Recent Progresses on Self-incompatibility Research in *Brassica* Species

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Many hermaphrodite plant species have evolved mechanisms to prevent self-fertilization. One such mechanism is self-incompatibility (SI), which is defined as the inability of a fertile hermaphrodite plant to produce zygotes after self-pollination. SI prevents self-fertilization by rejecting pollen from plants with the same genotype. The SI system in *Brassica* is controlled sporophytically by multiple alleles at a single locus, designated as *S*, and involves cell-cell communication between male and female. When the *S* phenotype of the pollen is the same as that of the stigma, pollen germination and/or pollen tube penetration are disturbed on the papilla cells. On the female side, two genes (*SLG* and *SRK*) located at the *S* locus, are involved in the SI reaction. *SLG* is a secreted glycoprotein expressed abundantly in the papilla cell, and *SRK* is a membrane-spanning receptor-like serine/threonine kinase whose extracellular domain is highly similar to *SLG*. Gain-of-function experiments have demonstrated that *SRK* solely determines *S* haplotype specificity of the stigma, while *SLG* enhances the recognition reaction of SI. The sequence analysis of the *S* locus genomic region of *Brassica campestris* (syn. *rapa*) has led to the identification of an anther-specific gene, designated as *SPI1*, which encodes a small cysteine-rich basic protein. Pollination bioassay and gain-of-function experiments have indicated that *SPI1* is the male *S* determinant. When the sequence of *SPI1* was aligned, six cysteine residues were found to be completely conserved among alleles. These conserved cysteine residues could be important for the tertiary structure of *SPI1*. Recent biochemical analysis has suggested that *SPI1* operates as a sole ligand to activate its cognate SRK specifically. Because the activity of the *S* allele is controlled sporophytically, dominance relationships influence the ultimate phenotype of both the stigma and pollen. Molecular analysis has demonstrated that the dominance relationships between *S* alleles in the stigma were determined by SRK itself, but not by the relative expression level. In contrast, in the pollen, the expression of *SPI1* from the recessive *S* allele was specifically suppressed in the *S* heterozygote, suggesting that the dominance relationships in pollen were determined by the expression level of *SPI1*.

Key Words: *Brassica* species, cell-cell recognition, dominance relationships, self-incompatibility, *S* haplotype, *S* locus.

Introduction

Flower is important for reproduction in angiosperms, and its shape is highly differentiated among the plant species. More than 70% of the species have hermaphrodite flowers for reproduction (de Nettancourt 2001). However, the structure of hermaphrodite flowers makes self-pollination highly possible. Self-pollination could lead to a high-risk situation in which the genetic diversity would decrease in the species. To prevent inbreeding and promote outcrossing, angiosperms have developed well-organized systems such as self-incompatibility (SI). SI is defined as the inability of a fertile hermaphrodite plant to produce zygotes after self-pollination (de Nettancourt 2001). In other words, SI is a system of discrimination between self-pollen and non-self-pollen on/in the pistil. The SI system in *Brassica* species is sporophytically controlled by a single polymorphic locus, designated as the *S* locus (Bateman 1955). When the pollen has the same *S* phenotype on the stigma, pollen germination and/or pollen tube penetration are disturbed at the stigma surface on the papilla cells (Fig. 1). SI has attracted considerable attention since this phenomenon was observed in detail by Darwin (1876, 1877). Furthermore, SI has been skillfully utilized in the breeding of hybrid varieties of economically important vegetables of Brassicaceae, namely cabbage, broccoli, cauliflower (*Brassica oleracea*), Chinese cabbage, turnip (*B. campestris* (syn. *rapa*)), radish (*Raphanus sativus*), and others. Thus, as an agricultural trait, SI is also considered to be very important.

