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Gametophytic Self-Incompatibility Systems

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INTRODUCTION

Self-incompatibility (SI) is one of the mechanisms that have evolved to encourage outbreeding in flowering plants and is defined as "the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination" (de Nettancourt, 1977). The effectiveness of SI in promoting outbreeding is believed to be one of the most important factors that ensured the evolutionary success of flowering plants (Whitehouse, 1951). It is a genetically controlled phenomenon, and in many cases, the control is by a single locus (known as the *S* locus) with a large number of alleles, up to several hundred in some species (Ockendon, 1974; de Nettancourt, 1977). SI has been a favorite topic for botanists and geneticists since Darwin (1877) first discussed the phenomenon and suggested the idea of its central significance during the evolution of flowering plants. During the century or more of work on the subject, there have been a number of key reviews, the most significant of which is the classic work by de Nettancourt (1977). Other early reviews are by Lewis (1949, 1979), Pandey (1979), Heslop-Harrison (1975, 1982, 1983), and de Nettancourt (1984). More recent reviews are by Ebert et al. (1989), Haring et al. (1990), Mau et al. (1991), Thompson and Kirch (1992), and Sims (1993).

In this review, we focus on the most widespread form of SI, homomorphic gametophytic SI. Homomorphic refers to the group of plants in which flowers of the interbreeding species have the same morphology. Gametophytic refers to the fact that the SI phenotype of the pollen is determined by its own (haploid) *S* genotype. This is in contrast to the sporophytic type of SI (see Nasrallah and Nasrallah, 1993, this issue), which is characterized by the fact that the behavior of the pollen is determined by the diploid *S* genotype of the pollen-producing plant.

DISTRIBUTION OF GAMETOPHYTIC SELF-INCOMPATIBILITY

Although SI is estimated to be present in more than half of all species of angiosperms (Brewbaker, 1959; de Nettancourt, 1977), it has been difficult in many cases to demonstrate SI unequivocally (Charlesworth, 1985). Despite this limitation, 15 families have examples of well-described gametophytic SI, and a number of others have SI of an undefined type (Mau et al., 1991). The widespread nature of SI systems suggests either that the SI systems arose early in angiosperm evolution, before the taxa diverged (Whitehouse, 1951), or that they arose independently on more than one occasion (Bateman, 1952). For a discussion of the evolution of gametophytic SI, see Mau et al. (1991).

Self-compatible taxa are also widespread and often closely related to taxa with SI. It is likely that they evolved from self-incompatible plants by mutation of the *S* locus or of genes involved in regulation of expression of that locus (Whitehouse, 1951; Jain, 1976; Mayo and Leach, 1987).

Gametophytic SI systems in the Solanaceae have been most intensively studied. Other families for which some information about gametophytic SI is available are the Papaveraceae (*Papaver rhoeas*, field poppy; Campbell and Lawrence, 1981; Franklin-Tong et al., 1989), the Rosaceae (*Pyrus serotina*, Japanese pear; Sassa et al., 1992; *Prunus avium*, cherry; Mau et al., 1982), and the Onagraceae (*Oenothera organensis*, evening primrose; Emerson, 1938). SI in these families is controlled by a single locus, but there are more complex systems, for example, those in some grasses, in which SI is controlled by two loci, *S* and *Z* (Lundqvist, 1964; Hayman and Richter, 1992). Four loci control SI in sugarbeet, *Beta vulgaris* (Lundqvist et al., 1973; Larsen, 1977) and in *Ranunculus acris* (Osterbye, 1975).

In this review, we will consider solanaceous plants as examples of a single-locus, gametophytic SI system.

BIOLOGY OF GAMETOPHYTIC SELF-INCOMPATIBILITY

In most gametophytic SI systems, incompatible pollen germinates successfully on the stigma surface, penetrates the stigma, and grows into the style. There, the tube grows between the longitudinal files of cells of the central transmitting tract, as shown in Figure 1, and at some point, pollen tube growth through the transmitting tract toward the ovary is arrested. This is in contrast to the sporophytic system, in which pollen tube growth in an incompatible mating is usually arrested on the surface of the stigma.

