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Review

Self-incompatibility: How plants avoid illegitimate offspring

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ABSTRACT **In some families of flowering plants, a single self-incompatibility (*S*) locus prevents the fertilization of flowers by pollen from the same plant. Self-incompatibility of this type involves the interaction of molecules produced by the *S* locus in pollen with those present in the female tissues (pistil). Until recently, the pistil products of the *S* locus were known in only two families, the Brassicaceae (which includes the cabbages and mustards) and Solanaceae (potatoes and tomatoes). A paper in this issue of the *Proceedings* describes the molecules associated with self-incompatibility in a third family, the Papaveraceae (poppies). We review current research on self-incompatibility in these three families and discuss the implications of the latest findings in poppy on the likely evolution of self-incompatibility in flowering plants. We also compare research into self-incompatibility with recent progress in understanding the mechanisms by which plants overcome infection by certain pathogens.**

Why Are Biologists So Interested in Self-Incompatibility?

Self-incompatibility, the inability of apparently healthy plants to produce seed when self-pollinated, was described by Charles Darwin as "one of the most surprising facts which I have ever observed" (1). A similar sense of wonder has led generations of plant biologists since Darwin to study self-incompatibility and more recently to characterize some of the molecules involved in this "surprising fact." In this issue of the *Proceedings* (2), Foote and others describe one of the molecules involved in self-incompatibility in field poppy (*Papaver rhoeas*), and in this brief review we place this new data in the context of our knowledge of other systems of self-incompatibility. It has been postulated that self-incompatibility arose a number of times during the evolution of flowering plants [angiosperms (3)], and the data presented by Foote *et al.* provides the first molecular insights into self-incompatibility in a family that diverged early in the evolution of the angiosperms, the Papaveraceae.

The strategies that plants use to recognize and reject "self" pollen while accepting "nonself" pollen are necessarily different from the strategies for "self" and "nonself" discrimination in animals. Plants lack both circulating lymphocytes and immunoglobulins and have no known equivalents to the tissue transplantation antigens [although some proteoglycans, the arabinogalactanproteins, may be analogous (4)]. Nonetheless, plant cells do recognize and respond to each other. The way in which this recognition is mediated is of profound interest, not only to an understanding of plant fertility and reproduction but also to an understanding of the way in which plants respond to pathogenic or symbiotic microorganisms in the environment.

Systems in Which Self-Incompatibility Is Studied

Self-incompatibility is a relatively simple and genetically defined example of cell–cell recognition in plants. Self-incompatible plants are able to distinguish between self pollen and nonself pollen within the female reproductive tissue (the pistil) and arrest the further growth of self pollen (see ref. 5 and the references therein). By recognizing and rejecting self pollen before fertilization, self-incompatible plants promote outbreeding and maintain genetic variability, a factor considered important in the evolutionary success of flowering plants (6). The molecular genetics of two types of self-incompatibility, gametophytic and sporophytic self-incompatibility, have been studied intensively (5, 7).

Gametophytic self-incompatibility is well-characterized in plants from the family Solanaceae—such as the ornamental tobacco (*Nicotiana alata*), petunia (*Petunia inflata* and *Petunia hybrida*), potato (*Solanum tuberosum* and *Solanum chacoense*), and wild tomato (*Lycopersicon peruvianum*). Some molecular information is also available for gametophytically self-incompatible species from the Papaveraceae, Rosaceae, and Scrophulariaceae (Fig. 1, see below). In each case, self-incompatibility is controlled by a single genetic locus (*S* locus) with many alleles. Rejection of pollen occurs when the single *S* allele present in the haploid pollen grain matches either of the *S* alleles present in the diploid tissues of the pistil. Anderson and others (10) showed that the *S* loci of *N. alata* and *L. peruvianum* encode extracellular glycoproteins that are abundant within the pistil, and the discovery that these glycoproteins are ribonucleases related to extracellular ribonucleases of some fungi was a major surprise (11). Until recently, evidence for the involvement of these glycoproteins (now called S-RNases) in self-incompatibility was indirect and relied on a number of correlations: for example, the genes that encode S-RNases cosegregate with alleles of the *S* locus, and the timing of expression of S-RNases is coincident with the onset of self-incompatibility in the pistil (5). There is now direct evidence that S-RNases determine the self-incompatibility phenotype of the pistil and that the ribonuclease activity of these glycoproteins is required for rejection of incompatible pollen (refs. 12 and 13; J. Royo, Y. Kowyama, and A.E.C., unpublished work). These findings strengthen the view that if S-RNases enter incompatible pollen tubes, then they would act as cytotoxins by degrading RNA, including ribosomal (r) RNA (14). As rRNA genes do not appear to be expressed in mature pollen (15), degrading the fixed amount of rRNA synthesized during early pollen development would be an effective way of arresting pollen-tube growth.

