Self-Incompatibility in Plants

Seiji Takayama and Akira Isogai

Laboratory of Intercellular Communications, Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan; email: takayama@bs.naist.jp, isogai@bs.naist.jp

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self-/nonself-recognition, signal transduction, receptor kinase, F-box protein, Ca^{2+} signaling

Abstract

Sexual reproduction in many flowering plants involves self-incompatibility (SI), which is one of the most important systems to prevent inbreeding. In many species, the self-/nonself-recognition of SI is controlled by a single polymorphic locus, the *S*-locus. Molecular dissection of the *S*-locus revealed that SI represents not one system, but a collection of divergent mechanisms. Here, we discuss recent advances in the understanding of three distinct SI mechanisms, each controlled by two separate determinant genes at the *S*-locus. In the Brassicaceae, the determinant genes encode a pollen ligand and its stigmatic receptor kinase; their interaction induces incompatible signaling(s) within the stigma papilla cells. In the Solanaceae-type SI, the determinants are a ribonuclease and an F-box protein, suggesting the involvement of RNA and protein degradation in the system. In the Papaveraceae, the only identified female determinant induces a Ca^{2+}-dependent signaling network that ultimately results in the death of incompatible pollen.

Contents

INTRODUCTION

SI: self-incompatibility

GSI: gametophytic self-incompatibility

SSI: sporophytic self-incompatibility

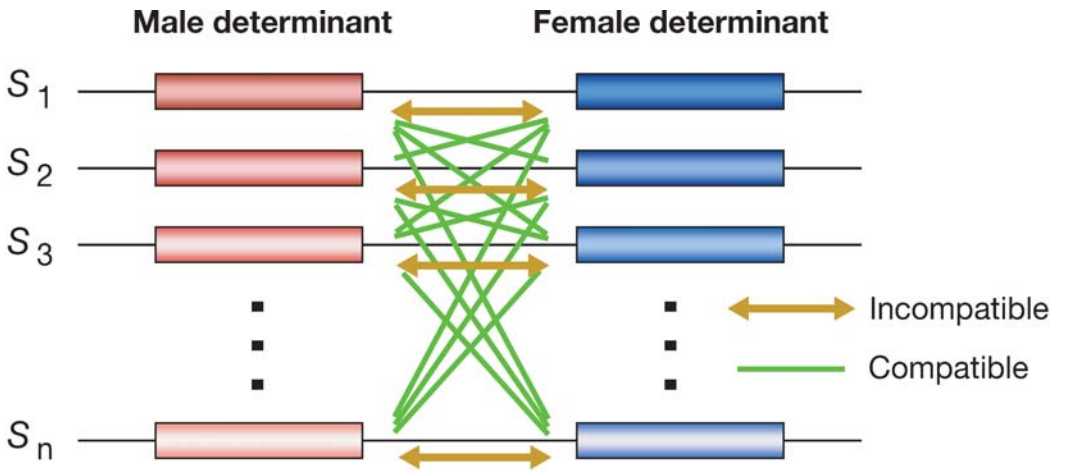
Self-incompatibility (SI) is one of the most important systems used by many flowering plants to prevent self-fertilization and thereby generate and maintain genetic diversity within a species (6). The SI response is comprised of a self- and nonself-recognition process between pollen and pistil that is followed by selective inhibition of the self-pollen (tube) development. Classic genetic studies have established that the self-/nonself-recognition in most species is controlled by a single multiallelic locus, the *S*-locus, and that pollen inhibition occurs when the same "*S*-allele" specificity is expressed by both pollen and pistil.

After 20 years of intense molecular studies focused on the entities of the *S*-locus, the molecules involved in the SI recognition were finally identified in certain plant species. These identified determinant genes have diverse structures, suggesting that the SI does not represent one mechanism but encompasses a collection of divergent systems. The only unifying scheme that has emerged from these studies is that the *S*-locus consists of at least two linked transcriptional units arranged in pairs, with one functioning as the female determinant and the other as the male (**Figure 1**). This multigene complex at the *S*-locus is inherited as one segregating unit, and therefore the variants of the gene complex are called "*S*-haplotypes." Self-/nonself-recognition operates at the level of protein-protein interaction of the two determinants and the SI response occurs when both determinants are issued from the same *S*-haplotypes.

Both the female and male determinants were first identified in the Brassicaceae (**Figure 1**). Recent studies also identified the male determinant in the Solanaceae, Rosaceae, and Scrophulariaceae, all of which share the same female determinant molecule. Additionally, the female determinant was identified from studies on the Papaveraceae. In spite of this unifying scheme of a multiallelic two-gene recognition system, the identified determinants bear no similarity to one another, suggesting that SI evolved independently and probably multiple times in different lineages of the angiosperms. There are numerous excellent reviews of SI (6, 10, 12, 20, 27, 32, 47, 68, 80, 93). The present review highlights recent works to update the reader on our current understanding of the molecular mechanisms of SI in these plant species.

BRASSICACEAE-TYPE SELF-INCOMPATIBILITY

Classic genetic studies in the early 1950s unraveled two distinct forms of SI, the gametophytic (GSI) and the sporophytic (SSI), which were distinguished by the genetic behavior of the pollen's SI phenotype (6, 47, 68). The pollen SI phenotype in GSI is determined by its own haploid genome, whereas in SSI the pollen SI phenotype is determined by the diploid genome



Family	Type of SI	Male determinant	Female determinant
Brassicaceae	SSI	SP11/SCR	SRK
Solanaceae, Rosaceae, Scrophulariaceae	GSI	SLF/SFB	S-RNase
Papaveraceae	GSI	(Unknown)	S-protein

Figure 1

A schematic drawing of the *S*-locus and a list of the identified female and male determinant genes. The *S*-locus contains at least two genes, one encoding the male determinant that is carried by the pollen grain, and the other encoding the female determinant that is expressed in the pistil. Both the male and female determinants are polymorphic and inherited as one segregating unit. The variants of this gene complex are called *S*-haplotypes. The recognition of self/nonself operates at the level of the protein-protein interactions between the two determinants and an incompatible response occurs when both determinants are issued from the same *S*-haplotype. Thus far, both determinants have been identified in the Brassicaceae and Solanaceae. Only the female determinant has been identified in the Papaveraceae.

of its parent (sporophyte). According to this classification, the SI in the Brassicaceae belongs to SSI and, so far, is the only SSI system in which the mechanism has been characterized at the molecular level (20, 27, 80, 93). More than 30 and 50 *S*-haplotypes have been identified in *B. rapa* (syn. *campestris*) and in *B. oleracea*, respectively (54, 56). In the self-incompatible plants of this family, pollen tubes do not develop properly on the stigma that express the same *S*-haplotypes as the pollen's parent. Self-pollen rejection results in abrogated pollen hydration, or a rapid arrest of the pollen tube growth at the stigma surface.

Female Determinant

Searches for the female determinant began with the immunological identification of an *S*-haplotype-specific antigen in the stigma, followed by the biochemical identification of stigma glycoproteins called *S*-locus glycoproteins (SLGs) that cosegregate with *S*-haplotypes. SLGs are 50–60-kDa secreted glycoproteins with several *N*-linked oligosaccharides and twelve conserved cysteine residues (52, 81). The identification of SLGs led to the isolation of the second *S*-locus gene, the *S*-locus receptor kinase (SRK) gene (72). SRK consists of an SLG-like extracellular domain (*S*-domain), a transmembrane domain, and an intracellular serine/threonine kinase domain.

