Review

Molecular mechanism for the recognition reaction in the self-incompatibility of *Brassica* species

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Abstract: Many higher plants have developed systems to prevent self-fertilization. The self-incompatibility (SI) system has been well known since the time of Darwin. In *Brassica*, SI is controlled by a single polymorphic locus called *S*. Discrimination of self and non-self pollen by the pistil should occur on the surface of the stigma. We have investigated the molecular mechanism underlying the SI system and have determined the *S*-gene products in pistil and pollen. The *S*-determinant of pollen (SP11) is a small cysteine-rich protein and is the ligand of a receptor complex in papilla cells, which is composed of *S*-receptor kinase (SRK) and *S*-locus glycoprotein (SLG). Ligand binding induces the autophosphorylation of SRK, which triggers the signaling cascade that results in the rejection of self-pollen. The ligand-receptor interaction occurs in an *S*-haplotype specific manner. This is the fundamental reaction of self-recognition system underlying SI in *Brassica*.

Key words: Self-incompatibility; self-recognition; *Brassica*; *S*-receptor kinase; SLG; SP11.

Introduction. Because of the energy system on which their existence depends, angiosperms are not mobile. More than 70% of them have hermaphrodite flowers for reproduction, undoubtedly for securing progeny. The structure of hermaphrodite flowers, however, makes inbreeding easily possible. To escape inbreeding, angiosperm species have developed several well-organized systems, one of which is self-incompatibility. This is defined as "the inability of a fertile hermaphrodite seed-plant to produce zygotes after self-pollination".[1] This phenomenon has been well known since its introduction by Darwin.[2] Self-incompatibility involves a recognition reaction of self or non-self, occurring between pollen grains and pistils at the earliest stages in the process of pollination and pollen-tube growth.

A number of classical genetic studies have been conducted on the recognition mechanism of self-incompatibility. In many cases in homomorphic self-incompatibility system, recognition is controlled by a multi-allelic single locus, the *S*-locus (S_1, S_2, S_3, and so on).[1] When both types of pollen have the same *S*-allele, pollen growth is rejected. These studies suggested that recognition of self or non-self could be replaced by recognition of the products of *S*-multi-alleles.

Currently, two systems mediating homomorphic self-incompatibility are known, the sporophytic and gametophytic systems, which are defined by the action of the *S*-locus. Brassicaceae, Asteraceae, and Convolvulaceae plants have the sporophytic system, in which the behavior of pollen tubes is determined by the phenotype of the sporophyte producing the pollen. A schematic diagram illustrating sporophytic self-incompatibility is shown in Fig. 1. When one of the two *S*-alleles of the pollen producing plant is the same as one of the two *S*-alleles of the pistil, pollen is recognized as self, and germination and tube elongation is inhibited. The seed set is successful when both *S*-alleles of the pollen parent are different from those of the pistil. Rosaseae and Solanaceae plants have the gametophytic system, in which the behavior of the pollen tube is determined by its *S*-allele. Molecular studies on these different systems are ongoing,[1] but the recognition system in Brassicaceae has been the first to be analyzed.

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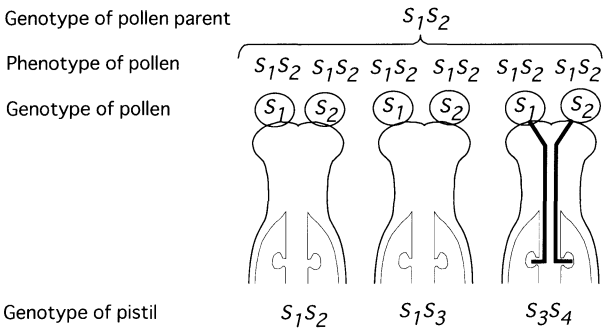

Fig. 1. Sporophytic self-incompatibility system. In this diagram, the *S*-alleles are presumed to act independently (co-dominant).

According to Bateman's survey,[3] out of 182 Brassicaceae species, 80 express self-incompatibility. This family includes a number of economically important vegetables such as *Brassica oleracea*, *B. rapa* (syn. *B. campestris*), and *Raphanus sativus*. When *Brassica* species and its closest relatives were surveyed, 50 out of 57 species were self-incompatible.[4] By using self-incompatibility, a number of hybrid varieties of *Brassica* vegetables have been successfully released in Japan since 1950, and they are highly valued in the rest of the world. Early studies on breeding practices showed that when young pistils, prior to flower opening, were pollinated by mature pollen from the same individual, self-fertilized seeds could be obtained to make pure lines. This indicated that self-incompatibility did not exist in young stigmas and was expressed over the course of stigma maturation, generally one or two days before flower opening. This finding gave a clue towards identifying an important protein in the stigma, as will be described later.

