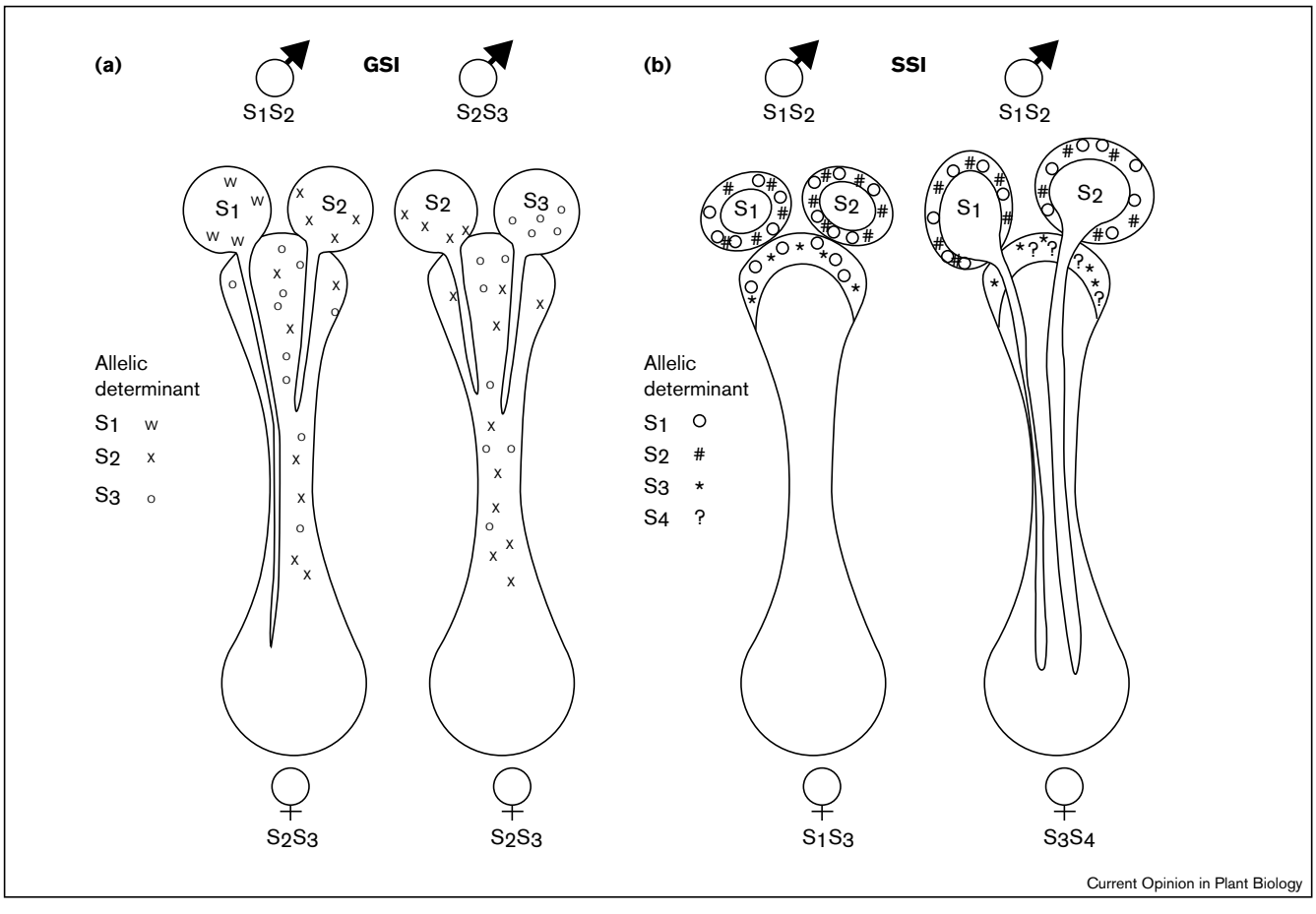
In the Solanaceae (and Roseaceae) the *S* locus encodes polymorphic RNases that are secreted into the style matrix. RNase activity is required for the incompatible interaction. The most favored model (the cytotoxic model) [3] proposes that the RNase is taken up by the pollen tubes and that, because ribosomal RNA is not synthesized in pollen tubes, the RNase degrades the existing rRNA, thereby causing protein synthesis and pollen tube growth to be inhibited in the upper part of the style. In a recent, provocative paper [8••], however, the results of grafting experiments between incompatible and compatible styles of *Nicotiana alata* were used to challenge some of the underlying premises of this model. This work showed that pollen tubes are not necessarily arrested in the upper third of the style and that incompatible pollen tubes can be inhibited at various times during pollen tube growth and at different locations along the style. Furthermore, at least some of the arrested pollen tubes in an incompatible style could re-initiate growth if the incompatible style was grafted to a compatible style. These results conflict with the idea that RNA synthesis is irretrievably blocked by the uptake of the RNase.