SLG and SRK exhibit a number of characteristics that would be expected for the female determinant of SI. First, they are predominantly produced in the stigma papilla cells, which come into direct contact with pollen. Second, their expression occurs just prior to flower opening and coincides with the timing of SI acquisition by the stigma. Third, they exhibit allelic sequence diversity among all of the *S*-haplotypes examined.

A gain-of-function experiment clarified the involvement of SLGs and SRKs in the SI response (79). Transgenic *B. rapa* expressing SRK_{28} (*SRK* of the S_{28}-haplotype) acquired S_{28}-haplotype specificity in the stigma and rejected the S_{28} pollen. In contrast, transgenic plants expressing SLG_{28} did not display S_{28}-haplotype specificity. When both SLG_{28} and SRK_{28} were introduced, however, the transformants exhibited stronger incompatibility against S_{28} pollen and produced fewer seeds. These results demonstrate that *SRK* alone determines the *S*-haplotype specificity of the stigma, and that *SLG* enhances the activity of *SRK*. In another gain-of-function experiment performed in *B. napus*, the role of SRK as the female determinant was also confirmed (69). However, no enhancing role for SLG was detected in the experiment. Thus, the requirement for SLG in the SI response may be variable among the different *S*-haplotypes. In support of this view, several *S*-homozygous lines of *Brassica*, and other genera of the Brassicaceae, *Arabidopsis lyrata*, lack *SLG* expression, even though they still exhibit a strong SI phenotype (35, 77, 78).

Male Determinant

The first important clue for identifying the male determinant was obtained from a pollination bioassay, which demonstrated that the biological activity responsible for SI resides in a small protein fraction (<10 kDa) of the pollen coat (73). Although isolating the active component was unsuccessful, two genetic approaches, the cloning and sequencing of the *S*-locus region and the polymorphic gene search using fluorescent differential display, succeeded in identifying the male determinant genes, which were named *SP11* (*S-locus protein 11*) or *SCR* (*S-locus cysteine-rich*) (62, 76, 82).

SP11/SCR encodes the secreted forms of small, basic, cysteine-rich proteins. *SP11/SCR* exhibits an extensive *S*-haplotype-associated polymorphism in which the alleles share a relatively conserved signal sequence but have mature proteins that are highly variable (19.5% to 94% amino acid identity), suggesting a strong positive selection for diversification. Only a few residues are highly, but not absolutely, conserved among most *S*-haplotypes. Namely, these include eight cysteine residues (hereafter designated as C1 through C8), a glycine residue between C1 and C2, and an aromatic residue between C3 and C4 (63, 82, 92).

The identity of SP11/SCR as the male *S*-determinant was definitively established by gain-of-function experiments and by direct activity testing using a pollination bioassay (62, 65, 82, 83). In all of the gain-of-function experiments, pollen from the transformants with the *SP11/SCR* transgene acquired the *S*-haplotype specificity of the transgene. In the pollination bioassay, the pretreatment of the stigma with bacterially expressed or chemically synthesized "self" SP11/SCR inhibited the hydration and penetration of "cross" pollen. These results clearly suggest that SP11/SCR is the sole male

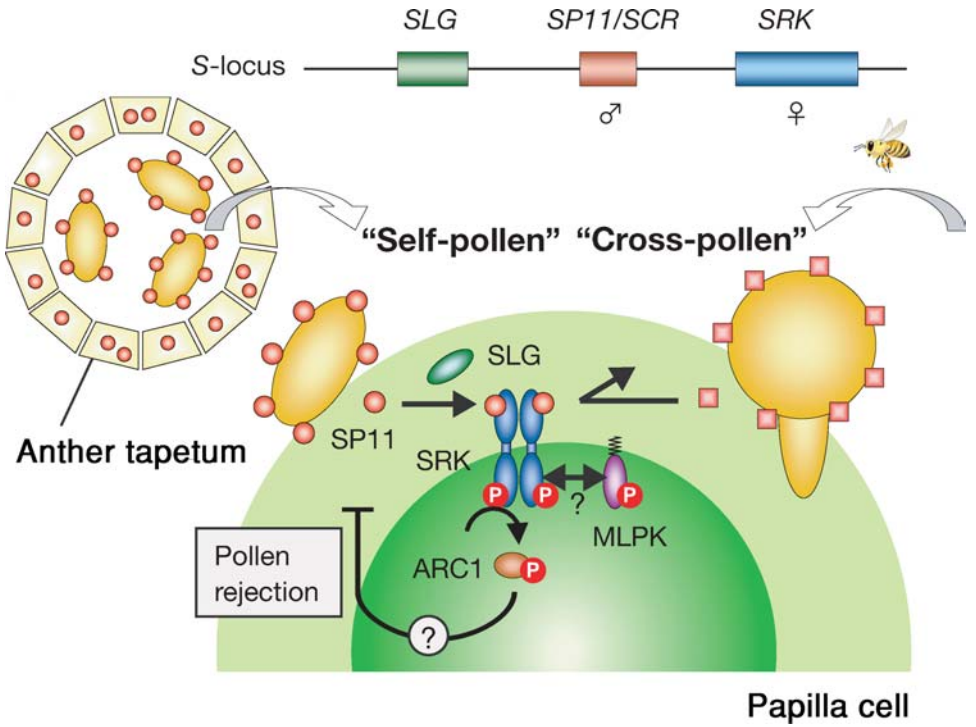


Figure 2

Molecular model of the self-incompatibility (SI) response in the Brassicaceae. The *S*-locus consists of three genes, *SRK*, *SP11*, and *SLG*. The SRK receptor kinase is the female determinant and spans the plasma membrane of the stigma papilla cell. SP11 is the male determinant and is predominantly expressed in the anther tapetum and accumulates in the pollen coat during pollen maturation. Upon pollination, SP11 penetrates the papilla cell wall and binds SRK in an *S*-haplotype-specific manner. This binding induces the autophosphorylation of SRK, triggering a signaling cascade that results in the rejection of self-pollen. SLG is not essential for the self-/nonself-recognition but localizes in the papilla cell wall and enhances the SI reaction in some *S*-haplotypes. The signaling cascade downstream of SRK has not yet been characterized, but the essential positive effectors include MLPK and ARC1. MLPK localizes papilla cell membrane and may form a signaling complex with SRK. ARC1, an E3 ubiquitin ligase, binds to the kinase domain of SRK in a phosphorylation-dependent manner and may target unknown substrates for ubiquitination. The proteasomal degradation of these substrates could result in pollen rejection.

determinant that directly induces incompatible reactions in the stigma papilla cells.

In situ hybridization analyses demonstrate that *SP11/SCR* is expressed sporophytically in the anther tapetum cells and gametophytically in microspores (82) (**Figure 2**). In some *S*-haplotypes, the *SP11/SCR* expression is detected only in the anther tapetal cell layer (63). Because the tapetal cell layer is a diploid tissue that nourishes developing pollen grains and provides the components of pollen coating, the expression pattern of *SP11/SCR* easily explains the sporophytic nature of *Brassica* SI. In fact, immunohistochemical studies suggest that the SP11/SCR protein is secreted in a cluster from the tapetal cells into the anther locule and translocated to the pollen surface (25).

