Self-Incompatibility: A Self-Recognition System in Plants

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Self-incompatibility (SI), a genetically controlled mechanism to prevent inbreeding in plants, offers a relatively simple model system for studying the interactions between plant cells or between a plant cell and the secreted product or products of another cell. Examples of two major types of SI, gametophytic and sporophytic, have been studied by cloning cDNAs corresponding to glycoproteins of the female tissues that segregate with particular variants encoded by the putative *S* locus. These secreted glycoproteins are envisaged to interact with the currently undescribed pollen component to cause arrest of pollen tube growth.

OUTBREEDING WITHIN A POPULATION OF FLOWERING plants is favored by specific mechanisms that prevent self-fertilization. One of the most important of these mechanisms is self-incompatibility (SI), which ensures that a plant is fertilized by a genetically distinct individual of the same species (*1*). Self-incompatibility is a mechanism for self-recognition that results in rejection of self pollen by the female somatic tissues. This rejection of self is in contrast to the animal histocompatibility system, in which nonself tissue is recognized and rejected.

In many plant species, the incompatibility reaction is mediated by the interaction of a product of the haploid genome of the male gametophyte (carried within the pollen grain) and a product of the diploid genome of the female tissue of the sporophyte, the pistil. If the pollen carries the same allele as one of the two in the pistil, fertilization is not achieved. This system is known as gametophytic SI. Another, less widely distributed type is sporophytic SI; in these cases, the incompatibility reaction is between factors carried by the pollen but specified by the diploid tissues of the pollen parent and a product of the female pistil. In the simplest case, pollen tube growth is arrested if either of the alleles of the pollen parent is also present in the pistil.

The pathway of pollen tube growth through the pistil is shown in Fig. 1 (A to C). Pollen carried to the stigma surface of the pistil hydrates and germinates to produce tubes that penetrate the stigma and grow through the style to the ovary, where fertilization occurs. In plants with a solid style, the pollen tubes grow intercellularly, between the cells of the central transmitting tract. These cells are arranged in longitudinal files, which are separated by mucilage produced by the cells of the transmitting tract. In some species the pistils have hollow styles and, in these cases, pollen tubes grow through the central, mucilage-lined canal.

In gametophytic systems, pollen tube growth is commonly arrested within the style and involves contact between the pollen tube and the mucilage secreted by cells of the transmitting tract. In sporophytic systems, tube growth is arrested at the stigma surface or soon after penetration and involves contact between the pollen grain or emerging pollen tube and material secreted into the cell walls or onto the surface of the stigmatic papillae. The products of the presumptive *S*-locus of the pistil are secreted into the style mucilage in *Nicotiana alata* (*2*), a species with a gametophytic SI system. In *Brassica oleracea*, a species with a sporophytic SI system, the products of the presumptive *S*-locus in the pistil are present in the cell walls of the stigmatic papillae (*3*). In both systems, arrest of tube growth involves changes in the morphology of the tube wall. A major component of the tube wall, (1 → 3)-β-glucan (callose) gives a characteristic fluorescence when stained with a specific fluorochrome, revealing, in a pollinated pistil, the profiles of the growing pollen tubes. In a compatible pollination, the tubes are interrupted at regular intervals by cross walls of heavily staining material (Fig. 1, D and E). This material is thought to be laid down to isolate the tube tip, which contains the cytoplasm and sperm cells, from the spent grain. Self (incompatible) pollination in species with gametophytic SI commonly results in changes in this staining pattern, which include an irregular appearance of the cross walls and swelling of tips of arrested tubes. In species with sporophytic SI, self pollination results in deposition of callose in the pollen grain, the rudimentary tube, and the stigmatic papilla. Thus, in both types of SI, self (incompatible) mating results in arrest of tube growth accompanied by changes in the wall structure of the pollen tube.

Models (*4*) proposed to account for the arrest of growth of self (incompatible) pollen tubes are built on the knowledge that the simplest systems are controlled by a single locus, the *S* locus, with multiple alleles. This highly polymorphic locus may have more than 40 *S* alleles in natural populations (*5*). Further, SI is developmentally regulated and the SI barrier can often be overcome by hand-pollination of immature flowers with mature pollen from the same plant (in this way, homozygous plants can be produced). Observations that SI is overcome or weakened by increased temperature and by treatment with inhibitors of protein or RNA synthesis are also encompassed by these models. Of all the models proposed, variations of an oppositional hypothesis have been most generally accepted. These models assume that the pollen and pistil products of identical *S* alleles interact in some way to interfere actively with growth of the pollen tube. Direct interaction of like products of the same *S* allele and indirect interaction involving different products of the same *S* allele in pollen and pistil have both been proposed (*4*). In contrast, a complementary hypothesis is based on an assumption

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that pollen tube growth requires factors only supplied by nonself styles. However, no one model accommodates all the genetic and physiological observations. During the last 5 years, the techniques of molecular biology have been applied to investigations of both gametophytic and sporophytic SI. This approach is giving a new dimension to understanding SI, but no system can yet be described in sufficient detail to support any one of the theoretical models proposed.