In *Brassica* species, during the last two decades, molecular analysis of the *S* locus has been performed for identifying the male and female *S* determinants of SI, and as a result, both *S* determinants have been isolated and characterized. This review focuses on the recent studies dealing with the molecular mechanisms of SI in *Brassica* species. Many review articles published recently have also provided a general

**Identification and functional characterization of female S determinant**

Identification of the S-specific antigen or the S locus-specific glycoprotein (later designated as S locus glycoprotein; SLG) in the stigma has enabled the analysis of this SI system by using molecular biological, molecular genetic, and biochemical techniques (Nasrallah and Wallace 1967, Nishio and Hinata 1977). SLGs encoded by respective S alleles were found to have different pl values (Nishio and Hinata 1977), and they co-segregated with S alleles without exception (Nou et al. 1993). They accumulated in the mature papilla cell wall, where the inhibition of self-pollen tube development occurs (Kandasamy et al. 1989, Kishi-Nishizawa et al. 1990). The SLGs consisted of a cleavable signal peptide, several N-glycosylation sites, three hypervariable regions, and twelve conserved cysteine residues towards the C-terminus (Takayama et al. 1987, Nasrallah et al. 1987). The function of SLG could not be predicted based on the sequence similarity to the other proteins.

The finding of ZmPK1 in maize, which encodes a receptor-like protein kinase with an extracellular domain similar to SLG (Walker and Zhang 1990), led to the identification of the second S-linked gene, the S receptor kinase gene (SRK; Stein et al. 1991). The extracellular domain (designated as S domain, SD) of SRK showed a high similarity to SLG, and was connected via a single-pass transmembrane domain to a serine/threonine type protein kinase catalytic domain (Stein et al. 1991). The expression of SRK was specifically detected in stigma tissues, like the SLG gene (Watanabe et al. 1994). Based on the structural characteristics of SRK, the interaction between the S domain of SRK and the unidentified male S determinant is expected to trigger a phosphorylation cascade in the papilla cell that leads to the rejection of self-pollen.

Nucleotide sequence similarity between SLG and the region encoding the S domain of SRK derived from the same S allele was about 90%, and in some cases exceeded 98% (Stein et al. 1991, Watanabe et al. 1994, Hatakeyama et al. 1998c). The phylogenetic analysis of the SLG and SRK genes demonstrated that diversification of these genes pre-dated the speciation in Brassica species (Hinata et al. 1995, Kusaba et al. 1997), and that intragenic recombination and point mutations have contributed to the generation of their sequence variation (Kusaba et al. 1997). Furthermore, gene conversion might occur frequently between the SLG and SRK genes (Watanabe et al. 1994, Suzuki et al. 1997a, Hatakeyama et al. 1998c).

Because the S locus consists of genes encoding both female and male S determinants within one segregational unit as described below, “S allele” is referred to as “S haplotype.” From the nucleotide sequence similarity of SLGs/SRKs, S haplotypes have been classified into two groups, class I and class II (Nasrallah et al. 1991). The sequence similarity among SLGs/SRKs within each class is about 80–90%, but that between classes is about 65%. In spite of the specific expression of both SLG and SRK genes in stigma tissues, the classification is correlated with dominant/recessive relationships on the pollen side (Hatakeyama et al. 1998a, 1998b): that is, class-II S haplotypes tend to be recessive to class-I S haplotypes on the pollen side.

Loss-of-function and gain-of-function experiments have been conducted to determine the role of SLG and SRK in the SI recognition reaction. In the case of the loss-of-function experiments, transgenic plants with antisense SLG gene and self-compatible mutants were analyzed. In transgenic B. campestris with antisense SLG driven by the SLG promoter, the level of transcripts of SLG and SRK decreased, and the transformants became self-compatible (Shiba et al. 1995, Shiba et al. 2000, Takasaki et al. 2001). In self-compatible mutants of Brassica species, the transcripts of SLG and/or SRK were down-regulated or undetectable (Nasrallah et al. 1992, Goring et al. 1993, Nasrallah et al. 1994, Watanabe et al. 1997). These results indicate that SLG and/or SRK are necessary for the SI recognition reaction. In contrast, in some S haplotypes of Brassica species, deletion of SLG from the genome and mutation in the SLG sequence were observed, indicating that SRK solely functions in the SI recognition reaction at least in these S haplotypes (Okazaki et al. 1999, Suzuki et al. 2000, Suzuki et al. 2003).