Sporophytic self-incompatibility, the other extensively studied system of self-incompatibility, is found in members of the Brassicaceae, and molecular information is available for three species, *Brassica oleracea, Brassica napus*, and *Brassica campestris* (7). Sporophytic self-incompatibility in the Brassicaceae, like gametophytic self-incompatibility in the Solanaceae, is controlled by a single, multiallelic *S* locus. However, rejection in the sporophytic system is controlled by the interaction of the self-incompatibility genotype of the pistil with the genotype of the pollen parent and not with the haploid genotype of the pollen, as is the case in the gametophytic system. Thus, each pollen grain in plants with sporophytic self-incompatibility presents the products of two *S* alleles, and rejection occurs when either one of these alleles matches either of the *S* alleles

Abbreviations: SLG, *S*-locus glycoprotein; SRK, *S*-locus receptor kinase.

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expressed in the pistil; complex dominant or codominant interactions often occur between *S* alleles, affecting the outcome of particular crosses.

At least two multiallelic genes are found within the *Brassica S* locus, and it has been suggested that the complement of allelic genes at the *S* locus be described as a "haplotype", a term also applied to a complex of highly allelic genes within the major histocompatibility locus of mammals (7, 16). One of the genes within the S haplotype of the Brassicaceae encodes an extracellular glycoprotein called the *S*-locus glycoprotein (SLG) and the other, called the *S*-locus receptor kinase (SRK), encodes a membrane-associated protein able to phosphorylate serine/threonine residues (17, 18). The two genes are within a few hundred kilobases of each other in the genome (16), and a number of independent lines of evidence have implicated both in sporophytic self-incompatibility (19–21). The genes for SLGs and SRKs are expressed within the reproductive structures of the flower (7), and SLG is particularly abundant in stigmatic papillae, the cells of the pistil that receive the pollen. The predicted sequence of the SRK contains at the N terminus a potentially glycosylated, extracellular domain with extensive similarity to SLG (17): indeed, on the basis of sequence comparisons of SLG/SRK gene pairs from the same S haplotype, it appears that the SLGs may be derived by duplication from the SRKs (20). The C terminus of the SRK encodes a domain with similarity to serine/threonine kinases, and it is thought that this domain is located within the cytoplasm and joined to the extracellular SLG-like domain by a membrane-spanning domain (17).

Evolution of Self-Incompatibility

The new data from field poppy (*P. rhoeas*; Papaveraceae) published in this issue of the *Proceedings* (2) can be compared to data from other systems and allows us to reexamine the question of the evolution of self-incompatibility. Foote and others describe a small glycoprotein associated with self-incompatibility in *P. rhoeas* that is unrelated in sequence to either the S-RNases of solanaceous plants or the SLGs and SRKs from *Brassica* spp. (see below). This result is consistent with earlier findings that showed no correlation between self-incompatibility and ribonuclease activity in *P. rhoeas* (22), even though the genetics of self-incompatibility in this species are similar to that of solanaceous plants, such as *N. alata* (23). The new molecular data indicates that the self-incompatibility systems found in the Brassicaceae, Papaveraceae, and Solanaceae are unrelated and supports the conclusion of Bateman (3) that self-incompatibility arose independently many times within the angiosperms.

Most flowering plants (angiosperms) are self-compatible, which is generally considered to be the primitive condition (3). Self-incompatibility, the presumed derived condition, is found scattered in most major lineages. Fig. 1 shows the broad taxonomic relationships within angiosperms, together with the location of some families in which self-incompatibility is controlled by a single locus (monofactorial). Only one type of self-incompatibility (gametophytic or sporophytic) is found within any one family (24): for example, gametophytic self-incompatibility is found within the Solanaceae, Scrophulariaceae, Rosaceae, and Papaveraceae, whereas sporophytic self-incompatibility is found within the Brassicaceae, Asteraceae, and Convolvulaceae. The Papaveraceae are included in the ranunculids, a group that is basal in nonmagnolid dicotyledons and quite widely separated from the Asteridae (see Fig. 1). However, within the Asteridae, the closest family to the Solanaceae is the Convolvulaceae (8, 25), and yet these two families have gametophytic and sporophytic self-incompatibility systems, respectively. It thus appears that self-incompatibility arose quite late in the evolution of the families, as closely related families do not share the same system of self-incompatibility.