Distribution and Evolution of SI

SI has been estimated to be present in more than half of all angiosperm species (*6, 7*); of 275 families of angiosperms, SI has been described in 91 families (*8*). However, it is often difficult to demonstrate whether or not a species is self-incompatible, and there is little information for many taxa (*8*). Notwithstanding these uncertainties, SI is clearly present in diverse taxa, and may have arisen early in angiosperm evolution (*7*). It is envisaged that the self-compatible taxa, which are often closely related to taxa with SI, arose by mutation of the *S* locus or of genes involved in regulation (*7, 9*). Success of self-compatible plants is dependent on the strength of inbreeding depression in the population (*10*). It has been suggested that sporophytic SI was derived during evolution from the more widely distributed gametophytic SI (*11*) or alternatively that sporophytic SI arose earlier than gametophytic SI (*12*). Other possibilities are that the two systems arose independently during evolution (*13*) or that there has been a monophyletic origin of the SI system for each family (*8*). The sequences of the *S*-glycoproteins of the SI species of the Solanaceae and the *S* locus–specific glycoproteins (SLSGs) of *Brassica* species have no apparent homology, supporting the suggestion of independent origin of the two systems. Recent evidence for the presence of gametophytic control as well as sporophytic control in the Brassicaceae (*14*) further complicates the issue. More detailed knowledge of the molecular genetics of the two systems will give greater insight into the evolution of the systems.

Molecular Genetics of SI

Study of the molecular biology of both gametophytic and sporophytic SI systems began with the cloning of complementary DNA (cDNAs) encoding the protein component of pistil glycoproteins that segregate with *S*-phenotype (*S*-glycoproteins) (*15, 16*). Plants with gametophytic SI for which style glycoproteins segregating with *S*-genotype are known are from three families, the Solanaceae, Rosaceae, and Papaveraceae (*17, 18*). These *S*-glycoproteins have relative molecular mass (M_r) in the range of 20,000 to 40,000 and have a characteristically high pI (*19*). *Nicotiana alata* (Solanaceae) is the most extensively studied species with gametophytic SI. Studies of sporophytic SI have been focused on two species of *Brassica* (*20*), *B. oleracea* (*16*) and *B. campestris* (*21*). The SLSGs of these systems are also characteristically of high pI, but they are of higher M_r, in the range of 50,000 to 65,000. The only report of *S* allele–related pollen components is an early immunological study of *Oenothera organensis* (Onagraceae) (*22*).

Molecular genetics of gametophytic SI in the Solanaceae. A cDNA encoding the 32-kD style glycoprotein that segregated with the S_2 allele of *N. alata* was described in 1986 (*15*). Information from the sequence of this cDNA was then used to obtain cDNAs corresponding to the S_3 and S_6 alleles of *N. alata* (*2*). Alignment of the derived amino acid sequences showed a conserved hydrophobic region of 15 amino acids at the NH_2-terminus of the mature protein, as well as two other conserved regions separated by hypervariable regions. In addition, for the S_2 and S_6 alleles, 5′ sequences encoding signal peptides were obtained, consistent with the expectation that the proteins are secreted into the style mucilage. Information from these sequences lead to cloning of cDNAs corresponding to additional *S* alleles of *N. alata* (*23*), as well as *S* alleles from *Petunia inflata* (*24*) and *Solanum tuberosum* (*25*). The deduced amino acid sequences are compared in Fig. 2A. The *N. alata* sequences fall into two groups, one comprised of the S_1, S_2, S_3, and S_6 sequences (51.5% identity between sequences) and the other of S_Z and S_{F11} (61.2% identity). The sequence identity between the two groups is 33%. The latter group seems to be more related to the *P. inflata* sequences with which they share 46.0% (S_{F11}) and 55.2% (S_2) identity (*24*). These relations might reflect the phylogeny of these *S*-glycoproteins in that their