The determination of the function of SLG and SRK by using gain-of-function experiments took more than 10 years of research after the isolation of the SLG and SRK genes. The difficulty in determining the function of SLG and SRK was due to the following two problems. One is that the transformation efficiency of Brassica species, especially B. campestris, was very low. Based on the screening of several cultivars of B. campestris, Takasaki et al. (1997) identified a suitable cultivar, Osome, for use as a plant material for transformation experiments. Another problem was that when the SLG or SRK genes were introduced into self-incompatible

![Fig. 1. Representative SI reaction in B. campestris (syn. rapa) L.](image-url)
Brassica species, co-suppression between the SLG/SLG

transgenes and the endogenous SLG/SLG genes occurred, and the transformants became self-compatible without acquiring new S haplotype specificity (Conner et al. 1997, Stahl et al. 1998, Takasaki et al. 1999). Takasaki et al. (1999, 2000) reported that this co-suppression depends on the sequence similarity between the SLG/SLG-transgenes and the endogenous SLG/SLG genes based on the following experiment. That is, when a class-I SLG<sup>α</sup> gene was introduced into a self-incompatible cultivar, Osome, which is the S heterozygous plant derived from class-I (S<sup>2</sup>) and class-II S haplotype (S<sup>0</sup>), the expression of endogenous SLG<sup>32</sup> was specifically suppressed in the transgenic plants, unlike that of SLG<sup>80</sup> (Takasaki et al. 1999). Thus, in order to avoid the co-suppression between transgene and endogenous gene, SLG<sup>α</sup> and SRK<sup>α</sup> of B. campestris were independently introduced into S<sup>60</sup> homozygous plants of B. campestris (Takasaki et al. 2000). The transgenic plants expressing the SRK<sup>α</sup>-transgene acquired the S<sup>α</sup> haplotype specificity in the stigma, but not in the pollen. In contrast, the transgenic plants expressing the SLG<sup>α</sup>-transgene did not acquire the S<sup>α</sup> haplotype specificity in the stigma nor in the pollen. These data clearly show that SRK is the sole determinant of the S haplotype specificity of the stigma (Takasaki et al. 2000). As previously described, SLG is also specifically expressed in the stigma tissues (Watanabe et al. 1994). What function does SLG have in the SI recognition reaction? When the number of seeds per pod obtained after crossing with S<sup>α</sup> pollen was examined precisely, the plant harboring both SRK<sup>α</sup> and SLG<sup>α</sup>-transgenes set few seeds (about 0.3 seeds per pod), a number comparable to that of the S<sup>α</sup>S<sup>60</sup> heterozygous plants (about 0.2 seeds per pod), and this number was significantly lower than that of the plants harboring the SRK<sup>α</sup>-transgene alone (about 1.9 seeds per pod) (Takasaki et al. 2000). These results suggested that SLG acts to promote the full manifestation of the SI response. Some self-compatible mutants, in which the SLG gene was deleted from the genome, were found to accumulate lower amounts of SRK protein compared with the self-incompatible plants, in spite of the similar level of SRK transcript (Dixit et al. 2000). In contrast, in an independent gain-of-function experiment using a related species, B. napus, the enhancing role of SLG was not observed (Cui et al. 2000, Silva et al. 2001). Studies on the difference of the SLG function in the SI response observed in these two experiments and on the more precise function of SLG need to be conducted in the future.

Identification and functional characterization of male S determinant

After the SLG and SRK genes were isolated and characterized, many SI researchers have tried to identify the male S determinant by using several strategies (reviewed in Hinata et al. 1993, Watanabe and Hinata 1999). The male S determinant is expected to exhibit the following four properties. First, the gene encoding the male S determinant must be located at the S locus, suggesting that the gene should be close to the SLG and SRK genes. Secondly, the gene should be expressed before meiosis in the pollen mother cell, or expressed later in the anther tapetum cells, which are nurse cells investing the developing pollen grains, because the regulation of Brassica SI is sporophytic (Bateman 1955). Thirdly, the male S determinant gene must show allelic diversity among the S haplotypes, as observed in the stigma-specific genes, SLG and SRK (reviewed in Watanabe et al. 2001). Fourthly, the male S determinant should physically interact with SLG and/or SRK in an S haplotype-specific manner.