Despite their apparently independent origins, gametophytic self-incompatibility systems in the Scrophulariaceae and Rosaceae appear to involve ribonucleases that are biochemically similar to those described for solanaceous plants (Y. Xue, H. Dickinson, and E. Coen, personal communication; ref. 26); the Solanaceae and Scrophulariaceae are closely related within Asteridae, whereas the Rosaceae are more distantly related and lie within the Rosidae. This result suggests that at least one and possibly two families other than the Solanaceae have acquired gametophytic self-incompatibility mechanisms that involve ribonucleases, although it is not known whether other aspects of the signal-transduction pathway in these families are related. Secreted ribonucleases have been described in the flowers, leaves, and seeds of many unrelated plant species (27, 28) and could possibly have been recruited to a cell-recognition role on more than one occasion.

One interesting possibility is that the molecules involved in self-incompatibility in the Convolvulaceae and Solanaceae are related, despite these families having different self-incompatibility sys-

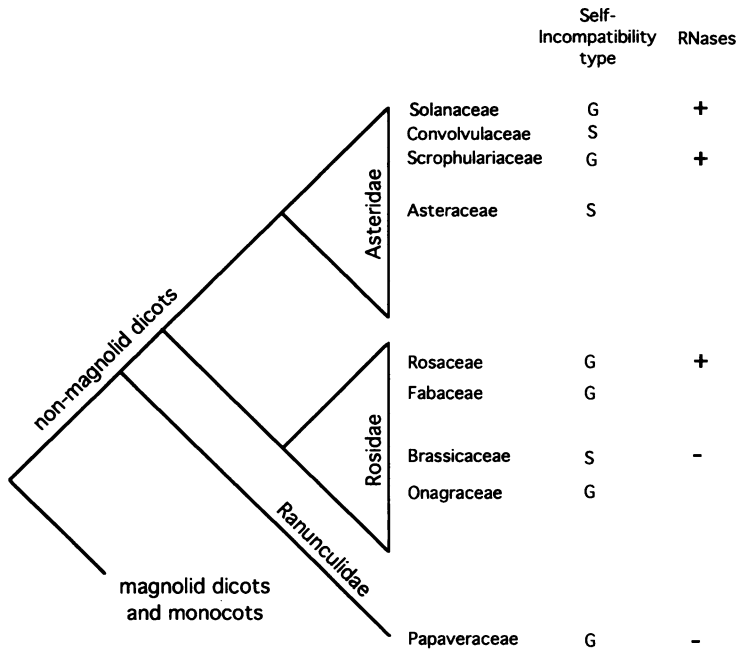


FIG. 1. Simplified version of the family tree of major angiosperm clades as given by Chase *et al.* (8). For clarity, the Hamamelidae have been omitted from the nonmagnolid dicots. The names of the other orders and of some of the families in which simple (monofactorial) self-incompatibility occurs are shown. The position of family names approximates their location in the order as given by Chase *et al.* (24), and the distance between names approximates their taxonomic relationship. The type of self-incompatibility in each family is indicated: G, gametophytic self-incompatibility; S, sporophytic self-incompatibility. A second column indicates whether ribonucleases (RNases) have been implicated (+) or are known not to be involved (−) in self-incompatibility in each family. The *S*-locus products of the Convolvulaceae, Asteraceae, Fabaceae, and Onagraceae are not known. The classification of the Brassicaceae in the Rosidae is debatable (9).