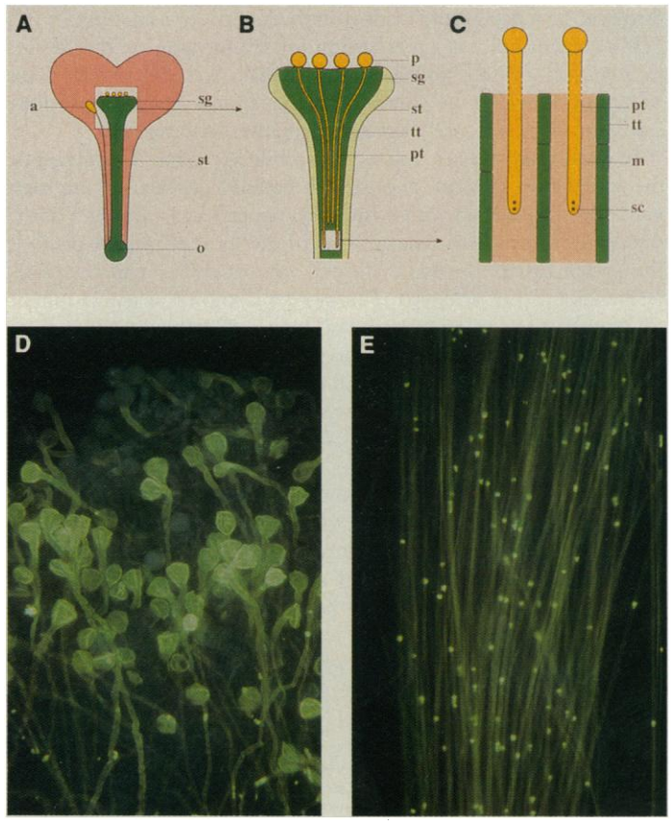

Fig. 1. Pathway of pollen tube growth through the female sexual tissues of a flower. (**A**) Flower, cut away to show the sexual organs. The female pistil consists of the stigma (sg), style (st), and ovary (o). The anthers (a) bear the pollen grains, which contain the sperm cells. (**B**) Stigma and upper part of the style. Pollen grains (p) from a compatible flower have been brought to the stigma surface where they germinate to produce pollen tubes, which grow through the central transmitting tract (tt) of the style. (**C**) Part of the transmitting tract tissue through which the pollen tubes (pt) grow. The transmitting tract consists of files of elongated cells, which are separated at maturity by a secreted mucilage (m). The pollen tubes grow in the mucilage between files of cells. The sperm cells (sc) are contained within the tips of the pollen tubes. The mucilage contains mixtures of proteins, glycoproteins, and proteoglycans as high molecular weight components. (**D** and **E**) Micrographs showing pollen tubes within the style of *N. alata* after a compatible pollination, stained with the synthetic fluorochrome specific for (1 → 3)-β-glucans and viewed by fluorescence microscopy (*38*). (**D**) Section including stigma. Pollen grains and tube walls fluoresce. (**E**) Section within the style adjoining that shown in (D). [Reprinted from (*39*) with permission, ©1990 Macmillan.] The pollen tube walls fluoresce. Intensely fluorescent deposits containing (1 → 3)-β-glucan are present at regular intervals.

allelic separation may have occurred before the species separated.

In total, 46 amino acids (22.4%) are conserved in all ten sequences. These residues are likely to play important roles in general functions associated with all *S*-glycoproteins from the Solanaceae. Since the conserved residues include seven of the eight to ten cysteines of each protein, it seems likely that these proteins have similar tertiary structures. There is only one hypervariable region of significant length in both the *N. alata* and *P. inflata* *S*-glycoproteins (around position 60 in Fig. 2A). A peptide sequence from this region of the S_2-glycoprotein has been used to generate antibodies that were specific to the S_2-glycoprotein of *N. alata* when tested by protein blot analysis of style extracts. These antibodies were used in immunocytochemical studies of the distribution of the S_2-glycoprotein within the pistil (*2*). As anticipated, the S_2-glycoprotein was present in the extracellular mucilage of the central transmitting tract of the style. It was also present in the walls of a specific layer of cells in the ovary, the inner epidermis of the placenta. This distribution of the S_2-glycoprotein mirrors the in situ hybridization pattern seen from the use of S_2-cDNA probe (*26*). The expression of these products, both the RNA and the protein, coincide with the pathway of the pollen tubes as they grow through the transmitting tract, over the inner epidermis of the placenta to the ovules.