Looking for the pollen S component

What is the factor from the pollen side of the interaction? Although the S-RNase is very weakly expressed in anthers [9], it is generally believed that the pollen S component is not also the RNase, but a closely linked gene. Evidence in support of this idea comes from analysis of a self-compatible mutation in Japanese pear, in which the pistil self-incompatibility response was lost. In this mutation the S-RNase gene was deleted from the genome, but the pollen S component was still functional [10].

The Solanaceae S-RNases of different alleles have both highly variable and conserved domains. To identify the recognition domain for the pollen S component, chimeras between two different S-RNases of *N. alata* (S_{a2} and S_{c10}) were constructed [11]. None of the resulting transgenic plants, however, rejected either S_{a2} or S_{c10} pollen. These results suggested that there is no single allele specificity domain, but rather that the whole RNase moiety determines specificity. Perhaps these *N. alata* RNases were too different from each other. Exciting work [12••] with *Solanum chacoense* has now shown that allelic specificity can be changed, and that the hypervariable region is necessary and sufficient. In *S. chacoense* the S_{11} and S_{13} RNases differ by only four amino acids in the

Figure 1



Genetics of self-incompatibility. **(a)** In gametophytic self-incompatibility (GSI) in the Solanaceae, pollen can germinate and grow all the way through the style if the genotype of the pollen does not match the genotype of the female parent. Thus S_2 and S_3 pollen grains are arrested in the upper part of the style, but the S_1 pollen tube is able to grow the length of the style. **(b)** In sporophytic self-incompatibility (SSI) in the Brassicaceae, the pollen determinants are sporophytically expressed and are presumed to be deposited on the pollen coat by the male parent. Pollen grains can germinate only if they are of a completely different genotype from the female parent. In the pistil at the left, the genotype of the S_2 pollen grain does not match either of the pistil alleles (S_1 and S_3), but it carries determinants derived from the male parent (S_1 and S_2) and, therefore, cannot germinate. In contrast, the pistil at the right is of a completely different genotype than that of the pollen grains (S_3 and S_4 as opposed to S_1 and S_2), thus enabling the pollen to germinate.

hypervariable regions. Transgenic plants with chimeric gene constructs in which the S_{11} RNase had those four amino acids changed to those in the S_{13} RNase were converted to the S_{13} phenotype (the plants arrested S_{13} pollen and accepted S_{11} pollen).

To determine if the S-RNase interacts with a pollen-expressed S allele product, a single amino acid change in the S_3 RNase that inactivated the RNase activity was introduced into S_2/S_3 plants of *Petunia inflata* [13••]. Three plants expressed the mutant S_3 RNase at a level equivalent to the endogenous S_2 and S_3 RNases. In these plants, the expression of the mutant S_3 RNase inhibited rejection of S_3 pollen but had no such effect on S_2 pollen. These results suggest that the mutant S_3 RNase is still recognized by the pollen S component and support the idea that the mutant S_3 RNase competes with the wild-type S_3 RNase for binding to the pollen S component.

