

Review

Molecular genetics, physiology and biology of self-incompatibility in Brassicaceae

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Abstract: Self-incompatibility (SI) is defined as the inability to produce zygotes after self-pollination in a fertile hermaphrodite plant, which has stamens and pistils in the same flower. This structural organization of the hermaphrodite flower increases the risk of self-pollination, leading to low genetic diversity. To avoid this problem plants have established several pollination systems, among which the most elegant system is surely SI. The SI trait can be observed in *Brassica* crops, including cabbage, broccoli, turnip and radish. To produce hybrid seed of these crops efficiently, the SI trait has been employed in an agricultural context. From another point of view, the recognition reaction of SI during pollen-stigma interaction is an excellent model system for cell-cell communication and signal transduction in higher plants. In this review, we describe the molecular mechanisms of SI in Brassicaceae, which have been dissected by genetic, physiological, and biological approaches, and we discuss the future prospects in relation to associated scientific fields and new technologies.

Keywords: Brassicaceae, cell-cell communication, pollen-stigma interaction, self-incompatibility, signal transduction

1. Introduction

In most species of plants the flowers are hermaphrodite that is both stamen and pistil are present in the same flower. Self-pollination occurs easily in hermaphrodite flowers, and leads to inbreeding depression and decreased genetic variation; therefore plants have evolved several mechanisms to avoid self-pollination. Self-incompatibility (SI) is defined as “the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination”, which is surely the most elegant pollination system.¹⁾ This trait attracted the attention of Charles Darwin, who

published detailed descriptions of pollination and the form of flowers during his studies of evolution.^{2),3)} In the past, the SI phenotype was mostly evaluated by seed formation after open pollination (artificial self-pollination and cross-pollination). However, from the evaluation of seed formation, the phenotype of “incompatibility” and “sterility” cannot be distinguished. However, in relation to biological events, “incompatibility” and “sterility” are clearly different. “Sterility” is caused by non-functional male and/or female components, whereas in “incompatibility” there is a lack of seed formation in a specific male and female combination, both of which are functional. Because the final phenotypes in “incompatibility” and “sterility” are quite similar, this phenomenon was originally termed “self-sterility”. Taking the initial letter of “sterility”, the genetic locus regulating SI was termed the “*S* locus”, which is still used as the authorized locus name.

SI is classified morphologically into heteromorphic SI and homomorphic SI. Heteromorphic SI is related to different flower shapes; for example, *pin* (long pistil and short stamen length) and *thrum* (short pistil and long stamen length) forms are

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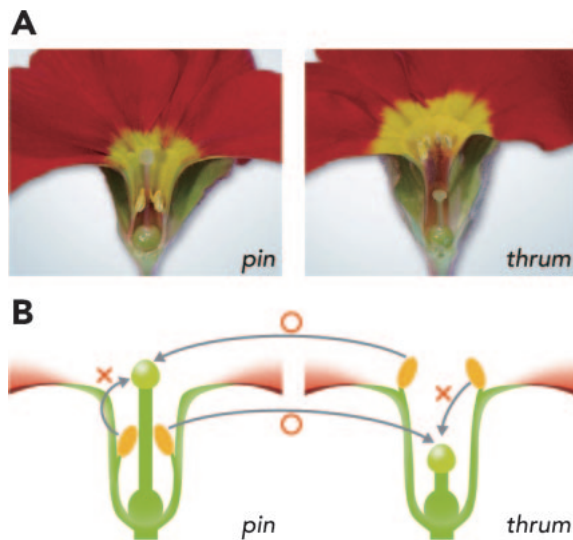


Fig. 1. Photograph (A) and schematic model (B) of heteromorphic SI in primrose. (A) The *pin* (long pistil and short stamen length) form is shown on the left, and *thrum* (short pistil and long stamen length) is on the right. (B) Selfed pollen and pollen derived from the same flower form are incompatible. Crossed pollen and pollen derived from the different flower form are compatible.

present in primrose (*Primula* species), buckwheat (*Fagopyrum* species) and star fruit (*Averrhoa* species). Cross pollination between different flower types is compatible and is regulated by a single locus with two alleles, S and s (Fig. 1). The genotype of *pin* is ss homozygote, and that of *thrum* is Ss heterozygote. Thus, on cross-pollination, a frequency of 50% each of *pin* and *thrum* should be maintained in a wild population under SI regulation. Interestingly, this S gene controls both the incompatibility phenotype and the flower shape (that is length of pistil and stamens). Although several attempts have been made to dissect genes residing at the S locus in plants with heteromorphic SI, no S genes or their products have been identified to date.¹⁾

On the other hand, homomorphic SI species have a single flower shape and are classified by the inheritance pattern of the S gene into gametophytic SI and sporophytic SI.^{1),4)} Both SI phenomena are controlled by a single locus, with multiple S alleles ($S^1, S^2, S^3, \dots, S^n$) in most of the plant species. As described in Fig. 2, the incompatible phenotype is apparent in self-pollination and in cross-pollination between pollen and stigma of two different plants carrying the same S allele. To represent alleles, superscripts are commonly used in general genetic research; however, traditionally, S alleles are denoted by subscripts. In most of our papers and review

