CMLS, Cell. Mol. Life Sci. 58 (2001) 1988–2007
1420-682X/01/141988-20 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Mechanisms of self-incompatibility in flowering plants

N. F. Silva and D. R. Goring*,†

Biology Department, York University, Toronto, Ontario M3J 1P3 (Canada)

Received 10 May 2001; received after revision 20 June 2001; accepted 20 June 2001

Abstract. Self-incompatibility is a widespread mechanism in flowering plants that prevents inbreeding and promotes outcrossing. The self-incompatibility response is genetically controlled by one or more multi-allelic loci, and relies on a series of complex cellular interactions between the self-incompatible pollen and pistil. Although self-incompatibility functions ultimately to prevent self-fertilization, flowering plants have evolved several unique mechanisms for rejecting the self-incompatible pollen. The self-incompatibility system in the *Solanaceae* makes use of a multi-allelic RNase in the pistil to block incompatible pollen tube growth. In contrast, the *Papaveraceae* system appears to have complex cellular responses such as calcium fluxes, actin rearrangements, and programmed cell death occurring in the incompatible pollen tube. Finally, the *Brassicaceae* system has a receptor kinase signalling pathway activated in the pistil leading to pollen rejection. This review highlights the recent advances made towards understanding the cellular mechanisms involved in these self-incompatibility systems and discusses the striking differences between these systems.

Keywords. Self-incompatibility; *S* locus; S-RNase; S protein; *S* locus glycoprotein; S receptor kinase.

Introduction

Flowering plants (angiosperms) are sessile by nature and unable to actively search out mating partners. Moreover, many species of flowering plants produce hermaphroditic flowers, having the male (anther) and female (pistil) reproductive organs within close proximity on the same flower [1]. At first glance, these traits which promote self-fertilization and inbreeding are often associated with a decline in genetic variability and may have been considered deleterious for the evolution of flowering plants. However, angiosperms are among the most successful groups of terrestrial flora [2]. This reproductive success is in part due to the evolution of several mechanisms which limit and prevent self-fertilization. Self-incompatibility represents the most widespread strategy employed by plants, which enables the pistil of a flower to recognize and reject self-pollen or pollen from genetically related individuals, thereby preventing inbreeding and promoting outcrossing [3].

During a compatible pollination (non-self pollen), fertilization relies on a series of complex cellular interactions initiated when a pollen grain lands and adheres to the receptive stigmatic papillae on the surface of the pistil (fig. 1). The pollen grain hydrates and germinates by extruding a pollen tube, which penetrates the stigmatic cell wall and continues to grow through the transmitting tissue of the style to reach the ovary where fertilization ultimately occurs [4]. The self-incompatibility system allows pistils to discriminate between the genetically diverse range of pollen landing on the stigma surface, so that only compatible pollen (non-self) but not incompatible pollen (self) is able to effect fertilization (fig. 1).

While many flowering plants rely on self-incompatibility to ensure genetic variability within a plant population, not all species depend upon the same self-incompatibility system.

* Corresponding author.
† Current address: Department of Botany, University of Toronto, Toronto, Ontario M5S 3B2 (Canada), Fax: +1 416 978-5878, e-mail: goring@botany.utoronto.ca

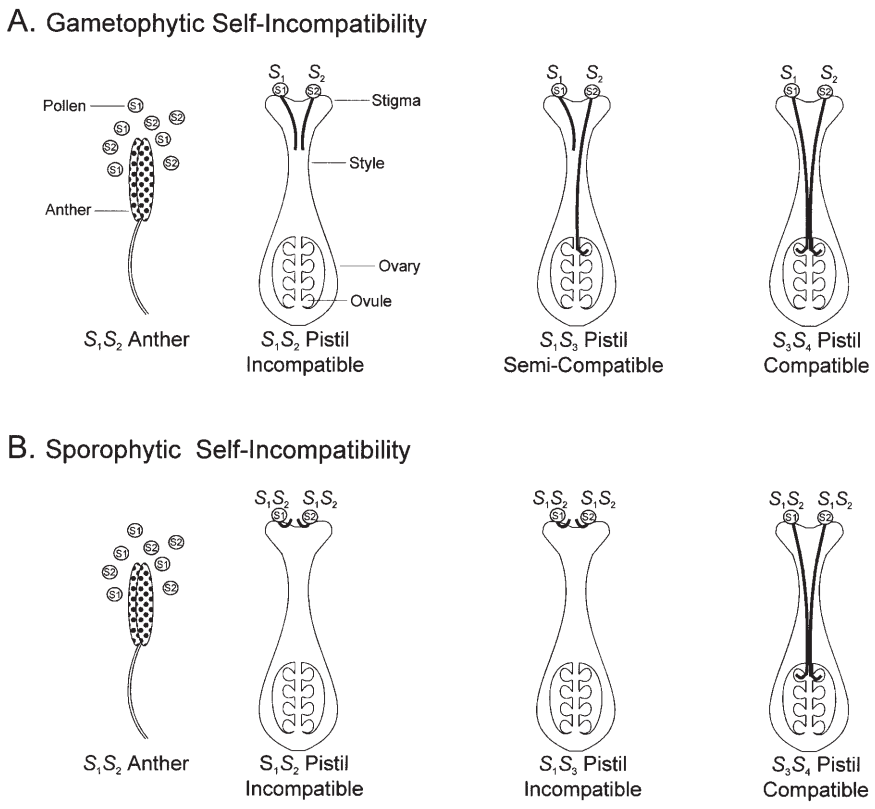

Figure 1. Gametophytic and sporophytic self-incompatibility in flowering plants. (*A*) In gametophytic self-incompatibility, the phenotype of the pollen is determined by its own haploid genome. Thus, pollen tube growth is inhibited when the pollen *S* allele matches one of the *S* alleles expressed in the pistil. Pollen grains from the S_1S_2 anther are self-incompatible with the S_1S_2 pistil (left pistil). When only one of the pollen alleles, S_1, is shared with those in the pistil, then half of the pollen grains, the S_2 pollen, are compatible on the S_1S_3 pistil (centre pistil). The pollen grains from the S_1S_2 anther bear different *S* alleles from the S_3S_4 pistil (right pistil), and are compatible. Consequently, the S_1 and S_2 pollen can germinate, grow down the style to the ovary and effect fertilization. (*B*) In sporophytic self-incompatibility, the phenotype of the pollen is determined by the diploid genome of the parent plant. Thus, when one of the *S* alleles in the pollen parent matches that of the pistil, pollen germination is arrested at the stigma surface. Pollen grains from the S_1S_2 anther are rejected on both the S_1S_2 pistil (left pistil) and the S_1S_3 pistil (centre pistil) due to matching alleles. However, the pollen grains from the S_1S_2 anther are fully compatible on an S_3S_4 pistil (right pistil), and fertilization occurs.

Some self-incompatible flowering plants produce morphologically distinct flowers, in which the relative positions of the reproductive organs within a flower pose an additional topological barrier to the already existing intra-specific barrier of self-incompatibility [1, 5]. In other self-incompatible species, the flowers possess the same morphological character, but the phenotype of the pollen can be either sporophytically or gametophytically derived [6]. In the gametophytic systems, the self-incompatibility phenotype of the pollen is determined by its own haploid genotype, whereas in sporophytic self-incompatibility systems, the self-incompatible behaviour of the pollen is determined by the genotype of the pollen parent. Gametophytic self-incompatibility is the most common system, and has been described in more than 60 families of flowering plants [1]. Despite its widespread prevalence, gametophytic self-incompatibility has only been studied in detail at the molecular level in the *Papaveraceae* (poppy), and in members of the *Solanaceae* family including *Nicotiana* (tobacco), *Petunia*, *Solanum* (potato), and *Lycopersicon* (tomato). Sporophytic self-incompatibility, which is less common, has been studied in detail in the *Brassicaceae* (mustard) family.

The existence of self-incompatibility systems as strategies to promote genetic variability has been documented since Darwin's classical genetic studies dating back to the end of the 18th century. Only within the last two decades have scientists been able to complement these genetic observations with molecular and biochemical analyses which have significantly contributed to elucidating the complex series of interactions occurring at the pollen-stigma interface. From an evolutionary perspective, it is interesting to note that many families of flowering plants have evolved different mechanisms of self-incompatibility, even through they share the function of preventing self-fertilization. This review will discuss some of the founding work and recent advances concerning essentially three different models of self-incompatibility: the *Solanaceae*, *Papaveraceae* and *Brassicaceae* self-incompatibility systems.

Self-incompatibility in the *Solanaceae*

The self-incompatibility system governing pollen-pistil interactions in the *Solanaceae* family is characterized by the ability of both compatible (non-self) and incompatible (self) pollen grains to hydrate and undergo normal germination on the stigmatic surface of the pistil. As the pollen tubes grow down the transmitting tract of the style, callose plugs are deposited at regular intervals giving the tubes a 'ladder-like' appearance [5]. Initially, compatible and incompatible tubes appear morphologically similar. However, incompatible tube growth arrest is manifested by a slower rate of tube growth, irregular deposition of callose and thickening of tube cell walls along with the swelling and eruption of the tip in the upper one-third of the style [5, 6]. In this way, incompatible pollen tubes are unable to effect fertilization.

In the *Solanaceae* family, classical genetic studies have shown that the self-incompatibility trait is genetically controlled by a single polymorphic locus called the *S* locus [6]. Since the phenotype of the pollen is gametophytically determined, fertilization is prevented when the *S* allele expressed by the haploid pollen grain matches one of the *S* alleles expressed in the pistil [6]. For example, a plant heterozygous for two different *S* alleles, such as the S_1 and S_2 alleles, would produce pollen carrying either the S_1 or the S_2 allele (fig. 1 A). If these pollen grains land on the stigmatic surface of the same flower, they would germinate, but pollen tube growth would ultimately arrest in the style due to the matching *S* alleles in the pistil (fig. 1 A). However, if S_1 and S_2 pollen land on the pistil of an S_1S_3 plant, the pollination outcome would be different. As expected, the S_1 pollen would be rejected due to the matching S_1 allele; however, the S_2 pollen would be recognized as non-self pollen, and be allowed to germinate, grow down the style to the ovary and effect fertilization [1]. Therefore, when only one *S* allele in the pollen parent is shared with those in the pistil, only half the pollen is rejected (semi-compatible) [7].

Molecular studies investigating the cellular interactions occurring during the self-incompatibility response in *Solanaceae* suggest that self-incompatibility is in fact controlled by two tightly linked genes in the *S* locus region, one controlling the function of the *S* protein in the pistil and the other, as yet unidentified, *S* gene controlling the pollen function.

Identification and structure of the S-RNase: the pistil component of self-incompatibility

For many years, scientists have geared research efforts to identify of the *S* genes which control self-incompatibility interactions. A major contribution to the elucidation of candidate proteins involved in *Solanaceae* self-incompatibility stemmed from work by Bredemeijer and Blaas [8] who established a connection between pistil proteins that were observed to co-segregate with the respective *S* alleles during genetic crosses in *Nicotiana alata* [8]. The identification of these pistil *S* proteins was facilitated by their abundance in pistils and their high degree of sequence diversity, which allowed allelic forms to be easily distinguished based on differences in isoelectric point and molecular mass [8]. Subsequently, the first gametophytic self-incompatibility *S* gene which segregated with the S_2 allele of *N. alata* was cloned [9]. Different alleles of this pistil *S* gene were then cloned from a variety of other *Solanaceae* species including *Petunia* [10–12], *Lycopersicon* [13] and *Solanum* [14, 15]. Similar pistil *S* proteins were identified in other gametophytic self-incompatibility families such as the *Rosaceae* [16, 17], and the *Scrophulariaceae* [18].

Insight into the potential function of the pistil *S* protein stemmed from the observation that it shared a striking degree of sequence similarity with the catalytic site of the fungal RNase T2 [19]. The pistil *S* proteins from *N. alata*, *Petunia hybrida* and *P. inflata* were then shown to possess ribonuclease activity [20–22]. These pistil *S* proteins were therefore appropriately renamed S-RNases. Overall, S-RNases were found to be basic glycoproteins of approximately 30 kDa in size possessing several characteristics consistent with their role in self-incompatibility. These glycoproteins were abundantly expressed in the pistil and localized primarily to the stigma and transmitting tract of the style, corresponding with the site of pollen tube rejection [23–25]. Furthermore, an increase in *S* protein expression from initially low levels in the pistils of immature flowers unable to reject self-incompatible pollen, to higher levels in the developing flowers coincided with the pistil's acquisition of self-incompatibility [23, 24].