Recently, NMR analysis revealed the tertiary solution structure of the SP11 protein of the S_8-haplotype (S_8-SP11) (48), which specifically induces the SI response on S_8 stigma at a dose of as little as 50 fmol per stigma (83). S_8-SP11 folds into an α/β sandwich structure

made up of a twisted three-stranded β-sheet layer backed by another layer formed by an α-helix with flanking loops. Four disulfide bonds (C1–C8, C2–C5, C3–C6, and C4–C7) stabilize the structure. Although S_8-SP11 adopts a fold similar to plant defensins, the edges of the loop region are extensively stabilized by disulfide bonds and hydrophobic packing. This feature seems to be unique to SP11/SCR. The conserved aromatic (tyrosine) residue makes hydrophobic contact with the sulfur atom of C7, stabilizing the L1 loop structure between the α helix and the $\beta 2$ strand. Another conserved glycine residue helps form a stable type II β-turn at the flanking segment, connecting the $\beta 1$ strand to the α helix. Structure-based sequence alignment and homology modeling of allelic SP11/SCR suggest that the L1 loop region forms the hypervariable (HV) domain that bulges out from the protein body and serves as an *S*-haplotype-specific site. However, the L1 loop region contributes only the protein surface area of 470 Å^2, which is too small to confer the high affinity observed with the stigma receptor (see below), suggesting that an additional interface(s) is required (48). In support of this was an experiment that used site-directed (alanine-scanning) mutagenesis, and the results suggest that both the C3-C4 region (corresponding to L1 loop region) and the C5-C6 region (corresponding to L2 loop region) contribute to the SRK binding (4). Similarly, mutations of each of the conserved cysteines or a tyrosine abolished the activity of SP11/SCR, whereas the replacement of a conserved glycine residue with valine was tolerated. A domain-swapping experiment was also conducted, but the results were more perplexing. For example, the SCR_6 (SCR from the S_6-haplotype) protein in which the C5-C6 region is derived from SCR_{13} acquired the S_{13}-haplotype specificity, whereas the SCR_{13} protein in which the C5-C6 region is derived from SCR_6 retained the S_{13}-haplotype specificity. Although further studies are required, these results support the fact that the arrangement of specificity determinants might vary significantly between SCR variants.

Interaction between Male and Female Determinants

Two different biochemical approaches demonstrated *S*-haplotype-specific interactions between the male (SP11/SCR) and the female (SRK) determinants. In one experiment, the interactions between tagged versions of recombinant SRK and SP11/SCR were analyzed. The extracellular domain of SRK_6-FLAG ($eSRK_6$-FLAG) was expressed in tobacco leaves, and the SCR (SCR-Myc-His_6) was expressed in bacteria. The $eSRK_6$-FLAG was shown by both pull-down and enzyme-linked immunosorvent assay (ELISA) to interact more strongly with SCR_6-Myc-His_6 than with SCR_{13}-Myc-His_6 (28). In another experiment, ^{125}I-labeled S_8-SP11 (^{125}I-S_8-SP11) was used to monitor the interaction with the stigmatic receptor (83). The ^{125}I-S_8-SP11 specifically bound the stigmatic microsomal membranes of the S_8 homozygote. Scatchard analysis indicated the presence of both a high-affinity (K_d = 0.7 nM, B_{max} = 180 fmol/mg protein) and a low-affinity (K_d = 250 nM, B_{max} = 3 pmol/mg protein) binding site. Cross-linking experiments revealed that the high-affinity binding site consisted of proteins of 110 and 60 kDa. Immunoprecipitation experiments suggested that the 110-kDa protein is SRK_8. The 60-kDa protein is assumed to be SLG_8 or a truncated form of SRK (designated eSRKs), but currently its identity is not clear. Furthermore, in an in vitro phosphorylation assay, autophosphorylation of SRK_8 on the stigma plasma membrane was induced by S_8-SP11, but not by S_9-SP11, at a kinetically relevant concentration. This result clearly indicates that SP11/SCR alone can activate SRK in an *S*-haplotype-specific manner (83).

Signal Transduction in the Incompatible Stigmatic Cell

Now that the molecular basis of self-pollen recognition, which is the first step of the self-incompatibility response, has been established, the present focus of research has turned to characterizing the downstream signaling

pathway(s) (**Figure 2**). The question that needs to be answered is how is the SRK activation transduced into the inhibition of self-related pollen? Presently, two molecules have been identified as positive signaling mediators of this pathway.

One is the arm repeat–containing protein, ARC1 (Armadillo-repeat-containing 1), a stigma protein first identified in a yeast two-hybrid screen as a protein interacting with the cytoplasmic domain of SRK (17, 51). This interaction, which requires the *C*-terminal arm repeats region of ARC1 and an active SRK kinase domain, results in the phosphorylation of ARC1 in vitro. Suppression of ARC1 expression by antisense cDNA causes a partial loss of the SI response, suggesting that ARC1 functions as a positive effector of SI signaling (75). However, the incomplete loss of the SI response implies that another branch of the signaling pathway exists, although it could also be attributed to residual *ARC1* expression in the transgenic stigmas. Scrutinizing the ARC1 sequence has suggested that it contains a U-box motif, a modified RING-finger; furthermore, recent analyses demonstrated that ARC1 has U-box-dependent E3 ubiquitin ligase activity (74). When expressed in cultured tobacco cells, ARC1 was distributed throughout the cytosol, but localized to the proteasome/COP9 signalosome in the presence of an active SRK kinase domain. In the pistil, levels of ubiquitinated protein increased after incompatible pollinations, but the increase was not apparent in ARC1 antisense-suppressed pistils. Furthermore, proteasome inhibition disrupts the SI response. Therefore, it was proposed that ARC1 is activated by SRK to promote the ubiquitination and proteasomal degradation of stigmatic proteins that support pollen germination and/or pollen tube growth. Other scenarios are possible, however, because ubiquitination has functions unrelated to protein degradation, such as subcellular targeting of proteins. Identifying ARC1 substrates will therefore be an essential next step in further dissecting the process of pollen rejection.

Another molecule is the *M* locus protein kinase (MLPK), which was recently identified after re-examining the *modifier* (*m*) gene, a recessive mutant gene responsible for the self-compatibility of *B. rapa* var Yellow Sarson (49), that was once thought to encode an aquaporin-like protein, MIP-MOD (24), although this turned out not to be the case (14). MLPK is a protein kinase belonging to the subfamily of the receptor-like cytoplasmic kinase (RLCK), which has a common monophyletic origin with receptor-like kinases but has no apparent signal sequence or transmembrane domain (66). MLPK from Yellow Sarson has a missense mutation in the conserved kinase subdomain VIa, resulting in the loss of kinase activity. The *mm* plants exhibit a completely self-compatible phenotype, and the transient expression of MLPK can restore the ability of *mm* papilla cells to reject self-pollen. These results suggest that MLPK is a positive mediator of SI signaling, and that MLPK localizes upstream in the signaling pathway, assuming that the pathway from SRK divides into multiple routes. In addition, MLPK has a typical plant *N*-myristoylation motif [MGXXXS/T(R)] at the *N* terminus, and is present in the plasma membrane of the stigma, suggesting that MLPK acts in the vicinity of SRK. If ARC1 is also the primary component of SRK signaling, then the SRK signal transferred to MLPK must return to SRK because ARC1 is a direct downstream effector of SRK. Taking the various factors together, MLPK may form a signaling complex with SRK that mediates the rejection response (49). Very little is known about the function of RLCKs, although the genome of *Arabidopsis thaliana* contains as many as 150 RLCKs (66). MLPK is the first example showing that a RLCK member mediates the signaling of receptor-like kinases. Further studies will address the precise relationship between SRK and MLPK in SI signaling.