Analysis of *N. alata* genomic DNA from plants containing the S_1, S_2, S_3, S_6, and S_7 alleles, with the use of the S_2, S_3, and S_6 cDNAs as probes, showed that most restriction enzymes produced single fragments that hybridized strongly (*2, 27*). In some cases, weakly hybridizing bands could also be detected, but the significance of these bands is not known. The major, strongly hybridizing bands were only large enough to accommodate one or a few copies of the coding sequence, suggesting a single-locus, single-copy genetic system. The abundance of restriction fragment length polymorphisms between *S* alleles revealed by these analyses indicates variability in flanking regions. The weak cross-hybridization between alleles reflects the variability within the coding region. The locus is thus highly polymorphic.

A pollen component that corresponds to a particular *S* allele has not been identified by probes from or antibodies to *S*-glycoproteins, combined with pollen RNA or pollen protein blot analyses. Another approach to understanding the basis of specificity is to reproduce the allele-specific inhibition of pollen tube growth in vitro with *S*-glycoproteins isolated from style extracts and pollen tubes grown in vitro. Such a system would allow examination of the changes in gene expression in pollen induced by contact with *S*-glycoproteins corresponding to the same and to different alleles as that borne by the test pollen. However, pollen tubes grown in vitro are not normal

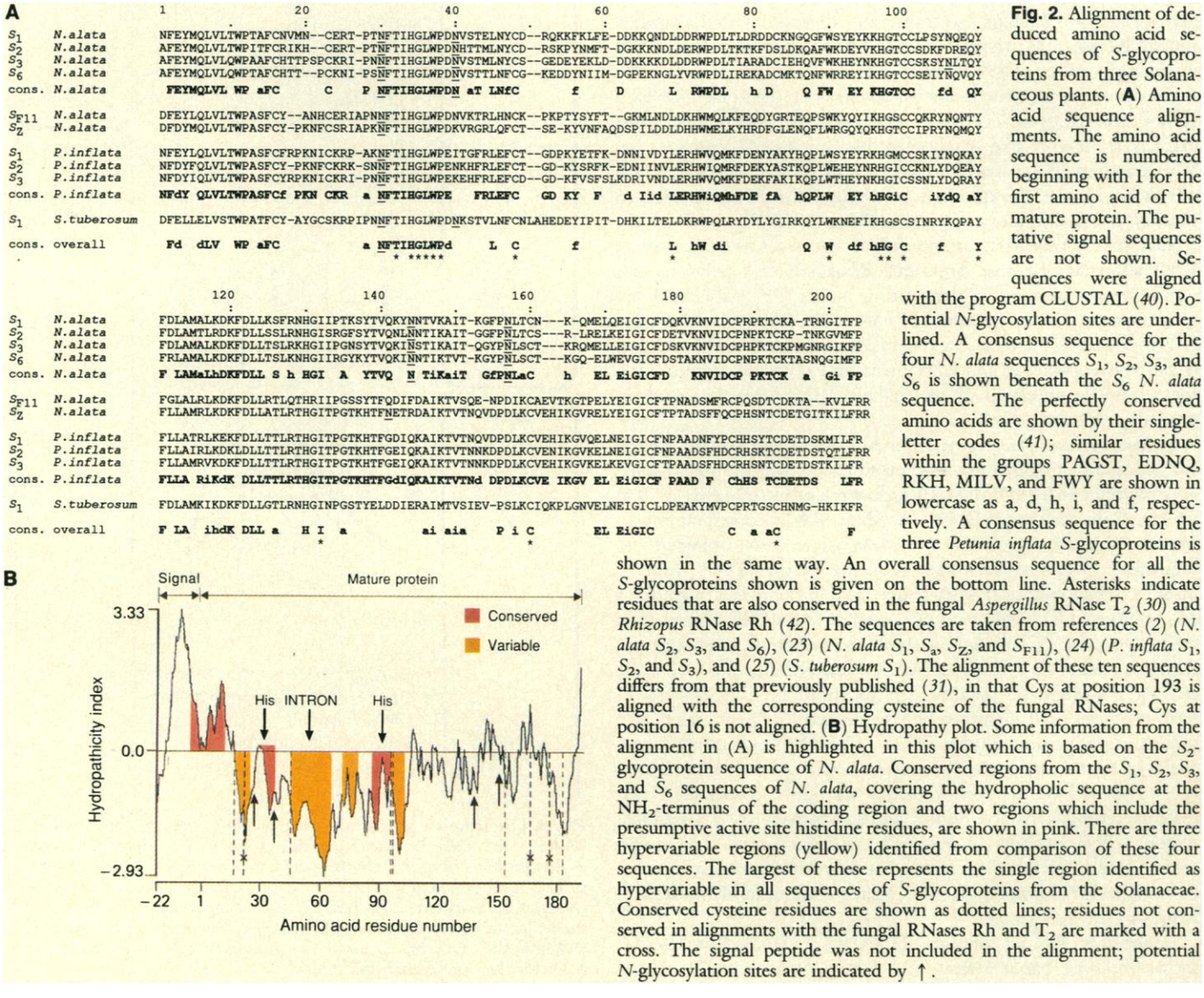

Fig. 2. Alignment of deduced amino acid sequences of *S*-glycoproteins from three Solanaceous plants. (**A**) Amino acid sequence alignments. The amino acid sequence is numbered beginning with 1 for the first amino acid of the mature protein. The putative signal sequences are not shown. Sequences were aligned with the program CLUSTAL (*40*). Potential *N*-glycosylation sites are underlined. A consensus sequence for the four *N. alata* sequences S_1, S_2, S_3, and S_6 is shown beneath the S_6 *N. alata* sequence. The perfectly conserved amino acids are shown by their single-letter codes (*41*); similar residues within the groups PAGST, EDNQ, RKH, MILV, and FWY are shown in lowercase as a, d, h, i, and f, respectively. A consensus sequence for the three *Petunia inflata* *S*-glycoproteins is shown in the same way. An overall consensus sequence for all the *S*-glycoproteins shown is given on the bottom line. Asterisks indicate residues that are also conserved in the fungal *Aspergillus* RNase T_2 (*30*) and *Rhizopus* RNase Rh (*42*). The sequences are taken from references (*2*) (*N. alata* S_2, S_3, and S_6), (*23*) (*N. alata* S_1, S_a, S_Z, and S_{F11}), (*24*) (*P. inflata* S_1, S_2, and S_3), and (*25*) (*S. tuberosum* S_1). The alignment of these ten sequences differs from that previously published (*31*), in that Cys at position 193 is aligned with the corresponding cysteine of the fungal RNases; Cys at position 16 is not aligned. (**B**) Hydropathy plot. Some information from the alignment in (A) is highlighted in this plot which is based on the S_2-glycoprotein sequence of *N. alata*. Conserved regions from the S_1, S_2, S_3, and S_6 sequences of *N. alata*, covering the hydropholic sequence at the NH_2-terminus of the coding region and two