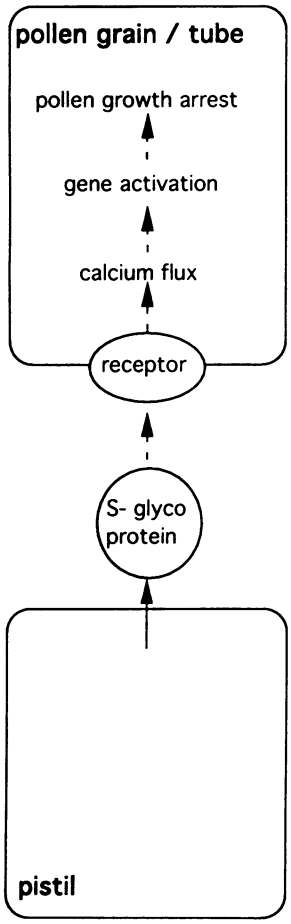
tems. This idea is supported by the closeness of the two families and by the observation that allelism of the *S* locus appears to predate speciation of the Solanaceae (29). Thus, the alleles present at the solanaceous *S* locus could have arisen in a species that was ancestral to both the Convolvulaceae and Solanaceae. The possibility that gametophytic and sporophytic self-incompatibility systems may, in fact, be more closely related than is at first apparent has been discussed previously (for example, see ref. 30): thus, a shift in the timing of expression of the *S* locus in pollen from before meiosis to after meiosis could convert a sporophytic incompatibility system to a gametophytic one. The limited molecular information available for the Convolvulaceae indicates that the molecules involved in sporophytic self-incompatibility in this family differ from those in the Brassicaceae (31). At this stage, any association between ribonucleases and self-incompatibility in the Convolvulaceae is untested.

Models of Self-Incompatibility

Most of the studies on self-incompatibility focus on the identification and characterization of the molecules associated with self-incompatibility present in the pistil. The complete process of signal perception and transduction is, however, not understood for any one system because of the lack of data on the pollen part of the *S* locus. Nevertheless, on the basis of the limited information available, the postulated pathways for cell recognition in self-incompatibility in the Papaveraceae, Brassicaceae, and Solanaceae show striking differences.

The availability of an *in vitro* assay for self-incompatibility in *P. rhoeas* put Franklin-Tong and coworkers (2, 32, 33) in a position to identify the *S*-locus product of the pistil directly and then to study the signal-transduction mechanism. They showed that pollen tubes grown in culture respond to the presence of extracts from pistils of the same *S* genotype with a transient increase of calcium and, subsequently, altered gene expression and protein phosphorylation. Using their pollen-tube bioassay to monitor purification, they isolated a small glycoprotein from pistil extracts and cloned the corresponding cDNA. This cDNA hybridized to a single gene in the poppy genome that cosegregated with the *S* locus. Remarkably, the recombinant protein isolated from a strain of *Escherichia coli* expressing this *S*-locus gene elicited the same effects on pollen-tube growth in culture as did the glycoprotein isolated from pistils, indicating that the carbohydrate component of the glycoprotein is not required for biological activity. Based on the involvement of calcium, they speculate that the S glycoprotein binds to a receptor in the pollen tube and induces a cellular response via the inositol phosphate pathway (34), but there are no experimental data at this stage for the existence of a receptor (which may be the product of the *S* locus in pollen) or the involvement of this pathway. *P. rhoeas* is perhaps the best system so far for studying the incompatibility responses of pollen. Fig. 2 shows the essential features of the model and uncertainty in correlating the *in vitro* data and the *in vivo* situation.

For *Brassica* spp., Nasrallah and colleagues (7) have identified two gene products of the *S* locus—namely, SLG and SRK (see above). These genes are expressed both in the pistil and in the pollen grain and anther, although the level of expression found in these latter two tissues is much lower than that found in the



level of confidence	S-locus products	perception and recognition	response
known	secreted glycoprotein produced by pistils		arrest of pollen growth
implicated	secreted glycoprotein functions as a ligand for a receptor on pollen tubes		* pollen gene activation * calcium flux in pollen tube * activation of calcium dependent enzymes
unknown	Is product of the S-locus in pollen a receptor?	* basis of allelic specificity * pollen-stigma signalling leading * to callose deposition in the stigma	correlation between *in vitro* and *in vivo* effects

FIG. 2. Schematic representation of the signal-transduction pathway governing self-incompatibility in field poppy (*P. rhoeas*). Solid arrows in the figure indicate known steps in the pathway, and dashed arrows indicate hypothetical steps. The accompanying table highlights some of the stages of the signal-recognition process listed according to the degree of certainty.

pistil. A specific interaction between the pollen and pistil S products may be mediated by another compound present in the pollen coat (Fig. 3), and an interaction between a coat-derived peptide from *Brassica* pollen and the pistil SLG has been reported (35). Nasrallah and Nasrallah (7) have postulated that protein-phosphorylation events in the pistil lead to inhibition of pollen germination and ultimately deposition of callose (a polysaccharide essentially composed of β, 1–3 glucan) on both pollen and the surface of the pistil. However, in this system, callose deposition may not be causally related to self-incompatibility (36). The main events and points of uncertainty in the pathway of cell recognition in sporophytic self-incompatibility of *Brassica* spp. are summarized in Fig. 3.