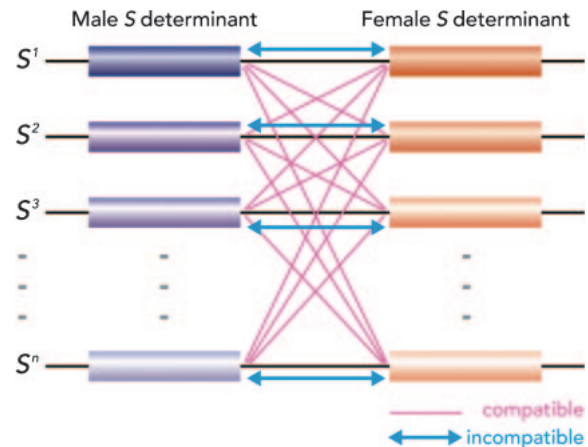


Fig. 2. Schematic model of the S locus. The S locus contains at least two genes, one encoding the male S determinant, which is expressed in anther tapetum surrounding the pollen grains during development, and the other encoding the female S determinant, which is expressed in the pistil (stigma). Both the genes encoding male and female S determinants are inherited as one segregating unit. The number of S alleles is thought to be over 50 to 100 in *Brassica* species.^{167)–169)} When both male and female S determinants are derived from the same S alleles, the incompatible response occurs. In contrast, in the case of a combination of different S alleles from the male and female parents, the compatible response is observed.

articles, we have used superscript allele symbols, according to the general genetic convention.^{4)–8)} Therefore, in this article, we have again used superscripts for allelic gene representation.

In gametophytic SI (GSI), the S phenotype of pollen is determined by its own haploid S gene. The self-pollen inhibition occurs during pollen tube elongation in the style of the pistil in most GSI. Plant species in families Fabaceae, Onagraceae, Poaceae, Rosaceae, Plantaginaceae and Solanaceae exhibit GSI (Fig. 3A). On the other hand, in sporophytic SI (SSI), the S phenotype of pollen is determined by its parental diploid S gene interaction, so that dominance relationships occur between S alleles (Fig. 3B).^{9),10)} The landmark genetic SSI model, established by Bateman from a series of experiments, is an excellent explanation of S gene behavior, and is still valid today.^{11)–13)} The molecular mechanisms of S allele dominance relationships will be described below. In most SSI plant species, from families Asteraceae, Brassicaceae and Convolvulaceae, pollen tube inhibition occurs on the stigma surface (Fig. 4).

SI is interesting in two respects: firstly, SI involves cell-cell communication between pollen/pollen tube and pistil, and signal transduction in

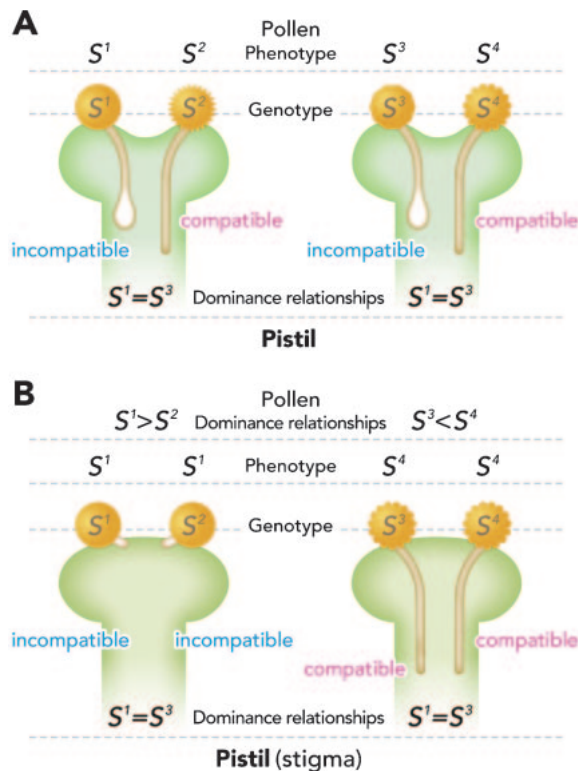


Fig. 3. Relationships between phenotype and genotype in GSI (A) and SSI (B). The pistil (stigma) phenotype presented here is S^1S^3 codominance, to clearly demonstrate the difference between the GSI and SSI. In both types of SI, pollen is rejected when the SI phenotype of pollen is identical to that of the pistil (stigma). (A) In the case of GSI, the pollen phenotype is identical to the genotype. The pistil phenotype of the GSI is always expressed in codominance. (B) In the case of SSI, the pollen phenotype is determined by interaction of the parental genotype; therefore, there is a dominance relationship at the pollen side. If S^1 is dominant over S^2 , all pollen grains produced from the S^1S^2 pollen parent show S^1 phenotype, and are rejected on the S^1S^3 ($S^1=S^3$) stigma. In contrast, on the same stigma ($S^1=S^3$), if S^3 is recessive to S^4 at the pollen side, all pollen grains, which show S^4 phenotype, germinate and pollen tube penetration is observed.

the stigma/style; and, secondly, SI is an important trait for F_1 hybrid seed production in *Brassica rapa* (syn. *campestris*, e.g. Chinese cabbage and turnip), and *B. oleracea* (e.g. cabbage, cauliflower, broccoli, kohlrabi, kale and Brussels sprouts), which was established in a Japanese seed company before the 2nd World War.⁶⁾ In other words, we are interested in *Brassica* SI from the viewpoint of both fundamental biology and agricultural applications. Thus, an understand of the molecular mechanisms of SI in *Brassica* species is an important research target. In this review article, we will summarize SI research in Brassicaceae species, including molecular cloning of the male and female S determinants, epigenetic