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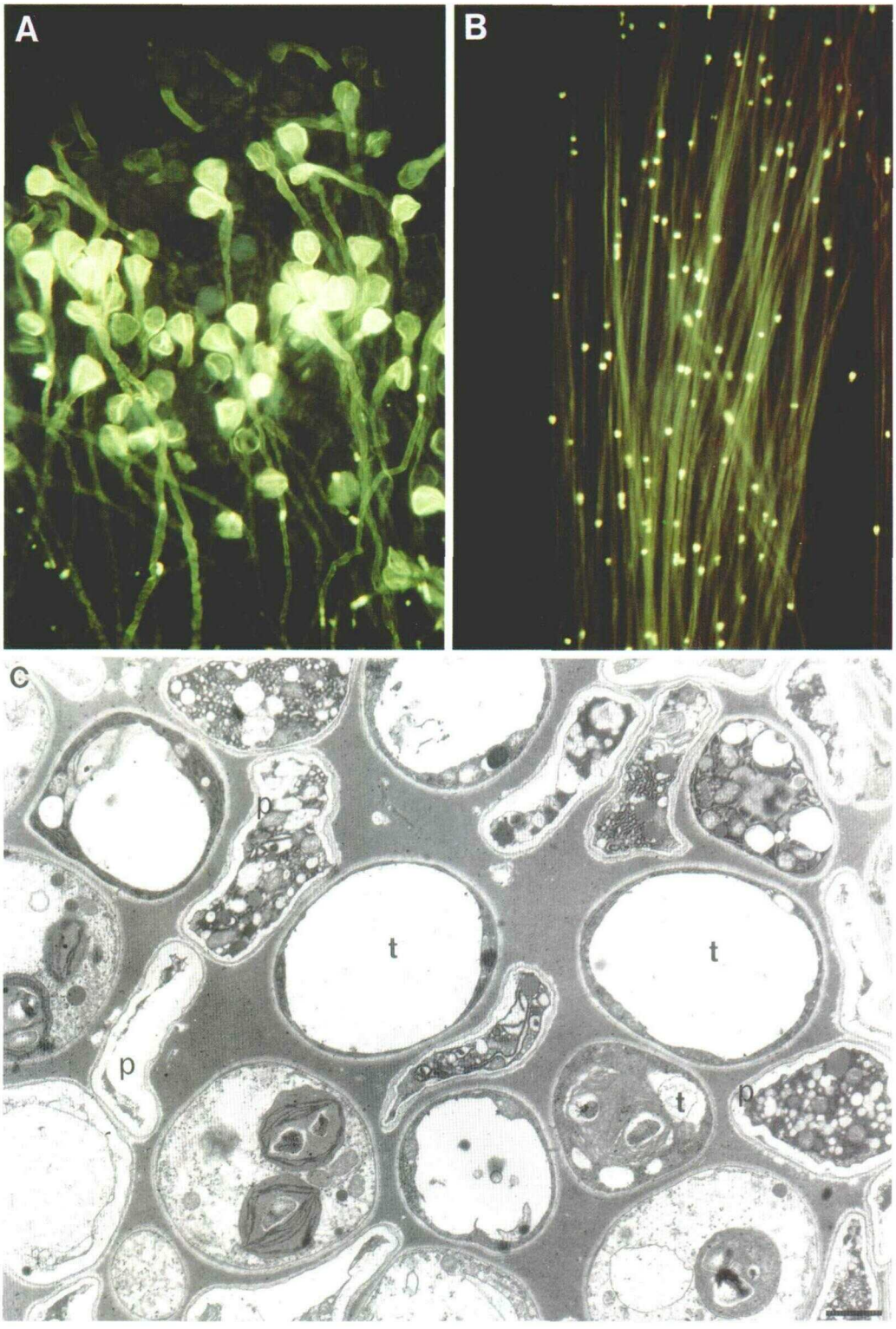

Figure 1. Pollen Tube Growth through the Pistil.

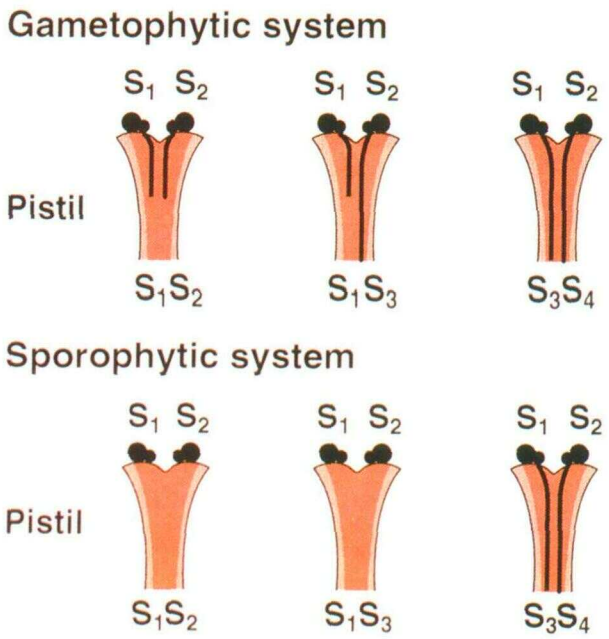

Figure 2. Behavior of Pollen in the Two Major SI Systems.