regions which include the presumptive active site histidine residues, are shown in pink. There are three hypervariable regions (yellow) identified from comparison of these four sequences. The largest of these represents the single region identified as hypervariable in all sequences of *S*-glycoproteins from the Solanaceae. Conserved cysteine residues are shown as dotted lines; residues not conserved in alignments with the fungal RNases Rh and T_2 are marked with a cross. The signal peptide was not included in the alignment; potential *N*-glycosylation sites are indicated by ↑.

in that they have thickened walls and grow to a fraction of the length of tubes grown in vivo. Nonetheless, an in vitro system for the poppy *Papaver rhoeas* showed specific interactions between pollen and isolated *S*-glycoprotein and will be extremely valuable for studies of the pollen component (*28*). With *N. alata*, absolute specificity was not obtained: each isolated *S*-glycoprotein inhibited tube growth of each genotype to a certain extent, although in some cases there was greater inhibition of the pollen bearing the same allele as that of the source of the *S*-glycoprotein (*29*).

Ribonuclease function of the S*-glycoproteins in* N. alata. An unexpected finding, that the *S*-glycoproteins of *N. alata* are ribonucleases (RNases), resulted from sequence comparison of the *S*-glycoproteins with sequences of the secreted fungal RNases from *Aspergillus oryzae* (RNase T_2) and *Rhizopus niveus* (RNase Rh) (*30, 31*). The sequence homology between the Solanaceous *S*-glycoproteins and the fungal RNases is not extensive. Of 122 amino acids conserved among three *N. alata* *S*-glycoproteins (S_2, S_3, and S_6), 30 were aligned with identical amino acids in the fungal RNases, and another 22 were aligned with related amino acids. However, the conserved regions include residues surrounding two putative active site histidine residues and five cysteine residues. These sequence homologies suggested structural and functional similarity with the fungal RNases, so we assayed the isolated *S*-glycoproteins for intrinsic RNase activity. The specific RNase activities measured in absorbance units at 260 nm (A_{260}) ranged from 170 (S_1-glycoprotein) to 2200 (S_2-glycoprotein) compared with the purified fungal RNase T_2 activity of 3900 (A_{260} units per minute per milligram) (*31*). The RNase activity associated with the *S*-glycoproteins accounted for 40 to 80% of the total recovered RNase activity of style extracts, depending on *S*-genotype. Style extracts from a related self-compatible species, *N. tabacum*, had RNase activity of less than 1% of that of the *N. alata* extracts. These observations raise the question of whether the RNase activity is directly involved in arrest of pollen tube growth (Fig. 3). Does the RNase, for example, pass into the pollen tube to act on a cytoplasmic RNA substrate? Because ribosomal RNA (rRNA) genes are not transcribed in mature or germinating pollen (*32*) viability of the growing tube would be reduced by entry of an RNase that degraded rRNA. Possibly, the RNase activity is not related to the SI function of the *S*-glycoproteins, and RNase was "recruited" during evolution for a purpose unrelated to its catalytic activity. Further study is needed in this area.