In Brassicaceae, the stigma of flowers belongs to the so-called "dry stigma" group and its surface is covered with one layer of papilla cells. The papilla is a typical secretory cell, in which the endoplasmic reticulum and Golgi apparatus are developed.[5] The cell wall of papilla cells is composed of an inner cellulose layer and an outer cuticle layer, on which waxes are deposited.

Pollen grains have three germ slits, and the exine is covered with a lipoidal coating, which is called a pollen kit, tryphine or pollen coat.[6] The coating, is transferred, at least in part, from tapetum cells of the anther wall just before pollen maturation, and includes some substances that may provide the signal for pollen identity. In cross-pollination, pollen grains absorb water from papilla cells, resulting in germination. The pollen tubes, penetrating the outer cuticle layer, grow in the cellulose layer toward the stylar tissue. In self-pollination, absorption of water and consequent germination are disturbed.[7] These phenomena are shown in Fig. 2. When ambient humidity is sufficiently high, pollen grains can germinate and the pollen tubes can penetrate the cuticle layer, but they cannot grow into the cellulose layer.[8]

This observation indicated that the self-recognition reaction between pollen and papilla cells should occur on the surface of a papilla cell. Elucidating the mechanism for plant self-recognition is not only of broad interest to basic science but is also practically useful for plant breeding. To understand the *Brassica* SI system on a molecular basis, it is essential to clarify the structure and function of the *S*-gene products in the pistil and pollen. Based on the *S*-gene hypothesis, the *S*-determinants of pistil and pollen should interact and induce an inhibitory reaction to self-pollination. Through joint research over the past twenty years, we have succeeded in identifying the pistil and pollen determinants of the *Brassica* SI system and established the fundamental mechanism for the self-recognition system. In this review, we describe our efforts to elucidate the *S*-determinants of pistil and pollen.

_S_-determinant of pistil. The first clue to the identity of the pistil *S*-determinant was obtained by biochemical analysis of stigma extract from various *S*-known plant materials by isoelectric focusing electrophoresis.[9),10)] We found proteins which were linked to the *S*-locus and expressed one or two days before flower opening. These proteins proved to be glycoproteins, which were later termed *S*-locus glycoproteins (SLG).[11),12)] We then purified these SLGs from over 30 thousand stigma from *Brassica rapa* S_8, S_9 and S_{12}-strains. We analyzed their amino acid sequences and determined the structures of the saccharide chains, using several milligrams of purified SLGs. Independently, Nasrallah *et al.* identified a cDNA clone encoding a part of a *B. oleraceae* SLG.[13)] Based on the amino acid sequences of the fragment peptides and the published cDNA sequence, we proposed the fundamental structure of SLG, along with the structure of the saccharide chains.[14),15)] As shown in Fig. 3, SLGs consist of around 400 amino acids. A characteristic property is the presence of 12 cysteine residues at the C-terminal site, which are conserved among SLGs. In spite of the high homology, highly variable regions are present in the middle portion of SLG amino acid sequences. Furthermore, SLGs have 6 to 7 N-glycosylation sites, mainly at N-terminal sites. In addition, the structures of the saccharide chains are common among the three SLGs.[16)] This was