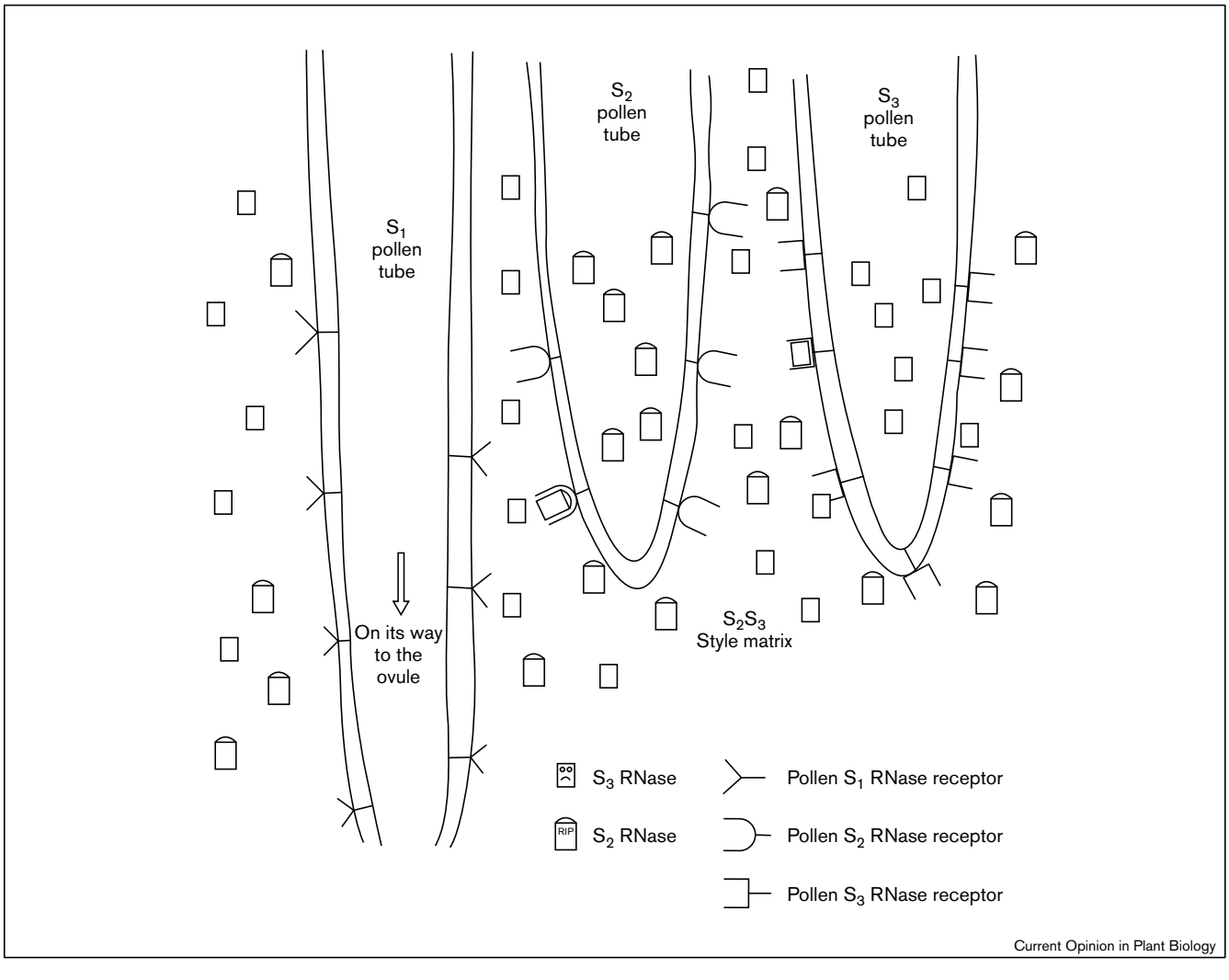
Because an S-RNase is capable of degrading pollen RNA both from the same and from different alleles, the difference that determines allele-specificity in RNA degradation must lie in the entry of the RNase into the pollen tube or in the access to the RNA once in the pollen tube. There are two theories for the mechanism of allele-specific RNase inhibition [3]. One holds that both S_2 and S_3 RNases can enter the pollen grain, but that there is an inhibitor in the pollen tube cytoplasm that inhibits all non-self RNases but not the self RNase. The other holds that there is an allele-specific receptor on the pollen tube surface that only allows the self RNase to enter. There is genetic evidence from tetraploid plants that is consistent with the inhibitor model (reviewed in