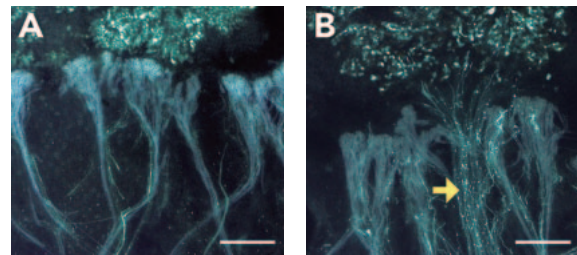


Fig. 4. Micrographs showing (A) the inhibition of self-pollen and the inability of the emerging pollen tubes to invade the papilla cell wall (incompatibility), and (B) the penetration of cross-pollen tubes into the papilla cells (compatibility) in *Brassica rapa* L. Yellow arrow indicates the penetrated pollen tubes. With aniline blue staining, pollen tubes appear as distinct tubes with prominent dots, because callose plugs in the pollen tubes fluoresce brightly. In contrast, vascular bundles and other tissues do not fluoresce brightly, as shown in (A). Bar = 200 μ m. (Courtesy of Masaaki Osaka and Ken-ichiro Hiroi, unpublished data).

regulation of dominance relationships between S alleles, and evolution of the SI system. Finally, we will discuss the future prospect for SI research. Many review articles have been published, and are helpful in understanding this field.^{1),4)-8),14),15)}

2. Identification of the S -locus-specific proteins from stigma

In the early SI research, *Oenothera* (Onagraceae), *Malus*, *Prunus* (Rosaceae), *Nicotiana*, *Solanum*, *Petunia* (Solanaceae), *Trifolium* (Leguminosae), *Secale*, *Pahalaris*, *Festuca* (Poaceae), *Cardamine*, *Iberis*, *Brassica* (Brassicaceae), *Crepis*, *Cosmos* (Asteraceae), *Theobroma* (Sterculiaceae), *Ipomoea* (Convolvulaceae) and *Antirrhinum* (Plantaginaceae), among others, were studied.¹⁾ These species were well suited to the establishment of genetic models of SI systems, as described above. However, for the next step, that is identification of S gene products at the protein level, only *Brassica* and *Nicotiana* species were used during the 1970s, because the protein products derived from their female S genes are highly expressed in stigma/style tissues. Thus, the SI researchers who selected these species for study had a great advantage for molecular dissection of the S -locus.¹⁾

In the case of the *Brassica* species, immunological cross-reactivity and isoelectric focusing (IEF) were applied to identify the allelic diversity of the S gene products.^{16),17)} These two methodologies had advantages for the identification of genetic diversity of multi-allelic gene products. For example, in the case of IEF, the molecular masses of allelic gene

products are similar. However, they differ in the isoelectric point (pI) because the amino acid composition of their products must be different as a consequence of the allelic diversity and tertiary structure of the proteins. Because the *S*-locus-specific protein was found to react with concanavalin A (Con A), which is plant lectin,¹⁸⁾ it was concluded that this protein has a carbohydrate moiety, and it was named *S*-locus glycoprotein (SLG).¹⁾

In the 1980s, SLG proteins were extracted and purified from plant material. Furthermore, the cDNA clones encoding *SLG* genes were obtained from *Brassica rapa* (syn. *campestris*) and *B. oleracea*.^{19),20)} At this time, the SLG was proposed to be the female *S* gene based on several characteristics, as described below. Firstly, the glycoprotein is directed to be secreted into the cell wall by a hydrophobic signal peptide.¹⁹⁾⁻²¹⁾ Secondly, twelve cysteine residues were found to be completely conserved in the C-terminal region and variable regions were found in the central and C-terminal regions, from comparisons of over 30 SLGs derived from different *S* alleles (Fig. 5). When the nucleotide sequences of allelic *SLGs* were aligned, three highly divergent regions were identified and other regions were found to be highly conserved among *S* alleles.⁵⁾ These variable regions were proposed to contribute to *S* allelic specificity, and could interact with the male *S* determinant. Thirdly, the glycosyl residues were similar among *S* alleles, indicating that the *S* allelic specificity is due to variable regions of SLG itself.^{1),20)} Fourthly, identification of the *S* allele by PCR methods using primers positioned in the conserved regions has contributed to the breeding of *Brassica* F₁ hybrid varieties.^{10),22),23)} However, this method sometimes resulted in the false amplification of the many other *SLG*-like genes in the Brassicaceae genome.²⁴⁾⁻²⁷⁾ To exclude non-*S*-locus *SLG*-like sequences, linkage to the *S* locus had to be confirmed by test pollinations using a *Brassica* breeding system.⁵⁾

Furthermore, in the 1980s, to construct cDNA libraries and determine the amino acid sequence of SLG, tens to hundreds of thousands of stigmas, the top part of the pistil, were necessary, because SLG expression is spatiotemporally controlled in mature stigma papilla cells.^{21),28)} Thus, this identification of *SLG* residing at the *S* locus gave a great advantage to *Brassica* SI researchers in the further molecular dissection of the *S* locus, and subsequently, during the 1990s, in determining the sets of male and female SI genes, as described below. However, at that time, comparisons of SLG to sequences in public databases

did not provide any evidence as to the function of SLG in SI recognition.⁵⁾

3. Identification and characterization of female *S* determinants

Following *SLG* identification, molecular dissection of the *S* locus was advanced by several important findings. One of the most important milestones was the discovery of *ZmPK1*, encoding receptor-like protein kinase of maize, whose receptor domain has homology to SLG.²⁹⁾ After this finding, several allelic *SRK* genes, encoding *S*-locus receptor-like kinases, located at the *S* locus, were isolated and characterized from *B. rapa*, *B. oleracea* and *B. napus*. *SRK* contains three functional domains: an extracellular *S* domain (receptor domain), a transmembrane domain, and an internal serine/threonine protein kinase domain (Fig. 5).^{28),30)-32)} Interestingly, sequence similarity at the amino acid level of SLG and the *S* domain of *SRK* within *S* alleles is over 80%,⁵⁾ indicating that these two regions arose by duplication within the *S* locus.^{28),33)}