(Top) Behavior of pollen in a single-gene gametophytic system. The pollen parent genotype is S_1S_2. When an allele in the individual haploid pollen grain matches either allele in the diploid style tissues, growth of the pollen tube is arrested, usually in the style. For example, both S_1 pollen and S_2 pollen are inhibited in an S_1S_2 style, whereas S_2 pollen will grow successfully through the S_1S_3 style. When there is no match of alleles (e.g., pollen grains from an S_1S_2 plant on an S_3S_4 pistil), the pollen tubes of both genotypes grow through the style to the embryo sac.

(Bottom) Behavior of pollen in a single-gene sporophytic system. The pollen parent genotype is S_1S_2. When an allele in the pollen parent matches that of the pistil (e.g., S_1S_2 or S_1S_3), pollen germination is arrested at the stigma surface. Where there is no match (S_3S_4), the pollen may germinate and grow through the style to the embryo sac. The central panel applies only if the S_1 allele is dominant to or codominant with S_2 in the pollen and if S_1 is dominant to or codominant with S_3 in the style. If S_3 is dominant to S_1 in the style, or if S_2 is dominant to S_1 in the pollen, pollen from the S_1S_2 parent will be compatible. (**[Top]** and **[Bottom]** are modified from Anderson et al., 1983; with permission of John Wiley and Sons.)

The morphology of incompatible pollen tubes differs from that of compatible tubes. The pollen tube wall consists of two main layers of polysaccharide. The inner "callosic" wall contains predominantly (1,3)-β-glucan (Stone and Clarke, 1992), and the outer wall contains predominantly arabinan consisting of α-L-arabinofuranosyl residues in 1 → 5 linkage, with some branching through C(O)2 and/or C(O)3 (Rae et al., 1985). The inner glucan layer fluoresces intensely when stained with the aniline blue fluorochrome (Evans and Hoyne, 1982). Staining of pollen tubes with aniline blue (Figures 1A and 1B) reveals the presence of callosic cross walls that give the tubes a ladderlike appearance. These cross walls effectively cut off the growing tip of the tube, which contains the sperm cells and other cytoplasmic contents, from the spent pollen grain. In incompatible tubes, the pattern of growth is similar to that initially seen in a compatible pollination, but at some stage, growth becomes irregular, the pollen tube walls become thicker, and the tips may burst. Often, there is a large deposit of callose close to the swollen tip. Although many gametophytic self-incompatible species share these general features, there are exceptions. In rye (*Secale cereale*) and field poppy, the morphological features of pollen tube arrest resemble that of the sporophytic SI system, in that the tube arrest occurs close to the stigma surface.

GENETICS OF GAMETOPHYTIC SELF-INCOMPATIBILITY

In gametophytic SI systems, an incompatible mating occurs when the *S* allele carried by the haploid pollen matches either of the *S* alleles present in the diploid style, as diagrammed in Figure 2. That is, the reaction is between a property expressed by the haploid genome of the male gametophyte

Figure 1. (continued).

(A) and **(B)** Fluorescence micrographs showing pollen tubes within the style of *N. alata* after compatible pollination, stained with a synthetic fluorochrome specific for (1,3)-β-glucans and viewed by fluorescence microscopy. (Original photographs are the work of Professor Elizabeth Williams, CSIRO Division of Horticulture.)

(A) The upper segment of the pistil, which includes the stigma. The pollen grains and tube walls fluoresce, indicating the presence of the (1,3)-β-glucan–containing material known as callose. (From Haring et al., 1990; copyright 1990 by the AAAS and reproduced with permission.)

(B) Section within the style adjoining that shown in **(A).** The pollen tube walls fluoresce and at regular intervals there are deposits of intensely fluorescent material known as callose plugs. These plugs are believed to cut off the cytoplasm that is carried in the tip of the growing tube from the spent grain. (From Peacock, 1990, reproduced with permission of Macmillan Magazines, Ltd.)