Molecular genetics of sporophytic SI in Brassica *species*. *S* locus–specific glycoproteins of stigma extracts separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) appear as a group of bands at 50 to 65 kD. Complementary DNAs encoding SLSGs corresponding to the S_6, S_{13}, and S_{14} alleles of *B. oleracea* have been obtained and sequenced (*33*). The nucleotide sequence of the cDNA from S_6 SLSG corresponds to a 436–amino acid protein and includes a 31–amino acid hydrophobic signal peptide at the NH_2-terminus of the polypeptide. Consistent with this observation, immunocytochemical studies show that the SLSG is secreted into the wall of the cells of the stigmatic papillae (*3*). In addition, SLSGs from *B. campestris* have been identified and sequenced by direct amino acid sequencing of peptide fragments (*21*). The cDNAs encoding four *S* alleles of *B. oleracea* have 90% sequence homology at the DNA level (*20*). The derived peptide sequences include a conserved NH_2-terminal region, a variable region, and a conserved COOH-terminal region, which contains 11 invariant cysteine residues. The amino acid sequences of the *S*-glycoproteins from the *Brassica* species are more than 80% homologous (*34*), which is significantly more than is seen in the Solanaceae.

Analysis of *Brassica* genomic DNA with cDNA from SLSGs shows multiple bands reflecting a family of at least 11 related sequences (*16, 33*). Two of these have been isolated and sequenced; one is the structural gene and the other is *S*-locus related (*SLR1*). Both are expressed in the mature stigma. The *SLR1* sequences are highly conserved and are present in plants homozygous for different *S* alleles, whereas the SLSG sequences have some variation. *SLR1* is not considered to be involved in determination of allelic specificity (*20*). Gene-specific probes for the SLSG sequences will allow further analysis of this locus. Two recent interesting observations may have some bearing on the mechanism of sporophytic SI. (i) Transformation of the self-compatible species *N. tabacum* with genomic clones corresponding to the *B. oleracea* S_{13} and S_{22} alleles resulted in gene expression in the style transmitting tract (*35*). This pattern of expression closely parallels the pattern observed for the style product of the *S* locus in *N. alata*, implying similarities in the promoter regions of the two genes. (ii) A cDNA from *Zea mays* that encodes a putative serine/threonine–specific receptor protein kinase is apparently linked through a transmembrane domain to an extracellular domain with homology to the *Brassica* SLSG sequence (*36*). The significance of this observation is not clear but the sequence homology between these molecules in such divergent species suggests a role of fundamental importance.