In addition to these positive mediators of SI signaling, several identified components are expected to negatively regulate the pathway. Two thioredoxin-h proteins, THL1 and THL2, are interacting stigma proteins that were

identified in a yeast two-hybrid screen. THL1 and THL2 interact with a conserved cysteine at the transmembrane domain of SRK in a phosphorylation-independent manner (1, 51). In vitro phosphorylation experiments demonstrate that THL1 inhibits the autophosphorylation activity of SRK in the absence of an "activating" component of the pollen coat (presumably SP11/SCR) (3). These results suggest that THL1 and THL2 may function as negative regulators, preventing the constitutive activation of the SI pathway. However, the relationship between THL1/2 and SRK activation requires further study because a different experimental system has not revealed any involvement of thioredoxins in the SP11/SCR-induced SRK activation process (83). Another negative-regulator candidate is a protein phosphatase, KAPP (kinase-associated protein phosphatase), which interacts with the kinase domains of many receptor-like kinases. In *Arabidopsis*, a series of transformation experiments suggest that KAPP plays a general role in the downregulation of various receptor kinases. Recently, a KAPP homolog was isolated from a stigma cDNA library of *B. oleracea* (90). This KAPP homolog interacts with, and is phosphorylated by, the kinase domain of SRK in vitro. In addition, KAPP dephosphorylated SRK as a substrate, suggesting its negative role in SRK downregulation. Recently, a yeast two-hybrid screen identified two kinds of additional interacting proteins, calmodulins 1 and 2, and a sorting nexin, SNX1. The calmodulins interact with an amphiphilic helix in the SRK subdomain VIa. In animal cells, both calmodulin and sorting nexin are implicated in the downregulation of receptor kinase activity. As with KAPP, the calmodulins and SNX1 interact in vitro with diverse members of plant receptor kinases, suggesting their general role in the receptor-kinase-mediated signaling pathways.

Another important finding was obtained from the comparative analysis of the self-compatible *A. thaliana* and its close relative the self-incompatible *A. lyrata* (35). Despite the fact that the *S*-locus region of *A. lyrata* contained functional *SRK* and *SP11/SCR* genes, the relevant genomic region of *A. thaliana* contained truncated and nonfunctional *SRK* and *SP11/SCR*, suggesting that the self-compatibility of *A. thaliana* might be due to the inactivation of the *S*-determinant genes. To support this, the transfer of *SRK* and *SCR* genes of *A. lyrata* are sufficient to impart a self-incompatible phenotype in *A. thaliana* (53). This transformation clearly demonstrates that the entire signaling cascade leading to inhibition of self-related pollen is retained in *A. thaliana*. Thus, the self-incompatible line of *A. thaliana* is an ideal tool for future genetic and molecular dissection of the SRK-mediated signal transduction cascade.

SOLANACEAE-TYPE SELF-INCOMPATIBILITY

The Solanaceae, Rosaceae, and Scrophulariaceae families all share a female *S*-determinant, an S-RNase (12, 32). The S-RNase was first identified in the Solanaceae so we refer to this S-RNase-mediated type of SI as Solanaceae-type SI. The Solanaceae-type SI is under gametophytic control (GSI) and the rejection of self-pollen occurs during pollen tube growth in the style. Recently, the genomic sequences around the S-RNase genes were thoroughly analyzed in these taxa, with the net result of finally identifying the elusive male *S*-determinant. The molecular nature of the identified male *S*-determinant suggests a new model of how these determinants are involved in the specific rejection of self-pollen.

Female Determinant

The female determinants were first identified in the self-incompatible *Nicotiana alata* as the style glycoproteins of ~30 kDa that cosegregate with the *S*-haplotype in genetic crosses. This enabled the identification and cloning of many related proteins from members of the Solanaceae and other families. Sequence data revealed that the style proteins contain a region homologous to the catalytic domain of the fungal T2-type ribonucleases. Further studies confirmed that

these proteins possess ribonuclease activity and thus are referred to as S-RNases (45). S-RNases are expressed exclusively in the pistil, with the protein localized mostly in the upper segment of the style where inhibition of the self-pollen tubes occurs. The function of the S-RNases in SI was directly confirmed by gain- and loss-of-function experiments (38, 50). These experiments demonstrated that the S-RNase is the sole female determinant responsible for the *S*-haplotype specificity of the pistil. S-RNases are glycoproteins with one or more *N*-linked glycan chains. An engineered S_3-RNase of *Petunia inflata* that was engineered so that only the *N*-glycosylation site was knocked out, retained its activity to reject S_3 pollen, suggesting that the *S*-haplotype specificity determinant of S-RNases resides in the protein backbone and not in the glycan chains.

The S-RNases are highly divergent, with amino acid sequence identity ranging from 38% to 98% in the Solanaceae species (47). Despite this high sequence diversity, the S-RNases contain a number of conserved regions. S-RNases from the Solanaceae contain five highly conserved regions, designated C1 through C5, and those from the Rosaceae and the Scrophulariaceae have similar structural features except that the C4 region is absent. C2 and C3 are the regions similar to fungal RNase T2, and each contains a conserved catalytic histidine residue. There are two hypervariable regions in the S-RNases from the Solanaceae, termed HVa and HVb, whereas only one hypervariable region, corresponding to HVa of the solanaceous S_{f11}-RNases, has been detected in the rosaceous S-RNases. The crystal structures of a solanaceous S_{f11}-RNase from *Nicotiana alata* and a rosaceous S_3-RNase from *Pyrus pyrifolia* have been determined (23, 41). Both S-RNases have very similar structures consisting of eight helices and seven β-strands, and this topology is typical of the RNase T2 family of enzymes. The amino acid residues constituting the substrate-binding sites of these S-RNases can be geometrically superimposed over those of the RNase T2 enzymes. The most remarkable difference between the two S-RNases is in their HV regions. The HVa and HVb regions of solanaceous S_{f11}-RNase are composed of a long, positively charged loop followed by a part of an α-helix and a short, negatively charged α-helix, respectively. Because these HV regions are geometrically close to one another and exposed to the molecular surface, they both are expected to form a domain interacting with the male *S*-determinant. Domain-swapping experiments of S-RNases support this model (42, 43). S_{11}-RNase and S_{13}-RNase of *S. chacoense* differ only in 10 amino acids, 3 of which are located in the HVa and 1 in the HVb region. When the amino acids of HVa and HVb of S_{11}-RNase were changed to those of S_{13}-RNase, transgenic plants expressing this hybrid S-RNase rejected S_{13} pollen but not S_{11} pollen. These results clearly suggest that the HVa and HVb regions play a key role in determining the *S*-haplotype specificity, despite the fact that the involvement of other regions cannot be ruled out. In contrast to the solanaceous S-RNases, the HV region of the rosaceous S_3-RNase comprises a positively charged long loop followed by a short α-helix, but the α-helix corresponding to HVb does not exist (41). In the rosaceous S-RNase, the HVa region alone may form the interacting domain corresponding to the HVa and HVb regions of the solanaceous S-RNases.

S-haplotype-specific pollen rejection requires high levels of S-RNase expression. The concentration of S-RNase in the extracellular matrix is estimated at 10–50 mg/ml, and only the transformants with an equivalent amount of S-RNase expression are able to acquire new *S*-haplotype specificities. The ribonuclease activity of S-RNases is essential for pollen rejection (18). Furthermore, a radioactive tracer experiment showed that pollen RNA is degraded specifically after incompatible pollination (44). Thus, S-RNases function as highly specific cytotoxins that inhibit the growth of incompatible pollen (**Figure 3**).