A comparison of the predicted amino acid sequences of *Solanaceae* S-RNases from different species or within the same species revealed an unusually high degree of sequence diversity, with sequence identities ranging from 38 to 93% [13, 26, 27]. The sequence comparisons also revealed five highly conserved regions (C1–C5) clustered throughout separate regions of the S-RNase proteins [28] (fig. 2 A). Three of these conserved regions, C1, C4 and C5, are primarily comprised of hydrophobic amino acids implicated in the formation of the core structure of S proteins [1, 28]. The two other conserved regions, C2 and C3, exhibit striking sequence similarity with the active site of the fungal ribonucleases RNase T2 and RNase Rh and in particular, there are two histidine residues which correspond to histidine residues in the active site of RNase T2 that are required for RNase activity [19, 20, 29–33] (fig. 2 A). The different allelic forms of the S-RNase also share seven to ten conserved cysteine residues which are involved in the formation of disulphide linkages and in the stabilization of the tertiary

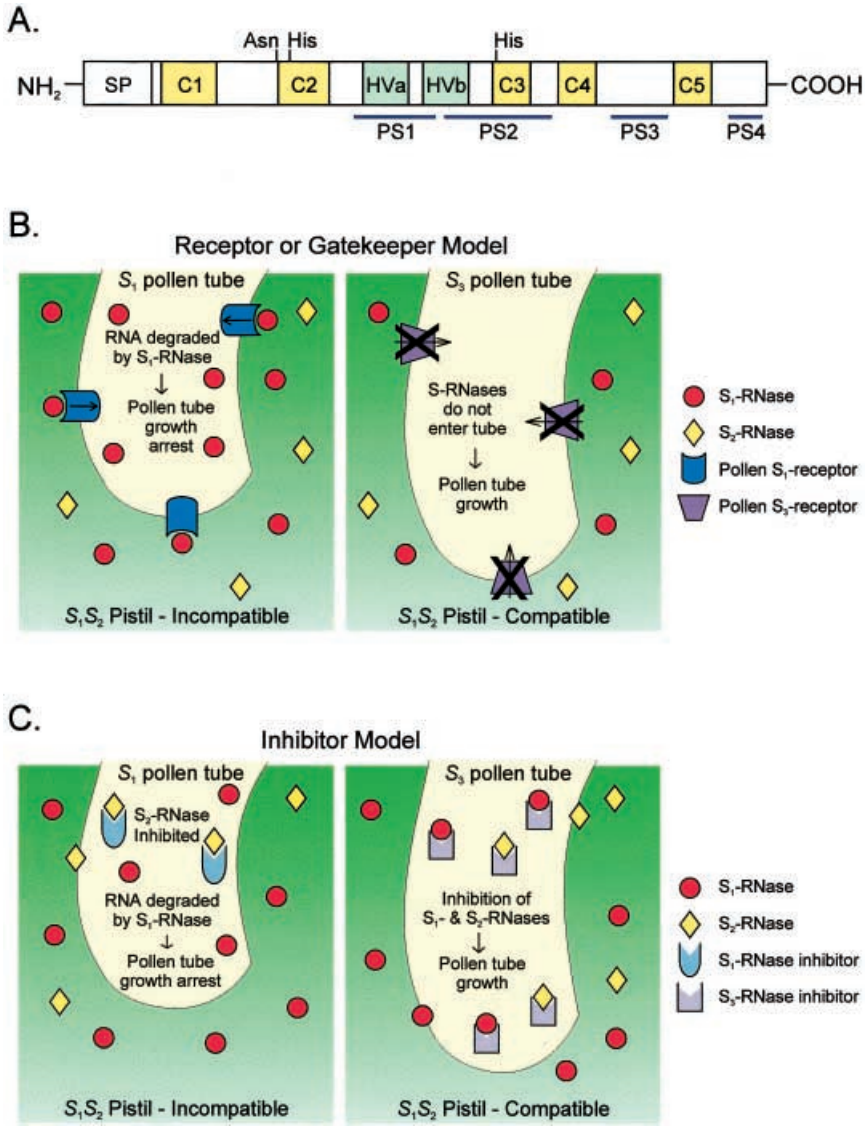

Figure 2. The self-incompatibility response in the *Solanaceae*. (*A*) Schematic representation of the primary structure of S-RNases. The five conserved regions are denoted C1–C5. The two histidine (His) residues essential for RNase activity, and the asparagine (Asn) residue involved in N-glycosylation are shown. The regions identified as the hypervariable regions (HVa and HVb) by Ioerger et al. [28] and positive selection domains (PS1–PS4) by Ishimizu et al. [40] are shown. SP, signal peptide. (*B*) Receptor or gatekeeper model of S-RNase-mediated pollen rejection. An S_1S_2 pistil produces and secretes S_1- and S_2-RNases into the transmitting tissue of the pistil. The pollen S_1 protein is predicted to be a receptor that selectively allows only S_1-RNases to enter the S_1 pollen tube. Once inside the pollen tube, the S_1-RNase is predicted to degrade the pollen RNA, inhibiting pollen tube growth. An S_3 pollen tube growing through an S_1S_2 pistil would continue to grow down the style of the pistil to the ovary since the pollen S_3 receptor would exclude both the S_1- and S_2-RNases from the pollen tube. (*C*) Inhibitor model of S-RNase-mediated pollen rejection. The S_1S_2 pistil produces and secretes S_1- and S_2-RNases into the transmitting tissue of the pistil. However in this model, both RNases enter the S_1 and S_3 pollen tubes. The pollen *S* proteins are proposed to act as cytosolic RNase inhibitors of all non-self RNases. Thus, inside the S_1 pollen tube, the S_1-RNase inhibitor would inhibit the activity of the S_2-RNase but not the S_1-RNase. The S_1-RNase would then degrade the pollen RNA, inhibiting pollen tube growth. In the S_3 pollen tube, the S_3-RNase inhibitor would inhibit both the S_1 and S_2-RNases, allowing the continued growth of the S_3 pollen tube.

structure of the protein [13, 34–36]. Lastly, all identified S-RNases are glycoproteins, having several potential sites for the attachment of N-glycans [1, 28, 37, 38].

The conserved regions of the S-RNase are separated by regions showing differing degrees of variability between S-RNase alleles (fig. 2A). There are two 'hypervariable' regions, HVa and HVb, which exhibit the highest degree of sequence diversity [28]. Since these two hypervariable regions are also the most hydrophilic in nature, they may potentially mediate interactions with pollen *S* allele products and thereby determine *S* allele specificity. The designation of a variable region varies according to different analyses performed, some of which identify fairly restricted areas as 'hypervariable' regions [28], while others

refer to all sequences outside the conserved regions as variable [39], and others yet which identify larger regions, PS1–PS4, as being under 'positive selection' and involved in determining the allelic specificity of self-incompatibility [40] (fig. 2A).

The predicted topology for the secondary structure of S-RNases is similar to that of the fungal RNase Rh whose tertiary structure is known [36, 40, 41]. This similarity between secondary structures suggests similarity at the tertiary level as well. A molecular model was therefore proposed for the S_3-RNase of *Lycopersicon peruvianum* based on the three-dimensional structure of the RNase Rh from *Rhizopus niveus* [36]. In this model, the two hypervariable regions, HVa and HVb, form a continuous surface on one side of the S-RNase despite these sequences being separated in the primary structure of the molecule. Furthermore, studies by Ishimizu et al. [40] propose a second model whereby an alignment based on the predicted secondary structures of S-RNases and the RNase Rh sequence places the four PS variable regions on two corresponding surface sites of the tertiary structure of RNase Rh [40]. The PS1 and PS2 regions together encompass the HVa and HVb regions identified by Ioerger et al. [28] as 'hypervariable' and correspond to one surface site, whereas the PS3 and PS4 regions form the second cluster at the opposite end of the molecule. In both cases, these variable regions clustered on the surface of S-RNase molecules may be involved in the interaction with or determination of the allele-specific binding site for the pollen *S* product.

Involvement of pistil S-RNases in the recognition and rejection of self-incompatible pollen

Conclusive evidence for the role of S-RNases in the recognition and rejection of self-incompatible pollen relied on several transgenic analyses utilizing loss- and gain-of-function approaches. Lee et al. [42] used a loss-of-function approach to investigate whether inhibiting the production of a particular S-RNase would render the pistil incapable of rejecting self-incompatible pollen. Indeed, they successfully demonstrated that the introduction of an antisense S_3-RNase transgene into S_2S_3 *P. inflata* plants resulted in the inhibition of both S_2- and S_3-RNase mRNA (due to sequence similarity), and a corresponding failure in the rejection of both S_3 and S_2 pollen by the transgenic plant. Gain-of-function experiments, on the other hand, addressed whether the pistil S-RNases alone were sufficient to recognize and reject self-incompatible pollen by introducting a foreign S_3-RNase gene into S_1S_2 *P. inflata* plants. Transgenic S_1S_2 plants expressing the S_3-RNase were able to reject S_3 pollen, indicating they had acquired S_3 allele specificity [42]. Similarly, Murfett et al. [43] introduced the S_{A2}-RNase cDNA of *N. alata* into a hybrid between the self-compatible *N. alata* × *N. langsdorffi* and showed that the transgenic plants expressing high levels of the S_{A2}-RNase mRNA successfully rejected S_{A2} pollen. Taken together, these experiments unequivocally demonstrated that S-RNases are necessary for the pistil to reject self-pollen and that they are necessary and sufficient to determine *S* allele specificity in the pistil. However, there are other factors in the pistil that are required for the rejection of self-incompatible pollen, as demonstrated by work on modifier loci and genes like *HT* (discussed below).

McClure et al. [44] were the first to provide evidence in support of ribonuclease activity as the mechanism governing gametophytic self-incompatibility when they showed that pollen rRNA was degraded following only self-incompatible pollinations in *N. alata* [44]. Further support for the requirement of ribonuclease activity in the rejection of self-incompatible pollen tubes was provided by transgenic S_1S_2 *P. inflata* plants transformed with a mutant S_3-RNase gene, in which the codon for His^{93}, one of the two catalytic histidines implicated in RNase activity, was replaced with a codon for asparagine [45]. Transgenic S_1S_2 plants carrying a mutant S_3-RNase gene produced a level of mutant S_3-RNase comparable to that produced in self-incompatible S_1S_3 and S_2S_3 plants; however, these plants failed to acquire the ability to reject self-incompatible pollen. In addition, these transgenic plants produced a mutant S_3-RNase that lacked any detectable RNase activity. In contrast, transgenic S_1S_2 plants expressing normal levels of the wild-type S_3-RNase were shown to completely reject S_3 pollen [45].

Determinants of S-RNase allele specificity

While S-RNases are well-established as the determinants of *S* allele specificity in the pistil, how the S-RNase allele specificity is determined is not precisely known. To examine whether the carbohydrate chains of S-RNases are involved in *S* allele specificity [21, 37, 46], Karunanandaa et al. [47] engineered a mutant *P. inflata* S_3-RNase by changing the codon for the only asparagine involved in N-glycosylation to an aspartic acid. Transgenic S_1S_2 *P. inflata* plants expressing normal levels of the non-glycosylated S_3-RNase completely rejected S_3 pollen, as well as S_1 and S_2 pollen, thereby suggesting that the determinants of S-RNase allele specificity must reside in the protein backbone and not in the N-linked glycan side chains [47].

Efforts to study the role of the S-RNase hypervariable regions in determining *S* allele specificity have been somewhat inconclusive. To examine the possible role of HVa and HVb regions as determinants of allele specificity, two chimeric *P. inflata* S-RNases were constructed [1]. In the S_3(HVa-HVb)-RNase, both the HVa and HVb regions of the S_3-RNase were replaced with the corresponding regions from the S_1-RNase, whereas the S_3(HVb)-RNase had only the HVb region of the S_3-RNase replaced with

the corresponding region from the S_1-RNase [1]. Transgenic S_2S_2 *P. inflata* plants expressing either chimeric S-RNase were assayed for their ability to reject S_1 or S_3 pollen, and were unable to reject either pollen. The chimeric S-RNases had lost the S_3 allele specificity and had failed to acquire S_1 allele specificity. Results from this study suggested that both hypervariable regions were necessary, but not sufficient for *S* allele specificity.

In another study by Zurek et al. [48], a series of nine chimeric constructs were generated by exchanging domains from *N. alata* S_{A2}- and S_{C10}-RNases in various combinations [48]. The nine chimeras along with wild-type S_{A2}- and S_{C10}-RNase genes were transformed into the hybrid *N. langsdorfii* × self-compatible *N. alata*. In the transgenic plants, the chimeric S-RNases were expressed at levels comparable to the control transformants, and had retained enzymatic activity. However, none of the chimeric S-RNases were able to reject either S_{A2} or S_{C10} pollen. Based on these domain-swapping experiments, Zurek et al. [48] suggested that S-RNases do not possess a specific domain required for allelic recognition but, instead, the sequences necessary for allelic recognition appear to be scattered throughout the molecule [48].

Matton et al. [49] investigated the role of the HV regions in allelic specificity using another *Solanaceous* species, *Solanum chacoense*. This study differed from those described above in that the S-RNases used, the S_{11}- and S_{13}-RNases, are almost identical, with only ten different amino acids. Three of these amino acids are localized to the HVa region and one to the HVb region [50]. Using site-directed mutagenesis, Matton et al. [49] substituted all four amino acids in the HV regions of an S_{11}-RNase with those of an S_{13}-RNase. Transgenic $S_{12}S_{14}$ *S. chacoense* plants expressing the chimeric S_{11}-RNase successfully rejected S_{13} pollen but not S_{11} pollen, therefore suggesting that the HVa and HVb regions together are sufficient to control *S* allele specificity [49]. Furthermore, a subsequent experiment demonstrated that the modification of only three of the four amino acid residues in an S_{11}-RNase to match those of an S_{13}-RNase resulted in transgenic $S_{12}S_{14}$ *S. chacoense* plants that expressed a dual specific S-RNase capable of rejecting both S_{11} and S_{13} pollen [51]. Matton et al. [49] proposed that the HV regions alone were sufficient for allelic recognition. However, the substitution of the S_{13}-RNase HV domains into another S-RNase exhibiting less pairwise identity than that observed for the S_{11}-/S_{13}-RNase pair would not likely produce the same result. As suggested by Verica et al. [52], the possibility that conserved amino acids outside the variable regions of S_{11} and S_{13} may also be involved in determining *S* allele specificity cannot be dismissed. Nevertheless, these experiments clearly demonstrate that the HVa and HVb regions play a crucial role in *S* allele specificity.

Identity of the pollen S gene

Several lines of evidence clearly suggest that the male component of the self-incompatibility response is not the S-RNase. For example, in all of the aforementioned transgenic experiments, only the self-incompatibility phenotype of the pistil but not that of the pollen was affected [1, 42, 43, 45, 48, 49, 51]. In an attempt to investigate this possibility further, Dodds et al. [53] transformed *L. peruvianum* plants with sense and antisense constructs of the S_3-RNase gene driven by a pollen-specific promoter. The resulting transgenic plants showed no change in the self-incompatibility phenotype of the pollen [53]. In addition, the deletion of a chromosomal region containing the S_4-RNase gene from the self-compatible *Pyrus serontina* also affected only the pistil but not the pollen function [54].

Numerous predictions about characteristics inherent to the pollen *S* gene have been made based on these findings. The pollen *S* gene must be genetically very tightly linked to the pistil S-RNase gene since recombination between the pollen and pistil components has never been observed and would likely result in a breakdown of self-incompatibility in future progeny [3]. In addition, the pollen *S* gene product is predicted to exhibit a high degree of sequence diversity at least in the *S* allele specificity domain and interact with S-RNases in an allelic-specific manner [3]. The requirement for such a specific interaction between the pollen *S* allele products and the pistil S-RNases during a self-incompatibility response was supported by a study by McCubbin et al. [55]. A mutated *P. inflata* S_3-RNase gene encoding an S-RNase lacking RNase activity was transformed into S_2S_3 plants. This transgene rendered the transgenic S_2S_3 plants unable to reject S_3 pollen but did not affect their ability to reject S_2 pollen. The mutated S_3-RNase is believed to compete with the wild-type S_3-RNase for binding to the pollen S_3 product, but does not compete with the wild-type S_2-RNase for binding to the S_2 allele pollen product [55].

Several approaches have been attempted to identify the pollen *S* gene and are based on either protein-protein interactions or on sequence polymorphisms [56]. Attempts to identify the pollen *S* gene based on protein interactions have relied on methods such as affinity chromatography, the yeast two-hybrid system and peptide display library screening, none of which proved to be successful [56]. The second approach based on sequence polymorphisms utilized primarily two methods, RNA differential display and subtractive hybridization to identify pollen expressed genes that exhibited polymorphisms between *S* genotypes. This has successfully led to the identification of 11 pollen expressed genes of *P. inflata* that exhibit *S* allele specific sequence polymorphism and are tightly linked to the S-RNase gene [56, 57]. Whether any of these genes encode the pollen *S* product remains to be determined.