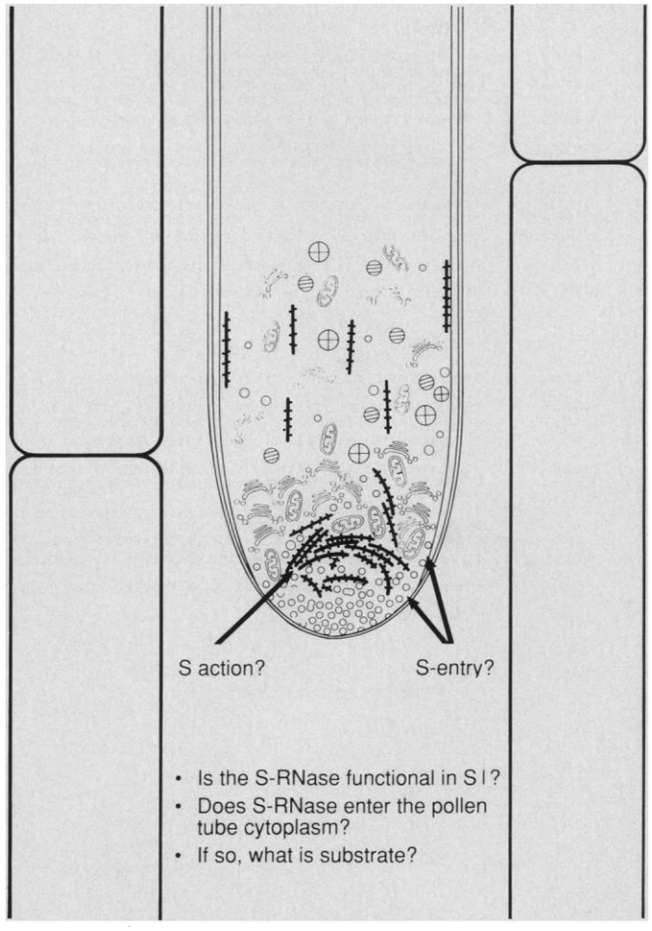

Fig. 3. Diagram of a pollen tube tip growing in the extracellular mucilage between the cells of the transmitting tract. The mucilage contains *S*-glycoproteins secreted from these cells, which is in direct contact with the surface of the pollen tube. There is zonation of organelles including vesicles, endoplasmic reticulum, mitochondria, and Golgi bodies in the tip region of the pollen tube. The questions of whether *S*-RNases enter the pollen tube and how they function to arrest the growth of tubes after incompatible pollination are presently being studied.

Directions for the Future

The study of SI is at an exciting stage with many leads to be followed from the current information. A major unanswered question is that of the nature of the pollen product of the *S* locus. Although RNA and protein blot analysis with specific cDNAs and antisera have not revealed any pollen component corresponding to *S*-genotype, the possibility that a homologous species is present in low abundance or that there is limited homology between the pollen and style products remains. Polymerase chain reaction (PCR) techniques can be used to test these possibilities. Another possibility is that the products of the *S* locus in pollen and style are distinct; in this case, the two must be closely linked as the two functions have never been separated by conventional breeding. An approach to testing this possibility would be chromosome walking.

There is a cluster of questions relating to how the products of the *S* locus in pollen and style interact; is a protein-protein interaction involved? Do the style *S*-locus products move into the pollen tube? If so, how do they cross the barriers of the pollen tube cell wall and plasma membrane? What role does the glycosyl component of the style *S*-glycoproteins play? How is the specificity of the interaction controlled? What is the mechanism of signal transduction? Is RNase activity found for the *S*-glycoproteins of *N. alata* present in other *S*-glycoproteins? Is it involved in SI function? In relation to this question, it was recently found that *S* allele–specific degradation of rRNA occurs in vivo (*37*).

Relating the knowledge that will come from further studies of the molecular genetics of SI to the physiology of pollen tube growth will be difficult because of the gaps in our knowledge of structure and biosynthesis of cell walls in general, and that of pollen tubes in particular. What is the normal process of nutrition of the tube? What are the cell wall components present in pollen tubes? How are these components synthesized and deposited in the growing tube? There is only fragmentary knowledge available relevant to these questions. An overriding uncertainty is the possibility that different SI systems may operate in different plant families, and that differences are not restricted to those between families with gametophytic or sporophytic control. This possibility highlights the pitfalls in drawing general conclusions as to the mechanism of SI from what is, at present, a very narrow knowledge base.