Thus, at that time, two stigma-specific genes, *SLG* and *SRK*, were known to be present at the *S* locus. However, it was difficult to determine the physical distance between the *SLG* and *SRK* genes precisely. In the case of *B. oleracea*, the size of the *S* locus was estimated to be 350 kb by PFGE analysis in the early 1990s,³⁴⁾ although this depended on the location of recognition sites of restriction enzymes around the *S* locus. An *S*-locus region as large as 350 kb suggested that cloning and sequencing of the *S*-locus might be quite difficult. However, this was overcome by the second important breakthrough, the successful cloning of the 76-kb genomic fragment containing both *SLG* and *SRK* genes in the *S*⁹ allele of *B. rapa* by use of the PAC vector.³⁵⁾ Based on this success, molecular dissection of the whole *S* locus was possible at the nucleotide sequence level. *B. rapa* had an advantage over *B. oleracea* for molecular dissection of the *S*-locus region because the size of the *S* locus of *B. rapa* was smaller than that of *B. oleracea*.³⁶⁾ Thus, the physical genomic structure of the *S*-locus region was determined in *B. rapa*, and also in *B. napus* (Fig. 6).³⁷⁾⁻⁴⁰⁾ Interestingly, in the case of *B. napus*, the *S*-locus region was found to have originated from the introgressed *B. rapa* genome and not from *B. oleracea*.^{31),37)}

Next, it was important to determine whether *SLG* and/or *SRK* are necessary for SI recognition at the stigma side, and for this several gene transformation studies were performed in the 1990s. Loss-

of-function experiments showed that *SLG* and/or *SRK* are important for the SI recognition reaction.^{5),6),41)–43)} In most cases, following introduction of *SLG* or *SRK* a change to the self-compatibility (SC) phenotype was observed, which might have been induced by co-suppression as shown by the change in petunia flower color.^{6),44)} In order to overcome co-suppression between endogenous and exogenous *SLG* and/or *SRK* genes, Takasaki and co-workers used lower-homology *SLG*/*SRK* genes from the two groups of *S* alleles, class I and class II.⁴⁵⁾ As described below, *S* alleles (*S* haplotypes) of class I (e.g. *S*²⁸) are dominant over those of class II (e.g. *S*⁶⁰) at the pollen side.^{10),32)} Takasaki and co-workers introduced *SLG*²⁸ and *SRK*²⁸ into recipient plant lines, *S*⁶⁰ homozygote or *S*²⁸*S*⁶⁰ heterozygote of *B. rapa*. As a result, the transgenic plants harboring *SLG*²⁸ did not show a change in *S* phenotype at the stigma side, while the transgenic plants harboring *SRK*²⁸ showed the *S* allele specificity at the stigma

side, but not in pollen. Interestingly, transgenic plants harboring both *SLG*²⁸ and *SRK*²⁸ showed the rigid SI phenotype comparable to non-transgenic *S*²⁸*S*⁶⁰ heterozygotes, indicating that *SRK* is the female *S* determinant and *SLG* is required for full manifestation of the SI response (Fig. 7).⁴⁵⁾ This additional effect of *SLG* was not observed in *B. napus*;⁴⁶⁾ the difference in *SLG* effect may be a consequence of the genome organization of the diploid (*B. rapa*) and amphidiploid (*B. napus*) species.

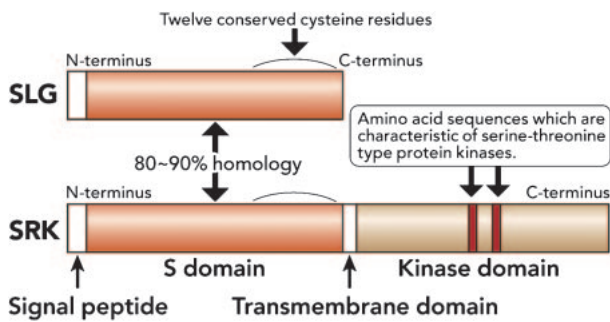


Fig. 5. Schematic structure of *SLG* and *SRK*. *SLG* is an *S* allele-specific secreted glycoprotein, having a hydrophobic signal peptide in the N-terminus and 12 cysteine residues at the C-terminus. *SRK* is a receptor protein kinase, whose extracellular domain (receptor domain) is similar to *SLG*. *SRK* consists of a signal peptide, an *SLG*-like domain (S-domain), a hydrophobic transmembrane domain and a cytoplasmic catalytic domain (kinase domain) of the serine/threonine protein kinase type.

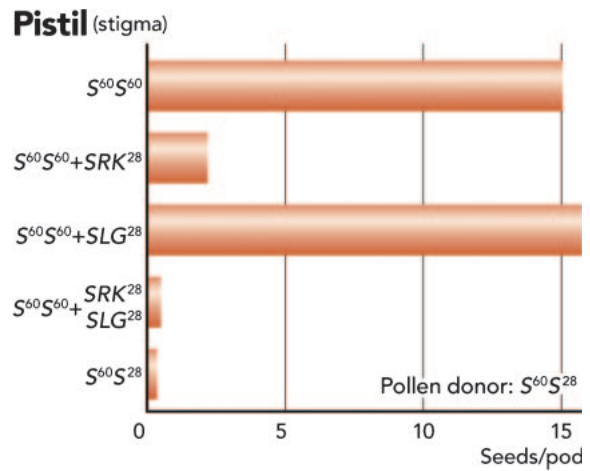


Fig. 7. Test pollinations for evaluation of *SLG* and *SRK* transgenic plants by seed formation. Line *S*⁶⁰ was used as the recipient of the transgene and line *S*²⁸*S*⁶⁰ was used as the pollen tester. In this case, the *S* phenotype of all pollen was *S*²⁸ because *S*²⁸ is dominant over *S*⁶⁰. The phenotype of transformants carrying *SLG*²⁸ was coincident to that of the non-transformant recipient plant, that is *S*⁶⁰. In contrast, the seed formation of transformants having *SRK*²⁸ decreased compared to that of transformants having *SLG*²⁸ and recipient *S*⁶⁰ line. After genetic crossing of the two transformants, which carried *SLG*²⁸ and *SRK*²⁸ transgenes, the F₁ plant showed a rigid SI phenotype, like the *S*⁶⁰*S*²⁸ heterozygote. SI phenotype was assessed by the number of seeds formed per pod.