Although S-RNase is the sole female factor determining the *S*-haplotype specificity of the pistil, a requirement of other stylar factors for the full function of S-RNase has been suggested (5). One such factor is HT-B, a

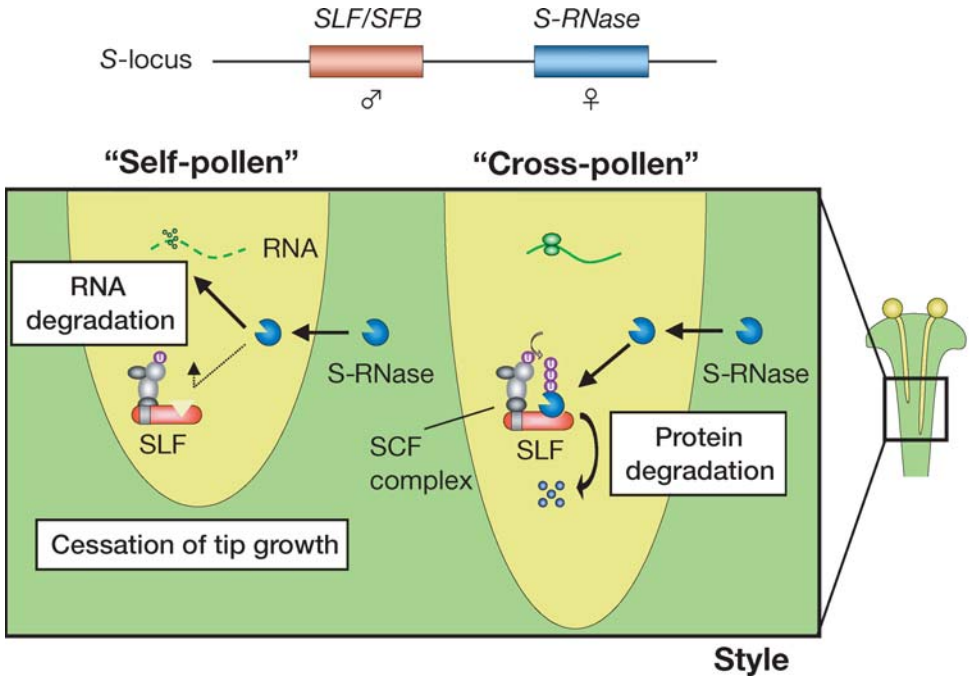


Figure 3

Molecular model of the self-incompatibility response in the Solanaceae, Rosaceae, and Scrophulariaceae. The *S*-locus consists of two genes, *S-RNase* and *SLF/SFB*. S-RNase is the female determinant and is secreted in large amounts into the extracellular matrix of the style. In a pollinated style, S-RNase is incorporated into the pollen tubes and functions as a cytotoxin that degrades pollen RNA. Although the S-RNase enters the pollen tubes regardless of their *S*-haplotypes, RNA degradation occurs only in self-pollen tubes. SLF/SFB is the male determinant and is a member of the F-box family of proteins, which generally function as a component of an E3-ubiquitin ligase complex. Thus, SLF/SFB is expected to be involved in ubiquitin-mediated protein degradation of nonself-S-RNases.

small asparagine-rich protein that was originally identified during a differential screen performed to identify stylar genes expressed in the self-incompatible *Nicotiana alata* but not in the self-compatible *Nicotiana plumbaginifolia* (46). Homologs of HT-B were also identified in two other genera of the Solanaceae, *Lycopersicon* and *Solanum* (33, 34, 55). In a comparative analysis of self-incompatible and self-compatible taxa of *Lycopersicon*, the expression of *HT-B* gene was not detected in all self-compatible taxa (33, 34). A requirement for HT-B protein was demonstrated by an RNAi suppression experiment in self-incompatible *Solanum chacoense*. Two *HT-B*-suppressed transformants expressed S-RNase normally but did not show *S*-haplotype-specific pollen rejection. These results suggest that the HT-B protein is implicated in the SI response, although its exact function remains unclear (55).

Male Determinant

The molecular nature of the male determinant and the molecular mechanisms of how S-RNases degrade pollen RNA in an *S*-haplotype-specific manner were long-standing mysteries of S-RNase-mediated SI. One plausible model was the "inhibitor model," in which the pollen *S*-determinant was postulated to be an inhibitor that could inhibit all S-RNases with the exception of the cognate S-RNase (31,

45). The immunocytochemical observations that all S-RNases could enter the pollen tube regardless of *S*-haplotype supported this model (40). Some refined "inhibitor models" were also proposed (39, 40), but such an S-RNase inhibitor has yet to be identified.

The male determinant was recently identified through genomic analyses of the *S*-locus region. Genomic analyses were first conducted on solanaceous species such as *Petunia inflata*, *Petunia hybrida*, *Lycopersicon peruvianum*, and *Nicotiana alata*. However, the *S*-locus of these species is located in the subcentromeric region and surrounded by abundant repetitive sequences that have hampered chromosomal walking (8, 91). The first clue for the male determinant was obtained from sequence analysis of the *S*-locus region of *Antirrhinum hispanicum*, a member of the Scrophulariaceae. The region of the S_2-haplotype contained a novel F-box protein gene, *AhSLF-S*$_2$ (*A. hispanicum S*-locus F-box of S_2-haplotype), which is specifically expressed in anther and pollen grains of S_2-haplotype (36). However, no gene allelic to *AhSLF-S*$_2$ has been identified in other *S*-haplotypes, and a gene with an extremely high sequence similarity (97.9% amino acid sequence identity) has been found in other lines with different *S*-haplotypes. It was thus unclear whether *AhSLF-S*$_2$ encoded the pollen *S*-determinant. Genomic analysis of the *S*-locus of *Prunus mume*, a member of the Rosaceae, reveals that the ~60-kb genomic region around the *S-RNase* gene contains as many as four F-box genes (7). Among them, only one F-box gene, termed *PmSLF*, fulfills the conditions of a pollen *S*-determinant gene: (*a*) it is located within the highly divergent genomic region of the *S*-locus, (*b*) it exhibits *S*-haplotype-specific diversity (78% to 81% amino acid identity), and (*c*) it is specifically expressed in pollen (7). Around the same time, polymorphic F-box genes were also found in the *S*-locus region of *Prunus dulcis*, *Prunus avium*, and *Prunus cerasus*, and were independently named SFB (*S*-haplotype-specific F-box) (86, 95). SLF/SFB from *Prunus* species fulfilled all conditions required of the pollen *S*-determinant. Aligning deduced amino acid sequences of SLF/SFBs of these *Prunus* species revealed the presence of two hypervariable regions, HVa and HVb, at the *C* terminus (32, 86). Two self-compatible haplotypes of *P. avium* and *P. mume* encoded partial loss-of-function mutations in SLF/SFB, which lack both HVa and HVb regions (87). This fact provides additional evidence that the SLF/SFB is the pollen *S*-determinant.