Modifier loci and the self-incompatibility response

There is evidence to suggest that in the *Solanaceae* self-incompatibility system, other genes or factors (modifiers) are required to modulate the self-incompatibility response in addition to the pollen and pistil *S* genes [6, 58, 59]. For example, when a putatively non-functional *S* allele from a self-compatible cultivar of *P. hybrida* was crossed into a self-incompatible *P. hybrida* background, it was shown to be functional suggesting that the self-compatible background lacked some modifier locus required for self-incompatibility [60]. Similarly, Bernatzky et al. [61] discovered that other factors required for self-incompatibility exist when they crossed chromosomal fragments bearing the *S* locus region from the self-incompatible *L. hirsutum* into the self-compatible *L. esculentum* and recovered self-compatible plants expressing S-RNases. In addition, Tsukamoto et al. [62] examined populations of *Petunia axillaris* comprised of both self-incompatible and self-compatible plants and implicated a modifier locus as the factor responsible for the breakdown in self-incompatibility caused by an allelic-specific effect on S_{13}-RNase expression. McClure et al. [63] relied on a differential screening approach to identify pistil expressed non-RNase factors required in *Nicotiana* for the self-incompatibility response, and the screen identified a cDNA designated HT that encodes a small 100 amino acid asparagine rich protein [63]. In the self-incompatible *N. alata* $S_{C10}S_{C10}$, accumulation of HT transcripts is slower than of the S_{C10}-RNase; however, it is well correlated with the onset of *S* allele-specific rejection in the style. To investigate the role of HT in pollen rejection, an antisense-HT construct was transformed into the hybrid *N. plumbaginfolia* × the self-incompatible *N. alata* $S_{C10}S_{C10}$. The transgenic plants expressing reduced levels of the HT protein continued to express normal levels of S_{C10}-RNase but were defective in S_{C10}-pollen rejection. While the antisense results directly implicate the HT protein in pollen rejection, its precise function and mode of action remain unknown.

Models on the molecular basis of self-incompatibility in Solanaceae

The self-incompatibility system enables the pistil to distinguish between self- and non-self pollen based on identity of the *S* alleles carried by both the pollen and pistil counterparts. In the *Solanaceae*, the inherent RNase activity carried by the pistil *S* proteins is required for the self-incompatibility response, but many aspects regarding the precise molecular mechanisms by which S-RNases inhibit the growth of self-incompatible pollen tubes remain unclear. Two models have been proposed to explain the molecular basis of self-incompatible pollen rejection, both of which, however, rely on S-RNases exhibiting an inhibitory effect on the growth of pollen tubes bearing the same *S* alleles as the S-RNases [1, 64, 65] (fig. 2B–C).

The 'receptor or gatekeeper' model proposes that growth arrest of self-incompatible pollen tubes may be due to the allele-specific uptake of S-RNases proteins into the pollen tubes [1, 64] (fig. 2B). This model envisions the pollen *S* product as a surface receptor localized to the cell wall or plasma membrane of the pollen tube, capable of allele-specific recognition and translocation of stylar S-RNases into the pollen tube [1, 64] (fig. 2B). For example, a pistil carrying the S_1 and S_2 alleles would synthesize and secrete stylar S_1- and S_2-RNases into the transmitting tract of the pistil where S_1 and S_3 pollen tubes are growing. The pollen S_1 protein would recognize and allow the S_1-RNase to enter the S_1 pollen tube where degradation of its RNA and arrest of pollen tube growth would occur (fig. 2B). In contrast, the pollen S_3 protein unable to recognize either S_1- or S_2-RNases would inhibit entry of these S-RNases into the S_3 pollen tube thereby allowing pollen tube growth (fig. 2B).

Support for the 'receptor' model of pollen tube rejection comes from the McCubbin et al. [55] study in which an inactive form of the *P. inflata* S_3-RNase exerted a dominant-negative effect on the wild-type S_3-RNase. Transgenic plants producing wild-type S_2- and S_3-RNases as well as the mutant S_3-RNase were unable to completely reject S_3 pollen but were able to reject S_2 pollen completely, thereby suggesting that the mutated S_3-RNase may compete with the wild-type S_3-RNase for binding to the pollen S_3 receptor. This would explain the observed partial inhibition of S_3 pollen, given that the mutated S_3-RNase lacks RNase activity and is unable to inhibit S_3 pollen [55].

The second 'inhibitor' model proposes that the pollen *S* product functions as a general S-RNase inhibitor localized in the cytosol of the pollen tube, interfering with the RNase activity of all S-RNases except the allele-specific S-RNase [1, 64, 65]. As illustrated in figure 2C, uptake of the S-RNases is not allele specific; therefore, both S_1- and S_2-RNases would enter the S_1 and S_3 pollen tubes. However, once inside the S_1 pollen tube, only S_1-RNase would be active since the pollen S_1-inhibitor would inhibit the S_2-RNase activity. The active S_1-RNase would be sufficient to cause growth arrest of the S_1 pollen tube. In the example of the S_3 pollen tube, growth would continue since the pollen S_3-inhibitor would inhibit both the S_1 and S_2-RNases (fig. 2C).

Genetic evidence in support of the 'RNase inhibitor' model was based on mutational studies to investigate the phenomenon referred to as 'competitive interaction', which refers to a breakdown of self-incompatibility on the pollen side resulting from the presence of more than one pollen *S* allele in a pollen grain. This phenomenon has been described in tetraploid plants [66] and was induced by irradiation in *N. alata* plants [67]. The 'RNase

inhibitor' model can be used to explain pollen mutations arising only as a result of *S* allele duplication events, since loss-of-function mutations in the pollen *S* gene would be lethal, resulting in the inability of pollen *S* proteins to inhibit any S-RNase [67]. Further support for the inhibitor model stemmed from work by Luu et al. [68] demonstrating that S-RNase accumulation inside the growing pollen tubes is not allele specific [68].

Self-incompatibility in the *Papaveraceae*

Gametophytic self-incompatibility has also been extensively studied in *Papaver rhoeas* (field poppy), and at first glance does share similarities with the *Solanaceae* system. The gametophytic nature of this system again leads to the phenotype of the pollen being determined by the haploid pollen genotype. In addition, while the *P. rhoeas* self-incompatibility system was initially shown to be genetically controlled by a single multi-allelic *S* locus, the molecular studies are predicting the existence of two tightly linked, multi-allelic *S* genes encoding a stigmatic *S* protein and a pollen *S* protein [69]. However, the similarities to the *Solanaceae* self-incompatibility system break down at this point. Rejection of the self-incompatible pollen tubes in *P. rhoeas* occurs on the stigmatic surface of the pistil and not in the style as observed with *Solanaceae* [70] (fig.1 A). In addition, the gametophytic self-incompatibility system in *P. rhoeas* is mechanistically quite distinct from the *Solanaceae* system. Significant progress has been made in understanding the cellular mechanisms involved in the rejection of self-incompatible *P. rhoeas* pollen. This is in part due to the development of a bioassay which has allowed the faithful reproduction of the *P. rhoeas* self-incompatibility reaction in vitro [71].

Identification and structure of the stigmatic S protein: the pistil component of self-incompatibility

Given the similarities between the *P. rhoeas* and *N. alata* self-incompatibility systems, the stigmatic *S* proteins of *P. rhoeas* were initially predicted to be RNases. However, Franklin-Tong et al. [72] first cast doubt on this prediction with several experiments which failed to give any evidence of RNase activity being associated with the *P. rhoeas* self-incompatibility response. Conclusive evidence that the stigmatic *S* gene encoded something other than an RNase came from the cloning and sequencing of the first stigmatic S_1 gene [73]. Since then, several other stigmatic *S* proteins corresponding to different *S* alleles have been isolated from *Papaver rhoeas* (S_3, S_7, S_8) and from a close relative, *P. nudicaule* (S_{n1}) [73–75]. These stigmatic *S* genes were expressed in *Escherichia coli* and the resulting recombinant proteins were shown in vitro to possess the ability to inhibit pollen tube growth in an *S* allele-specific manner [73, 74]. Thus, the multi-allelic stigmatic *S* gene represents the pistil component of the *Papaver* self-incompatibility system. Of interest is that sequence database searches using the *P. rhoeas* stigmatic *S* gene have identified a large family of predicted *Arabidopsis* proteins known as *S* protein homologues (SPH) [76]. Since *Arabidopsis* does not employ a self-incompatibility system, these *SPH* genes likely have other functions in *Arabidopsis*.

In general, the stigmatic *S* proteins encode small 15-kDa secreted proteins, some of which are N-glycosylated. Comparisons of the predicted amino acid sequences from the stigmatic *S* genes reveal a high degree of amino acid polymorphism, with the sequences sharing between 51.3–63.7% amino acid identity [74, 75]. The stigmatic *S* proteins are predicted to adopt identical secondary structures, predominantly comprised of six β-strands followed by two C-terminal α-helical regions all linked together by seven hydrophilic loops that are likely exposed to the surface of the protein [74, 75]. In addition, stigmatic *S* proteins contain four invariant cysteine residues which are likely involved in the formation of disulphide bridges [74, 75]. An amino acid substitution in one of these cysteines results in a completely inactive stigmatic *S* protein [77].

Using site-directed mutagenesis, Kakeda et al. [77] investigated the molecular basis of the stigmatic *S* protein allele specificity. Several mutant forms of the stigmatic S_1 protein were generated and assayed for their ability to specifically inhibit S_1 pollen when compared to the wild-type S_1-recombinant protein. Substitutions of the aspartic acid residues located in the predicted second hydrophilic loop caused some reduction in the inhibitory activity of the stigmatic *S* proteins [77]. More dramatically, amino acid substitutions in the sixth hydrophilic loop resulted in a complete loss of stigmatic S_1-protein activity. Among the stigmatic *S* protein sequences available for the various alleles, only one hypervariable amino acid was identified in the sixth hydrophilic loop. A substitution of this hypervariable amino acid in the stigmatic S_1-protein, Asp^{79}, for the corresponding His^{80} in the stigmatic S_3-protein resulted in a complete loss of biological activity, in that the altered stigmatic S_1-protein was unable to inhibit S_1 pollen tube growth. However, this substitution failed to confer S_3 inhibitory activity on the altered stigmatic S_1-protein. This result clearly suggests that this residue within loop 6 plays a critical role in the recognition of self-incompatible pollen, but is not sufficient for defining allele specificity [77].

Identity of a putative pollen S protein in the *P. rhoeas* self-incompatibility system

In a working model for the *Papaver* self-incompatibility system, the small secreted stigmatic *S* protein is thought

to bind to a pollen-specific *S* protein (receptor) present on the growing pollen tube. In an attempt to identify this pollen protein, Hearn et al. [78] performed binding experiments by adding recombinant stigmatic *S* proteins to membrane filters containing pollen extracts, followed by immunodetection of the bound stigmatic *S* protein [78]. This technique successfully led to the identification of a 70–120-kDa pollen plasma membrane protein called *S* protein binding protein (SBP) present in the pollen of all *S* genotypes tested [78]. Although binding of SBP was specific for stigmatic *S* proteins, binding was evident regardless of the *S* genotype, thereby suggesting that the interaction between SBP and the stigmatic *S* protein is not allele specific [78].

The functional significance of the SBP-stigmatic *S* protein interaction was further studied by examining the effects of amino acid substitutions in the stigmatic S_1-protein on its SBP-binding activity and the inhibition of S_1 pollen [79]. Amino acid substitutions within loop 6 completely destroyed pollen inhibitory activity and caused a variable reduction in SBP-binding activity. Loop 2 mutants differed in that they only exhibited a moderate decrease in pollen inhibition activity, proportional to their reduced SBP-binding ability [79]. Thus, there was some correlation between SBP binding and the inhibition of incompatible pollen [79]. The apparent lack of *S* allele specificity first suggested that SBP may function as an accessory receptor by facilitating the binding of *S* proteins to an *S*-specific receptor [78]. However, Jordan et al. [79] further proposed that SBP may in fact encode the *S* pollen receptor itself, and that the non-allele-specific binding of the stigmatic *S* proteins to SBP may indicate the presence of both *S* allele-specific and *S* allele-independent stigmatic *S* protein-binding sites on SBP [79]. Cloning of the *SBP* gene in the future will provide important insights into the role of this protein in the *P. rhoeas* self-incompatibility system. If *SBP* does encode the pollen *S* receptor, then polymorphisms in the SBP gene would be expected as seen for *S* genes in other self-incompatibility systems.

Response genes and signalling events in the *P. rhoeas* self-incompatibility response

For the *P. rhoeas* self-incompatibility system, extensive work has been carried out on the identification of cellular events occurring in the incompatible pollen tube, following treatment with the allele-specific stigmatic *S* protein, that ultimately lead to growth inhibition of the incompatible pollen tube. As described in more detail below, evidence has been found for the involvement of a phosphoinositide signalling pathway and transient increases in intracellular calcium levels in the pollen tube as part of the self-incompatibility response. Other events include protein phosphorylation and possibly gene expression and, more recently, evidence has been presented for the involvement of programmed cell death in the inhibition of incompatible pollen tubes.

Earlier studies investigated the effects of various metabolic inhibitors using the in vitro pollen bioassay [80]. Treatment with tunicamycin (a glycosylation inhibitor) and actinomycin D (a transcription inhibitor) demonstrated that both processes of N-glycosylation and transcription are required for the inhibition of pollen tube growth in the presence of self-incompatible stigmatic extracts [80]. The detection of several newly synthesized RNA transcripts specifically produced following a self-incompatible response, in addition to the identification of pollen genes found to be either ‘up’ or ‘down’-regulated during a self-incompatible response confirms this finding [70, 80, 81].

The involvement of cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) as a secondary messenger in the regulation of pollen tube growth is well established [82, 83]. However, analysis of the self-incompatibility response in *P. rhoeas* has provided an additional role for regulation of pollen tube growth by $[Ca^{2+}]_i$. Through a series of $[Ca^{2+}]_i$ imaging experiments, self-incompatible stigmatic extracts were found to induce a transient increase in $[Ca^{2+}]_i$ levels in the pollen tube which preceded inhibition of pollen tube growth [84, 85]. This rapid increase in $[Ca^{2+}]_i$ levels was detected in the shank of the pollen tube, along with a detectable decrease in the tip-focussed $[Ca^{2+}]_i$ gradient, and was then followed by a re-establishment of the basal $[Ca^{2+}]_i$ levels within 1 minute. This type of change in $[Ca^{2+}]_i$ levels is often referred to as a ‘calcium wave’ [86]. The rapidity of the $[Ca^{2+}]_i$ response likely represents the initial step in a signalling pathway occurring within the self-incompatible pollen tube in response to the stigmatic *S* protein [79, 82, 83]. One striking observation was the nature of the $[Ca^{2+}]_i$ increase which was restricted to the shank of the pollen tube and corresponded to the ‘nuclear complex’ region, suggesting that $[Ca^{2+}]_i$ may be involved in the regulation of gene expression [85].