Two different approaches, biochemical and molecular genetic analyses, were adopted for the identification of the male S determinant. First, biochemical studies provided an important clue for the identification of the male S determinant. By using a band-shift assay in isoelectric focusing gels, a 7-kDa PCP (pollen coat protein), which was designated as PCP7 (later renamed PCP-A1), was identified as an SLG-interacting protein. However, its interaction was not S haplotype-specific and its gene was not linked to the S locus, indicating that PCP-A1 was not a real male S determinant (Doughty et al. 1993, Doughty et al. 1998). The PCP-A1 was a cysteine-rich basic protein, and a member of a large protein family, the PCP family (Doughty et al. 1998). A cDNA clone homologous to the PCP-A1 gene was also isolated from a cDNA library of immature anthers with polyclonal antisera against the PCPs (Toriyama et al. 1998). By using a surface plasmon resonance sensor (BIAcore) along with chromatographic methods, many PCP-like molecules, which can interact with SLG, were identified in the proteins extracted from the pollen coat, though their interaction was not S haplotype-specific, like PCP-A1 (Takayama et al. 2000a). Furthermore, a pollination bioassay system monitoring the hydration of pollinated pollen grains was developed for the identification of the male S determinant from a pollen coat. By using this bioassay system, it was demonstrated that a small basic protein fraction (<10 kDa) containing PCP-A1 induced the SI response on the papilla cells in an S haplotype-specific manner, indicating that the male S determinant must be a PCP-A1-like peptide in the pollen coat (Stephenson et al. 1997).

Based on these biochemical experiments, two different molecular biological experiments were performed to identify the male S determinant. Firstly, in the S<sup>α</sup> haplotype of B. campestris, a 76-kb genomic fragment containing the SLG and SRK genes was cloned into a P1-derived artificial chromosome (PAC) vector (Suzuki et al. 1997b). After complete sequencing of this fragment, 12 additional expressed genes were identified in this 76-kb SLG/SLG region (Suzuki et al. 1999, Watanabe et al. 1999). One of these genes, designated as SP11 (S locus protein 11), which was located between the SLG and SRK genes, was specifically expressed in the anther tissues, and encoded a small basic protein, which had eight cysteine residues partially similar to the PCP-A1 protein (Suzuki et al. 1999). From S<sup>32</sup> haplotype, a cDNA clone ho-
mologous to $S^8$-SP11 was also isolated by using $S^8$-SP11 as a probe (Takayama et al. 2000b). As a second approach, a fluorescent differential display (FDD) was used to compare anther cDNA populations between $S^8$ and $S^{12}$ haplotypes to identify the $S$ haplotype-specific genes. Among 26 $S$ haplotype-specific clones, one clone, $S^8$-SP11, exhibited characteristics specific for SP11 gene. With the information on the nucleotide sequence of $S^8$-SP11, a cDNA clone homologous to $S^8$-SP11 was also isolated from $S^2$ haplotype by using RT-PCR. When the sequences of the deduced amino acids of these four clones ($S^8$-SP11, $S^8$-SP11, $S^{12}$-SP11 and $S^{12}$-SP11) were aligned, the hydrophobic region in the amino-terminal, which was expected to be a signal peptide, was highly conserved among the genes, though the region of the mature protein markedly diverged except for the cysteine residues (Takayama et al. 2000b). As determined in the $S^9$ haplotype, these genes were located upstream or downstream of the $S^9$ haplotype in each $S$ haplotype, indicating that the genes are alleles at the $S$ locus (Suzuki et al. 1999, Takayama et al. 2000b, Watanabe et al. 2000a). The temporal and spatial expression analysis of the SP11 gene clearly demonstrated that $S^8$-SP11 was sporophytically expressed in the tapetum cells at early developmental stages and also gametophytically in microspores at late developmental stages (Takayama et al. 2000b), explaining easily the sporophytic nature of Brassica SI. Independently, a group at Cornell University identified the same $S^8$-SP11 gene during the analysis of the SLG/ SRK region of the $S^8$ haplotype of B. campestris, and it was designated as SCR ($S$ locus cysteine-rich protein). By using SCR as a probe, two genes orthologous to SCR were isolated from a related species, B. oleracea (Schopfer et al. 1999).