REFERENCES AND NOTES

1. D. de Nettancourt, *Incompatibility in Angiosperms* (Springer-Verlag, Berlin, 1977).
2. M. A. Anderson *et al.*, *Plant Cell* **1**, 483 (1989).
3. M. K. Kandasamy, D. J. Paolillo, J. B. Nasrallah, C. D. Faraday, M. E. Nasrallah, *Dev. Biol.* **134**, 462 (1989).
4. D. Lewis, *Genet. Today* **3**, 656 (1964); J. A. W. van der Donk, *Mol. Gen. Genet.* **141**, 305 (1975); D. L. Mulcahy and G. B. Mulcahy, *Heredity* **54**, 139 (1985).
5. M. J. Lawrence, *Proc. R. Soc. London Ser. B* **188**, 275 (1975).
6. E. M. East, *Proc. Am. Philos. Soc.* **82**, 449 (1940).
7. M. L. K. Whitehouse, *Ann. Bot. (London) New Ser.* **14**, 198 (1950).
8. D. Charlesworth, in *Evolution—Essays in Honour of John Maynard Smith*, P. J. Greenwood, P. H. Harvey, M. Slattern, Eds. (Cambridge Univ. Press, Cambridge, 1985), pp. 237–268.
9. S. K. Jain, *Annu. Rev. Ecol. Syst.* 7, 469 (1976); O. Mayo and C. R. Leah, *Theor. Appl. Genet.* **24**, 789 (1987).
10. D. Charlesworth and B. Charlesworth, *Annu. Rev. Ecol. Syst.* **18**, 237 (1987); D. A. Levin, *Evolution* **43**, 1417 (1989).
11. J. L. Brewbaker, *Indian J. Genet. Plant Breed.* **19**, 121 (1959); K. K. Pandey, *Nature* **187**, 1220 (1958); *Evolution* **14**, 98 (1960); *New Phytol.* **84**, 381 (1980); L. K. Crowe, *Heredity* **19**, 437 (1964).
12. M. S. Zevada, *Plant Syst. Evol.* **147**, 63 (1984).
13. A. J. Bateman, *Heredity* **6**, 285 (1952).
14. M. I. Zuberi and D. Lewis, *ibid.* **61**, 367 (1988).
15. M. A. Anderson *et al.*, *Nature* **321**, 38 (1986).
16. J. B. Nasrallah, T.-H. Kao, M. L. Goldberg, M. E. Nasrallah, *ibid.* **318**, 263 (1985).
17. E. C. Cornish, M. A. Anderson, A. E. Clarke, *Annu. Rev. Cell Biol.* **4**, 209 (1988).
18. V. E. Franklin-Tong, E. Ruuth, P. Marmey, M. J. Lawrence, F. C. H. Franklin, *New Phytol.* **112**, 307 (1989).
19. W. Jahnen, M. P. Batterham, A. E. Clarke, R. L. Moritz, R. J. Simpson, *Plant Cell* **1**, 493 (1989).
20. J. B. Nasrallah and M. E. Nasrallah, *Annu. Rev. Genet.* **23**, 121 (1989).
21. S. Takayama *et al.*, *Nature* **326**, 102 (1987).
22. D. Lewis, *Proc. R. Soc. London Ser. B* **140**, 127 (1952).
23. A. Kheyr-Pour *et al.*, *Sex. Plant Reprod.* **3**, 88 (1990).
24. Y. Ai *et al.*, *ibid.*, p. 130.
25. H. Kaufmann, C. Gebhardt, E. Ritter, F. Salamini, R. D. Thompson, in preparation.
26. E. C. Cornish, J. M. Pettitt, I. Bönig, A. E. Clarke, *Nature* **326**, 99 (1987).
27. R. Bernatzky, M. A. Anderson, A. E. Clarke, *Dev. Genet.* 9, 1 (1988).
28. V. E. Franklin-Tong, M. J. Lawrence, F. C. H. Franklin, *New Phytol.* **110**, 109 (1988).
29. W. Jahnen, W. M. Lush, A. E. Clarke, *Plant Cell* **1**, 501 (1989).
30. Y. Kawata, F. Sakiyama, H. Tamaoki, *Eur. J. Biochem.* **176**, 683 (1988).
31. B. A. McClure *et al.*, *Nature* **342**, 955 (1989).
32. J. P. Mascarenhas, *Annu. Rev. Plant Physiol.* **41**, 317 (1990).
33. J. B. Nasrallah, T.-H. Kao, C.-H. Chen, M. L. Goldberg, M. E. Nasrallah, *Nature* **326**, 617 (1987).
34. A. Isogai *et al.*, *Plant Cell Physiol.* **28**, 1279 (1987).
35. H. M. Moore and J. B. Nasrallah, *Plant Cell* **2**, 29 (1990).
36. J. C. Walker and R. Zhang, *Nature* **345**, 743 (1990).
37. B. A. McClure, J. E. Gray, M. A. Anderson, A. E. Clarke, *ibid.* **347**, 757 (1990).
38. A. L. Rae, P. J. Harris, A. Bacic, A. E. Clarke, *Planta* **166**, 128 (1985); N. A. Evans, P. A. Hoyne, B. A. Stone, *Carbohydr. Polym.* **4**, 215 (1984).
39. J. Peacock, *Nature* **347**, 714 (1990).
40. D. G. Higgins and P. M. Sharp, *Comput. Appl. Biosci.* **5**, 151 (1989).
41. Single-letter abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
42. H. Horiuchi *et al.*, *J. Biochem.* **103**, 408 (1988).

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