The conclusive evidence that SLF/SFB encodes the pollen *S*-determinant was finally obtained from transformation experiments in *Petunia inflata* (67). A thorough search of the pollen *S*-determinant in a huge *S*-locus region (328-kb BAC contig of S_2-haplotype) identified a polymorphic F-box gene, named *PiSLF*, ~161-kb downstream of the *S-RNase* gene. Although the genomic region outside this contig contained two more polymorphic F-box genes that were genetically linked to the *S*-locus, the *PiSLF* exhibited the highest sequence diversity. To ascertain whether *PiSLF* encodes the pollen *S*-determinant, a well-documented phenomenon termed "competitive interaction" was utilized. Competitive interaction is often observed in tetraploid plants. Among the diploid pollen grains produced, those carrying two different *S*-haplotypes (heteroallelic pollen), but not two of the same *S*-haplotypes (homoallelic pollen), fail to function in SI, although the molecular mechanism of the breakdown is unknown (6, 9). Consistent with this phenomenon, the transformation of S_1S_1, S_1S_2, and S_2S_3 plants with the S_2-allele of *PiSLF* (*PiSLF*$_2$) caused breakdown of their pollen function in SI. Furthermore, genotypic analyses of the progeny from self-pollinations of S_1S_2/*PiSLF*$_2$ and S_2S_3/*PiSLF*$_2$ revealed that only S_1 and S_3 pollen carrying the *PiSLF*$_2$ transgene (corresponding to heteroallelic pollen), but not S_2 pollen carrying *PiSLF*$_2$ (corresponding to homoallelic pollen), became self-compatible. These results conclusively demonstrate that SLF/SFB is the long-sought pollen *S*-determinant.

Mechanisms of *S*-haplotype-Specific Pollen Inhibition

In spite of the fact that both female and male determinants have been identified, the molecular mechanisms regulating how these molecules interact and specifically inhibit self-pollen growth remain unclear. The fact that RNase activity is required for the function of S-RNases, and that S-RNases are taken up by both self- and nonself-pollen tubes, suggests that S-RNases function inside pollen tubes as specific cytotoxins degrading the RNA of self-pollen (**Figure 3**). On the other hand, SLF/SFB contains a motif, called the F-box, which is best known for mediating interactions with other proteins that make up an enzyme complex referred to as the E3 ubiquitin ligase complex (15). E3 ubiquitin ligases act in conjunction with the E2 enzymes to ubiquitinate target proteins, which in many cases are degraded by the 26S proteasome. Recent biochemical studies suggest the involvement of AhSLF-S_2 in this protein degradation pathway, although it remains to be clarified whether *AhSLF-*S_2 from *Antirrhinum* is an ortholog of *PiSLF* (57, 67). AhSLF-S_2 interacts with ASK1- and CULLIN1-like proteins, which are the expected components of the SCF complex. AhSLF-S_2 interacts with both self- and nonself-S-RNases, but appears to mediate degradation of only nonself-S-RNases. Although such interaction and degradation have not been reported for *Prunus* and *Petunia* SLF/SFBs, if this is the case then SLF/SFBs should interact with all S-RNases but ubiquitinate only nonself-S-RNases.

To explain the molecular mechanisms for this specificity, some hypothetical models that are compatible with the "inhibitor models" have been presented (7, 57, 67, 87). One model postulates that SLF/SFBs contain two separate interaction domains, like the classical "inhibitor model." One domain would bind to the hypervariable domain of its cognate S-RNase in an *S*-haplotype-specific way, and the other domain would bind to a domain common to all S-RNases. The *S*-haplotype-specific interaction is expected to somehow stabilize, or at least not alter, the S-RNase activity, and the general interaction would lead to the polyubiquitination and degradation of S-RNases. Another mechanism postulates the involvement of another molecule, such as a general inhibitor in a modified "inhibitor model." To support this, a pollen-expressed RING-finger protein, PhSBP1 (*P. hybrida* S-RNase-binding protein), interacts specifically with S-RNases in an *S*-haplotype-nonspecific manner (70). Because many RING-finger domain proteins also function as E3 ubiquitin ligases, PhSBP1 is postulated to be involved in the general degradation of S-RNases. In such cases, SLF/SFB is expected to bind to its cognate S-RNase as a pseudosubstrate and protect it from ubiquitination and subsequent degradation (12).

However, none of these models can explain the phenomenon of "competitive interaction." These models assume that the *S*-haplotype-specific binding between S-RNase and its cognate SLF/SFB is thermodynamically favored over general binding between S-RNase and nonself-SLF/SFB (or PhSBP1), and that the *S*-haplotype-specific binding somehow precludes S-RNases from ubiquitination, permitting RNase activity. In "competitive interaction," two SLF/SFBs in the heteroallelic pollen should each preferentially bind to their respective S-RNases in an *S*-haplotype-specific manner, leaving the RNases active. Therefore, in contrast to experimental observations, these models predict incompatibility for heteroallelic pollen. To explain the "competitive interaction," a refined version of the "inhibitor model" has been proposed in which the male *S*-determinants are predicted to form a multimer prior to interacting with the *S*-haplotype-specific binding site of S-RNases (39). However, SLF/SFBs are not likely to form a multimer during the interaction process. Thus, although both the female and male determinants have been identified, exactly how *S*-haplotype-specific pollen inhibition is achieved remains a mystery.

PAPAVERACEAE-TYPE SELF-INCOMPATIBILITY

SI in the field poppy, *Papaver rhoeas*, is also under gametophytic control (GSI) in that the *S*-phenotype of pollen is determined by its haploid *S*-genotype. However, the identified S-protein (female determinant) and the mechanisms involved in pollen inhibition differ dramatically from those in the Solanaceae. The overall number of *S*-haplotypes in *P. rhoeas* is estimated at around 66 (37). Although the exact nature of the male determinant is not known, a reliable in vitro bioassay system was developed in which pollen germination and pollen tube growth can be inhibited by the recombinant S-protein in an *S*-haplotype-specific manner, thus allowing the biochemical events that take place in the pollen following self-recognition to be studied in detail.

Female Determinant

The *bioassay* system that reproduces the SI reaction of *P. rhoeas* in vitro allowed the identification of biologically active stigmatic *S*-determinants (11). The S-proteins are small, secreted proteins (~15 kDa), some of which are modified by *N*-glycosylation (12, 47). Thus far, five allelic stigmatic *Papaver* S-protein genes have been cloned. The S-proteins are highly polymorphic and share between 51.3% and 63.7% amino acid sequence identity. Nevertheless, they have four conserved cysteine residues and a predicted conserved secondary structure that is comprised of six β-strands and two α-helices connected by seven hydrophilic surface loops. In contrast to the *S*–determinants in the Brassicaceae and Solanaceae, amino acid sequence variation is not found in hypervariable blocks, but rather throughout the S-proteins.

Because some recombinant S-proteins produced in *Escherichia coli* inhibit pollen germination in an *S*-haplotype-specific manner, the S-proteins must be the sole female determinant in *Papaver* SI. This also suggests that the glycan chains are not required for the *S*-determinant function. Further studies, using site-directed mutagenesis, revealed that some residues located in predicted surface loop 6 are crucial for pollen recognition. Mutations of the only amino acid residue (Asp-79 in loop 6 of S_1) that is variable across five available *S* alleles, and of adjacent highly conserved amino acids (Asp-77 and Asp-78), resulted in complete loss of ability of S_1-protein to inhibit S_1 pollen (30).

Male Determinant

When S-proteins were challenged on pollen tubes, the fastest change occurred in the shank region approximately 50 μm behind the pollen tube tip, where the concentration of intracellular free calcium ($[Ca^{2+}]_i$) increased within only a few seconds (**Figure 4**). Because this rapid alteration in $[Ca^{2+}]_i$ is induced by Ca^{2+} influx (13), the pollen *S*-determinant is expected to be a membrane-located receptor that is somehow associated with channels that conduct Ca^{2+}. Biochemical studies suggest the presence of a candidate receptor, which was named SBP (S-protein binding protein). SBP is a pollen-specific integral membrane proteoglycan of 70–120 kDa that binds specifically to stigmatic S-proteins but apparently does so in a non-*S*-haplotype-specific manner. Therefore, it has been proposed that SBP acts as an accessory receptor rather than as the pollen *S*-determinant (19). Biochemical analysis using site-directed mutagenesis reveals that all S-protein mutants that exhibit a reduced ability to inhibit incompatible pollen also exhibit reduced SBP binding activity, suggesting a direct involvement of SBP in the SI reaction (26).