[4]), but the receptor model is more attractive because it is simpler. The inhibitor model requires that the pollen tube be able to inhibit all the different S-RNases that it might encounter — except for the RNase from the same S allele. An interesting test of these models would involve specific labeling of the S_2, S_3 and mutant S_3 RNases in some way, incubating them with *in vitro* grown pollen tubes from different S genotypes, and measuring their transport into or binding to pollen tubes. For example, it is now possible to image individual green fluorescent protein (GFP)-tagged molecules [14] and this method could be applied to monitor expression of individual RNAases. It has recently has been suggested that [13••] the mutant S_3 RNase and its dominant-negative activity in transgenic plants will be useful in identifying the pollen partner.

Other gametophytic self-incompatibility systems

In poppy, the style glycoprotein encoded by the *S* locus has no similarity to any sequence in the databases. There is evidence that the S-protein interacts with a pollen protein, and that changes in the phosphorylation state of pollen proteins occur during the incompatible reaction. Thus far, however, no allelic specificity for these interactions has been shown [5,6]. In contrast to all other self-incompatibility systems that have been studied at the molecular level, the gene encoding the pollen component has been cloned in the grass *Phalaris coerulescens* [7]. This predicted pollen *S* gene product is composed of two domains, a presumed allele specificity domain and a thioredoxin catalytic domain. Analysis of a self-compatible

Figure 2



The RNase-receptor model for allele-specific inhibition of pollen tube growth. The schematic shows that S_1 pollen is able to grow through an S_2S_3 style because it has no receptors for the S_2 or S_3 RNases that are present in the style matrix. In contrast, germination of the S_2 and S_3 pollen grains is slowed or arrested because the S_2 and S_3 RNases are internalized by the S_2 and S_3 pollen tubes, respectively.

mutant suggests that the thioredoxin activity is essential for the self-incompatibility response [7], but the role of thioredoxin in the self-incompatibility response is not known. Attempts to identify similar genes in other grass species were not successful using the presumed allele specificity domain as a probe, although sequences in other grasses could be identified using PCR and primers designed against conserved regions of the thioredoxin domain [15]. Until these thioredoxin sequences are shown to be contiguous with S-allele specificity domains it is premature to conclude that the S-allele specificity domain exists in other grasses.

Sporophytic self-incompatibility

The sporophytically controlled *S* locus (or haplotype) in *Brassica* species encodes several genes involved in the self-incompatibility response (Figure 3); these include a secreted glycoprotein (SLG; S-locus glycoprotein) and a transmembrane receptor kinase (SRK; S-receptor kinase) whose extracellular domain is similar to the SLG. Both the extracellular domain of the SRK and the SLG are polymorphic between haplotypes, which is in keeping with the prediction for self-incompatibility determinants [1]. It was deduced that the kinase activity was required for the self-incompatibility response because two self-compatible lines had deletions that prevented expression of an active kinase [16,17]. In an attempt to demonstrate this requirement, a chimeric kinase was introduced into the self-compatible Sf_1 line. Unfortunately the chimeric kinase was only weakly expressed and it could not restore self-incompatibility [18•]. Thus there is as yet no direct proof of a requirement for SRK. In some haplotypes downregulation of SLG is correlated with a loss of self-incompatibility [1]. There are also cases, however, where self-compatible haplotypes express high levels of SLG and where self-incompatible haplotypes have nearly undetectable levels of SLG [19]. Thus it is still not proven that SLG is important for self-incompatibility. In addition, there is evidence in at least one haplotype for a membrane-anchored SLG [1], and in other haplotypes, for a secreted glycoprotein (eSRK) that is an alternatively spliced product of the SRK gene [20]. The most widely discussed model for sporophytic self-incompatibility [1,2] predicts that the pollen ligand somehow interacts with SRK to initiate a signal transduction chain in the stigma papillar cell, thus preventing self pollen from growing a pollen tube. The roles played by SLG and eSRK in this scheme are still not clear.