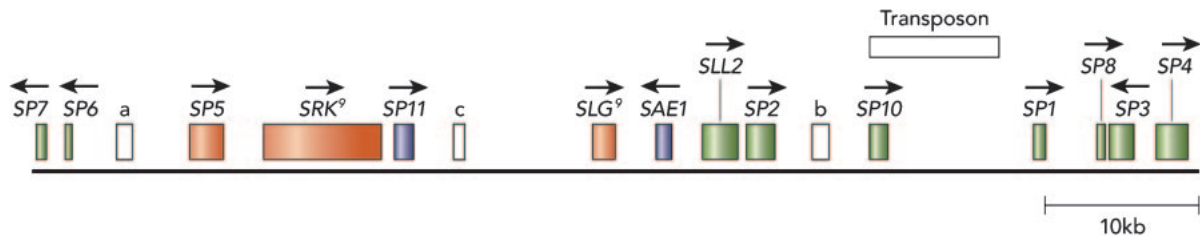


Fig. 6. Physical map of the *S* locus of the *S*⁹ line of *B. rapa*, showing the location of 14 genes, a transposon-like sequence and three open reading frames (ORFs). Filled boxes indicate locations of the 14 genes, and horizontal arrows above the genes indicate directions of their transcription. Three long ORFs (a, b and c) and a transposon-like sequence are represented by open boxes labeled “a,” “b,” “c” and “Transposon.” Three genes represented by orange-colored boxes are specifically expressed in stigma tissues. Two genes represented by purple-colored boxes are specifically expressed in anther tissues. The other nine genes represented by green-boxes are expressed in both vegetative and reproductive tissues.

4. Identification and characterization of male *S* determinants

In the course of investigating the identification of the male *S* determinant, several *S*-linked genes were found to be unrelated to the male *S* determinant.^{47)–50)} However, another detailed approach, analysis of pollen coat proteins (PCPs), successfully lead to identification of the male *S* determinant. In *Brassica* species, PCPs, which are derived from anther tapetum, are essential for pollen development.⁵¹⁾ Initially, SLG-interactive PCPs were searched,⁵²⁾ and these interactive molecules were then characterized as cysteine-rich small proteins.^{53)–57)} In the course of these experiments, a bioassay system showed that a <10 kDa PCP is the male *S* determinant.⁵⁸⁾ Interestingly, a gene encoding a PCP-like protein, *SP11*, was found to be located at the *S* locus, near *SRK* in *B. rapa*; thus *SP11* was the most likely candidate for the male *S* determinant (Fig. 6).³⁸⁾ Subsequently, it was demonstrated that *SP11* is the male *S* determinant by using bioassay and transgenic experiments in *B. rapa*.^{59),60)} Around the same time, a gene termed *SCR* (*S*-locus cysteine-rich protein) in *B. oleracea* was also cloned, and identified as the male *S* determinant.⁶¹⁾ Because *SP11* and *SCR* are identical,^{38),59),61)} *SP11* will be used as the name of the male *S* determinant in this review article, in both *Brassica* and *Arabidopsis* species.

After identification of *SLG*, *SRK* and *SP11* genes, many allelic genes were isolated,^{5),6)} which made it possible for us to calculate the allelic diversity. As described above, *S* alleles were divided into two groups, class I and class II, based on the sequence similarity between allelic genes of the three *S*-locus genes, *SLG*, *SRK* and *SP11*. In the case of *SLG* and *SRK*, within a group, allelic diversity was from a few percent to around 20%. In contrast, between groups, the diversity was over 30%. In contrast, the allelic diversity of *SP11* was higher than that of *SLG* and *SRK*.^{5),6)} Particularly in the class I alleles of *SP11*, only certain amino acid residues (glycine, aromatic amino acid and cysteine residues) were conserved in the mature protein.²³⁾ From determination of the tertiary structure with 2-D NMR analysis, these amino acids were found to be important for recognition specificity.⁶²⁾ From comparison of *SP11* and *SRK* sequences, these two genes encoding the pollen ligand and stigma receptor, respectively, were found to have co-evolved in a *trans*-specific mode.^{6),23),39)}

5. Molecular mechanisms of the SI reaction

After identification of both male and female *S* determinants, the next research targets were the physical interaction between *SP11* and *SRK*, and the *SRK*-related signaling cascade following this interaction. For the physical interaction between *SP11* and *SRK*, different results have been reported by two laboratories. Shimosato *et al.*⁶³⁾ showed that both the S-domain and membrane-anchoring domain were necessary for allele-specific interaction by using cross-linking and immunological methods. In contrast, Kachroo *et al.*⁶⁴⁾ showed that only the S-domain of *SRK* could interact with *SP11* in the allele-specific manner by using an immunoprecipitation method. In the future, after determination of the tertiary structure of *SRK*, this inconsistency is likely to be resolved.