Signaling Cascade in the Incompatible Pollen

The *S*-haplotype-specific interaction between stigmatic S-protein and its putative pollen receptor somehow induces a rapid increase of $[Ca^{2+}]_i$ in the pollen grains (tubes). The $[Ca^{2+}]_i$ increase occurs in the shank region of the pollen tube within a few seconds after the challenge of an incompatible S-protein and continues for several minutes (13) (**Figure 4**). The

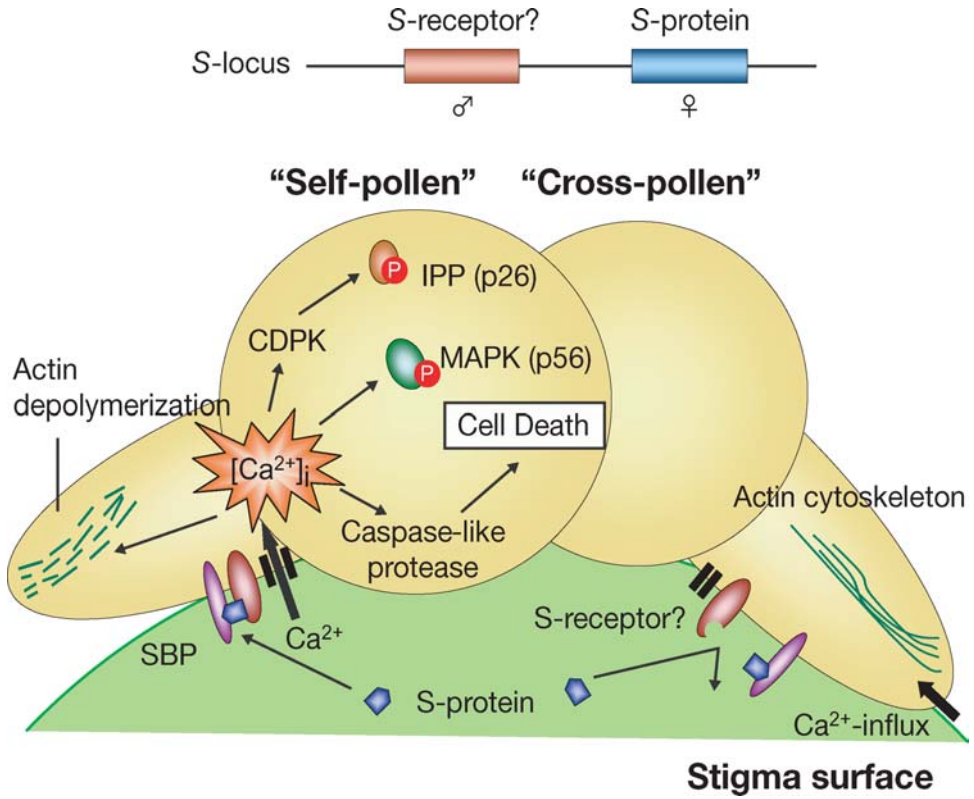


Figure 4

Molecular model of the self-incompatibility response in the Papaveraceae. Only the female determinant gene has been identified, which encodes a secreted stigma protein named S-protein. S-protein interacts with the assumed *S*-haplotype-specific pollen receptor (the putative male determinant) and induces Ca^{2+} influx in the shank of the pollen tube. SBP is an integral proteoglycan of the pollen plasma membranes and is expected to function as an accessory receptor. Ca^{2+}-influx stimulates increases in $[Ca^{2+}]_i$, with some contribution from the intracellular stores as well as from extracellular sources. These increases in $[Ca^{2+}]_i$ trigger the downstream signaling cascades that result in rapid growth inhibition and ultimately the death of incompatible pollen tubes.

increase of $[Ca^{2+}]_i$ in the subapical region accompanies the rapid loss of the oscillating high $[Ca^{2+}]_i$ gradient in the apical region of the pollen tubes. Most biochemical and physiological studies suggest that $[Ca^{2+}]_i$ acts as a second messenger that triggers multiple intracellular signaling cascades, resulting in rapid inhibition of pollen tube growth and ultimately the death of the incompatible pollen. Thus, recent studies have focused on identifying the signaling components downstream of the initial Ca^{2+} signals and their targets in pollen. These studies are expected to give insight into how the growth of the pollen tube is regulated, not only in the SI response, but also in a more general context.

The oscillating apical high $[Ca^{2+}]_i$ is typical of all tip-growing cells such as pollen tubes and neurons, although their biological significance is unclear. Thus, the loss of the oscillating $[Ca^{2+}]_i$ gradient will likely play a part in the initial inhibition of pollen tube growth.

One of the most rapid and dramatic physiological changes observed during the SI response is the dynamic rearrangement of the actin cytoskeleton in the pollen tube. Within 1–2 min postchallenge by an incompatible

S-protein, many F-actin bundles are lost and later punctate foci of actin accumulate in the cortex (16). Quantitative analysis of F-actin demonstrates that the SI-induced actin alterations are due to the depolymerization of F-actin, which starts within 1 min after SI challenge, with the reduction of F-actin reaching ~74% after 60 min (71). Similar depolymerization of actin was achieved in pollen by treatments that increase $[Ca^{2+}]_i$ artificially, suggesting that the actin depolymerization is not just a consequence of pollen tube growth arrest, but is part of the Ca^{2+}-mediated SI signaling cascade. Furthermore, recent biochemical studies have suggested that two kinds of actin-binding proteins, profilin and gelsolin, are involved in this Ca^{2+}-induced actin depolymerization process (22).

Another early target of the SI response is p26, a cytosolic 26-kDa pollen protein, the phosphorylation of which is induced within 90 sec of an incompatible S-protein challenge, with a further increase occurring during the next 400 sec. Because this timing coincides with the increase of $[Ca^{2+}]_i$ in SI-induced pollen and the protein kinase activity responsible for p26 phosphorylation is dependent on Ca^{2+} and calmodulin, the phosphorylation of p26 is also expected to be apart of the Ca^{2+}-mediated SI signaling cascade (12). Sequence analyses reveal that p26 shares approximately 80% amino acid sequence identity of plant-soluble inorganic pyrophosphatases, and its activity was confirmed by biochemical assays on the recombinant p26 protein. Furthermore, under conditions of raised $[Ca^{2+}]_i$, when p26 is phosphorylated, its pyrophosphatase activity is reduced (12, 59), indicating that p26 activity will likely be affected by the SI response. Soluble inorganic pyrophosphatases drive cellular biosynthetic reactions generating ATP and biopolymers, such as long-chain carbohydrates and proteins. Thus, it has been proposed that the inactivation of p26 during the SI response leads to inhibition of the pollen tube growth by depleting biopolymers required for tip growth. This proposal, however, needs to be experimentally tested.