Have the SLG and SRK genes of a haplotype co-evolved? Sequence comparisons of the SLG and SRK genes from S_2 (a class I haplotype) and S_6 (a class II haplotype) supported this idea. These sequence comparisons had shown that the SLG and SRK sequences were more similar when compared within a haplotype than when compared between haplotypes [1]. The previous comparisons, however, might have overemphasized the differences between haplotypes (as pointed out in [21••]). There are only a few class II haplotypes; they have a weak self-incompatibility response and are considered pollen recessive, and are thus quite different from class I haplotypes. Sequences from the SLG genes from 31 class I haplotypes were analyzed [21••]. Surprisingly, two different *S* haplotypes had identical sequences in the hypervariable region of the SLG, the part believed to be important for the S specificity. Secondly, the SLG sequence was not necessarily most closely related to the SRK of the same haplotype. In order to reconcile these contradictory findings with the model that holds that both SLG and SRK of a haplotype are required for the self-incompatibility response, it was proposed that perhaps the pollen ligand interacts with both SLG and SRK, but at different sites. It is also possible that these results support the idea that SLG is not a critical element for the self-incompatibility response. Further analysis of SLG and SRK sequences from a larger number of haplotypes might help resolve this controversy.

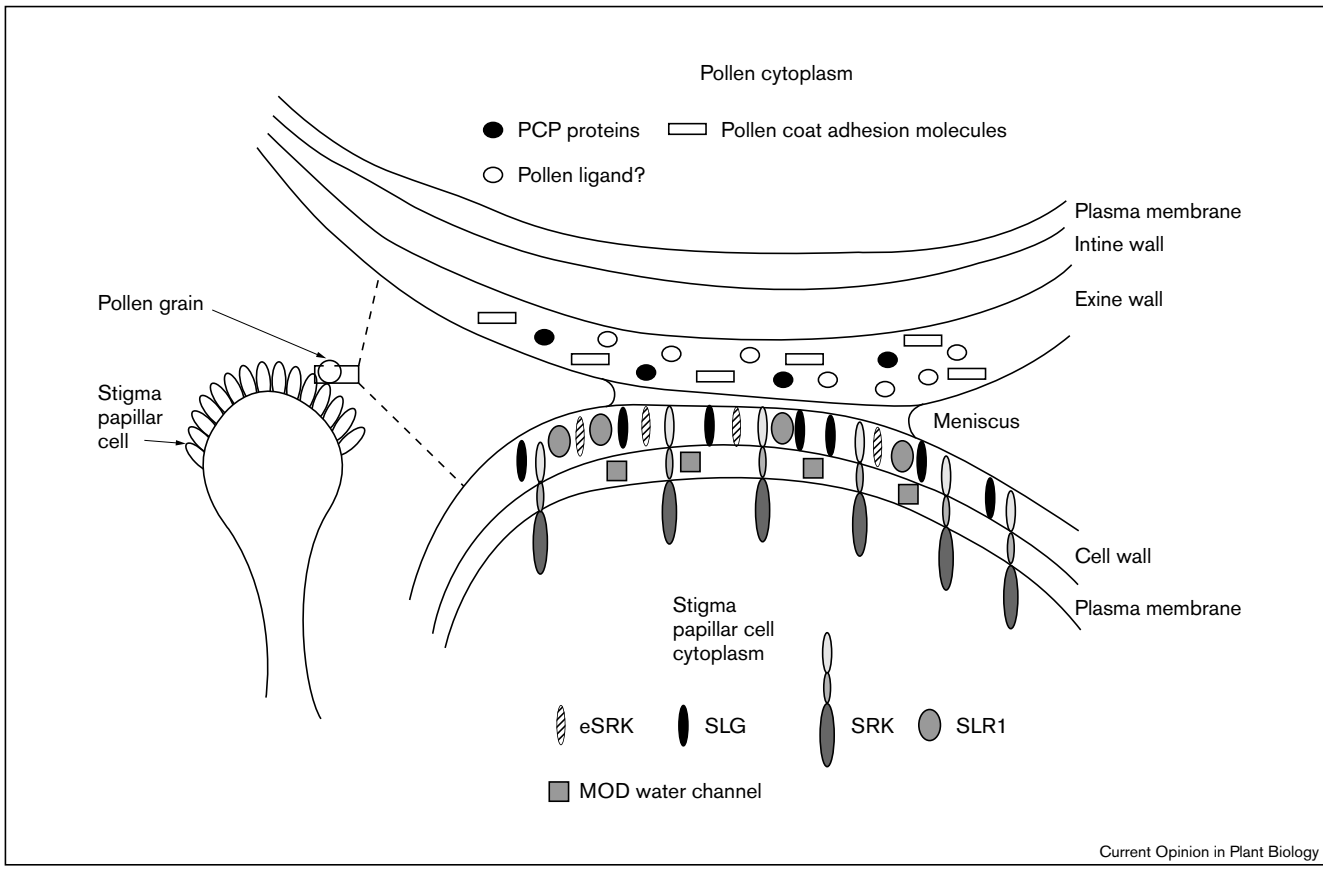
Recognition, adhesion and hydration

As in the gametophytic self-incompatibility systems in the Solanaceae, attempts to switch allelic specificity have been problematic in sporophytic self-incompatibility systems. For example, mutant forms of SRK and SLG that were predicted to act as dominant-negative alleles were introduced into *B. oleracea* [18•]. Although self-incompatibility breakdown was found in two cases, it was not due to expression of a transgene, but rather to homology-dependent gene silencing of the endogenous S haplotype genes.