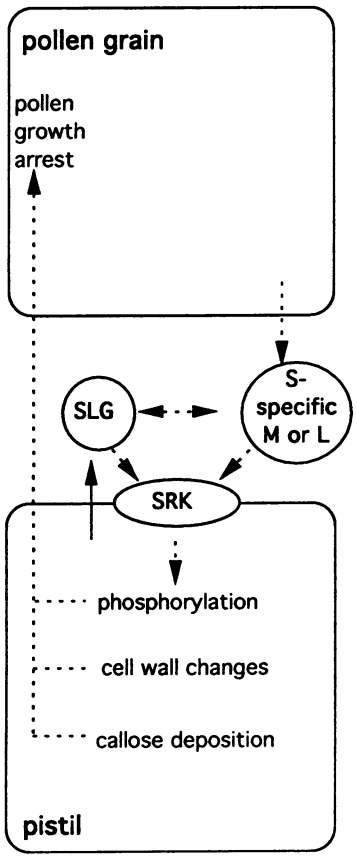
As mentioned above, the products of the *S* locus expressed in the pistil of solanaceous plants are active ribonucleases (S-RNases), and there is one report that low levels of S-RNases are produced during pollen development (37). The basis of the allelic interaction in this system is not understood, although our current thinking on the mode of action of S-RNases is that they are specifically taken up by incompatible pollen tubes. This specific uptake may involve domains on the surface of the protein that are very different in sequence between different S-RNase alleles ("hypervariable domains"; ref. 38). An alternative hypothesis is based on nonspecific uptake of S-RNases into the pollen tube followed by specific inactivation or other modification (39). In both cases, the RNase is thought to act as a cytotoxin and degrade the RNA essential for protein translation; arrest of pollen-tube growth would follow (Fig. 4).

Self-Incompatibility and Disease Resistance

All cell–cell interactions have certain features in common, including some kind of discrimination and differential response. Many authors have compared self-incompatibility and host–pathogen interactions, another process in plants that requires specific cell–cell signaling (for examples, see refs. 40 and 41).

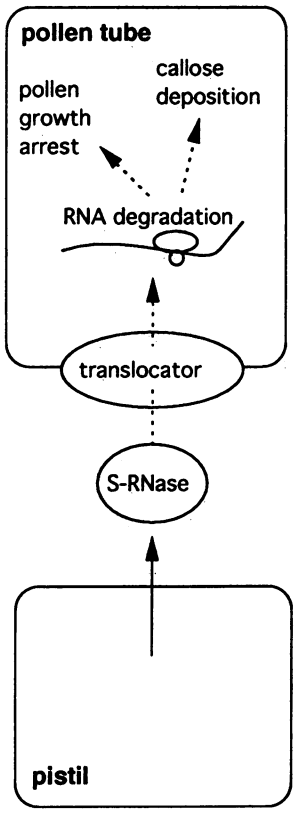
Plants lack an immune system and rely on several different strategies to resist or overcome infection. In many cases, resistance to a particular pathogen is controlled by a single, multiallelic locus (*R* locus). There can be many *R* loci in a single species, and these encode resistance to a variety of pathogens; there are also examples of multiple-resistance loci for a single pathogen. Some types of resistance loci direct a strategy to combat infection that results in the death of cells around the site of infection (a "hypersensitive response"). This response prevents further colonization of the plant by the pathogen. The genetic basis underlying the hypersensitive response of a plant to a specific race of a pathogen was originally provided by Flor (42), who described it as a gene-for-gene interaction (for reviews, see refs. 40, 41, and 43). Flor hypothesized that certain types of infection are controlled by two dominant genes, one gene for resistance in the host and another gene for avirulence in the pathogen. An incompatible (hypersensitive) response is triggered when the complementary products of the plant's resistance gene and the pathogen's avirulence gene interact. A single avirulence/resistance gene combination is sufficient to activate the hypersensitive response, regardless of how many other virulence and susceptibility combinations exist. A number of such avirulence/resistance gene pairs have been described for different plant–pathogen combinations.