In order to determine whether SP11/SCR is the male S determinant or not, gain-of-function experiments (Schopfer et al. 1999, Shiba et al. 2001) and a pollen bioassay (Takayama et al. 2000b) were performed. In the case of the gain-of-function experiments, when the $S^-$ and $S^8$-SP11 genes were introduced into self-incompatible B. campestris ($S^{25S^{60}}$ heterozygote), the pollen grains from transgenic plants expressing the $S^-$ and $S^8$-SP11 transgene acquired the $S^-$ and $S^8$-haplotype-specificity, respectively (Shiba et al. 2001). A similar result was obtained in the case of B. oleracea (Schopfer et al. 1999). In the case of the pollen bioassay, which was developed by Stephenson et al. (1997), when papilla cells were pre-treated with the chemically synthesized and/or recombinant $S^-$ and $S^8$-SP11 proteins, $S^-$ and $S^8$-SP11 induced the SI response only when applied to the $S^-$ and $S^8$-stigma, respectively, and inhibited the cross-pollen hydration and germination (Takayama et al. 2000b, Takayama et al. 2001). All these results taken together suggest that SP11/SCR is the real male $S$ determinant in SI.

Recently, the localization and translocation of SP11 during pollen development and pollination have been demonstrated by using immuno-electron microscopy. The SP11 protein was secreted from the tapetal cell into the anther locule as a cluster and translocated to the pollen surface at the early developmental stage of the anther tissues. During the pollination process, SP11 was translocated from the pollen surface to the papilla cell, and then penetrated the cuticle layer of the papilla cell to diffuse across the pectin cellulose layer. Furthermore, SP11 could only penetrate the cuticle layer of the papilla cell in the presence of pollen grains, suggesting that some factors different from pollen grains must be necessary for SP11 to penetrate the papilla cell wall (Iwano et al. 2003).

In order to determine the allelic diversity of SP11 among S haplotypes in Brassica species, several SP11 allelic genes were isolated from the class-I S haplotypes by using RT-PCR with a primer designed from the conserved signal peptide region (Watanabe et al. 2000a, Suzuki et al. 1999, Takayama et al. 2000b, Sato et al. 2002). The amplified PCR product was found to be closely linked to the SLG/ SRK genes, indicating that this PCR product was an allele of SP11 (Watanabe et al. 2000a). Although the physical distances between SLG/SRK and SP11 varied considerably among S haplotypes (Takayama et al. 2000b, Watanabe et al. 2000a, Suzuki et al. 2000, Kimura et al. 2002), the sizes of the S locus region of B. campestris were generally smaller than those of B. oleracea, probably because the multiple insertion events specifically occurred in the region of B. oleracea after speciation (Suzuki et al. 2000, Kimura et al. 2002). When the amino acid sequences of SP11 were aligned, several characteristic features were observed. First, eight cysteine residues (C1 to C8) were conserved in the mature protein. Secondly, a glycine residue between C1 and C2 was conserved. Thirdly, one aromatic amino acid residue between C3 and C4 was conserved (Watanabe et al. 2000a, Sato et al. 2002). SP11 genes derived from class-II S haplotypes were isolated and characterized in Brassica species (Shiba et al. 2002). These three characteristic features identified in class-I S haplotypes were also conserved in the SP11 of class-II S haplotypes, indicating that these common primary structural features are important for the tertiary structure of SP11 in general (Watanabe et al. 2000a, Shiba et al. 2002, Sato et al. 2002).

Recently, the SP11 protein has been purified from the pollen coat, and found to be present as a monomer stabilized with four disulphide bonds. By using the chemically synthesized SP11 protein, the disulphide linkage (C1-C8, C2-C5, C3-C6, C4-C7) of SP11 was determined (Takayama et al. 2001, Takayama and Isogai 2003). The synthesized form of SP11 was found to be biologically active by using a modified pollen bioassay system (Takayama et al. 2001).