Franklin-Tong et al. [87] investigated whether transient $[Ca^{2+}]_i$ increases in response to an incompatible challenge could result from the presence of a functional phosphoinositide signalling pathway [87]. Indeed, experiments involving $[Ca^{2+}]_i$ imaging of pollen tubes following the photoactivation of caged inositol (1,4,5)-triphosphate (IP_3) demonstrated that IP_3 could induce $[Ca^{2+}]_i$ release. Treatment with neomycin and heparin, known inhibitors of phosphoinositidase C and IP_3, respectively, blocked the release of $[Ca^{2+}]_i$ induced by photolysis of caged IP_3 [88]. Treatment with mastoparan which stimulates IP_3 production also resulted in increased $[Ca^{2+}]_i$ levels and inhibition of pollen tube growth [87]. These results suggest that the phosphoinositide signalling pathway is functional and involved in mediating IP_3-induced $[Ca^{2+}]_i$ release, thereby modulating pollen tube growth.

Following the self-incompatibility response in *P. rhoeas*, the phosphorylation of at least two pollen proteins, p26 and p68, has also been detected [88, 89]. The increase in p68 phosphorylation was delayed when compared to that of p26 and shown to be Ca^{2+} independent [89]. Phosphorylation of p26 occurs rapidly and is first detected within 90 seconds and persists to 400 seconds following a self-incompatible interaction [88]. In contrast to p68, the in vitro phosphorylation state of p26 is Ca^{2+}-dependent and sensitive to the effect of W7, a Ca^{2+}-dependent protein kinase (CDPK) inhibitor, which significantly decreased the level of p26 phosphorylation [88]. The p26 protein shares sequence similarity with soluble inorganic pyrophosphatases, and phosphorylation of p26 has been associated with the down-regulation of its activity [90]. A recent study by Clarke et al. [91] provided evidence to suggest that profilin, an abundant actin-binding protein, not only plays a structural role in the regulation of the actin cytoskeleton but that it also functions as a signalling molecule [91–94]. Interestingly, the addition of excess *P. rhoeas* profilin to pollen extracts in vitro was shown to alter the phosphorylation levels of several soluble pollen proteins, one of these being p26 [91].

The role of programmed cell death as a downstream signalling event involved in the self-incompatibility response was recently investigated by Jordan et al. [95]. Using the FragEL assay, DNA fragmentation was first detected in the nuclei of incompatible tubes 4 hours following challenge with the *S* proteins. Although inhibition of pollen tube growth occurs rapidly in response to an incompatible interaction, cell death is not apparent until several hours later. Treatment with mastoparan which is known to increase $[Ca^{2+}]_i$ [87] also elicited DNA fragmentation in pollen tubes, thereby suggesting that programmed cell death may be mediated by $[Ca^{2+}]_i$ signalling [95].

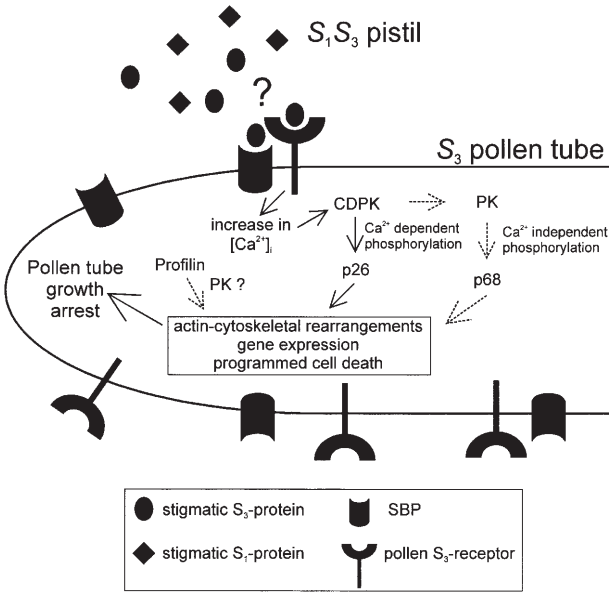

Figure 3. Model for stigmatic *S* protein mediated pollen rejection in *Papaver rhoeas*. Allele-specific recognition between the stigmatic S_3-protein and the putative pollen S_3-receptor activates an intracellular signalling pathway. Although the interaction with SBP appears to be required for the self-incompatibility response, whether SBP represents the pollen *S* receptor or acts as an accessory receptor is not known. The initial signalling cascade is marked by a rapid increase in cytosolic $[Ca^{2+}]_i$ which is thought to be mediated by an inositide signalling pathway, resulting in the activation of a Ca^{2+}-dependent protein kinase (CDPK) and phosphorylation of p26. Profilin, an actin-binding molecule, was shown to regulate actin-based cytoskeletal protein assembly and protein kinase (PK) and phosphatase activity [91] which suggests that this protein may be involved in a signalling pathway that regulates pollen tube growth. Later in the self-incompatibility response, Ca^{2+}-independent phosphorylation of p68 occurs. These signalling cascades are ultimately predicted to lead to events such are gene expression, actin-cytoskeleton rearrangements and programmed cell death, culminating in the inhibition of pollen tube growth.

Model for self-incompatibility in *P. rhoeas*

The self-incompatibility response in *P. rhoeas* involves complex cellular interactions and signalling cascades in the incompatible pollen tube leading to inhibition of tube growth (fig. 3). The stigmatic *S* protein is proposed to initiate this by interacting with a receptor molecule on the plasma membrane of the incompatible pollen tube [3, 83]. Whether SBP functions as an accessory receptor or represents the pollen *S* receptor itself is not yet known. The initial step in the inhibition of the incompatible pollen tube is a rapid and transient increase in $[Ca^{2+}]_i$ which appears to be mediated by a phosphoinositide signalling pathway [84–87]. This rise in $[Ca^{2+}]_i$ in turn results in the rapid Ca^{2+}-dependent phosphorylation of p26 [88]. The actin-binding protein, profilin, is also implicated in the regulation of p26 phosphorylation [91]. Finally, during this early stage, the actin cytoskeleton also undergoes rapid and dramatic rearrangements which may be regulated by $[Ca^{2+}]_i$ [94]. The end result of this early response is likely the inhibition of pollen tube growth. Subsequently, there is a second phase in which the Ca^{2+}-independent phosphorylation of p68 occurs. Whether this is a downstream event in the same pathway regulating phosphorylation of p26 or a result of the activation of a different pathway is unclear [83, 89]. Increased p68 phosphorylation may participate in gene expression and, ultimately, in the irreversible inhibition of pollen tube growth by programmed cell death [89, 95].

Self-incompatibility in the *Brassicaceae*

Molecular investigations into sporophytic self-incompatibility have centred on species within the *Brassicaceae* family, primarily *Brassica oleracea* (e.g. cabbage, cauliflower, kale, broccoli), *B. rapa* (chinese cabbage) and

B. napus (canola) [1, 96]. The self-incompatibility response in *Brassica* involves a series of complex cellular interactions that occur at the stigmatic surface of the pistil. Unlike the self-incompatibility systems prevalent among the *Solanaceae* and *Papaveraceae* families, the recognition and subsequent rejection of self-incompatible *Brassica* pollen is manifested rapidly and is marked by the inhibition of pollen hydration, pollen germination or pollen tube invasion of the stigma surface [97]. As in the previous systems, initial genetic studies identified a single highly polymorphic *S* locus controlling self-incompatibility in *Brassica*, but molecular studies have identified several tightly linked genes in the *S* locus region that play a role in this response [98]. The term '*S* haplotype' has been adopted to designate the different *S* alleles for this group of multi-allelic genes in the *S* locus region [99–102].

Due to the sporophytic nature of the *Brassica* self-incompatibility system, the phenotype of the pollen is not determined by its haploid genome, but by the *S* genotype of its diploid parent (fig. 1B). Consequently, pollen is recognized as being self-incompatible by the pistil if either of the two *S* haplotypes carried by the pollen parent matches one of the *S* haplotypes carried by the pistil. Therefore, successful fertilization occurs only when the pistil and pollen parent carry different *S* haplotypes [98] (fig. 1B). Considerable progress has been made over the years towards elucidating the mechanisms leading to the recognition and rejection of self-incompatible *Brassica* pollen. Both the pollen and pistil *S* gene counterparts have been identified, and several downstream genes involved in the self-incompatibility response have been characteriszed.

SLG and SRK: pistil S proteins and their involvement in self-incompatibility

The first step in the isolation of the pistil *S* protein was the identification of the *S* locus-specific glycoprotein (SLSG), an abundant *S* allele-specific glycoprotein detected in stigmatic protein extracts [103–105]. Based on the observation that approximately 5% of protein synthesis in self-incompatible stigmas was dedicated to the synthesis of SLSG, a stigma cDNA library was differentially screened and resulted in the isolation of the first *S* locus linked candidate cDNA from *B. oleracea* called the *S* locus glycoprotein (SLG) [106]. The predicted amino acid sequence of this *SLG* cDNA exhibited a high degree of sequence identity to peptide sequences derived from *B. rapa* SLSGs [107]. The SLG protein was subsequently shown to be an abundant glycoprotein localized to the stigmatic papillae cell wall [108]. All of the *S* haplotype-specific *SLG* cDNAs isolated to date are predicted to encode secreted glycoproteins consisting of a cleavable signal peptide, several potential N-glycosylation sites, three hypervariable regions and 12 conserved cysteine residues located towards the C terminus of the protein [109, 110] (fig. 4A).

Isolation of the *SLG* gene led to the identification of a second *S* locus-linked gene, the *S* locus receptor kinase (SRK) [111, 112]. Based on cDNA sequence analysis, *SRK* was predicted to encode a plasma membrane receptor kinase comprised of three distinct domains: an extracellular domain followed by a single transmembrane-spanning region and a cytoplasmic serine/threonine kinase domain [111, 112]. The extracellular domain of SRK which is implicated in ligand binding shares a high degree of sequence similarity with SLG implying that both genes co-evolved and that SLG perhaps is the result of a duplication of the SRK extracellular domain [113, 114]. The extracellular domain of SRK, like SLG, also encodes a signal peptide, potential N-glycosylation sites, three hypervariable regions and 12 conserved cysteine residues [111, 112, 115] (fig. 4A). In addition, SRK possesses several other characteristics consistent with its role in self-incompatibility: a pattern of expression restricted predominantly to the pistil and a high degree of *S* haplotype specific sequence polymorphism similar to SLG [111, 112].

SRK has been found to be membrane associated, as predicted for a receptor kinase, and has a functional serine/threonine kinase domain [112, 116–119]. Giranton et al. [119] conducted further biochemical studies on SRK using an insect/baculovirus system, and found that the phosphorylation of the recombinant SRK integral membrane protein was constitutive in the absence of pollen or stigma extracts. SRK phosphorylation was shown to occur in trans, whereby a kinase-inactive SRK became phosphorylated when co-expressed with a functional SRK construct likely as a result of SRK dimerization [119]. Experiments performed to address the physiological relevance of these results examined the oligomeric status of SRK in planta and showed that SRK forms complexes with either other SRK/SLGs or other stigmatic proteins in unpollinated flowers [119].

Since both *SLG* and *SRK* are highly polymorphic, the *S* haplotypes of both genes can be divided into two classes (class I and class II) based on sequence similarities between the SLG proteins and between the SRK proteins. Class I *S* haplotypes possess highly polymorphic *SLG* and *SRK* genes, and show a strong self-incompatibility phenotype. Class II haplotypes, however, possess *SLG* and *SRK* genes that are less polymorphic, exhibit a weaker self-incompatibility phenotype and are recessive to class I alleles [110, 120, 121]. Within a class of a given *S* haplotype, the SLG and the extracellular domain of SRK generally exhibit between 75–90% amino acid identity [4, 122, 123]. While the SLG/SRK pair for a particular *S* haplotype often share the highest amino acid