The physical interaction of *SP11* and *SRK* was found to trigger autophosphorylation of *SRK* in an allele-specific manner.⁶⁵⁾ The question was then, how does the SI signal transduce in the cytoplasm? In order to clarify the *SRK* signaling cascade, several *SRK*-interacting molecules were identified and characterized.^{66)–72)} *MLPK* (*M* locus protein kinase), identified from the self-compatible line *yellow sarson*, was found to encode a novel membrane-anchored cytoplasmic protein kinase,⁷¹⁾ which interacts with *SRK* directly.⁷²⁾ *ARC1* (arm repeat containing 1) and *THL1* (thioredoxin 1) were isolated using a yeast two-hybrid system with the kinase domain of *SRK* as a bait.⁶⁶⁾ *ARC1* interacts with the kinase domain of *SRK* in a phosphorylation-dependent manner and has E3 ubiquitin ligase activity, indicating that the proteasome protein degradation system is involved in SI signal transduction. Furthermore, an *ARC1* knock-out line showed the self-compatible phenotype.^{67),69)} At present, only *MLPK* and *ARC1* have been identified as positive effectors of the *Brassica* SI reaction. Other identified factors found to date may be negative effectors of SI.^{68),71)} Recently, novel self-compatible lines of *B. rapa* have been identified, which will enable progress to be made in understanding the role of other interactive molecules in the complete SI signaling cascade.⁷³⁾ A schematic model of the current understanding of *Brassica* SI is shown in Fig. 8.

Other approaches to studying the SI signaling cascade have used *Arabidopsis* species. Because *Arabidopsis thaliana* is a self-compatible model plant, a very large number of traits of *A. thaliana* have been studied.^{74)–77)} In addition, for research into the

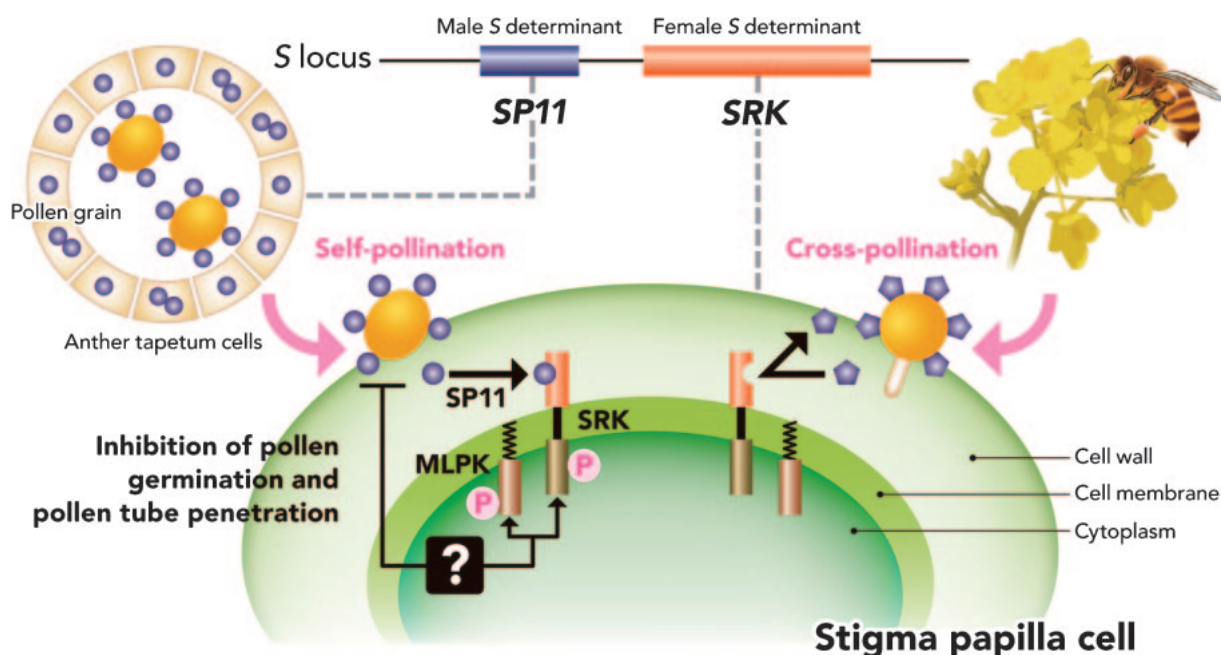


Fig. 8. Schematic model for self-pollen recognition in *Brassica* species. Male and female *S* determinant genes, *SP11* and *SRK*, are located at the *S* locus. *SP11* is predominantly expressed in the tapetum cells of anther locules, and accumulates on the pollen surface during pollen maturation. On self-pollination, *SP11* molecules penetrate into the papilla cell wall, and interact with *SRK* in an *S*-allele-specific manner. Phosphorylated *SRK* interacts with *MLPK*. After the subsequent signal transduction, which has not yet been determined, rejection of the self-pollen occurs.