(C) Electron micrograph showing a transverse section through a pollinated style. Pollen tubes (p) are indicated. The pollen tubes at the upper left and lower right both show the two layers of the cell wall close to the tip. The large number of organelles present is further evidence that both pollen tubes were sectioned close to their tips. The pollen tube at the lower left has been sectioned through a thickened callosic wall, which appears electronlucent; the section is behind the tip because there is little cytoplasm present. The transmitting tract cells (t) are generally highly vacuolate and contain chloroplasts. The pollen tubes are located in the mucilage or extracellular matrix that separates the transmitting tract cells. Bar = 1 μm.

contained in the pollen grain and the pistil tissue of the sporophyte. Successful (i.e., compatible) matings occur when the *S* allele carried by the haploid pollen is different from either of the alleles carried by the diploid style. This is in contrast to the sporophytic system, in which the outcome of a pollination event is determined by the alleles carried by the pollen parent (i.e., sporophytically) rather than by the pollen itself (Figure 2). Thus, the reaction involves factors carried by the pollen that originated from the diploid tissues of the pollen parent and factors on the pistil tissue of the sporophyte. Pandey (1958) suggested that the difference in behavior between pollen from plants with gametophytic and pollen from plants with sporophytic SI is due to a shift in timing of gene action, as shown in Figure 3, the *S* genes in the sporophytic system being activated before meiosis, whereas those in the gametophytic system are activated after meiosis.

MOLECULAR GENETICS OF GAMETOPHYTIC SELF-INCOMPATIBILITY

The molecular genetics of SI was opened up by the observation that extracts of pistils of certain species contained glycoproteins that segregate with particular *S* genotypes. The first glycoprotein isolated for N-terminal amino acid sequencing was the glycoprotein that segregates with the S_2 allele of *Nicotiana alata* (Anderson et al., 1986). From the sequence data, a cDNA encoding the protein component was identified; subsequently, cDNAs corresponding to the glycoproteins of the S_3 and S_6 alleles of *N. alata* were obtained (Anderson et al., 1989). Comparison of these cDNAs showed that the derived proteins include a putative signal sequence and a conserved 15–amino acid hydrophobic region at the N terminus

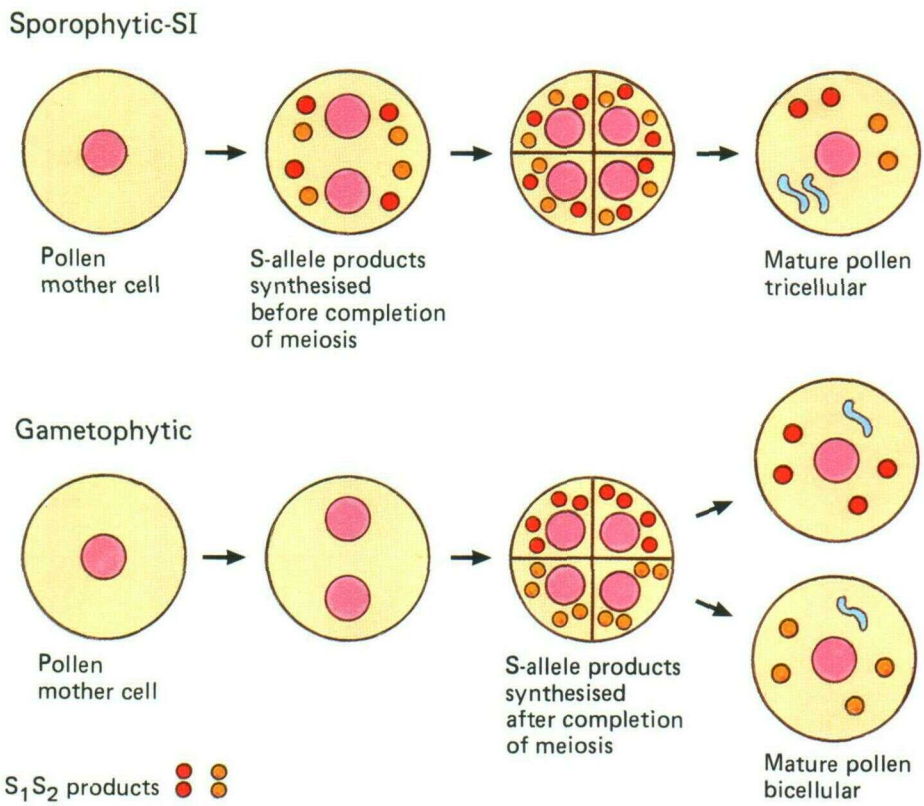

Figure 3. Possible Developmental Basis for the Difference between Sporophytic and Gametophytic SI.