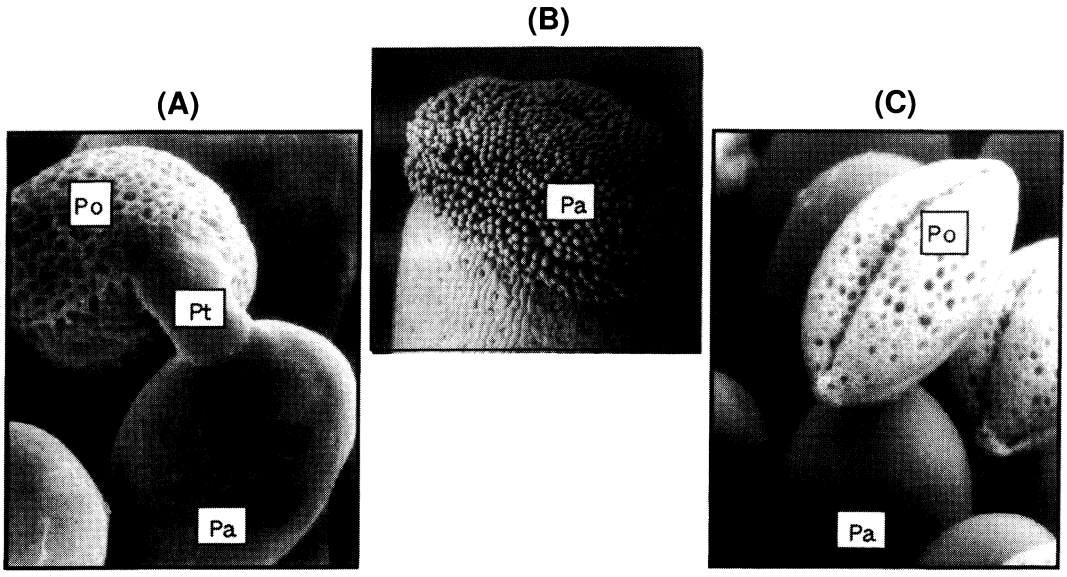

Fig. 2. Scanning electron microscopic profiles of stigma, pollen, pollen tube, and papilla. In cross-pollination (A), a cylidrical pollen tube penetrates a papilla smoothly, whereas in self-pollination (C), the pollen does not hydrate on the surface of the papilla. (B) Papilla cells at the stigma of the pistil. Pa, papilla cell; Po, pollen; Pt, pollen tube.

the first description of the fundamental structure of SLGs and clearly indicated that the *S*-specificity of SLGs should be present in their amino acid sequences. Later, we isolated the corresponding cDNAs and compared the sequences of the SLGs.[17]

We analyzed the subcellular localization of SLGs, with electron microscopy using anti-SLG antiserum, and showed that SLGs accumulate in the mature papilla cell wall, where inhibition of self-pollen tube formation occurs. SLGs are secreted via a route that involves the ER lumen, Golgi bodies, and small vesicles.[5] This circumstantial evidence, including temporal and spatial expression patterns, suggested that SLG is the product of the *S*-gene, but its function was still unclear at that time.

Important information on SLG was obtained by Walker and Zhang,[18] who were searching for plant receptor kinase genes similar to mammal ones. They found a gene in maize encoding a transmembrane receptor kinase ZmPK1, which has an extracellular domain homologous to SLG. This suggested the presence of such a receptor kinase in *Brassica*. Based on this data, similar genes were isolated from *Brassica* species.[19),20] These genes proved to be linked to corresponding *SLG* genes and were termed *S*-receptor kinase (*SRK*). The extracellular domain (*S*-domain) of SRK shows high similarity to the SLG of the respective *S*-locus, and is connected via a single-pass transmembrane domain to a serine/threonine type protein kinase catalytic center in the cytoplasm, as shown in Fig. 3. The expression of *SRK*, like *SLG*, is detected specifically in stigma tissues. Studies on several SRK/SLGs showed that they are highly polymorphic.[21),22] Thus, the *S*-locus contains at least two genes, *SLG* and *SRK*, and the *S*-locus was later referred to as an *S*-haplotype, as is the case for the mammalian MHC complex.

The involvement of the SLG/SRK in the *Brassica* SI system was confirmed with transformation experiments. We succeeded in transforming *B. rapa* with antisense-oriented *SLG*, driven by the *SLG* promoter.[23),24] The transformant became self-compatible and the levels of *SLG* and *SRK* transcripts were markedly decreased. This phenotypic change into self-compatibility was only observed in the stigma, whereas the pollen phenotype was unchanged. This clearly indicated that SRK and/or SLG are likely to be involved in the *Brassica* pistil SI system, but it was not yet certain whether both SRK and SLG were necessary.

In other studies on transformation, introduction of a sense *SLG* gene resulted in production of self-compatible *Brassica* plants and in decreased *SLG* transcripts.[25] This phenotypic change was considered to be due to co-suppression of the transgenes and endogeneous *SLG*.