Despite the problems with transgenic analyses with the *S* haplotype genes, antisense constructs of the related but unlinked gene, SLR1, demonstrated that this protein is not required for the self-incompatibility response or for compatible crosses [22]. In more recent studies, however, the adhesive forces between pollen and stigma papillar cells in SLR1 antisense plants and in compatible and incompatible crosses were measured [23•,24•]. These studies showed that the pollen coat and SLR1 (and perhaps SLG) play a role during the second phase of pollen adhesion, that is to say, the point at which the pollen coat and stigma papillar cell surface components coalesce and when water transfer from the stigma to the pollen grain occurs [25].

In *B. campestris*, a recessive mutation (*mod*) in an unlinked modifier gene (*MOD*) eliminates the self-incompatibility response in the stigma. *MOD* encodes an aquaporin [26•]. Because pollen hydration of self-incompatible pollen grains can occur in the *mod* mutant, it was hypothesized that the lack of the aquaporin would block a signal transduction pathway that normally leads to pollen tube arrest in a functional self-incompatibility system. This is counter-intuitive, because it means that hydration is the default state. To explain these results, it was proposed that self pollen triggers the SRK response, that the kinase then activates MOD channels, thereby preventing the flow

Figure 3



Schematic of contact between a *Brassica* stigma papillar cell and an incompatible pollen grain. Some of the protein players involved in pollen adhesion, recognition and hydration are shown. It is believed that the SRK is phosphorylated after interaction with a pollen ligand, and that this triggers a signal transduction pathway, somehow involving the MOD water channel, eventually leading to a block in pollen hydration. SRK may bind the pollen ligand in conjunction with SLG and/or eSRK. Pollen coat proteins are likely candidates for the pollen ligand. SLR1 and pollen coat components are involved in adhesion.

of water from the stigma into the pollen grain and thus preventing pollen hydration. According to this hypothesis, therefore, in the *mod* mutant, SRK would be activated but the MOD channel would not. It is also possible that the MOD channel regulates the movement of small molecules between the pollen coat and the stigma, and that these molecules might alternatively inhibit or promote pollen hydration.