One major difference in the two systems is believed to be the timing of *S* gene expression. In the sporophytic system, the *S* allele products are considered to be synthesized before the completion of meiosis (upper panel), whereas in gametophytic systems, the *S* allele products are considered to be synthesized after completion of meiosis. This difference in timing of expression is one explanation for the fact that pollen in the sporophytic system displays its maternal genotype, whereas pollen in the gametophytic system displays its own (haploid) genotype. (Diagram prepared with Dr. J.M. Pettitt, Monash University, Australia.)

of the mature protein as well as several other conserved sequences, as depicted in Figure 4. This information allowed the cloning of cDNAs corresponding to further *S* alleles of *N. alata* (Kheyr-Pour et al., 1990) and *S* alleles from *Petunia inflata* (Ai et al., 1990), *Solanum chacoense* (Xu et al., 1990), *P. hybrida* (petunia; Clark et al., 1990), *Solanum tuberosum* (potato; Kaufmann et al., 1991), and *Lycopersicon peruvianum* (Tsai et al., 1992). Alignment of all these sequences (Tsai et al., 1992) shows that, overall, ~16% of the amino acids are identical, including eight cysteine residues. Apart from the cysteine residues, there are five short conserved regions, two of which include a histidine residue (Figure 4).

DNA gel blot analysis of *N. alata* genomic DNA shows that these cDNAs are the allelic products of a single locus and that each is encoded by a single gene (Anderson et al., 1989). Genomic DNA from plants homozygous for the S_1, S_2, S_3, S_6, or S_7 alleles was digested with restriction enzymes and probed with the S_2, S_3, and S_6 cDNAs. The hybridizing fragments from the different *S* genotypes were dissimilar in size and constituted a series of restriction fragment length polymorphisms (RFLPs). The behavior of these RFLPs was examined in a population of plants segregating for four alleles of the *S* locus (Anderson et al., 1989). The *S* genotype of the plants was also determined by conventional breeding experiments. It was possible to correlate the DNA gel blot analysis of an individual plant with its breeding behavior, and it was found that each RFLP was associated with only one *S* allele.

Together, these data show that the cDNAs encoding the style-specific glycoproteins are the allelic products of the *S* locus. Because of this, the proteins encoded by these cDNAs are referred to as *S*-glycoproteins, with individual proteins named after the *S* genotype from which they are obtained (e.g., S_2-glycoprotein is the product of the S_2 allele). Subsequently the RFLP linkage map of potato, another self-incompatible solanaceous plant, has been produced (Gebhardt et al., 1991). The study indicates that the *S* locus resides on chromosome 1 and that the genes encoding the *S*-glycoproteins of potato are alleles of this locus. This confirmed earlier studies by Tanksley and Loaiza-Figueroa (1985) who mapped the *S* locus to chromosome 1 of *L. peruvianum*. Although the single *S* locus controls the specificity of the SI reaction, genes at other loci are also required for effective expression of SI. The genetics of these "modifier" genes have been described in *L. peruvianum* (Martin, 1967), petunia (Ai et al., 1991), and potato (Thompson et al., 1991).

RIBONUCLEASE ACTIVITY OF THE *S*-GLYCOPROTEINS

Sakiyama and coworkers, while studying fungal ribonucleases (RNases), recognized two short stretches of amino acid homology between the fungal RNase and the S_2-glycoprotein, which at that time was the only *S*-glycoprotein sequence published. In the absence of any other information, these short

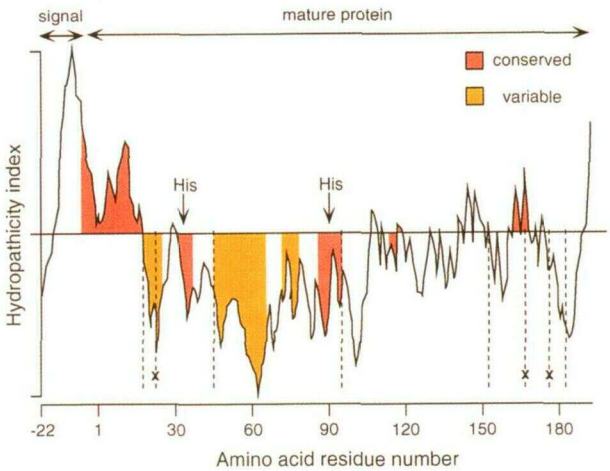

Figure 4. Hydropathy Plot Based on the *N. alata* S_2-Glycoprotein Sequence.