To avoid co-suppression due to the high homology among *SLG*s and *SRK*s, SLG_{28} and SRK_{28} were independently introduced into the *B. rapa* S_{60}-haplotype,

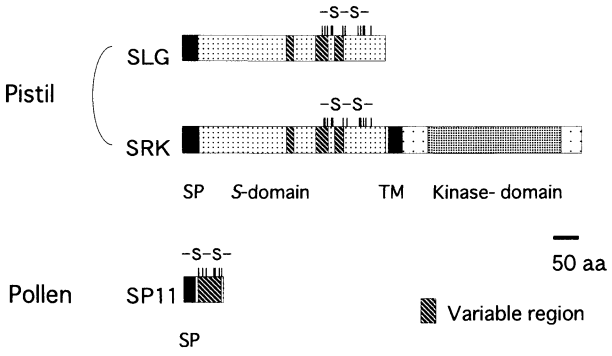

Fig. 3. Schematic drawing of predicted amino acid sequences of SLG, SRK and SP11 of *B. rapa*. SP, signal peptide; TM, transmembrane domain; -S-S-, cysteine rich region.

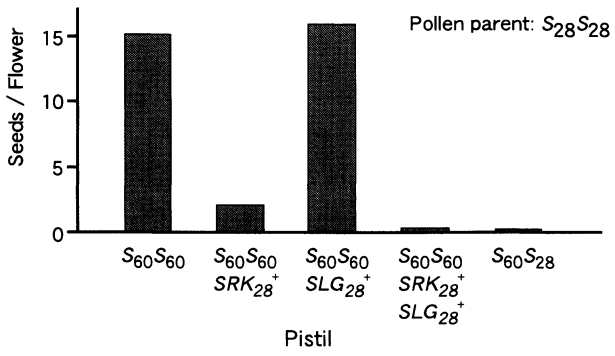

Fig. 4. Pollination analysis of *SRK* and *SLG* transformants. SRK_9 and/or SLG_9 transgenes were introduced into S_{60}-homozygotes, and the stigmas of the transformants were pollinated with pollen from S_9-homozygotes. The ordinate represents the average number of seeds per flower obtained after pollination.

whose *SLG* and *SRK* have relatively low homology to the introduced genes.[26),27)] As shown in Fig. 4, the expression of SRK_{28} alone, but not SLG_{28} alone, conferred the ability to reject S_{28}-pollen in transgenic plants. In addition, the ability of SRK_{28} to reject S_{28}-pollen was enhanced by SLG_{28}. The transformation of these genes did not have any effects on the pollen side. These findings clearly show that SRK is the sole determinant of pistil *S*-haplotype specificity, and that SLG promotes the full manifestation of the recognition response.

S-determinant of pollen. Screening for the pollen factor, the *S*-determinant of pollen and the counterpart of SRK in the *Brassica* SI system, was performed based on the expected properties of the pollen factor: 1) the gene encoding the pollen factor should be at the *S*-locus and linked to the *SRK* and *SLG* genes, 2) the pollen factor should be expressed on pollen and/or anther, 3) the pollen factor should have sequence diversity depending on the *S*-haplotype, and 4) the pollen factor should bind to SRK or SLG proteins in an *S*-specific manner.

Our first approach was to search for proteins that can bind to SLG or SLG-related protein (SLR1).[28),29)] Purified SLG and SLR1 were anchored on a tip of the BIAcore system and the binding ability was assayed in the pollen extract. We isolated a small peptide termed SLR1BP, which binds to SLR1 protein.[30)] In addition, we noticed that many similar proteins which bind to SLG were present in the extract, but we could not isolate any *S*-specific SLG binding proteins. Doughty *et al.* searched for an SLG-binding protein using isoelectric focusing electrophoresis and found a small cysteine-rich protein (PCP7, later renamed PCPA1) homologous to plant defensin.[31),32)] This protein, however, was not the pollen factor, because it lacked *S*-diversity. They then devised a bioassay method for the pollen factor and showed that the pollen factor should be present in the pollen extract preparation.[33)] This suggested that a small peptide might be the pollen factor, and that the levels of this factor are very low in the pollen extract.