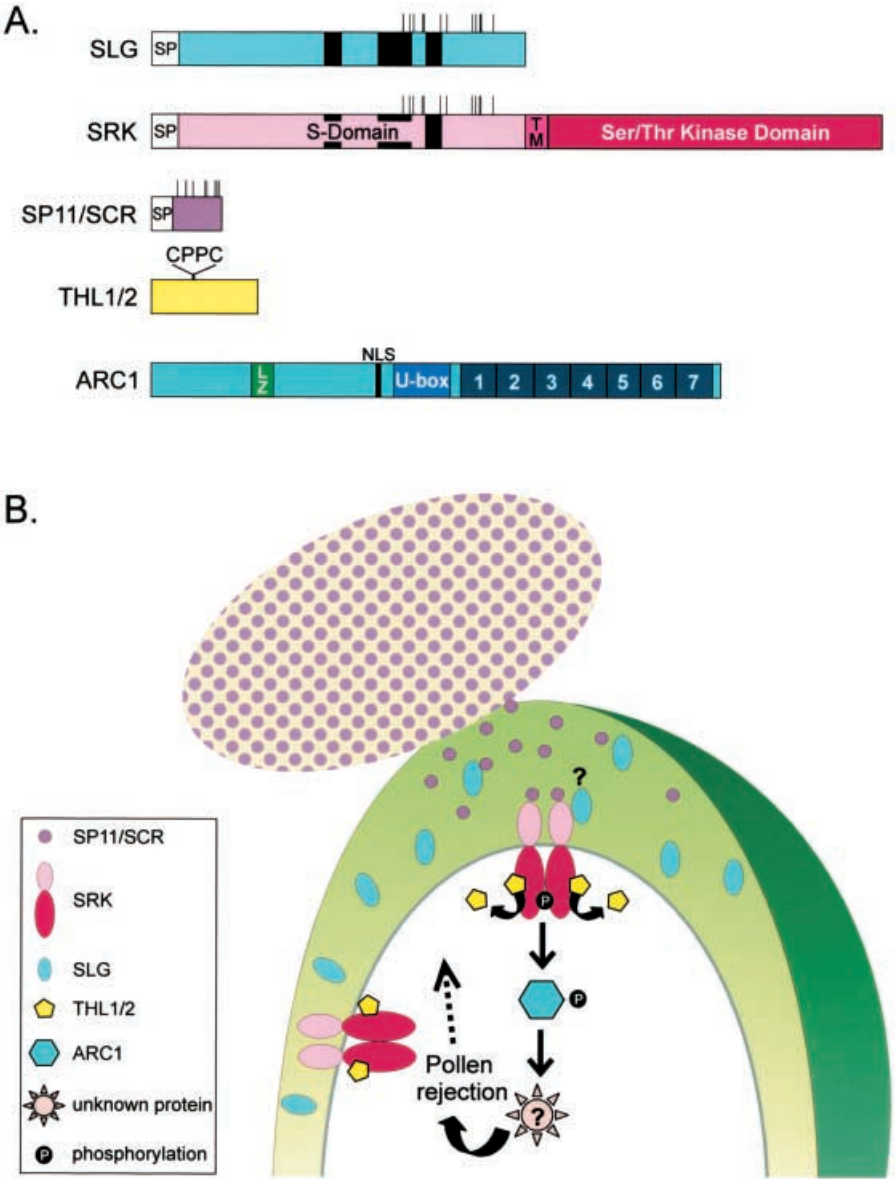

Figure 4. The self-incompatibility response in the *Brassicaceae*. (*A*) Schematic representation of genes involved in the *Brassica* self-incompatibility response. SP11/SCR represents the pollen *S* protein. Pistil proteins involved in the self-incompatibility response include the *S* locus glycoprotein (SLG), *S* receptor kinase (SRK), THL1/2 (thioredoxin *h*), and ARC1 (arm repeat containing-1). In SLG and the extracellular domain of SRK, black boxes denote the three hypervariable regions shared by these two proteins. In SLG, SRK and SCR, black lines denote conserved cysteine residues. SP, signal peptide; TM, transmembrane domain in SRK. The active site of THL1/2 required for redox activity is represented by CPPC (C, cysteine; P, proline). In ARC1, LZ is a predicted leucine zipper; NLS is a predicted nuclear localization signal, the U-box is a predicted motif implicated in ubiquitination, and the numbered boxes (1–7) represent the seven predicted arm repeats. (*B*) Model for the rejection of self-incompatible pollen in *Brassica*. In an incompatible pollination, the SP11/SCR ligand is proposed to bind to and activate SRK, suggested to exist as oligomeric complexes in the stigmatic membranes [119]. In the absence of incompatible pollen, SRK is proposed to be inhibited by THL1 (and THL2), and this inhibition is released upon the addition of haplotype-specific SP11/SCR pollen ligand. Whether SLG participates in the SRK complex is unclear since the allele-specific SLG is not always required [132, 133]. Activated SRK is proposed to autophosphorylate on serines and threonines, and some of these phosphorylated sites represent docking sites for downstream signalling proteins such as ARC1. ARC1 interacts with the activated SRK kinase domain through its arm repeat domain, and may become phosphorylated by SRK. How ARC1 leads to the rejection of incompatible pollen is unknown. ARC1 may play a role in the ubiquitination of other signalling proteins (negative regulators of self-incompatibility?) and may end up in the nucleus as part of its function.

identities, there are examples of SLGs sharing higher amino acid identities with other SLGs [110, 124]. Between the class I and class II SLGs/SRK, there is a lower degree of sequence conservation with approximately 65–68% amino acid identity [4, 122, 123].

Given that both SLG and SRK were present in the pistil, several transgenic experiments were carried out in an attempt to elucidate their functions in the pistil during the self-incompatibility response. Several lines of evidence have established SRK as the primary determinant of self-incompatibility in the pistil; however, the requirement for SLG in this response has been somewhat controversial. Early attempts to demonstrate a direct role for SLG in the self-incompatibility response were largely inconclusive [125–128]. In the transgenic experiments demonstrating a correlation between the loss of SLG expression and the loss of the self-incompatible phenotype in the pistil, the breakdown in self-incompatibility could not be attributed solely to SLG since transgene-induced gene silencing may have also affected the levels of SRK expression [125, 126, 129].

Stahl et al. [130] demonstrated that the transformation of a kinase-inactive form of SRK_{910} into the self-incompatible *B. napus* W1 line resulted in a breakdown of self-incompatibility [130]. This kinase-inactive SRK_{910} was proposed to have a dominant-negative effect on the endogenous wild-type SRK_{910}, and thereby provided evidence that SRK is required for the self-incompatibility response [130]. Furthermore, in a gain-of-function experiment Cui et al. [131] demonstrated that SRK and SLG were sufficient for the self-incompatibility phenotype of the pistil but not of the pollen. Two overlapping *S* region genomic clones carrying the SLG_{910} gene, SRK_{910} gene and an anther- expressed gene were transformed into self-compatible *B. napus* cv. Westar. Transgenic plants expressing SLG_{910} and SRK_{910} similar to the wild-type W1 control were shown by cross-pollination studies to have acquired the ability to reject W1 pollen but not pollen from a different self-incompatible *Brassica* line [131].

Two independent research groups conducted a series of transgenic experiments in an attempt to confirm the role of SRK and investigate the function of SLG in the self-incompatibility response [132, 133]. Both groups showed definitively that SRK is the sole *S* specificity determinant in the pistil; however, their findings differed as to the possible role of SLG. Takasaki et al. [132] transformed the self-incompatible *B. rapa* line carrying the S_{60} haplotype with either SLG_{28} or SRK_{28} of *B. rapa*. Transgenic plants expressing SRK_{28} alone but not those expressing SLG_{28} acquired the ability to reject self (S_{28}) pollen. The function of SLG was further examined by crossing the SLG_{28} transgene into the SRK_{28} transgenic lines. The resulting plants expressing both SLG_{28} and SRK_{28} showed an enhanced ability to reject S_{28} pollen. Therefore, these results concluded that SLG may function to enhance the recognition events in the self-incompatibility response [132].

Silva et al. [133] used a transgenic approach to examine the function of SLG whereby the SLG_{910} and SRK_{910} cDNAs from the *B. napus* self-incompatible W1 line were transformed independently into the *B. napus* self-compatible Westar line. The resulting transgenic plants expressing only SRK_{910} acquired the ability to reject W1 pollen, whereas the plants expressing the SLG_{910} transgene did not reject W1 pollen and remained fully fertile. These results clearly demonstrated, as seen with Takasaki et al. [132], that the SRK transgene alone could confer the self-incompatibility trait in the pistil. Transgenic plants expressing both SLG_{910} and SRK_{910} did not exhibit any further decrease in seed production when compared to the transgenic plants expressing SRK_{910} alone. This suggests that unlike the enhancing effect of SLG reported by Takasaki et al. [132], SLG_{910} in this study had no detectable effects on the self-incompatibility phenotype [133].

Other types of studies have also produced mixed conclusions on the role of SLG. Dixit et al. [134] characterized two mutant self-compatible *Brassica* lines which expressed low levels of SLG. While these lines were found to synthesize wild-type levels of SRK transcripts, they were unable to produce SRK protein [134]. Furthermore, the analysis of transgenic *Nicotiana* plants transformed with either SRK_6 alone or with both SLG_6 and SRK_6 indicated that SLG_6 was required for the stabilization and proper maturation of SRK_6 [134]. In contrast, Gaude et al. [135] found no correlation between SLG expression levels and self-incompatibility. They characterized a self-compatible *B. oleracea* line which had abundant expression of SLG in stigmas, and self-incompatible lines that weakly expressed SLG. Furthermore, Suzuki et al. [136] identified two self-incompatible *B. oleracea* haplotypes, S_{18} and S_{60}, which produced no SLG protein, yet exhibited a strong self-incompatibility phenotype. Thus, the requirement for SLG in the self-incompatibility response appears to be variable with only some of the *S* haplotypes requiring the allele-specific SLG.

Further support for the enigmatic role of SLG in the self-incompatibility response stems from investigations of the related self-incompatibility system in *Arabidopsis lyrata*. Kusaba et al. [137] characterized tightly linked genes that were orthologous to *SRK* and the *SP11*/*SCR* pollen determinant (described in the next section); however, an *SLG* orthologue was not identified [137]. Similarly, Schierup et al. [138] characterized the genetics of the self-incompatibility system in *A. lyrata* and identified several *SRK* alleles; however, they could not find any evidence for the presence of a tightly linked SLG gene. Thus, these two studies suggest that SLG is not required for the self-incompatibility system in *A. lyrata*.

Identity and function of the pollen S gene in self-incompatibility

The arrival of self-incompatible pollen on the stigmatic surface of the pistil rapidly initiates the self-incompatibility response, suggesting that the male component of this reaction is deposited on the pollen grain surface and is readily accessible to the stigmatic papillae [139]. Several strategies have been used to identify proteins in the pollen coat that may function in self-incompatibility. Fractionation studies of the pollen coat proteins led to the identification of a basic, cysteine-rich, 7-kDa protein (PCP7/PCP-A1) which was shown to interact with SLGs and belongs to a larger family of similar proteins [139–143]. However, PCP7/PCP-A1 was unlikely to encode the pollen *S* determinant given that there was no *S* allele specificity to the interaction between PCP7/PCP-A1 and SLG, and that the *PCP7/PCP-A1* gene is unlinked to the *S* locus [138, 140]. Using a pollination bioassay, Stephenson et al. [144] provided experimental evidence confirming that the pollen *S* determinant did reside in the pollen coat and was likely to be a small basic protein.

Mapping and sequencing of the *S* locus region was another approach used by several groups searching for the pollen *S* gene [100–102, 114, 145, 146]. Although several anther-expressed genes were identified in the genomic region defined by the *SLG* and *SRK* genes [100–102, 114, 145, 146], only recently has one of these genes been implicated in the self-incompatibility response . This gene, termed *SP11* [101] or *SCR* [147], has been shown to encode the male determinant of self-incompatibility [101, 147–149]. *SP11* was first identified by Suzuki et al. [101] as an anther expressed S_9-specific gene from *B. rapa* predicted to encode a protein with characteristics similar to those of pollen coat proteins [101]. Subsequently, Schopfer et al. [147] isolated different alleles of a gene named *SCR* (*S* locus cysteine rich) which turned out to be the same as *SP11*. In a gain-of-function experiment, Schopfer et al. [147] introduced a transgenic construct consisting of the SCR_8 promoter fused to the SCR_6 cDNA into S_2S_2 *B. oleracea* plants. The resulting transgenic S_2S_2 *B. oleracea* plants expressing SCR_6 were used in subsequent pollinations of S_6S_6 and $S_{22}S_{22}$ *B. oleracea* plants. The pollen from transgenic plants expressing SCR_6 was rejected by S_6S_6 but not by $S_{22}S_{22}$ pistils. Therefore, these results confirmed that *SCR* is required and sufficient to confer the *S* allele-specific male trait in the self-incompatibility system [147].

Takayama et al. [148] demonstrated that *SP11*/*SCR* encoded the pollen *S* determinant by using pollination bioassays in which recombinant SP11/SCR of the S_9 haplotype was applied to the papillae cells of S_9 and S_8 stigmas. They observed that the SP11/SCR protein elicited the self-incompatibility response only when applied to S_9 (self-) stigmas, resulting in the inhibition of pollen hydration. Furthermore, in situ hybridization of anther sections clearly showed that SP11/SCR was expressed in the tapetum of the anther, consistent with the sporophytic control of the self-incompatibility response in *Brassica* [148]. Shiba et al. [149] then used a gain-of-function transgenic approach to further confirm the role of SP11/SCR as the sole male determinant of self-incompatibility in *B. rapa*.

Other pistil proteins involved in self-incompatibility

In the *Brassica* self-incompatibility system, SRK, SP11/SCR and perhaps SLG are the determinants that initiate the allele-specific rejection of self-incompatible pollen. However, other components are required to carry out cellular responses leading to pollen rejection; that is, the signalling cascade that is initiated by the activated SRK in the stigmatic papillae. One approach used in a search for these downstream signalling molecules was a yeast two-hybrid screen for proteins which interacted with the SRK kinase domain [150]. Screening of a *B. napus* pistil cDNA library led to the identification of three proteins, THL1, THL2 and ARC1 [151, 152] (fig. 4A).

THL1 and THL2 belong to the thioredoxin *h* family and are most similar to the *Arabidopsis* thioredoxin *h* members TRX3 and TRX4, respectively [151]. Thioredoxin *h* is a cytoplasmic protein involved in the reduction of disulphide bonds [153]. Expression studies determined that both THL1 and THL2 appear to be ubiquitously expressed in a variety of *B. napus* tissues, although THL2 is mainly expressed in floral tissues [151]. Yeast two-hybrid studies have demonstrated that both THL1 and THL2 interact specifically with the kinase domain of different allelic forms of SRK [151, 154]. Furthermore, a conserved cysteine at the end of the predicted transmembrane domain of SRK is required for this interaction. This conserved cysteine is not found in other related plant receptor kinases, consistent with the interaction being specific to the SRKs [154]. Conflicting results were obtained regarding the requirement of a phosphorylated SRK kinase, suggesting that the interaction may be phosphorylation independent [151]. Finally, site-directed mutagenesis was used to substitute the conserved flanking cysteines in the active site of THL1 to serine residues. Mutation of these cysteine residues rendered THL1 unable to interact with SRK_{910}, thereby suggesting that the inherent redox activity of thioredoxins is required for this interaction [154].

A biological role for THL1/2 in the self-incompatibility response was recently proposed in a study by Cabrillac et al. [155]. They found that stigmatic extracts contained a soluble inhibitor of SRK, which was removed during membrane purifications leading to the constitutive activation of SRK in the absence of self-incompatible pollen. This result also explains a previous study where SRK produced in the insect/baculovirus system was found to be

constitutively active [119]. When a soluble stigmatic extract was added to membrane fractions purified from either stigmas or from the SRK expressing insect/baculovirus system, SRK no longer had constitutive phosphorylation activity. Through a series of experiments, Cabrillac et al. [155] then went on to demonstrate that the soluble inhibitor in the stigmatic extracts was thioredoxin, and that recombinant THL1 could also inhibit the in vitro autophosphorylation of SRK. With the addition of allele-specific pollen coat proteins, this inhibition effect was overcome and SRK became autophosphorylated [155]. Thus, these results suggest that thioredoxin acts as a negative regulator of SRK in the absence of the SP11/SCR ligand.