The *N. alata* sequence was aligned with cDNAs encoding other *S*-glycoproteins from *N. alata*, *P. inflata*, and potato. The variable regions are shown in yellow, and the conserved sequences are shown in red. Conserved cysteine residues are shown as dotted lines; cysteine residues that are not conserved in alignments with fungal ribonucleases are marked with a cross. The signal peptide was not included in the alignment.

stretches would not be considered significant. However, because the stretches correspond to the sequences surrounding the catalytic domains of the fungal ribonucleases and include the histidine residues that are essential for enzymatic activity (Kawata et al., 1990), this observation assumed a new significance. These short stretches were also found in two of the conserved regions identified in the S_3- and S_6-glycoproteins, which were unpublished at that time. Overall, 30 of the 122 amino acids conserved between three *N. alata* *S*-glycoproteins could be aligned with identical amino acids in the fungal RNases, and another 22 aligned with related amino acids. Apart from the two putative active site histidine residues, five cysteine residues are conserved in the fungal RNases and the *S*-glycoproteins (McClure et al., 1989). This led us to test the purified *S*-glycoproteins for RNase activity (McClure et al., 1989). Style extracts were fractionated using a cation exchange chromatography procedure that had been developed for isolation of *S*-glycoproteins (Jahnen et al., 1989). Testing of individual fractions for RNase activity showed that the activity eluted precisely with the individual *S*-glycoproteins. Furthermore, the RNase activity of the *S*-glycoproteins accounted for 40 to 80% of the total RNase activity of style extracts of different *S* genotypes.

The finding that the *S*-glycoproteins have RNase activity has led to their being referred to as *S*-RNases. Subsequently, *S*-glycoproteins from other solanaceous species have been shown to have RNase activity (Broothaerts et al., 1991; Kaufmann et al., 1991; Singh et al., 1991; Ai et al., 1992). In addition,

RNases have been detected in the styles of Japanese pear (a member of the family Rosaceae), and it is possible that these are also S-related glycoproteins (Sassa et al., 1992). This is of particular interest because it is the first indication that RNases may be associated with SI outside the Solanaceae. However, this is not a universal phenomenon, because the S-glycoproteins of poppy, a member of the Papaveraceae, do not have RNase activity (Franklin-Tong et al., 1991).

LOCALIZATION OF THE S-RNASES WITHIN THE PISTIL TISSUE

Immunocytochemical studies using S-RNase–specific antibodies raised to a synthetic peptide corresponding to one of the "hypervariable" regions (Figure 4) showed that S-glycoproteins are present on the surface of the stigmatic papillae and the extracellular matrix that separates the files of transmitting tract cells of the style. S-RNases are also present in the ovary but are restricted to the single layer of cells that makes up the inner epidermis of the placenta (Anderson et al., 1989). As might be expected, in situ hybridization studies show that S-RNase mRNA accumulates in the same tissues in which the protein is found (Cornish et al., 1987). A most interesting aspect of this localization is that it coincides with the path that the pollen tube follows as it grows through the style and across the inner epidermis of the placenta to individual ovules. Therefore, the pollen tube is in direct contact with RNase throughout its growth through the stigma and style to the ovules (Jahnen et al., 1989).

ROLE OF S-RNASES IN THE ARREST OF POLLEN TUBE GROWTH

The obvious question that arises from the discovery that stylar S-glycoproteins are RNases is whether the enzymatic function is involved in the SI response. One hypothesis is that the S-RNase is taken up by the incompatible pollen tube as it grows through the extracellular matrix. Once taken into the pollen tube, the RNase then degrades RNA, resulting in pollen tube death. In view of the fact that rRNA genes are not transcribed in pollen (Mascarenhas, 1990; McClure et al.,1990; Mascarenhas, 1993, this issue), this would be a very effective way of ensuring pollen tube death. In this case, the specificity of the interaction would be controlled either by the uptake of the S-RNase into the pollen tube or by the presence of an inhibitor that would act in an allele-specific manner to prevent RNA degradation in compatible pollen tubes.

However, it is possible that the RNase activity of the S-glycoproteins is unrelated to their function in SI. In this case, it could be envisaged that the enzyme was recruited during evolution for a role in SI unrelated to its catalytic activity. One way to test whether RNase activity is involved in SI is to follow the fate of pollen RNA in styles after compatible and incompatible pollinations. To do this, radioactive pollen prepared by growing *N. alata* plants in the presence of Phosphorus-32 (McClure at al., 1990) was placed on either compatible or incompatible stigmas, and RNA was prepared from styles 24 hr later.

Initially, it was observed that less radioactive (i.e., pollen-derived) RNA was recovered from incompatible pollinations than from compatible pollinations. When this RNA was fractionated on an agarose gel, it was apparent that the rRNAs from pollen were intact in samples from compatible crosses but degraded in samples from incompatible crosses. These results are consistent with the hypothesis that SI is mediated by degradation of pollen RNA in incompatible pollen tubes during their growth within the style. However, the experiments do not exclude the possibility that degradation of the rRNA is an effect rather than the cause of pollen tube death.