There are several parallels between self-incompatibility and gene-for-gene interactions. (*i*) Both are genetically simple and based on a specific interaction between the products of complementary genes expressed in separate cells. (*ii*) In both systems, there are only two possible outcomes of an interaction, either a compatible reaction (leading to pollen growth



level of confidence	S-locus products	perception and recognition	response
known	secreted glycoproteins (SLGs), and membrane bound receptor kinases (SRKs)		* inhibition of pollen germination
implicated	allelic specificity results from sequence differences in the SLGs and SRKs	SRKs interact with SLGs and a pollen product (either a ligand (L) or modifier (M))	*protein phosphorylation * callose deposition in stigma and pollen
unknown	S-locus product(s) in pollen	other interacting molecules	substrate for SRK

FIG. 3. Schematic representation of the signal-transduction pathway governing self-incompatibility in *Brassica*. Details of the figure are described in the legend to Fig. 2.



level of confidence	S-locus products	perception and recognition	response
known	Secreted, active RNases produced by pistil		arrest of pollen growth associated with changes in cytoplasm and pollen-tube wall callose content
implicated	allelic specificity is due to hyper-variable regions on S-RNase	* uptake of RNase into pollen tube * degradation of pollen RNA * function of S-RNase in pollen grain wall	RNA degradation leading to disruption of protein synthesis and pollen-tube elongation
unknown	nature of the S-locus product in pollen		connection between RNase activity, pollen-tube growth arrest and callose deposition

FIG. 4. Schematic representation of the signal-transduction pathway governing self-incompatibility in solanaceous plants. Details of the figure are described in the legend to Fig. 2.

or a systemic infection) or an incompatible reaction (leading to the arrest of pollen growth or a hypersensitive response). Additionally, there are some superficial similarities in the biochemistry and physiology of incompatible responses in the two systems (40).

Most models of gene-for-gene interactions postulate that the products of the avirulence gene of the pathogen and the resistance gene of the plant host are analogous to a ligand and a membrane-bound receptor (44, 45). Other models recognize the potential for intracellular processing and predict that resistance genes may play a critical role in preferential transport and/or processing of the avirulence gene product (43). Considerable progress has been made in characterizing avirulence genes from various bacterial pathogens (46), but the isolation of the first plant resistance gene has proved more elusive. Recently, the gene responsible for resistance to bacterial speck (*Pseudomonas syringae*) was cloned from the *Pto* locus of tomato using a map-based approach (47). Comparison of the sequence of the *Pto* gene with sequences in DNA data bases uncovered similarities with catalytic domains of a number of serine/threonine kinases, including SRKs from *Brassica* spp. However, unlike SRKs, the putative Pto protein contains no obvious membrane-spanning or extracellular domains and may be localized in the cytoplasm. Thus, both sporophytic self-incompatibility in *Brassica* and resistance to this bacterial pathogen probably involve phosphorylation, although the nature of the substrates and the subsequent steps in the signal-transduction pathways are not known in either case.

The *pto* gene is only one of many *R* genes used by plants to recognize avirulence specificities of different pathogens. Although the initial events in these interactions may be quite different, it is thought that all the pathways eventually converge into one or a few pathways that mediate the hypersensitive response (43). The pathways that mediate the cellular responses found during incompatible pollinations may prove to share some components with those involved in disease resistance.

Summary

The most important contribution of the paper by Foote *et al.* (2) is to describe at the molecular level a system of self-incompatibility in a representative of a family of angiosperms for which no molecular information was previously available. It allows us to extend our view of the evolution of self-incompatibility and confirms the conclusion, based on other data, that self-incompatibility arose independently on several occasions during evolutionary history. The molecules associated with self-incompatibility from different plant families appear quite dissimilar, but the possibility that the subsequent pathways converge and share some features with pathways that mediate disease resistance remains open. A major quest in self-incompatibility research in many laboratories is the identification of *S*-locus product in pollen. For reasons that are not entirely clear, the experimental approaches that led to the cloning of the stylar products of the *S* locus, the SLGs from *Brassica* spp., S-RNases from solanaceous plants, and the small glycoproteins from *P. rhoeas*, have not proved useful in identifying the pollen products. Possibly, map-based approaches, similar to that that led to the cloning of the *pto* gene from tomato (47), will be required. However, although identifying the product of the *S* locus in pollen will provide another valuable piece of the puzzle, it will not reveal the whole story. The questions of signal transduction and the steps that lead to the

arrest of pollen growth will be major areas of research for the future.

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