The third protein isolated from the yeast two-hybrid screen was ARC1, which was predicted to be a novel protein [152]. As with THL1/2, ARC1 specifically interacted with SRK kinase domains; however, in contrast to THL1/2, ARC1 clearly demonstrated a phosphorylation-dependent interaction [152]. This suggested that in vivo, ARC1 would only interact with the SRK kinase domain after the activation of SRK by SP11/SCR. The C-terminal region of ARC1 consists of seven predicted arm repeats which comprise the kinase-binding domain (fig. 4A). Motif searches of the N-terminal region of ARC1 lead to the identification of a putative leucine zipper, nuclear localization signal, and U-box [S. L. Stone and D. R. Goring, unpublished data] (fig. 4A). Whether these motifs are functional is not known. Interestingly, ARC1 has a very tissue specific pattern of expression with its mRNA localized to the stigma at the top of the pistil [152]. This suggests that ARC1 has a specialized role in pollen-pistil interactions. Confirmation for a biological role for ARC1 in self-incompatibility came from the analysis of ARC1 antisense transgenic plants [156]. Reduced levels of *ARC1* mRNA were correlated with a partial breakdown of the self-incompatibility response in the pistil [156]. This suggested that ARC1 is a positively acting downstream component of the self-incompatibility signalling pathway in the pistil.

Analysis of a naturally occurring mutation in *B. rapa*, called *mod*, which is unlinked to self-incompatibility and which causes a breakdown in self-incompatibility, led to the isolation of a candidate gene called *MIP-MOD* [157]. This gene was predicted to encode an aquaporin-like protein proposed to play a role in preventing pollen hydration by increasing water flow away from the incompatible pollen [157]. However, in a recent study by Fukai et al. [158], data were presented showing that *MIP-MOD* is not the *MOD* gene. Ikeda et al. [157] had suggested that decreased levels of *MIP-MOD* mRNA in the *B. rapa mod* plants was responsible for the breakdown in self-incompatibility. However, Fukai et al. [158] demonstrated that there is naturally occurring variation in levels of *MIP-MOD* mRNA, and that some self-incompatible lines contained low levels of *MIP-MOD* mRNA. Thus, while *MOD* is a locus involved in self-incompatibility, it does not appear to be the aquaporin-like *MIP-MOD* gene.

Model for *Brassica* self-incompatibility

Prior to pollination, SRK is present in the stigmatic papillae, ready to be activated when a self-incompatible pollen comes in contact with a stigmatic papilla. In the absence of the pollen stimulus, low basal levels of SRK activity are maintained by the presence of the THL1/2 inhibitor. The involvement of SLG in the self-incompatibility response remains ambiguous and if required, SLG may complex with SRK to stabilize it and thereby enhance the self-incompatibility response [132, 134].

When a self-incompatible pollen grain lands on a stigmatic surface, the pollen *S* determinant, SP11/SCR, is predicted to diffuse into the papillar cell wall where it interacts with the extracellular domain of SRK in an *S* haplotype-specific manner (fig. 4B). This leads to activation of SRK by overcoming the inhibitory effect of THL1/2. The activated SRK then initiates a signalling cascade in the stigmatic papillae ultimately leading to rejection of the incompatible pollen. Little is known about these cellular events. ARC1 has been demonstrated to be required for self-incompatibility and likely acts directly downstream of SRK (fig. 4B). The discovery of the U-box motif which is implicated in ubiquitination [159] suggests that ARC1 may be regulating the ubiquitination and degradation of another protein in the stigmatic papillae. If this is correct, then this protein would be predicted to be a negative regulator of the self-incompatibility response. Whether as part of this process ARC1 is being targeted to the nucleus is also unknown. Since only a partial breakdown of the self-incompatibility response was detected in the *ARC1* antisense transgenic plants, SRK may also be activating one or more other signalling pathways which may partially compensate for the absence of ARC1 (fig. 4B). Ultimately, these pathways may be blocking steps such as the release of water required for pollen hydration and the release of stigmatic papillar cell wall-degrading enzymes required for pollen tube growth.

One interesting feature of the *Brassica* self-incompatibility system observed by Thompson and Taylor [7] was the occurrence of some dominant and recessive interactions between *S* alleles, though many of the *S* alleles were co-dominant. These dominant-recessive interactions were not in a linear arrangement and the recessiveness of a particular *S* allele depended on the combination of *S* alleles and the site of the interaction (pistil or pollen). For example, if the interaction site was in the pollen, then pollen originating from a heterozygous pollen parent carrying a dominant-recessive *S* allele combination would be rejected by a pistil homozygous for the dominant *S* allele, but accepted by a pistil homozygous for the recessive *S*

allele [7]. If the interaction site was in the pistil, then a heterozygous pistil carrying a dominant-recessive *S* allele combination would reject pollen coming from a pollen parent homozygous for the dominant *S* allele and accept pollen coming from a pollen parent homozygous for the recessive *S* allele [7]. Therefore, in certain combinations, one *S* allele is able to block the function of another *S* allele. Now that the male and female determinants of *Brassica* self-incompatibility have been identified (SP11/SCR, SRK), it will be of great interest to begin investigating the molecular and biochemical basis of these dominant-recessive interactions.

Conclusion

Self-incompatibility is a system used by many flowering plant species to prevent self-fertilization and thereby promote outcrossing. Over the years, considerable insight into the mechanisms regulating self-incompatibility has been obtained for the *Solanaceae* and *Papaveraceae* gametophytic self-incompatibility systems as well as for the sporophytic self-incompatibility system of the *Brassicaceae*. A combination of genetic and molecular studies has resulted in the identification and characterization of the self-incompatibility genes involved in this response. Although substantial progress has been made, our understanding of how these systems work is far from complete. In the *Solanaceae* and *Papaveraceae* self-incompatibility systems, the identification of the pollen *S* genes will contribute towards establishing a more comprehensive model of self-incompatibility. In addition, careful investigation of the components in the signalling cascades of both the *Papaveraceae* and the *Brassicaceae* is required for a complete understanding of the self-incompatibility response in these families. Self-incompatibility has fascinated biologists for over a century and will continue to be an area of intense research focus.

Acknowledgements. We thank Mark Gagliardi and members of the Goring laboratory for critical reading of the manuscript. N.F.S. is supported by a graduate scholarship from the Ontario Graduate Scholarships in Science & Technology, and research in this laboratory is supported by grants from the Natural Sciences and Engineering Research Council (NSERC) to D.R.G.

1 Kao T.-H. and McCubbin A. G. (1996) How flowering plants discriminate between self and non-self pollen to prevent inbreeding. Proc. Natl. Acad. Sci. USA **93:** 12059–12065
2 Giranton J.-L., Passelegue E., Dumas C., Cock J. M. and Gaude T. (1999) Membrane proteins involved in pollen-pistil interactions. Biochimie **81:** 675–680
3 McCubbin A. G. and Kao T.-H. (2000) Molecular recognition and response in pollen and pistil interactions. Annu. Rev. Cell Dev. Biol. **16:** 333–364
4 Gaude T. and McCormick S. (1999) Signaling in pollen-pistil interactions. Cell Dev. Biol. **10:** 139–147
5 Ebert P. R., Anderson M. A., Bernatzky R., Altschuler M. and Clarke A. E. (1989) Genetic polymorphism of self-incompatibility in flowering plants. Cell **56:** 255–262
6 Nettancourt D. de (1977) Incompatibility in angiosperms. In: Monographs on Theoretical and Applied Genetics, No. 3, pp. 1–96, Frankel R., Gall G. A. E. and Linskens H. F. (eds), Springer, Berlin
7 Thompson K. F. and Taylor J. P (1966) Non-linear dominance relationships between *S*-alleles. Heredity **21:** 345–362
8 Bredemeijer G. M. M. and Blaas J. (1981) S-specific proteins in styles of self-incompatible *Nicotiana alata*. Theor. Appl. Genet. **59:** 185–190
9 Anderson M. A., Cornish E. C., Mau S.-L., Williams E. G., Hoggart R., Atkinson A. et al. (1986) Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana alata*. Nature **321:** 38–44
10 Ai Y., Singh A., Coleman C. E., Ioerger T. R., Kheyr-Pour A. and Kao T.-H. (1990) Self-incompatibility in *Petunia inflata*: isolation and characterization of cDNAs encoding three S-allele-associated proteins. Sex. Plant Reprod. **3:** 130–138
11 Clark K. R., Okuley J. J, Collins P. D. and Sims T. L. (1990) Sequence variability and developmental expression of S-alleles in self-incompatible and pseudo-self-compatible *Petunia*. Plant Cell **2:** 815–826
12 Ai Y., Tsai D.-S. and Kao T.-H. (1992) Cloning and sequencing of cDNAs encoding two S-proteins of a self-compatible cultivar of *Petunia hybrida*. Plant Mol. Biol. **3:** 130–138
13 Tsai D.-S., Lee H.-S., Post L. C., Kreiling K. M. and Kao T.-H. (1992) Sequence of an S-protein of *Lycopersicon peruvianum* and comparison with other Solanaceous S-proteins. Sex. Plant Reprod. **5:** 256–263
14 Xu B., Mu J., Nevins D. L., Grun P. and Kao T.-H. (1990) Cloning and sequencing of cDNAs encoding two self-incompatibility associated proteins in *Solanum chacoense*. Mol. Gen. Genet. **224:** 341–346
15 Kaufmann H., Salamini F. and Thompson R. D. (1991) Sequence variability and gene structure at the self-incompatibility locus of *Solanum tuberosum*. Mol. Gen. Genet. **226:** 457–466
16 Sassa H., Nishio T., Kowyama Y., Hirano H., Koba T. and Ikehashi H. (1993) Self-incompatibility (*S*) alleles of the Rosaceae encode members of a distinct class of the T2/S ribonuclease superfamily. Mol. Gen. Genet. **250:** 547–557
17 Tao R., Yamane H., Sassa H., Mori H., Gradziel T. M., Dandekar A. M. et al. (1997) Identification of stylar RNases associated with gametophytic self-incompatibility in almond (*Prunus dulcis*). Plant Cell Physiol. **38:** 304–311
18 Xue Y., Carpenter R., Dickinson H. G. and Coen E. S. (1996) Origin of allelic diversity in *Antirrhinum* S locus RNases. Plant Cell **8:** 805–814
19 Kawata Y., Sakiyama F. and Tamaoki H. (1988) Amino acid sequence of ribonuclease T2 from *Aspergillus oryzae*. Eur. J. Biochem. **176:** 683–697
20 McClure B. A., Haring V., Ebert P. R., Anderson M. A., Simpson R. J., Cornish E.C. et al. (1989) Style self-incompatibility gene products of *Nicotiana alata* are ribonucleases. Nature **342:** 955–957
21 Broothaerts W., Vanvinckenroye P., Decock B., Van Damme J. and Vendrig J. C. (1991) *Petunia hybrida* S-proteins: ribonuclease activity and the role of their glycan side chains in self-incompatibility. Sex. Plant Reprod. **4:** 258–266
22 Singh A., Ai Y. and Kao T.-H. (1991) Characterization of ribonuclease activity of three S-allele-associated proteins of *Petunia inflata*. Plant Physiol. **96:** 61–68
23 Cornish E. C., Pettitt J. M., Bonig I. and Clarke A. E. (1987) Developmentally controlled expression of a gene associated with self-incompatibility in *Nicotiana alata*. Nature **326:** 99–102

24 Anderson M. A., McFadden G. I., Bernatzky R., Atkinson A., Orpin T., Dedman H. et al. (1989) Sequence variability of three alleles of the self-incompatibility gene of *Nicotiana alata*. Plant Cell **1:** 483–491

25 Jahnen W., Batterham M. P., Clarke A. E., Moritz R. L. and Simpson R. J. (1989b) Identification, isolation, and N-terminal sequencing of style glycoproteins associated with self-incompatibility in *Nicotiana alata*. Plant Cell **1:** 493–499

26 Clark A. G. and Kao T.-H. (1991) Excess nonsynonymous substitution at shared polymorphic sites among self-incompatibility alleles of Solanaceae. Proc. Natl. Acad. Sci. USA **88:** 9826–9827

27 Ioerger T. R., Clark A. G. and Kao T.-H. (1990) Polymorphism at the self-incompatibility locus in Solananceae predates speciation. Proc. Natl. Acad. Sci. USA **87:** 9732–9735

28 Ioerger T. R., Gohlke J. R., Xu B. and Kao T.-H. (1991) Primary structural features of the self-incompatibility protein in Solanaceae. Sex. Plant Reprod. **4:** 81–87

29 Kawata Y., Sakiyama F., Hayashi F. and Kyogoku Y. (1990) Identification of two essential histidine residues of ribonuclease T2 from *Aspergillus oryzae*. Eur. J. Biochem. **187:** 255–262

30 Green P. J. (1994) The ribonucleases of higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **45:** 421–445

31 Royo J., Kunz C., Kowyama Y., Anderson M., Clarke A. E. and Newbigin E. (1994) Loss of a histidine residue at the active site of *S*-locus ribonuclease is associated with self-incompatibility in *Lycopersicon peruvianum*. Proc. Natl. Acad. Sci. USA **91:** 6511–6514

32 Ishimizu T. I., Miyagi M., Norioka S., Liu Y.-H., Clarke A. E. and Sakiyama F. (1995) Identification of histidine 31 and cysteine 95 in the active site of self-incompatibility associated S_6-RNase in *Nicotiana alata*. J. Biochem. **118:** 1007–1013

33 Parry S., Newbigin E., Currie G., Bacic A. and Oxley D. (1997) Identification of active-site histidine residues of a self-incompatibility ribonuclease from a wild tomato. Plant Physiol. **115:** 1421–1429

34 Oxley D. and Bacic A. (1996) Disulphide bonding in a stylar self-incompatibility ribonuclease of *Nicotiana alata*. Eur. J. Biochem. **242:** 75–80

35 Ishimizu T., Norioka S., Kanai M., Clarke A. E. and Sakiyama F. (1996) Location of cysteine and cystine residues in S-ribonuclease associated with gametophytic self-incompatibility. Eur. J. Biochem. **242:** 627–635

36 Parry S., Newbigin E., Craik D., Nakamura K. T., Bacic A. and Oxley D. (1998) Structural analysis and molecular model of a self-incompatibility RNase from wild tomato. Plant Physiol. **116:** 463–469

37 Woodward J. R., Bacic A., Jahnen W. and Clarke A. E. (1989) N-linked glycan chains on S-allele-associated glycoproteins from *Nicotiana alata*. Plant Cell **1:** 511–514

38 Oxley D., Munro S. L. A., Craik D. J. and Bacic A. (1996) Structure of N-glycans on the S_3- and S_6-allele stylar self-incompatibility ribonucleases of *Nicotiana alata*. Glycobiology **6:** 611–618

39 Zurek D. M., Mou B., Beecher B. and McClure B. (1997) Exchanging sequence domains between S-RNases from *Nicotiana alata* disrupts pollen recognition. Plant J. **11:** 797–808