evolution of SI and SC, *Arabidopsis* species are powerful research tools.^{78)–82)} As describe above, *A. thaliana* is a model cruciferous plant, which naturally has an SC phenotype, whereas other species, such as *A. halleri* and *A. lyrata*, are self-incompatible. *A. thaliana* modified to be self-incompatible is a powerful tool for genetic approaches to studying the SI signaling cascade, because the whole genome sequence of *A. thaliana* has been determined²⁶⁾ and other genetic tools (e.g. T-DNA tag lines, many ecotypes and microarray expression data) are available (<http://www.arabidopsis.org/>). To produce self-incompatible *A. thaliana* by transformation, two approaches have been used. Nasrallah and co-workers introduced *SP11* and *SRK* from self-incompatible *A. lyrata* into self-compatible *A. thaliana* (C24 ecotype) and the resulting transgenic *A. thaliana*, with both *SP11* and *SRK*, exhibited the SI trait.^{83),84)} On the other hand, Tsuchimatsu, Suwabe and co-workers introduced only *SP11* into the Wei-1 ecotype of *A. thaliana*, which has a functional *SRK* but shows SC, to produce a self-incompatible *A. thaliana*.⁸⁵⁾ In the course of sequencing analysis of the *S*-locus genes in self-incompatible *A. halleri*, the *SP11* gene of haplogroup A of *A. halleri* was found to be quite similar to the

disrupted *SP11* gene of haplogroup A of *A. thaliana*; it is suggested that a 213-bp inversion of *SP11* might have occurred in *A. thaliana*. Furthermore, from test-crosses using the pollen of the haplogroup A of *A. halleri*, it was found that several ecotypes (e.g. Wei-1) were functional on the female side of the SI system, indicating that these ecotypes may have a functional *SRK* and SI signaling cascade. By using Wei-1 as a representative of these ecotypes, self-incompatible transgenic *A. thaliana* plants carrying the restored *SP11* gene were successfully produced (Fig. 9).⁸⁵⁾ This transgenic *A. thaliana* plant with the restored *SP11* transgene, in which the recipient and donor are of the same origin, is an impressive achievement because this transformation represents the artificial retrograde evolution of the SI gene.

6. Molecular analysis of dominance relationships between *S* alleles

An important characteristic of *Brassica* SI is the dominance relationships between *S* alleles, which are a consequence of the sporophytic behavior of *S* genes.^{9)–13)} Genetic experiments have revealed four characteristics of the dominance relationships of cruciferous plants:^{9)–13)} (1) co-dominance is more frequent than dominance/recessiveness; (2) domi-

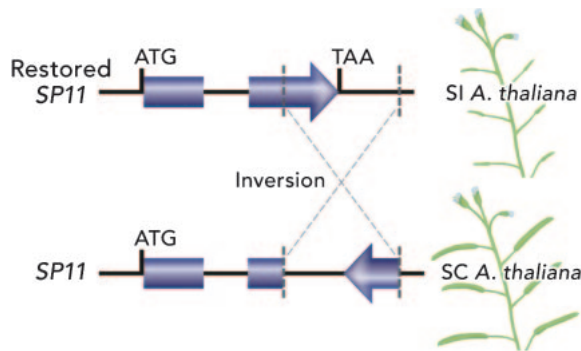


Fig. 9. Restoration to SI phenotype in *Arabidopsis thaliana*. The Wei-1 ecotype usually has a self-compatible phenotype (formation of large siliques and many seeds). The reason for the SC phenotype Wei-1 is a gene inversion within the 2nd exon of *SP11*, although *SRK* is functional. When transformed to restore an active *SP11*, the transformants showed the self-incompatible phenotype (formation of small siliques and no seeds).

nance/recessiveness in the pollen is observed more frequently than that in the stigma; (3) dominance relationships act independently in the pollen and stigma; and (4) non-linear dominance relationships are also observed, and are more frequent in the stigma than in the pollen. Discovery of the molecular mechanisms of dominance relationships has been based on these genetic features.

In *Brassica* pollen, dominance relationships of SI are regulated by transcription of *SP11*. In sporophytic tapetum cells of anthers of *S* heterozygous plants, *SP11* derived from the dominant allele is normally expressed, whereas expression of the recessive *SP11* is significantly suppressed (Fig. 10A).^{86,87} Interestingly, linear dominance relationships ($S^9 > S^{44} > S^{60} > S^{40} > S^{29}$) are also observed in *B. rapa*.^{10,87} In this case, dominance/recessiveness of S^{44} , S^{60} , S^{40} alleles could be altered, indicating involvement of epigenetic regulation.⁸⁷ From observation of the methylation level of the promoter region of *SP11* in several *S* heterozygotes, it was shown that the recessive *SP11* is specifically methylated in *S* heterozygotes.⁸⁸ Recently, it was further demonstrated that the small RNA produced from the dominant allele could activate methylation of the recessive allele. In transgenic experiments, S^{60} (class II) transformants with a class-I-derived small RNA region (S^9) showed SC, and their promoter regions were highly methylated, as in the S^9S^{60} heterozygote, indicating that the small RNA from the dominant allele functions in *trans* to induce transcriptional silencing of the recessive allele (Fig. 10B).⁸⁹

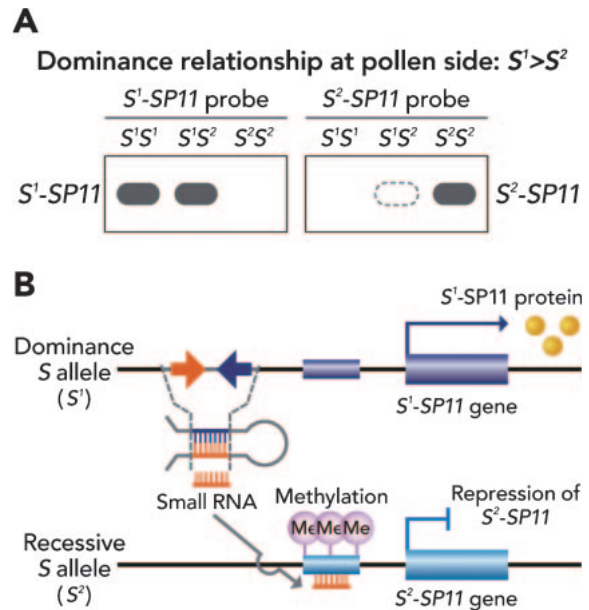


Fig. 10. Schematic model for molecular mechanisms of dominance relationships at the pollen side. (A) In the case where S^1 is dominant over S^2 , dominant transcripts of *SP11*, S^1 -*SP11*, are specifically expressed in the S^1S^2 heterozygote. However, S^2 -*SP11* transcripts are not detected in the S^1S^2 heterozygote on RNA gel blot analysis. The results demonstrate that the dominance relationship at the pollen side is regulated at the transcriptional level. (B) In the dominant *S* allele, small RNA, termed *Smi* (*SP11* methylation inducer), is specifically produced, and its nucleotide sequence is highly similar to the promoter region of the recessive *SP11* gene. This small RNA induces the methylation of recessive *SP11*, and represses the recessive *SP11* at the transcriptional level.