The trans-specific mode of polymorphism of the SP11 gene was observed from the phylogenetic tree (Watanabe et al. 2000a, Shiba et al. 2002, Sato et al. 2002), as was also the case with the SLG and SRK genes, as described above. The topology of the phylogenetic tree of SP11 is very similar to that of SLG and the S domain of SRK, though the branching pattern of SP11 is slightly different from that of SRK. Therefore, it appears that the three SI-related genes, SLG, SRK and SP11, have similar evolutionary histories (Watanabe et al. 2002).
Interaction between male and female S determinants

As described above, the molecular features of the male (\textit{SP11}) and female (\textit{SRK}) \textit{S} determinants were characterized, and the next question is to determine how the SP11 molecule physically interacts with SRK. In order to analyze the interaction between SP11 and SRK, \textit{125I}-labeled S\textsuperscript{8}-SP11 (125I-S\textsuperscript{8}-SP11) displaying the native biological activity of S\textsuperscript{8}-SP11 was used to perform cross-linking experiments with stigmatic microsomal membranes. Two protein bands (120 and 65 kDa), which were cross-linked to \textit{125I}-S\textsuperscript{8}-SP11, were detected on SDS-PAGE gel. Based on the molecular masses of the protein bands and the immunoreactivity to specific antibodies to SLG and SRK-KD (kinase domain), the 120-kDa protein was considered to correspond to SRK\textsuperscript{8} and the 65-kDa protein probably corresponded to SLG\textsuperscript{8} (Takayama et al. 2001). However, because the truncated soluble protein of SRK (eSRK), which was derived from alternative splicing of the SRK gene (Giranton et al. 1995, Suzuki et al. 1996), and several other SLG-like proteins were expressed in stigma tissues (Suzuki et al. 1997c, Kai et al. 2001), it is necessary to determine whether the 65-kDa protein band corresponds to SLG or not. Furthermore, S\textsuperscript{8}-SP11 could specifically induce the autophosphorylation of SRK\textsuperscript{8}, but not that of S\textsuperscript{8}-SP11, clearly indicating that the SP11 molecule solely can activate SRK in an \textit{S} haplotype-specific manner (Takayama et al. 2001).

Independently, by using tagged versions of recombinant SRK (eSRK-FLAG) and SCR (SCR-Myc-His\textsubscript{6}), the reactivity between SCR and SRK was investigated (Kachroo et al. 2001). In both the “pull-down” assay and ELISA experiments, eSRK\textsubscript{6}-FLAG interacted more strongly with SCR\textsubscript{6}-Myc-His\textsubscript{6} than with SCR\textsubscript{11}-Myc-His\textsubscript{6}, indicating that SRK-SD itself has an affinity to SP11 (Kachroo et al. 2001), though no interaction between SP11 and SRK-SD expressed in silkworm cells was observed (Takayama et al. 2001). Furthermore, eSRK-FLAG could interact with ca. 16-kDa PCP, suggesting that SP11 might function as a dimer (Kachroo et al. 2001), which is in contrast to our data showing that S\textsuperscript{8}-SP11 with biological activity is present as a monomer (Takayama et al. 2001). It is difficult to explain at present the discrepancies in the data derived from different laboratories because of the variations in the materials and methods used in these experiments. However, these should be clarified in the future.