40 Ishimizu T., Endo T., Yamaguchi-Kabata Y., Nakamura K. T., Sakiyama F. and Norioka S. (1998) Identification of regions in which positive selection may operate in S-RNase of Rosaceae: implication for S-allele-specific recognition sites in S-RNase. FEBS Lett. **440:** 337–342

41 Kurihara H., Mitsui Y., Ohgi K., Irie M., Mizuno H. and Nakamura K. T. (1992) Crystal and molecular structure of RNase Rh, a new class of microbial ribonuclease from *Rhizipus niveus*. FEBS Lett. **306:** 189–192

42 Lee H.-S, Huang S. and Kao T.-H. (1994) S proteins control rejection of incompatible pollen in *Petunia inflata*. Nature **367:** 560–563

43 Murfett J., Atherton T. L., Mou B., Gasser C. S. and McClure B. A. (1994) S-RNase expressed in transgenic *Nicotiana* causes S-allele-specific pollen rejection. Nature **367:** 563–566

44 McClure B. A., Gray J. E., Anderson M. A. and Clarke A. E. (1990) Self-incompatibility in *Nicotiana alata* involves degradation of pollen rRNA. Nature **347:** 757–760

45 Huang S., Lee H.-S., Karunanandaa B. and Kao T.-H. (1994) Ribonuclease activity of *Petunia inflata* S proteins is essential for rejection of self-pollen. Plant Cell **6:** 1021–1028

46 Woodward J. R., Craik D., Dell A., Khoo K. K-H., Munro S. L. A., Clarke A. E. et al. (1992) Structural analysis of the N-glycan chains from a stylar glycoprotein associated with the expression of self-incompatibility in *Nicotiana alata*. Glycobiology **2:** 241–250

47 Karunanandaa B., Huang S. and Kao T.-H. (1994) Carbohydrate moiety of the *Petunia inflata* S_3 protein is not required for self-incompatibility interactions between pollen and pistil. Plant Cell **6:** 1933–1940

48 Zurek D. M., Mou B., Beecher B. and McClure B. A. (1997) Exchanging sequence domains between S-RNases from *Nicotiana alata* disrupts pollen recognition. Plant J. **11:** 797–808

49 Matton D. P., Maes O., Laublin G., Xike Q., Bertrand C., Morse D. et al. (1997) Hypervariable domains of self-incompatibility RNases mediate allele-specific pollen rejection. Plant Cell **9:** 1757–1766

50 Saba-El-Leil M., Rivard S., Morse D. and Cappadocia M. (1994) The S_{11} and S_{13} self-incompatibility alleles in *Solanum chacoense* Bitt. are remarkably similar. Plant Mol. Biol. **24:** 571–583

51 Matton D. P., Luu D. T., Xike Q., Laublin G., O'Brien M., Maes O. et al. (1999) Production of an *S*-RNase with dual specificity suggests a novel hypothesis for the generation of new *S* alleles. Plant Cell **11:** 2087–2097

52 Verica J. A., McCubbin A. G. and Kano T.-H. (1998) Are the hypervariable regions of S-RNases sufficient for allele-specific rejection of pollen? Plant Cell **10:** 314–316

53 Dodds P. N., Ferguson C., Clarke A. E. and Newbigin E. (1999) Pollen-expressed S-RNases are not involved in self-incompatibility in *Lycopersicon peruvianum*. Sex. Plant Reprod. **12:** 76–87

54 Sassa H., Hirano H., Nisho T. and Koba T (1997) Style-specific self-compatible mutation caused by deletion of the S-RNase gene in Japanese pear (*Pyrus serontia*). Plant J. **12:** 223–227

55 McCubbin A. G., Chung Y.-Y. and Kao T.-H. (1997) A mutant S_3-RNase of *Petunia inflata* lacking RNase activity has an allele-specific dominant negative effect on self-incompatibility interactions. Plant Cell **9:** 85–95

56 Dowd P. E., McCubbin A. G., Wang X., Verica J. A., Tsukamoto T., Ando T. et al. (2000) Use of *Petunia inflata* as a model for the study of solanaceous type self-incompatibility. Ann. Bot. **85:** 87–93

57 McCubbin A. G., Wang X. and Kao T.-H. (2000) Identification of self-incompatibility (*S*-) locus linked pollen cDNA markers in *Petunia inflata*. Genome **43:** 619–627

58 Mather K. (1943) Specific differences in *Petunia* I. Incompatibility. J. Genet. **45:** 215–235

59 Martin F. W. (1968) The behaviour of *Lycopersicon* incompatibility alleles in an alien genetic milieu. Genetics **60:** 101-109

60 Ai Y., Kron E. and Kao T.-H. (1991) S-alleles are retained and expressed in a self-compatible cultivar of *Petunia hybrida*. Mol. Gen. Genet. **230:** 353–358

61 Bernatzky R., Galven R. H. and Rivers B. A. (1995) S-related protein can be recombined with self-compatibility in interspecific derivatives of *Lycopersicon*. Biochem. Genet. **33:** 215–225

62 Tsukamoto T., Ando T., Kokubun H., Watanabe H., Masada M., Zhu X. et al. (1999) Breakdown of self-incompatibility in

a natural population of *Petunia axillaris* (Solanaceae) in Uruguay containing both self-compatible and self-incompatible plants. Sex. Plant Reprod. **12:** 6–13
63 McClure B. A., Mou B., Canevascini S. and Bernatzky R. (1999) A small asparagine-rich protein required for S-allele-specific pollen rejection in *Nicotiana*. Proc. Natl. Acad. Sci. USA **96:** 13548–13453
64 Thompson R. D. and Kirch H. H. (1992) The *S* locus of flowering plants: when self-rejection is self-interest. Trends Genet. **8:** 381–387
65 Matton D. P., Nass N., Clarke A. E and Newbigin E. (1994) Self-incompatibility: how plants avoid illegitimate offspring. Proc. Natl. Acad. Sci. USA **91:** 1992–1997
66 Brewbaker J. L. and Natarajan A. T. (1960) Centric fragments and pollen part mutations of incompatibility alleles in *Petunia*. Genetics **45:** 699–704
67 Golz J. F., Su V., Clarke A. E. and Newbigin E. (1999) A molecular description of mutations affecting the pollen component of the *Nicotiana alata S* locus. Genetics **152:** 1123–1135
68 Luu D.-T., Qin X., Morse D. and Cappadocia M. (2000) S-RNase uptake by compatible pollen tubes in gametophytic self-incompatibility. Nature **407:** 649–651
69 Lawrence M. J., Afzal M. and Kenrick J. (1978) The genetic control of self-incompatibility in *Papaver rhoeas* L. Heredity **40:** 239–285
70 Franklin-Tong V. E., Thorlby G. J., Lawrence M. J. and Franklin F. C. H. (1992) Recognition signals and pollen responses in the incompatibility reaction in *Papaver rhoeas*. In: Angiosperm Pollen and Ovules: Basic and Applied Aspects, pp. 84–93, Mulcahy D. L., Bergamini-Mulcahy G°, Ottaviano E. (eds), Springer, New York
71 Franklin-Tong V. E., Lawrence M. J. and Franklin F. C. H. (1988) An in vitro bioassay for the stigmatic product of the self-incompatibility gene in *Papaver rhoeas* L. New Phytol. **110:** 109–118
72 Franklin-Tong V. E., Lawrence M. J. and Franklin F. C. H. (1991) Self-incompatibility in *Papaver rhoeas* L: there is no evidence for the involvement of stigmatic ribonuclease activity. Plant Cell Environ. **14:** 423–429
73 Foote H. G., Franklin-Tong V. E., Walker E. A., Lawrence M. J. and Franklin F. C. H. (1994) Cloning and expression of a novel self-incompatibility (S-)gene from *Papaver rhoeas* L. Proc. Natl. Acad. Sci. USA **91:** 2265–2269
74 Walker E. A., Ride J. P., Kurup S., Franklin-Tong V. E., Lawrence M. J. and Franklin F. C. H. (1996) Molecular analysis of two functionally identical S_3 homologues of the self-incompatibility gene of *Papaver rhoeas* from two different populations. Plant Mol. Biol. **30:** 983–994
75 Kurup S., Ride J. P., Jordan N. D., Fletcher G., Franklin-Tong V. E. and Franklin F. C. H. (1998) Identification and cloning of related self-incompatibility S genes in *Papaver rhoeas* and *Papaver nudicaule*. Sex. Plant Reprod. **1:** 192–198
76 Ride J. P., Davies E. M., Franklin F. C. H. and Marshall D. F. (1999) Analysis of Arabidopsis genome sequence reveals a large new gene family in plants. Plant Mol. Biol. **39:** 927–932
77 Kakeda K., Jordan N. D., Conner A., Ride J. P., Franklin-Tong V. E. and Franklin F. C. H. (1998) Identification of residues in a hydrophilic loop of the *Papaver rhoeas* S protein that play a crucial role in the recognition of incompatible pollen. Plant Cell **10:** 1723–1731
78 Hearn M. J., Franklin F. C. H. and Ride J. P. (1996) Identification of a membrane glycoprotein in pollen of *Papaver rhoeas* which binds stigmatic self-incompatibility (S-) proteins. Plant J. **9:** 467–475
79 Jordan N. D., Kakeda K., Conner A. C., Ride J. P., Franklin-Tong V. E. and Franklin F. C. H. (1999) S protein mutants indicate a functional role for SBP in the self-incompatibility reaction of *Papaver rhoeas*. Plant J. **2:** 119–125
80 Franklin-Tong V. E., Lawrence M. J. and Franklin F. C. H. (1990) Self-incompatibility in *Papaver rhoeas* L: inhibition of incompatible pollen tube growth is dependent on gene expression. New Phytol. **116:** 31–324
81 Franklin F. C. H., Atwal K. K., Ride J. P. and Franklin-Tong V.E. (1994) Progress towards elucidating the molecular basis of the *Papaver rhoeas* self-incompatibility system. In: Molecular and Cellular Aspects of Plant Reproduction, SEB Seminar Series 55, pp. 173–191, Scott R. J. and Stead A. D. (eds), Cambridge University Press, Cambridge
82 Franklin-Tong V. E. (1999) Signalling and the modulation of pollen tube growth. Plant Cell **11:** 727–738
83 Snowman B. N., Geitman A., Clarke S. R., Staiger C. J., Franklin F. C. H., Emons A. M. C. et al. (2000) Signalling and the cytoskeleton of pollen tubes in *Papaver rhoeas*. Ann. Bot. **85:** 49–57
84 Franklin-Tong V. E., Ride J. P., Read N. D., Trewavas A. J. and Franklin F. C. H. (1993) The self-incompatibility response in *Papaver rhoeas* is mediated by cytosolic free calcium. Plant J. **4:** 163-177
85 Franklin-Tong V. E., Ride J. P. and Franklin F. C. H. (1995) Recombinant stigmatic self-incompatibility (S-) protein elicits a Ca^{2+} transient in pollen of *Papaver rhoeas*. Plant J. **8:** 299–307
86 Franklin-Tong V. E., Hacket G. and Hepler P. K. (1997) Ratio-imaging of Ca^{2+}_{i} in the self-incompatibility response in pollen tubes of *Papaver rhoeas*. Plant J. **12:**1375–1386
87 Franklin-Tong V. E., Drøbak B. K., Allen A. C., Watkins P. A. C. and Trewavas A. J. (1996) Growth of pollen tubes of *Papaver rhoeas* is regulated by a slow-moving calcium wave propagated by inositol 1,4,5-triphosphate. Plant Cell **8:** 1305–1321
88 Rudd J. J., Franklin F. C. H., Lord J. M. and Franklin-Tong V. E. (1996) Increased phosphorylation of a 26 kDa protein is induced by the self-incomplatibility response. Plant Cell **8:** 713–724
89 Rudd J. J., Franklin F. C. H., Lord J. M. and Franklin-Tong V. E. (1997) Ca^{2+}-independent phosphorylation of a 68 kDa protein is stimulated by the self-incompatibility response. Plant J. **12:** 507–514
90 Jordan N. D., Ride J. P., Rudd J. J., Davies E. M., Franklin-Tong V. E. and Franklin F. C. H. (2000) Inhibition of self-incompatible pollen in *Papaver rhoeas* involves a complex series of cellular events. Ann. Bot. **85:** 197–202
91 Clarke S. R., Staiger C. J., Gibbon B. C. and Franklin-Tong V. E. (1998) A potential signaling role for profilin in pollen of *Papaver rhoeas*. Plant Cell **10:** 967–979
92 Drøbak B. K., Watkins P. A. C., Valenta R., Dove S. K., Llyod C. W. and Straiger C. J. (1994) Inhibition of plant plasma membrane phosphoinositide phospholipase C by the actin-binding protein, profilin. Plant J. **6:** 389–400
93 Goldschmidt-Clermont P. J., Machesky L. M., Baldassare J. J. and Pollard T. D. (1990) The actin-binding protein profilin binds to PIP_2 and inhibits its hydrolysis by phospholipase C. Science **247:** 1575–1578
94 Geitmann A., Snowman B. N., Emons A. M. and Franklin-Tong V. E. (2000) Alterations in the actin cytoskeleton of pollen tubes are induced by the self-incompatibility reaction in *Papaver rhoeas.* Plant Cell **12:** 1239–1251
95 Jordan N. D., Franklin F. C. H. and Franklin-Tong V. E. (2000) Evidence for DNA fragmentation triggered in the self-incompatibility response of *Papaver rhoeas*. Plant J. **23:** 471–479
96 Cock J. M., Cabriallac D., Giranton J.-L., Pastuglia M., Ruffio-Châbles., Miege C. et al. (2000) Investigating the molecular mechanisms of the self-incompatibility response in *Brassica*. Ann. Bot. **85:** 147–153
97 Dickinson H. (1995) Dry stigmas, water and self-incompatibility in *Brassica*. Sex. Plant Reprod. **8:** 1–10

98 Bateman A. J. (1955) Self-incompatibility systems in angiosperms. III. Cruciferae. Heredity **9:** 53–68

99 Boyes D. C. and Nasrallah J. B. (1993) Physical linkage of the *SLG SRK* genes at the self-incompatibility locus of *Brassica oleracea*. Mol. Gen. Genet. **236:** 369–373

100 Cui Y., Brugière N., Jackman L., Bi Y.-M. and Rothstein S. J. (1999) Structural and transcriptional comparative analysis of the S-locus regions in two self-incompatible *Brassica napus* lines. Plant Cell **11:** 2217–2231