Another strategy we used to find the pollen factor was the analysis of a large genomic fragment containing *SLG* and *SRK*. We isolated a 76-kb genomic fragment containing both *SLG* and *SRK* genes of the *B. rapa* S_9-haplotype by using a P1-derived artificial chromosome (PAC) vector.[34)] We analyzed the expression of this fragment's coding regions and identified ten genes in addition to the four previously identified genes in this fragment. One of these 14 genes is *SP11* (*S*-locus protein 11), which encodes a small cysteine-rich protein with a putative signal peptide of 24 hydrophobic amino acid residues at its N-terminus as shown in Fig. 3.[35)] This gene is located between *SLG* and *SRK* and is expressed mainly in pollen and anther.

We also used Fluorescent Differential Display (FDD) to screen pollen mRNA from the S_8 and S_{12}-haplotypes, which show different patterns on SDS-PAGE. We isolated several sets of genes with sequence differences between the two haplotypes. One encodes a lipid transfer protein, which is also a cysteine-rich small protein, but this was not diverse enough to be the pollen factor (Takayama, unpublished data). The next candidates were small basic proteins with eight cysteine residues. Comparison of the sequences of these genes in the S_8 and S_{12}-haplotype revealed that the mature protein regions are highly divergent, except for the positions of the eight cysteine residues. The putative 24 amino acid signal sequences, however, show high homology

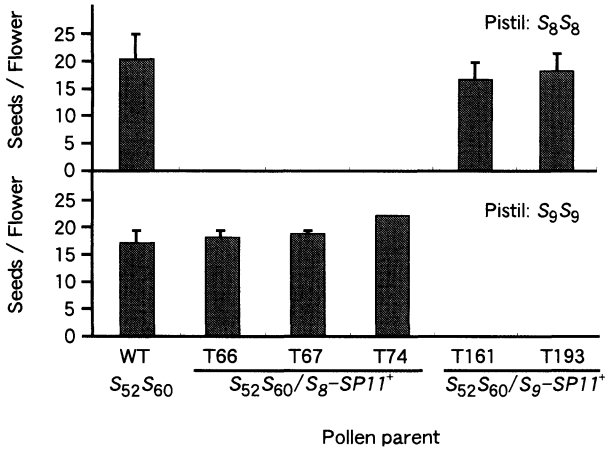

Fig. 5. Pollination analysis of *SP11* transformants. The S_8 or S_9-*SP11* gene was introduced into $S_{52}S_{60}$-heterozygotes, and the stigmas of S_8 or S_9-homozygotes were pollinated with transgenic pollen. The ordinate represents the average number of seeds per flower obtained after pollination.

between the two genes, suggesting that the two genes are allelic. Furthermore, the *SP11* gene in the S_9-haplotype also shares similarities with the gene in the S_8 and S_{12}-haplotypes. They seem to be allelic genes and were all termed to be *SP11*. We postulated that SP11 is the strongest candidate for the pollen factor and analyzed this molecule further based on its temporal and spatial expression patterns and its genomic sequence.

In situ hybridization analysis revealed that *SP11* mRNA is not only expressed in pollen, but also in anther tapetum around four or five days before flower opening.[36] Similarly, Western blot analysis indicated that *SP11* protein is present in pollen and anther.[37] As mentioned in the introduction, the *Brassica* SI system is sporophytic, so the phenotype of pollen is determined by the genotype of the pollen producing plant. The fact that SP11 is also expressed in the anther tapetum can explain the sporophytic nature of *Brassica* SI system and strongly supports that SP11 is the pollen factor.

The genomic constitution of the S_9-haplotype *S*-locus has already been discussed. We analyzed the genomic localization of *SP11* and *SRK*/*SLG* in two other haplotypes to confirm the allelic relation of the three *SP11*s. In the S_8, S_9, and S_{12}-haplotypes, the *SP11* genes are all located between *SLG* and *SRK*, though their location relative to *SLG*/*SRK* and their transcriptional orientation are divergent.[36] This complex structure should contribute to the suppression of recombination at the *S*-locus in meiosis, resulting in the conservation of function as an *S*-haplotype. This data also supports that SP11, itself, is the pollen factor. We confirmed this hypothesis with two methods, an SP11 bioassay and a transformation experiment.

Using the bioassay system developed by Stephenson *et al.*,[33] we tested the ability of recombinant S_9-SP11 to inhibit cross pollen hydration when SP11 is applied on papilla cells of the S_9-haplotype. The recombinant S_9-SP11 is able to elicit SI responses, resulting in the inhibition of cross-pollen hydration.[36] Later, we modified the bioassay system in order to see the penetration of pollen tubes in the stigma, as an indicator of cross pollination, and to observe the effects of chemically synthesized S_8-SP11 and recombinant S_9-SP11. These results were similar to those with the hydration assay system.[38] The modified method, however, is easier to perform than the original hydration bioassay.