Searching for the ligand

The ligand for the *Brassica* SRK should be anther-expressed, be linked to the SLG/SRK chromosomal region, and have sequence polymorphisms in different S haplotypes. An anther-expressed gene (SLA, for S-locus anther) that was isolated from the S_2 haplotype of *B. oleracea* seemed to fit these criteria [27]. Furthermore, because of a retrotransposon insertion, there was no SLA transcript present in a compatible strain carrying an S_2-like haplotype (in *B. napus*). Lastly, because sequences homologous to that of SLA could not be found in other S haplotypes, it was suggested that SLA is highly polymorphic and, therefore, might encode the pollen ligand. Contrary to this suggestion, however, SLA sequences nearly identical to the S_2 sequence have now been characterized from other *B. oleracea* haplotypes [28•]. Furthermore, the retrotransposon disruption of SLA is no longer correlated with self-incompatibility, because it is found in both compatible and incompatible haplotypes of *B. oleracea*. In conclusion, expression of SLA is not required for self-incompatibility [28•].

In similar attempts to identify the S ligand, the chromosomal region between the SRK and SLG of the S-910 haplotype in *B. napus* was chosen for cloning [29•]. In this haplotype the SRK and SLG genes are separated by only about 25 kb, while in other S haplotypes the distance between SLG and SRK can be as much as 250 kb [30•]. A cDNA library was screened with probes derived from the S-910 haplotype and two anther-expressed genes (SLL1 and SLL2) were identified. The SLL2 gene is a member of a multigene family, and other family members map elsewhere in the genome. SLL2 is expressed in both self-incompatible and self-compatible lines, making it a less likely candidate for the pollen ligand. The

SLL1 gene would only be capable of encoding two very small peptides (2 and 3 kDa), but intriguingly SLL1 is only expressed in self-incompatible lines. When SLL1 homologous sequences were cloned and sequenced from several different S-haplotypes, however, the sequences were identical to SLL1 from the S-910 haplotype [30]. As for the SLA gene discussed above, this sequence identity is not predicted for the pollen ligand.

The *S*-locus has been mapped [31] but neither the physical limits of the locus nor the true frequency of recombination within the locus is known [30•]. Without this information, researchers cannot know for sure if they have searched the correct regions of the genome for the pollen S component. It will be very important to obtain a more complete physical and molecular map for the *S* locus region.

In contrast to a map-based approach to identify the pollen S component, an extremely interesting approach has been taken by Stephenson *et al.* [32••]. In this work, pollen coatings from different S haplotypes were isolated and, by micromanipulation, coating from one haplotype was placed on a stigma papillar cell of the same or different haplotype, before placing single pollen grains of the same or different haplotypes on the stigma papillar cell. Significant and haplotype-specific changes in pollen hydration and germination were observed. For example, self pollen coating could inhibit the growth of non-self pollen, and non-self pollen coating could allow growth of self pollen. These results suggest that the pollen coat contains compounds that can be transferred to other pollen grains and that this transfer can mimic changes in allelic specificity. An enriched fraction with these activities contained proteins of the PCP (pollen coat protein) class [33,34]. Previously, a PCP was shown to interact *in vitro* with SLG [33], but no allelic specificity was found for this interaction. Because PCPs are encoded by a multigene family [35], however, this finding does not eliminate the possibility that a PCP is the pollen ligand. Further characterization should allow cloning of the genes that encode the PCPs present in the active fractions isolated by Stephenson *et al.* [32••]; it will be most important to determine if these genes map to the *S* locus.

Pollen tube adhesion and growth

In the past year several technical advances have been made which will facilitate the molecular dissection of pollen tube growth. It has long been known that pollen tubes grow faster *in vivo* through the style than in *in vitro* germination medium. In order to determine why, an adhesion assay for *in vitro* pollen tube growth was developed for lily, a plant with a hollow style [36•]. In this assay, exudate from styles is applied to nitro-cellulose membranes, to which it adheres. Pollen tube tips adhere to this matrix of exudate and grow faster than in liquid germination medium; interestingly, style exudate did not improve pollen tube growth rate if added to liquid medium. The style exudate is rich in arabinogalactan proteins, and lily pollen tubes adhered most strongly in regions of the nitro-cellulose membrane that were enriched for arabinogalactan proteins. More evidence for the importance of arabinogalactan proteins in pollen tube growth comes from antisense or sense suppression experiments with the TTS (transmitting tissue-specific) gene of tobacco, which encodes an arabinogalactan protein. Transgenic plants with reduced expression of TTS showed reduced pollen tube growth rates [37]. It will be important to test if the adhesion assay developed for lily can be adapted to other plants.