If the S-RNases do act to inhibit pollen tube growth by destruction of rRNA (as well as other types of RNA), then the question of allelic specificity arises. When the S-RNases from *P. inflata* are tested on homopolymeric substrates (Singh et al., 1991), they show a cleavage preference of poly(C) over poly(A) or poly(U). However, this is unlikely to be related to biological function because the S-RNases have no obvious differences in RNA substrate specificity and effectively degrade RNA from many sources. For example, RNA from pollen genotypes S_6S_6 and S_2S_2 as well as yeast and wheat germ RNA were all degraded in vitro by purified style S_2- and S_6-RNases (McClure et al., 1990). It is also unlikely that the S-RNases function in a way analogous to the DNA restriction endonucleases, i.e., that they cleave a specific sequence motif. The evidence is, first, the lack of base specificity on polymeric substrates, and, second, that S-RNase added to pollen tubes growing in vitro affects the synthesis of all pollen proteins rather than a specific subset (Gray et al., 1991). The existing data are therefore consistent with the idea that the S-RNases act to arrest pollen tube growth by degrading RNA. However, the cellular processes that lead to degradation occurring only in pollen tubes from incompatible pollinations but not those from compatible pollinations are unclear.

NATURE OF THE POLLEN S ALLELE PRODUCT

Lewis (1960) envisaged the gametophytic S locus as having three parts, a part expressed in the pistil, a part expressed in the pollen, and a third part, encoding allelic specificity. This "tripartite" theory is based on the findings that pollen and style SI functions can be mutated independently of each other. One possible mechanism by which SI might function is that the products of the pollen and stylar parts of the S locus are identical and come together in incompatible matings to form an active dimer. Careful experiments to search for a pollen protein that segregates with S genotype or that cross-reacts with antisera raised to a peptide corresponding to the hypervariable region

of the style S-RNase did not turn up candidate molecules (Mau, 1990; S.-L. Mau and A.E. Clarke, unpublished observations). Likewise, no candidate has been found in a search for pollen transcripts homologous to the S-RNase. If a molecule with homology or identity to the style S-RNase exists in pollen, then it must be present at levels below that detectable by the techniques used. Alternatively, the gene may be expressed only after contact with the style or at a particular stage of pollen development. There is some evidence that supports the latter suggestion (P.N. Dodds, I. Bönig, H. Du, J. Rödin, B. McClure, M.A. Anderson, E. Newbigin, and A.E. Clarke, manuscript submitted). It is also possible, however, that the pollen part of the SI interaction may be the product of a separate gene within the *S* locus. Establishing the nature of the pollen part of the *S* gene will probably require a concerted approach using molecular genetics to map the *S* locus and biochemical techniques to follow the interaction between style S-RNases and pollen tubes.

SIGNAL TRANSDUCTION DURING SELF-INCOMPATIBILITY

N. alata pollen extracts contain protein kinase activity that phosphorylates model proteins and a range of endogenous pollen proteins (Polya et al., 1986). However, to date, there are no reports of a protein kinase gene being involved in gametophytic SI. Recently, a gene encoding a putative protein kinase has been described in *Brassica oleracea* (Stein et al., 1991; see Nasrallah and Nasrallah, 1993, this issue), a plant with the sporophytic type of SI. This gene, which is expressed in the cells of the stigma and is within the *Brassica S* locus, may be involved in signal transduction, although this has not yet been demonstrated.

Whatever the nature of the signal transduction system that leads to pollen incompatibility, the effect of a self-incompatible mating is, ultimately, the arrest of pollen tube growth within the style. Several potential stages of pollen tube growth could be affected (Harris et al., 1984). For example, the rate of pollen tube extension depends on a balance between turgor pressure exerted by the contents of the pollen tube and the pressure exerted by the wall. If turgor pressure diminishes but cell wall biosynthesis proceeds, then wall abnormalities would result. Alternatively, direct interference with wall synthesis would lead to wall abnormalities. The processes of wall biosynthesis and pollen tube growth are not yet understood in detail (see Mascarenhas, 1993, this issue, for a discussion of pollen tube growth), but knowledge of these fields will be necessary for a complete understanding of how a self-incompatible mating results in arrest of pollen tube growth.