The results of the transformation experiment are summarized in Fig. 5. *B. rapa* of the $S_{52}S_{60}$-haplotype was transformed with the S_9 and S_8-*SP11* genomic or cDNA clones. Transformant pollen was obtained and applied to a stigma with the same haplotype. Control $S_{52}S_{60}$-haplotype pollen germinated and set seeds when applied to the stigma of the S_8-haplotype. On the other hand, S_8-*SP11* transformant pollen did not set seeds when applied to stigma of the S_8-haplotype. The result for S_9-*SP11* was similar to that obtained from transformation of the S_8-*SP11* gene.[37] These results finally confirmed that SP11 is the pollen factor which can solely determine the *S*-phenotype of the plant male organ.

The pollen factor was independently identified by Schopfer *et al.*[39] by genomic analysis of the *B. rapa* S_8-haplotype, and the gene was termed SCR (*S*-locus cysteine-rich protein). This gene is allelic to the *SP11* gene of *B. rapa* S_9-haplotype.

Further, we isolated another *SP11* gene from *B. rapa* by PCR. Comparison of the 14 SP11/SCR amino acid sequences demonstrates that most of the eight cysteine residues are conserved, but the sequence between these cysteine residues is highly diverse. The phylogenetic tree for *SP11*/*SCR* is topologically very similar to that of *SLG* and the *S*-domain of *SRK*, indicating that the three related genes, *SLG*, *SRK* and *SP11*/*SCR* may have similar evolutionary histories.[40]

Interaction between SP11 and SRK. As described above, the pollen *S*-determinant, SP11, is a small protein which can elicit the SI reaction in papilla cells of the same haplotype. On the other hand, the pistil *S*-determinant is a receptor kinase, *SRK*, which should accept some ligand from the self-pollen. SP11

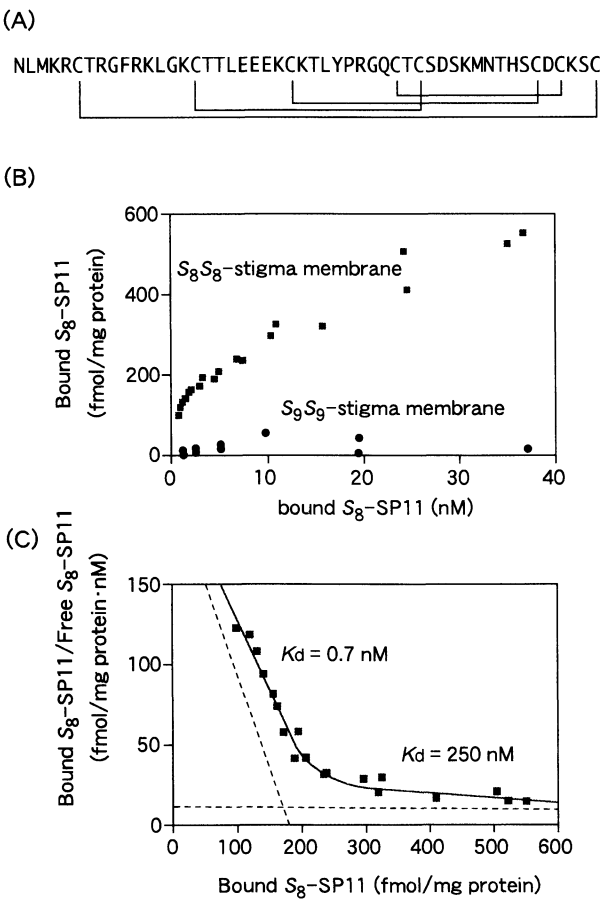

Fig. 6. Specific ^{125}I-labelled S_8-SP11 binding to microsomal membranes of the stigma. (A) Primary structure of S_8-SP11. The four intramolecular disulfide linkages are indicated by connecting lines. (B) ^{125}I-labelled S_8-SP11 specifically binds the stigmatic microsomal membranes of the S_8-homozygote, but not those of the S_9-homozygote. (C) Scatchard plot of the binding data shown in (B).