101 Suzuki G., Kai H., Hirose T., Fukui K., Nishio T., Takayama S. et al. (1999) Genomic organization of the S locus: identification and characterization of genes in SLG/SRK region of the S9 haplotype of *Brassica campestris* (syn rapa). Genetics **153:** 391–400

102 Casselman A. L., Vrebalov J., Conner J.A., Singhal A., Giovannoni J., Nasrallah M. E. et al. (2000) Determining the physical limits of the *Brassica* S locus by recombinational analysis. Plant Cell **12:** 23–33

103 Nasrallah M. E. and Wallace D. H. (1967) Immunogenetics of self-incompatibility in *Brassica oleracea*. Heredity **22:** 519–527

104 Nasrallah M. E., Wallace D. H. and Savo R. M. (1972) Genotype-protein-phenotype relationships in self-incompatibility of *Brassica*. Genet. Res. **20:** 151–160

105 Hinata K. and Nishio T. (1978) S-allele specificity of stigma proteins in *Brassica oleracea* and *B. campestris*. Heredity **41:** 93–100

106 Nasrallah J. B., Kao T.-H., Goldberg M. L. and Nasrallah M. E. (1985) A cDNA clone encoding an S-locus-specific glycoprotein from *Brassica oleracea*. Nature **318:** 263–267

107 Takayama, S., Isogai A., Tsukamoto C., Udea Y., Hinata K., Okazaki K. et al. (1987) Sequences of S-glycoproteins, products of the *Brassica campestris* self-incompatibility. Nature **326:** 102–105

108 Kandasamy M. K., Paolillo D. J., Nasrallah J. B., Nasrallah J. B. and Nasrallah M. E. (1989) The S-locus specific glycoproteins of *Brassica* accumulate in the cell wall of developing stigma papillae. Dev. Biol. **136:** 462–472

109 Dwyer K. G., Balent M., Nasrallah J. B. and Nasrallah M. E. (1991) DNA sequences of self-incompatibility genes from *Brassica campestris* and *B. oleracea*: polymorphism predating speciation. Plant Mol. Biol. **16:** 481–486

110 Kusaba M., Nishio T., Satta Y., Hinata K. and Ockendon D. (1997) Striking sequence similarity in inter- and intra-specific comparisons of class I *SLG* alleles from *Brassica oleracea* and *Brassica campestris*: implications for the evolution and recognition mechanism. Proc. Natl. Acad. Sci. USA **94:** 7673– 7678

111 Stein J. C., Howlett B., Boyes D. C., Nasrallah M. E. and Nasrallah J. B. (1991) Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. Proc. Natl. Acad. Sci. USA **88:** 8816–8820

112 Goring D. R. and Rothstein S. J. (1992) The *S*-locus receptor kinase gene in a self-incompatible *Brassica napus* line encodes a functional serine/threonine kinase. Plant Cell **4:** 1273–1281

113 Nasrallah J. B. and Nasrallah M. E. (1993) Pollen-stigma signaling in the sporophytic self-incompatibility response. Plant Cell **5:** 1325–1335

114 Yu K., Schafer U., Glavin T. L., Goring D. R. and Rothstein S. J. (1996) Molecular characterization of the *S* locus in two self-incompatible *Brassica napus* lines. Plant Cell **8:** 2369–2380

115 Galvin T. L., Goring D. R., Schafer U. and Rothstein S. J. (1994) Features of the extracellular domain on the S-locus receptor kinase from *Brassica*. Mol. Gen. Genet. **244:** 630–637

116 Stein J. C. and Nasrallah J. B. (1993) A plant receptor-like gene, the S-locus receptor kinase of *Brassica oleracea* L., encodes a functional serine/threonine kinase. Plant Physiol. **101:** 1103–1106

117 Delorme V., Giranton J. L., Hatzfeld Y., Friry A., Heizmann P., Ariza M. J. et al. (1995) Characterization of the S locus genes, SLG and SRK, of the *Brassica* S3 haplotype: identification of a membrane-localized protein encoded by the S locus receptor kinase gene. Plant J. **7:** 429–440

118 Stein J. C., Dixit R., Nasrallah M. E. and Nasrallah J. B. (1996) SRK, the stigma-specific *S*-locus receptor kinase of *Brassica*, is targeted to the plasma membrane in transgenic tobacco. Plant Cell **8:** 429–445

119 Giranton J.-L., Dumas C., Cock J. M. and Gaude T. (2000) The integral membrane *S*-locus receptor kinase of *Brassica* has serine/threonine kinase activity in a membranous environment and spontaneously forms oligomers in planta. Proc. Natl. Acad. Sci. USA **97:** 3759–3764

120 Cabrillac D., Delorme V., Garin J., Ruffo-Châble V., Giranton J. L. Dumas C. et al. (1999) The S_{15} self-incompatibility haplotype in *Brassica oleracea* includes three S gene family members expressed in stigmas. Plant Cell **11:** 971–986

121 Hakateyama K., Takasaki T., Watanabe M. and Hinata K. (1998) Molecular characterization of S-locus genes, SLG and SRK, in a pollen-recessive self-incompatibility haplotype of *Brassica rapa* L. Genetics **149:** 1587–1597

122 Nasrallah J. B. (1997) Signal perception and response in the interactions of self-incompatibility in *Brassica*. Essays in Biochem. **32:** 143–160

123 Goring D. R. and Rothstein S. J. (1996) S-locus receptor kinase genes and self-incompatibility in *Brassica napus.* In: Signal Transduction in Plant Growth and Development, pp. 217–230, Verma D. P. S. (ed.), Springer, New York

124 Kusaba M. and Nishio T. (1999) Comparative analysis of S haplotypes with very similar SLG alleles in *Brassica rapa* and *Brassica oleracea.* Plant J. **17:** 83–91

125 Toriyama K., Stein J. C., Nasrallah M. E. and Nasrallah J. B. (1991) Transformation of *Brassica oleracea* with an *S*-locus gene from *B. campestris* changes the self-incompatibility phenotype. Theor. Appl. Genet. **81:** 769–776

126 Shiba H., Hinata K., Suzuki A. and Isogai A. (1995) Breakdown of self-incompatibility in *Brassica* by the antisense RNA of the *SLG* gene. Proc. Jpn. Acad. **71:** 81–83

127 Nishio T., Toriyama K., Sato T., Kandasamy M. K., Paolillo M. K., Nasrallah J. B. et al. (1992) Expression of *S*-locus glycoprotein genes from *Brassica oleracea* and *B. campestris* in transgenic plants of self-incompatible *B. napus* cv. Westar. Sex. Plant Reprod. **5:** 101–109

128 Takasaki T., Hatakeyama K., Watanabe M., Toriyama K., Isogai A and Hinata K. (1999) Introduction of SLG (S locus glycoprotein) alters the phenotype of endogenous *S* haplotype, but confers no new *S* haplotype specificity in *Brassica rapa* L. Plant Mol. Biol. **40:** 659–668

129 Conner J. A., Tantikanjana T., Stein J. C., Kandasamy M. K., Nasrallah J. B. and Nasrallah M. E. (1997) Transgene-induced silencing of *S*-locus genes and related genes in *Brassica*. Plant J. **11:** 809–823

130 Stahl R. J., Arnoldo M. A., Glavin T. L., Goring D. R. and Rothstein S. J. (1998) The self-incompatibility phenotype in *Brassica* is altered by the transformation of a mutant S locus receptor kinase. Plant Cell **10:** 209–218

131 Cui Y., Bi Y.-M., Brugière, Arnoldo M. and Rothstein S. J. (2000) The *S* locus glycoprotein and the *S* receptor kinase are sufficient for self-pollen rejection in *Brassica*. Proc. Natl. Acad. Sci. USA **97:** 3713–3717

132 Takasaki T., Hatakeyama K., Suzuki G., Watanabe M., Isogai A. and Hinata K. (2000) The *S* receptor kinase determines self-incompatibility in *Brassica* stigma. Nature **403:** 913–916

133 Silva N. F., Stone S. L. Christie L. N., Sulaman W., Nazarian K. A. P., Burnett L. A. et al. (2001) Expression of the *S* receptor kinase in self-compatible *Brassica napus* cv. Westar leads to the allele-specific rejection of self-incompatible *Brassica napus* pollen. Mol. Genet. Genom. **265:** 552–559

134 Dixit R., Nasrallah M. E. and Nasrallah J. B. (2000) Post-transcriptional maturation of the S receptor kinase in *Brassica* correlates with co-expression of the S-locus glycoprotein in the stigmas of two *Brassica* strains and in transgenic tobacco plants. Plant Physiol. 2000 **124:** 297–311

135 Gaude T., Rougier M., Heizmann P., Ockendon D. J. and Dumas C. (1995) Expression of the *SLG* gene is not correlated with the self-incompatibility phenotype in the class II *S* haplotypes of *Brassica oleracea*. Plant Mol. Biol. **27:** 1003–1014

136 Suzuki T., Kusaba M., Matsushita M., Okazaki K and Nishio T. (2000) Characterization of *Brassica S*-haplotypes lacking *S*-locus glycoprotein. FEBS Lett. **482:** 102–108

137 Kusaba M., Dwyer K., Hendershot J., Vrebalov J., Nasrallah J. B. and Nasrallah M. E. (2001) Self-incompatibility in the genus *Arabidopsis*: characterization of the S locus in the outcrossing *A. lyrata* and its autogamous relative *A. thaliana*. Plant Cell **13:** 627–643

138 Schierup M. H., Mable B. K., Awadalla P. and Charlesworth D. (2001) Identification and characterization of a polymorphic receptor kinase gene linked to the self-incompatibility locus of *Arabidopsis lyrata*. Genetics **158:** 387–399

139 Doughty J., Dixon S., Hiscock S. J., Willis A. C., Parkin I. A. P. and Dickinson H. G. (1998) PCP-A1, a defensin-like *Brassica* pollen coat protein that binds the *S* locus glycoprotein, is a product of gametophytic gene expression. Plant Cell **10:** 1333–1347

140 Doughty J., Hedderson F., McCubbin A. and Dickinson H. (1993) Interaction between a coating-borne peptide of the *Brassica* pollen grain and stigmatic S (self-incompatibility)-locus-specific glycoproteins. Proc. Natl. Acad. Sci. USA **90:** 467–471

141 Hiscock S. J., Doughty J., Willis A. C. and Dickinson H. G. (1995) A 7-kDa pollen coating-borne peptide from *Brassica napus* interacts with S-locus glycoprotein and S-locus-related glycoprotein. Planta **196:** 367–374

142 Stanchev B. S., Doughty J., Scutt C. P., Dickinson H. and Croy R. R. D. (1996) Cloning of PCP1, a member of a family of pollen coat protein (PCP) genes from *Brassica oleracea* encoding novel cysteine-rich proteins involved in pollen-stigma interactions. Plant J. **10:** 303–313

143 Takayama S., Shiba H., Iwano M., Asano K., Minoru H., Che F.-S., Watanabe M. et al. (2000b) Isolation and characterization of pollen coat proteins of *Brassica campestris* that interact with *S*-locus-related glycoprotein 1 involved in pollen-stigma interactions. Proc. Natl. Acad. Sci. USA **97:** 3765–3770

144 Stephenson A. G., Doughty J., Dixon S., Elleman C., Hiscock S. and Dickinson H. G. (1997) The male determinant of self-incompatibility in *Brassica oleracea* is located in the pollen coating. Plant J. **12:** 1351–1359

145 Boyes D. C., Nasrallah M. E. Vrebalov J. and Nasrallah J. B. (1997) The self-incompatibility (*S*) haplotypes of *Brassica* contain highly divergent and rearranged sequences of ancient origin. Plant Cell **9:** 237–247

146 Conner J. A., Conner P., Nasrallah M. E. and Nasrallah J. B. (1998) Comparative mapping of the *Brassica S* locus region and its homeolog in *Arabidopsis*: implications for the evolution of mating systems in the Brassicaceae. Plant Cell **10:** 801–812

147 Schopfer C. R., Nasrallah M. E. and Nasrallah J. B. (1999) The male determinant of self-incompatibility in *Brassica*. Science **286:** 1697–1700

148 Takayama S., Shiba H., Iwano M., Shimosato H., Che F.-S., Kai N. et al. (2000) The pollen determinant of self-incompatibility in *Brassica campestris*. Proc. Natl. Acad. Sci. USA **97:** 1920–1925

149 Shiba H., Takayama S., Iwano M., Shimosato H., Funato M., Nakagawa T. et al. (2001) A pollen coat protein, SP11/SCR, determines the pollen *S*-specificity in the self-incompatibility of *Brassica* species. Plant Physiol. **125:** 2095–2103

150 Goring D. R. (2000) The search for components of the self-incompatibility signalling pathway(s) in *Brassica napus*. Ann. Bot. **85:** 171–179

151 Bower M. S., Matias D. D., Fernandes-Carvalho E., Mazzurco M., Gu T., Rothstein S. et al. (1996) Two members of the thioredoxin-h family interact with the kinase domain of a *Brassica S* locus receptor kinase. Plant Cell **8:** 1641–1650

152 Gu T., Mazzurco M., Matias D. D., Sulaman W. and Goring D. R. (1998) Binding of a novel arm repeat protein to the kinase domain of the *S* locus receptor kinase. Proc. Natl. Acad. Sci. USA **95:** 382–387

153 Buchanan B. B. (1991) Regulation of CO_2 assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Arch. Biochem. Biophys. **288:** 1–9

154 Mazzurco M., Sulaman W., Elina H., Cock J. M. and Goring D. R. (2001) Further analysis of the interactions between the *Brassica S* receptor kinase and three interacting proteins (ARC1, THL1 and THL2) in the yeast two-hybrid system. Plant Mol. Biol. **45:** 365–376

155 Cabrillac D., Cock J. M., Dumas C. and Gaude T. (2001) The *S*-locus receptor kinase is inhibited by thioredoxins and activated by pollen coat proteins. Nature **410:** 220–223

156 Stone S. L., Arnoldo M. and Goring D. R. (1999) A breakdown of *Brassica* self-incompatibility in ARC1 antisense transgenic plants. Science **286:** 1729–1731

157 Ikeda S., Nasrallah J. B., Dixit R., Preiss S. and Nasrallah M. E. (1997) An aquaporin-like gene required for the *Brassica* self-incompatibility response. Science **286:** 1564–1566

158 Fukai E., Nishio T. and Nasrallah M. E. (2001) Molecular genetic analysis of the candidate gene for *MOD*, a locus required for self-incompatibility in *Brassica rapa*. Mol. Genet. Genom. **265:** 519–526

159 Aravind L. and Koonin E. V. (2000) The U-box is a modified RING finger – a common domain in ubiquitination. Curr. Biol. **10:** R132–R134