Another target of SI signaling that displays a slightly delayed response is the putative mitogen-activated protein kinase (MAPK), p56 (60). In-gel kinase assays identified p56 as a 56-kDa protein kinase in pollen that is specifically activated after an SI challenge. The activation of p56 was detected 5 min after incompatible S-protein treatment, with peak activity at ~10 min. Pretreatment of growing pollen with the calcium channel blocker, lanthanum, inhibited the activation of p56, suggesting that p56 activation is also downstream of the Ca^{2+}-mediated SI signaling cascade. Although p56 has not yet been cloned, several pieces of biochemical evidence suggest that p56 is a MAPK. For example, p56 reacts with the TEY antibody that specifically binds activated MAPKs, and the kinase activity of p56 is abolished by apigenin, a specific inhibitor of MAPKs. Because the arrest of pollen tube growth precedes p56 activation, p56 is unlikely to play a role in the early inhibition events. One speculative hypothesis suggests that p56 might activate a PCD signaling cascade (see below) because some data have emerged suggesting a role for MAPK activation in the induction of PCD in plants.

PCD: programmed cell death

Recently, compelling evidence has suggested that SI challenge ultimately triggers PCD in incompatible pollen tubes (84). Nuclear DNA fragmentation, which is a hallmark of PCD, was observed in incompatible pollen tubes. Fragmentation was first detected 4 h after an incompatible S-protein challenge and increased to approximately 80% of affected pollen tubes within 14 h postchallenge. Recently, this DNA fragmentation was shown to be inhibited by pretreatment with the tetrapeptide DEVD, an inhibitor of caspase-3. Although no caspase homologue has been found in plant genomes, the result suggests the involvement of caspase-like activity in this signaling cascade. Furthermore, DEVD treatment revealed the biphasic nature of SI signaling. The SI-induced arrest of pollen tube growth is very rapid and occurs within 5 min of SI induction. The arrest was also observed in DEVD-pretreated pollen tubes. However, growth restarted in the

tubes 15–45 min after the arrest, suggesting that by inhibiting the caspase-like activity, the initial growth arrest can be recommenced. Thus, there seems to be a biphasic SI response: rapid inhibition of pollen tube growth, followed by caspase-like protease activation, which presumably makes inhibition irreversible. Cytochrome c leakage from the mitochondria, a classic marker for PCD in many organisms, was also stimulated by SI induction in incompatible pollen tubes. Cytochrome c leakage was detected as early as 10 min after SI induction and increased until 120 min. To obtain further evidence of the involvement of caspase-like activity in this SI signaling, the enzymatic activity that cleaves poly(ADP-ribose) polymerase (PARP) was analyzed. The PARP-cleavage activity was detected in incompatible pollen tubes 2 h after SI induction and increased over time. Finally, each of these PCD hallmarks, i.e., nuclear DNA fragmentation, cytochrome c leakage, and PARP-cleavage activity, can be sequentially induced by artificially increasing $[Ca^{2+}]_i$ in the pollen tubes, suggesting that these are all a part of the series of reactions that make up the Ca^{2+}-mediated SI signaling cascade.

CONCLUSIONS AND FUTURE PERSPECTIVES

It is clear that the Brassicaceae, Solanaceae, and Papaveraceae have developed completely different self-/nonself-recognition systems. Nonetheless, the *S*-loci controlling these recognition reactions have structural commonalities, i.e., they contain at least two polymorphic determinant genes surrounded by highly divergent intergenic sequences (7, 10, 64, 94). Significant sequence heterogeneity of the *S*-locus explains how intergenic recombination is suppressed between two determinant genes, which results in the breakdown of SI. A more difficult issue to resolve is how new *S*-haplotypes evolve. In these two-gene systems, the female and male determinants must coevolve to maintain their interaction. Phylogenetic analyses of SRK and SP11/SCR in *Brassica* produced almost identical tree topologies for these two genes (61, 63, 92). These analyses provide evidence for the coevolution of the female and male determinant genes and suggest that new *S*-haplotypes arise from pre-existing *S*-haplotypes. Several interesting schemes outlining how such a process might occur have been proposed (4, 10, 42, 47, 88), although all schemes are hypothetical. Detailed comparative analyses characterizing more *S*-haplotypes, and additional structure-function relationship analyses of mutated determinants, should provide clues to this difficult issue.

Similar multiallelic two-gene recognition systems are also evident in the mating-type loci of fungi, which prevent self-matings (2, 29). The mating-type loci can be diallelic or multiallelic, similar to that seen for the *S*-loci. For example, in the phytopathogenic basidiomycete *Ustilago maydis*, the fusion of haploid cells and the maintenance of dikaryotic filamentous growth are controlled by two unlinked mating-type loci, termed *a* and *b*. Multiallelic genes at these loci (*a* locus exists in diallelic forms and *b* locus exists in at least 18 allelic forms) specify a large number of different mating types, and mating can only be completed between individuals that differ at both loci. The *a* locus contains two linked genes for lipopeptide ligands and their receptors, and is therefore analogous to the *S*-locus of the Brassicaceae. The *b* locus contains two genes for homeodomain proteins (bE and bW), and bE and bW from different alleles can form an active bE/bW heterodimer that is able to maintain filamentous growth. In multiallelic two-gene recognition systems, two contrary means can exist to lead to the same end of self-rejection, namely, "self-recognition" versus "nonself-recognition," or in other words, "opposition" versus "complementation" (**Figure 1**). In the mating-type recognition of fungi, the latter means are used, i.e., when two genes from different alleles are present in the mating cells, their interaction leads to compatible reactions. However, in the plant SI systems, at least in the Brassicaceae and the Papaveraceae types, the former means are used, i.e., when

two genes from the same haplotypes meet, their interaction leads to incompatible reactions to reject self-pollen.

Classic genetic analyses reveal the presence of two SI types, GSI and SSI. Molecular analyses show that GSI contains at least two mechanisms, the Solanaceae type and the Papaveraceae type. For SSI, although the molecular mechanism has only been elucidated in the Brassicaceae, the presence of different mechanisms has been suggested. In the Convolvulaceae, the entire *S*-locus region has been cloned and sequenced in *Ipomoea trifida*, which has an SSI system (85). However, no homologous gene for the *Brassica SRK* or *SP11/SCR* has been found in the *S*-locus region. In the Asteraceae, a candidate gene for the female determinant, which was named SSP (stigma *S*-associated protein), has been identified, although it bears no resemblance to either SRK or SLG (21). Current evidence supports the view that SI evolved independently and probably multiple times in different lineages, with its recognition genes recruited in the family lineage by duplication and modification of preexisting genes that perform other functions (probably other cell-cell recognition or communication functions) in the plant. This view is also supported by the observation that the determinant genes identified thus far typically belong to large gene families that include members expressed in nonreproductive tissues (15, 58, 66, 89).

One great advantage of the studies on the self-/nonself-recognition mechanisms in SI is that both the female and male determinants involved in recognition are encoded in pairs in a single locus. Although not easy tasks due to the complex structures of the *S*-loci, several studies are attempting to identify novel *S*-determinant genes. These studies will not only lead to a greater understanding of how flowering plants discriminate self/nonself during fertilization, but should also shed light on the processes used by plants for cell-cell communication.

SUMMARY POINTS

1. In many species, the specificity of the SI response is determined by the haplotypes of the *S*-locus, which contains at least two separate multiallelic genes, the female and the male determinant genes.
2. SI does not represent one system, but rather a collection of divergent mechanisms, suggesting that SI evolved independently in several lineages.
3. In the Brassicaceae, the determinant genes encode a pollen ligand and its stigmatic receptor kinase, and their interaction induces incompatible signaling(s) within the stigma papilla cells.
4. In the Solanaceae, Rosaceae, and Scrophulariaceae, the determinants are a ribonuclease and an F-box protein, suggesting the involvement of RNA degradation and protein degradation within the system.
5. In the Papaveraceae, the only identified female determinant induces a Ca^{2+}-dependent signaling network that ultimately results in the death of incompatible pollen.

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