might be the ligand activating SRK. To confirm this directly, we first determined the mature form of SP11 and the nature of the eight cysteine residues of SP11. We extracted S_8-haplotype pollen and purified SP11 by immunoprecipitation. TOF-MS analysis of S_8-SP11 indicated a $(M+H)^+$ ion at m/z 5717, which corresponds to the molecular mass of the putative mature protein, assuming that all eight cysteine residues form internal disulfide bonds. This data indicated that SP11 is present as a monomer with four intramolecular disulfide bonds, and that the putative signal peptide is cleaved at the expected site when SP11 is secreted.[38]

Based on these data, we chemically synthesized S_8-SP11 by the fragment condensation method. The crude product was reduced once and purified with HPLC. The purified S_8-SP11 was then oxidized to form disulfide bonds and purified again with HPLC. Purified S_8-SP11 showed biological activity in the bioassay described above and had the same molecular mass as native S_8-SP11 by TOF-MS analysis. The locations of the four disulfide bonds were determined chemically using the synthetic S_8-SP11.[38] The structure of S_8-SP11 is shown in Fig. 6 (A).

S_8-SP11 contains one tyrosine residue, which was iodinated with ^{125}I and used to assay binding between SP11 and the microsomal membrane fraction of the stigma. Radioactive S_8-SP11 specifically binds to microsomal membranes from the stigma of S_8-haplotype with K_ds of 0.7 nM and 250 nM as indicated in Fig. 6 (BC).[38] Binding between the membrane substance and SP11 was fixed with a cross-linking reagent and the membrane was solubilized. SDS-PAGE analysis of the binding substances indicated two bands of 120 KD and 65 KD. The larger band corresponds to SRK bound to SP11 and the smaller band seems to be SLG bound to SP11. To confirm this, the binding substances were immunoprecipitated with antisera against SRK and SLG specific sequences and the data all suggested that the binding substances are SRK and SLG. Even when the concentration of SP11 was lowered, the two bands were detected in almost equal quantity.[38] This suggests that SP11 binds to a receptor complex, consisting of SRK and SLG, which data supports the observation that SLG can enhance the SI reaction in the transformation experiment,[27] and confirms *S*-specific binding between SP11 and SRK.

Similar experiments assaying for SP11 binding to the membrane fraction were performed using cold SP11 and ^{32}P-labeled ATP. The band corresponding to SRK was *S*-specifically radiolabeled, indicating that the receptor kinase of SRK in the membrane fraction is phosphorylated upon the addition of SP11. The above binding assay confirms that SP11 is the sole ligand for SRK, which is part of a receptor complex, probably with SLG. The binding of SRK and SP11 activates the SRK kinase activity resulting in SRK autophosphorylation. This series of reactions occurs *S*-specifically and is the fundamental mechanism underlying *Brassica* self-recognition. A current model illustrating the mechanism of self-recognition in the *Brassica* SI system is shown in Fig. 7 as are the expression patterns of genes encoded in the *S*-locus.

Perspective. Important clues to understanding the *Brassica* SI system have been obtained. We still have several unresolved questions regarding the reception system for the pollen factor SP11. We demonstrated that SRK is a component of a receptor complex, but the

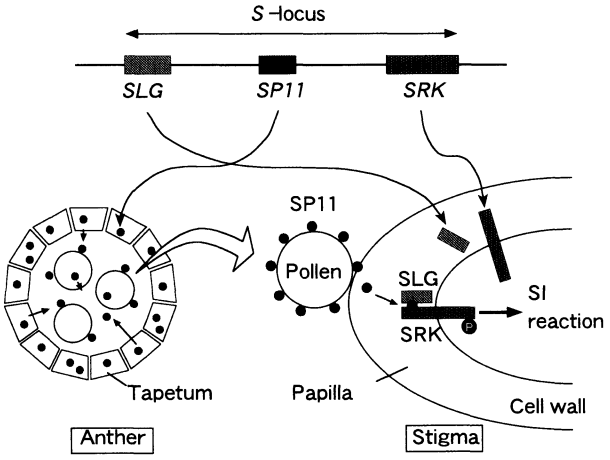

Fig. 7. Model for the self-pollen recognition in *Brassica*. The *S*-locus consists of three genes, *SLG*, *SRK*, and *SP11*. SP11 is predominantly expressed in anther tapetum and accumulates in the pollen coat during pollen maturation. On pollination, SP11 penetrates the papilla cell wall and binds to the receptor complex consisting of SRK and SLG (or its relatives). This binding induces the autophosphorylation of SRK, which triggers the signaling cascade that results in the rejection of self-pollen. This ligand-receptor interaction and receptor activation occur in an *S*-haplotype specific manner.