Signaling cascade downstream of SP11-SRK recognition

To date, three different molecules, which interact with the kinase domain (SRK-KD) and/or transmembrane domain of SRK (SRK-TM), have been identified by using different methodologies. These molecules are expected to offer important clues for understanding the SI downstream signaling pathway(s). By using a yeast two-hybrid system with SRK-KD as bait, two kinds of proteins (THL1/THL2 and ARC1) were isolated and characterized (Bower et al. 1996, Gu et al. 1998). The ARC1 (arm-repeat-containing protein 1) protein bound to SRK-KD with a phosphorylation-dependent manner (Gu et al. 1998). Furthermore, when the expression of the ARC1 gene was suppressed by its antisense gene, the transgenic plants became partially self-compatible, indicating that ARC1 is a positive effector of the SI signaling (Stone et al. 1999). Recently, it has been shown that ARC1 had displayed an E3 ubiquitin ligase activity, indicating that ARC1 promotes the ubiquitination and proteasomal degradation of some compatibility factors in the pistil, which in turn leads to pollen rejection (Stone et al. 2003). In contrast, THL1/THL2 (thioredoxin-h-1 and 2) proteins were found to interact with a conserved cysteine residue at SRK-TM in a phosphorylation-independent manner (Bower et al. 1996, Muzzurco et al. 2001). In vitro phosphorylation experiments showed that SRK was negatively regulated by THL1 and activated by pollen coat proteins (presumably SP11)(Cabrillac et al. 2001). As another strategy, by using recombinant SRK-KD and [\textgamma-\textsuperscript{32}P]ATP, a phosphorylation-positive clone designated as \textit{Bc-NDPK-III} (homologous to nucleoside diphosphate kinase) was isolated from the stigma cDNA library. Based on in vitro phosphorylation experiments, SRK-KD was found to be phosphorylated by Bc-NDPK (Matsushita et al. 2002). It is important to determine the precise roles of these interactive molecules in the SI signaling cascade.

Analyses of self-compatible lines, whose mutated genes are not linked to the \textit{S} locus, are important for understanding the signaling cascade downstream of SP11-SRK interaction. One well-studied self-compatible line is the \textit{B. campestris var. yellow sarson}, which was derived from a naturalized population in India. The compatibility of this line was found to be regulated by a recessive gene, designated as \textit{m} gene (Hinata and Okazaki 1986). The \textit{M} locus was independent of and epistatic to the \textit{S} locus, and the \textit{S\textsuperscript{8}mm} plants exhibited a complete loss of self-incompatibility response in the stigma, but not in the pollen, suggesting that the \textit{M} gene encodes a key effector working downstream of SRK. It was once suggested that the \textit{M} gene encoded an aquaporin-like protein, MOD (Ikeda et al. 1997). However, recent molecular analyses on the \textit{MOD} gene of several self-incompatible lines demonstrated that \textit{MOD} was not the real \textit{M} gene (Fukai et al. 2001). A schematic model of the recognition reaction in \textit{Brassica} SI is presented in Fig. 2.

Dominance relationships on the stigma and pollen

To date, more than 30 and 50 \textit{S} haplotypes have been identified in \textit{B. campestris} (Nou et al. 1993) and in \textit{B. oleracea} (Ockendon 2000), respectively. Because the \textit{Brassica} SI system is sporophytically controlled, the SI phenotype of the stigma and pollen of a plant is determined by whether the two \textit{S} haplotypes it carries are co-dominant or exhibit a dominant/recessive relationship (Thompson and Taylor 1966, 2000a, Sato et al. 2002).
Hatakeyama et al. 1998b). The characteristics of the dominance relationships in *Brassica* species include: (i) common co-dominant relationships; (ii) dominant/recessive relationships occurring more frequently in pollen than in stigma; (iii) dominant/recessive relationships differing between pollen and stigma; (iv) non-linear dominant/recessive relationships (Thompson and Taylor 1966, Hatakeyama et al. 1998b).

In the case of the dominance relationship on the stigma side, the regulating molecule was determined by analyzing the transformants carrying the *SRK* gene. The pollination experiments and detection of transcripts of *SRK* suggested that the dominance relationships between *S* haplotypes in the stigma were determined by *SRK* itself, but not by the relative transcript level (Hatakeyama et al. 2001).

As for the dominance relationship on the pollen side, it was found that class-I *S* haplotypes are generally dominant over class-II *S* haplotypes (Hatakeyama et al. 1998b). Comparison of the sequences of *SLG*, *SRK* and *SP11* between class-I and class-II *S* haplotypes, showed that the sequences in each of the three genes were highly different between classes (Hatakeyama et al. 1998b, Shiba et al. 2002). Based on molecular cloning of the male *S* determinant, *SP11*, from class-I and class-II *S* haplotypes (Schopfer et al. 1999, Takayama et al. 2000, Watanabe et al. 2000a, Shiba et al. 2002), it is considered that the correlation between the classification of *S* haplotypes and sequence similarity of *SLG* and *SRK* results from the co-evolution between male (*SP11*) and female (*SRK*) *S* determinant genes (Watanabe et al. 2000a, Sato et al. 2002).