An allergen from maize pollen was shown to have cell-wall-loosening activity characteristic of expansins [38]. The allergen protein is released from the wall of ungerminated pollen and might, therefore, play a role in loosening the tightly adhering cell walls of the stigma to allow initial pollen tube entry into the female tissue. Whether pollen tubes have easily released expansin was not tested, so it not clear whether pollen-derived expansins might also play a role during further pollen tube growth.

Pollen tube growth direction can be manipulated by asymmetrically modifying calcium levels in the tip. In the most recent work [39•], caged calcium or calcium chelators were released by photoactivation in discrete regions of the growing pollen tube; the point of highest calcium concentration determined the direction of growth. Malho and Trewavas [39•] speculate that ion channels in the tip of the pollen tube might be asymmetrically activated by interactions with arabinogalactan proteins or other components of the style matrix. In general, the importance of individual arabinogalactan proteins (AGPs) has been difficult to assess, because AGPs are highly glycosylated and resolve poorly in standard gel systems. A newly developed SDS-agarose gel procedure for separation of plasma-membrane-associated AGPs proteins should facilitate such experiments [40].

How is pollen tube growth initiated? Although in some species (e.g. maize) pollen grains have only one aperture, in most species pollen grains have more than one. Are all apertures equally competent to form pollen tubes? Two recent papers [41•,42•] describe *Arabidopsis* mutants which are relevant to this point. The *tetraspore* (four alleles) and *stud* (three alleles) mutants have similar phenotypes, map to the same region of chromosome 3, and are thus likely to be allelic. These mutants are primarily defective in meiotic cytokinesis, but they also exhibit several other phenotypes later during pollen development. For example, although the pollen grains in *stud* and *tetraspore* are formed from a coenocytic tetrad, and in principle multiple pollen tubes might be expected, only one pollen tube is formed. Another *Arabidopsis* mutant of interest in this context is *sidecar pollen* [43•]. In homozygous *sidecar pollen* plants, about 30% of the pollen grains have an extra vegetative cell, so that the exine encloses two vegetative cells (one

of which also carries two sperm). In these grains, either of the two vegetative cells can form a pollen tube, but no two-tubed grains were ever seen. The *stud* and *scp* pollen tube growth results were used to suggest [41•] that pollen tube formation must be determined at the level of the whole grain and that only one tube can form. We have frequently observed two-tubed pollen grains after *in vitro* germination of tomato, and occasionally after *in vivo* germination (J Muschietti, Y Eyal, S McCormick, unpublished data). It might be interesting to test if all the apertures in tomato and *Arabidopsis* are equally capable of forming tubes, perhaps by measuring or manipulating calcium levels. One obvious difference between the two species is that pollen of *Arabidopsis* has already undergone the second mitotic division, whereas tomato pollen has not. How or if the presence of sperm cells would restrict pollen to forming only one tube is not clear.

Conclusions

The past year has been frustrating because of the continued elusiveness of the pollen S component of the most studied self-incompatibility systems, but recent results and new experimental approaches offer hope. Because of the extensive rearrangements at the *Brassica* S haplotypes, it is important to define the physical limits of the S locus before additional attempts are made to locate the pollen S component via map-based approaches. The results suggesting that pollen coat compounds can mimic specificity changes should certainly be pursued. In *Petunia*, the best hope for identifying the pollen partner for the RNase lies with the mutant S_3 RNase and its use in biochemical or genetic screens. The roles of adhesive molecules in pollen tube growth can now be systematically tested using the assay for pollen tube adhesion.

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