nature of the other SP11 binding protein is somewhat ambiguous. We assume that the band corresponds to SLG, but the data depend on the properties of the immunoprecipitation. The SRK gene produces a so-called *eSRK* mRNA, which only consists of an extracellular domain.[41] Further, some strains lack SLG genes and are still self-incompatible.[42] Therefore, the role of SLG and the nature of the other SP11 binding protein require further analysis.

A receptor complex system was further suggested by a report that a thioredoxin-like protein is involved in pollen factor-induced phosphorylation of the SRK kinase domain.[43] In addition, the SRK kinase domain binds to the ARC1 protein, which might be involved in the *Brassica* SI system.[44] The properties of the receptor complex should now be clarified biochemically. Research to explore how the different components of the complex work together to recognize self-pollen and how the signal is transduced in the papilla cell are the next step to further understanding the *Brassica* SI system.

In *Brassica*, there are expected to be about 100 *S*-haplotypes,[45] and SP11 and SRK are highly divergent. The mode by which *S*-specificity of each *S*-haplotype is determined is an interesting problem to be clarified. For this purpose, tertiary structures of *S*-gene products might be useful. SRK and SLG, however, bear saccharide chains and their expression in a heterologous system has not yet been established. In our experiment, recombinant SRK receptor domain could not bind to SP11.[38] Therefore, structural analysis of SP11 might be the first approach to studying the function of the *S*-specific region. NMR analysis of SP11 is currently being done using S_8-SP11.

In the *Brassica* SI system, the pistil and pollen phenotypes are determined based on two alleles of *SRK* and *SP11*. The alleles are usually co-dominant, but sometime they are in a dominant-recessive relation. The dominant group of *S*-haplotypes in pollen is called class-I and the recessive group class-II. We have shown that *SP11* genes could be amplified with PCR using signal peptide sequences.[40] Class-II genes, however, could not be amplified with the same method. Recently, we isolated class-II type *SP11* genes[46] and found that the signal peptide sequences are rather different from those of class-I, and that the sequence diversity among class-II *SP11* genes is lower than in class-I genes. *In situ* analysis of class-II *SP11* mRNA revealed that the mRNA is mainly expressed in the anther tapetum in class-II homozygote plants. On the other hand, expression of class-II *SP11* mRNA is not observed in tapetum and in pollen of class-I and class-II heterozygote plants. This phenomenon indicates that the dominant/recessive relationship is determined at the mRNA level. The reason why mRNA of the recessive *SP11* gene is not detected is presently unknown. To our knowledge, such a reversible dominant/recessive relationship at the mRNA level has not been described, so research to elucidate the mechanism for this effect might lead to a new understanding of the dominant/recessive relation at the molecular level.

We also established the order of dominance in pistil and pollen.[47] Interestingly the order of dominance in the stigma is different from the order in pollen. The SRK mRNA of a recessive haplotype is expressed, even in heterozygotes with a dominant haplotype.[48] The dominant/recessive relation in the pistil, which should depend on SRKs, is different system from that of pollen.

In studying the SI system of *Brassica*, we are now beginning to dissect the signal transduction system through a serine/threonine receptor kinase. The total genomic sequence of *Arabidopsis*, which also belongs to Brassicaceae, revealed that over 300 serine/threonine receptor kinases.[49] In animal systems, many G-protein coupled receptors with seven-pass transmembrane sequence play an important role in transducing extra-

cellular signals. In plants, however, seven-pass transmembrane receptors are rare and receptor kinases are the main receptors. So plants and animals have developed separate systems to receive and transduce external signals. Among the 300 receptor kinases in plants, only five ligands have been clarified.[50),51)] With the exception of SP11, the receptors for these ligands have leucine-rich repeat receptor domains. But in all of these cases, the real receptor complexes have not yet been established. Furthermore, the signal transduction systems for these signals are still uncertain. The *Brassica* SI system should be an important example for studying serine/threonine receptor kinase signal transduction systems in plants.

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