Recent RNA gel blot and *in situ* hybridization analyses of the *SP11* gene in the *S* heterozygotes with a pair of class-I and class-II *S* haplotypes have shown that the expression of class-I *SP11* was predominantly observed, while that of class-II *SP11* was completely repressed, suggesting that the dominance relationships between class-I and class-II *S* haplotypes on the pollen side are determined by the expression level of *SP11* (Shiba et al. 2002, Kakizaki et al. 2003). Similar phenomena were observed in another cruciferous SI plant, *Arabidopsis lyrata* (Kusaba et al. 2002).

Within four class-II *S* haplotypes of *B. campestris*, a linear dominance relationship (*S*44 > *S*60 > *S*40 > *S*29) was observed on the pollen side. In all the six combinations of *S* heterozygotes derived from these four class-II *S* haplotypes, *SP11* from the dominant *S* haplotype was predominantly expressed, while *SP11* from the recessive *S* haplotype was always repressed. Therefore, the linear dominance relationship within class-II *S* haplotypes on the pollen side is also regulated by the RNA level of the *SP11* gene. Interestingly, *S*60 and *S*40 haplotypes can be both dominant and recessive depending on their *S* haplotype partner in the heterozygote, indicating that these linear dominance relationships are apparently reversible epigenetic phenomena (Kakizaki et al. 2003). More precise molecular mechanisms of expression of dominance relationships in both stigma and pollen remain to be elucidated in the future.

**Future perspectives—from *Brassica* to *Arabidopsis***

During the last decade, molecular genetic and biochem-
ical analyses have revealed that the male and the female S determinants of Brassica SI are SP11 and SRK, respectively. As described above, many problems still remain to be solved to fully understand the recognition reaction of Brassica SI. In order to elucidate the mechanism(s) controlling this function, the use of a cruciferous self-compatible plant, A. thaliana, is considered to be very important, because the genomic sequence of A. thaliana has already been determined (The Arabidopsis Genome Initiative 2000). In the genomic sequence of A. thaliana, the region homeologous to the Brassica S locus has been identified in the ETRI flanking region on chromosome I. In this region of A. thaliana, however putative genes orthologous to the Brassica SI genes, SLG, SRK and SP11, have not been found (Conner et al. 1998, Takada et al. 2001). Recently, it has been reported that SRK and SCR orthologous genes were mapped on an ARK3 region in chromosome IV of A. thaliana by comparison with the S locus region of A. lyrata, a self-incompatible species closely related to A. thaliana. In A. thaliana, the SRK and SCR orthologs encode a nonfunctional protein, whereas A. lyrata harbors the functional SRK and SCR genes, suggesting that the self-compatibility of A. thaliana might be due to the inactivation of the S locus genes by mutation (Kusaba et al. 2001). Based on these data, it was expected that the introgression of genes encoding both male and female S determinants (SP11/SCR and SRK) would lead to a change from the self-compatible A. thaliana to a self-incompatible one. Actually, by using the genomic clone of the SLG/SRK region of Brassica species, A. thaliana was transformed. However, the transformants did not exhibit the SI phenotype (Bi et al. 2000). In contrast, when A. thaliana was transformed with SRK and SCR derived from a closely related species, A. lyrata, the transformants partially exhibited a weak SI phenotype, suggesting that the downstream signaling cascade leading to the rejection of self-pollen is maintained in A. thaliana (Nasrallah et al. 2002). If A. thaliana successfully acquires the strong SI phenotype, the transformants could become very informative materials for the analysis of the signaling cascade of Brassica SI (The Arabidopsis Genome Initiative 2000, Nasrallah et al. 2002).

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Literature Cited