GLYCOSYLATION OF S-RNASES OF *N. ALATA*

All the isolated S-glycoproteins bear N-glycosyl chains. Four potential glycosylation sites (Asn-X-Thr/Ser) are conserved among the S_2-, S_3-, and S_6-glycoproteins of *N. alata*, and the S_3-glycoprotein has an additional site. Each of these S-glycoproteins also contains one unsubstituted glycosylation site: hydrolysis of the S_2-, S_3-, and S_6-glycoproteins with N-glycanase indicates that three of the four potential glycosylation sites in the S_2- and S_6-glycoproteins bear chains, as do four of the five sites in the S_3-glycoprotein. The single glycosylation site on the S_1-glycoprotein is occupied (Woodward et al., 1989). The structure of the N-glycan chains has been established by fast atom bombardment mass spectrometry and ^{1}H-nuclear magnetic resonance. Four different structures were found for both the S_1- and S_6-glycoproteins, with one form being dominant. The finding that S_1-glycoprotein has only one potential site indicates heterogeneity with respect to the chains at a single site (Woodward et al., 1992). Whether these N-glycosyl chains are involved in any way in the function of the *S* alleles is not known.

SEQUENCES RELATED TO S-RNASES FROM OTHER TISSUES

Recent experiments have shown that proteins similar to S-RNases are found in tissues other than the style of solanaceous plants. By using polymerase chain reaction techniques and primers corresponding to the conserved sequences around the presumptive active site histidine residues, fragments with sequence similarity to the S-glycoproteins were obtained from a cDNA library prepared from total green tissue from Arabidopsis (Taylor and Green, 1991). Proteins with sequence similarity to the S-RNases have also been isolated from seeds of *Momordica charantia* (bitter gourd; Ide et al., 1991) and from the culture medium of phosphate-starved *L. esculentum* cells (Jost et al., 1991). These findings prompt a review of the evidence that the S-RNases are indeed products of the *S* locus. The most compelling evidence is the absolute correspondence between genomic DNA fragments hybridizing to a particular cDNA encoding an S-RNase and the SI phenotype, as revealed by test crosses. This correspondence is also seen in populations examined for the correlation between the allelic glycoproteins separated by gel electrophoresis and the SI phenotype. Over 100 plants, the F_1 progeny of crosses between *N. alata* plants bearing different *S* alleles, have been examined in this way, as well as a smaller number of F_2 plants, and in no case has any anomalous behavior been detected (Anderson et al., 1989; M.A. Anderson and A.E. Clarke, unpublished observations). Plants from several other solanaceous species have also shown absolute correspondence between the RFLP and/or glycoprotein and breeding behavior (T.-H. Kao, unpublished observations).

The definitive experiment would be to induce a change of breeding behavior by transforming a self-compatible plant with a novel *S* allele. However, to date, these experiments have been unsuccessful. When the S_2-RNase gene of *N. alata* was introduced into the self-compatible tobacco, the amount of protein

in the style was very low compared with that found normally in the style of an *N. alata* plant expressing the S_2 allele (Murfett et al., 1992). Perhaps because of this lower protein level, the plants showed no change in the incompatibility phenotype.

PERSPECTIVE AND FUTURE DIRECTIONS

In this review, we have given a brief history of the molecular basis of gametophytic SI, concentrating on *N. alata* as an example. It is likely that the mechanism for recognition and rejection of self-pollen is common in all members of the Solanaceae. However, our knowledge of systems outside the Solanaceae is very limited, and we should be cautious in drawing broad principles from such a narrow data set.

The sequences of the allelic series *S*-RNases expressed in female tissue do not seem to have a direct counterpart in the sporophytic system of *Brassica* species; conversely, there is no evidence for systems in *N. alata* that correspond to the *S* locus glycoproteins or *S*-receptor kinases of *Brassica* species. It may be that the two systems operate by quite different means, although there are such wide gaps in our knowledge at present that we cannot unequivocally rule out some underlying similarities.

We have highlighted some of the key questions for the future. What is the nature of the pollen part of the *S* locus? How do the products of the pollen and style parts of the locus cause the incompatible response? What is the signaling mechanism? How are molecules transported from the extracellular matrix across the wall of the pollen tube to the cytoplasm? What is the mechanism of pollen tube wall biosynthesis? How is the required nutrient supply maintained? What is the relationship of the different macromolecules in the extracellular matrix of the transmitting tract? All these questions and others relevant to the pollen–pistil interactions have counterparts in more general aspects of plant molecular biology and physiology. The self-recognition implicit in SI may well have similarity with aspects of plant development, symbiosis, and pathogenesis. The mechanisms by which macromolecules are secreted through cell walls or taken up and transported through the cell wall to the cytoplasm are common to all types of cells. Cell wall biosynthesis, tip growth, and callose deposition are all fields of study with direct implications for other systems. Our knowledge in many of these areas is not particularly thorough, but we now have the experimental tools available to move forward and gain a greater insight into the cycle of reproduction and development in flowering plants.

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