In contrast, dominance relationships of SI at the female side appear to be post-transcriptionally regulated by *SRK*, unlike transcriptional suppression of *SP11*.⁹⁰ The different mechanisms operating in the male and female side in the dominance relationships of SI are consistent with the four genetic characteristics described above.^{9)–13)}

7. Future prospects

As outlined in this review, the *Brassica* SI reaction has several interesting biological features, including cell-cell communication, ligand-receptor interaction, signal transduction, phosphorylation cascade, molecular evolution, allelic polymorphism and epigenetic regulation by small RNAs. For an overall understanding of SI events, collaborative research with diverse biological approaches (metabolome, proteome, bioinformatics, phenome, transcriptome, etc.) is necessary (*e.g.* refs. 91–119).

SI is one of the most interesting phenomena in sexual reproduction. Building on recent progress in sexual plant reproduction research, the molecular processes in male and female gametophyte development have now been extensively dissected in several plant species (*e.g.* refs. 104, 118–136). Such accumulation of biological knowledge will also contribute to the understand of pollen germination and pollen tube behavior in the SI recognition reaction.

Interestingly, peptide signals similar to SP11 are common in several other related phenomena (fertilization, morphogenesis, plant-microbe interaction, etc.; *e.g.* refs. 15, 137–141). To elucidate a complete overview of the peptide signaling, further analysis of protein-protein interaction by use of the yeast two-hybrid system, protein cross-linking, etc. is required,^{142),143)} in addition to the genetic analysis described above. To date, binding of SP11 to the SRK receptor, the tertiary structure of SP11 and the variable regions of SP11 have been determined.⁶²⁾ However, the tertiary structure of SRK has not yet been established. When this has been carried out, the precise SP11-SRK allelic-specific interaction will provide new insight into SP11-induced SRK activation, as has been achieved for brassinosteroid and BRI1.¹⁴⁴⁾

As the next important topics for study in pollen-stigma interaction, genetic barriers between different species and interspecific incompatibility are interesting phenomena, and these are also related to the SI reaction. Although genetic analysis of interspecific incompatibility is complicated due to the difficulty or impossibility of obtaining hybrid seeds and their progenies,¹⁾ the understanding of molecular mechanisms in the SI recognition reaction will help to identify the molecular players in interspecific incompatibility. In addition, overcoming the barrier of interspecific incompatibility could lead to the establishment of new species. From observations of chromosome hybridization, it was determined that the amphidiploid *B. napus* originated from crosses between *B. rapa* and *B. oleracea*.¹⁴⁵⁾ Interestingly, the parental diploids, *B. rapa* and *B. oleracea*, show SI phenotype and their amphidiploid, *B. napus*, has the SC phenotype. In some SC *B. napus*, the mutated genes conferring SC phenotype have been identified and characterized.^{146),147)} Taken together, these points indicate that further studies of these *Brassica* SI and SC lines should contribute to our understanding of the molecular mechanism of evolution from SI to SC during polyploidy formation under cultivation.

Very many reports have been published of the early physiological studies.⁵¹⁾ In *Brassica*, breakdown of SI was reported to be caused by several treatments, including CO₂ gas, NaCl solution, high temperature, organic solvents and electrical stimulation.^{148)–154)} However, the molecular mechanisms involved in overcoming the SI recognition reaction are still unknown. Understanding the mechanisms in overcoming SI will contribute to the discovery of the missing links in our understanding of the physiological aspect of the *Brassica* SI reaction. Another factor is the cuticle layer of stigma papilla cells, which is important for *Brassica* SI. Even in an incompatible cross the pollen tube can penetrate into the cuticle layer,¹⁵⁵⁾ indicating that there is ‘cutinase’ activity on the surface of pollen grains. Recently, results of studies on cuticle wax in other tissues have been published,^{156),157)} and these are informative for dissecting *Brassica* SI. Thus, from earlier studies we can obtain many pointers to advance *Brassica* SI research in the future.

Finally, just as we were starting to write this review article, the huge earthquake (magnitude 9.0) hit the northern part of Japan, around Sendai, on March 11, 2011.¹⁵⁸⁾ The Fukushima Daiichi nuclear power station was severely damaged by the huge earthquake and tsunami, and radio-active elements (iodine-131, cesium-137 and other compounds) were released into the environment.¹⁵⁹⁾ For the remediation of soil contaminated by radioactive compounds, especially cesium-137, *Brassica* species are potentially very useful.^{160)–165)} Of course, *Brassica* crops, cabbage, turnip, broccoli, etc., are important as food crops for people in all parts of the world. In addition, the breeding of *Brassica* crops has the potential to contribute to production of bio-fuel, phytoremediation and phytomining.¹⁶⁶⁾ In view of these points, understanding molecular mechanism of the SI recognition reaction in *Brassica* species will be important in establishing F₁ hybrid seed production in the future.

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Profile

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Profile

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Profile

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