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Molecular Mechanisms of Self-Incompatibility in *Brassica*

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Abstract

In *Brassica* species, self-incompatibility has been mapped genetically to a single chromosomal location. In this region several closely linked genes have been identified. One of them, S-locus receptor kinase (*SRK*), determines S haplotype specificity of the stigma and it's the key protein for SI reaction. The role of the S locus glycoprotein (*SLG*) gene remains unclear. In the last decade approximately 15 additional genes linked to S-locus have been found. Recently, a gene has been identified (*SCR*) that encodes a small cysteine-rich protein which is a candidate for the pollen ligand. In addition to S locus linked genes there are unlinked *SLR* genes (S-locus related genes). In this review, we discuss the role of these genes and the current view on the self-incompatibility mechanism in *Brassica.*

Introduction

The commonest way in which plants avoid self-fertilisation is by self-incompatibility, a physiological barrier making it difficult or impossible for a flower to fertilise itself even though it may be abundantly pollinated with its own pollen. Self-incompatibility involves the ability of a plant to discriminate between its own pollen grains and those of another plant and only allow pollen from a different plant to grow and fertilise the ovules. It is unusual as a recognition system since most other systems (such as the immune system) involve recognition and rejection of a foreign organism or protein, such as a disease organism or a tissue transplant. In a self-incompatibility system, it is the *same* type that is rejected and a different type leads to acceptance and fertilisation. In the sporophytic form of self-incompatibility, the stigma surface is the site of recognition and the proteins in the outer coat of the pollen grain are recognised. This pollen surface material derives from the parent plant, the sporophyte or spore-bearing plant, not from the pollen grain itself, hence the name of this system.

Sporophytic self-incompatibility is a feature of important plant families, the crucifers (*Brassicaceae*). In this review, we discuss mechanisms of self-incompatibility in light of recent molecular genetic data derived from the analysis of three *Brassica* species, *B. oleracea*, *B. rapa* (synonym *campestris*), and *B. napus.* Both of the diploid *Brassica* species, *B. oleracea* and *B. rapa* possess this self-incompatibility system, whereas *B. napus,* an allotetraploid composed of the *B. rapa* and *B. oleracea* genomes, generally occurs as a self-compatible plant (Downey and Rakow, 1987). There are a few naturally occurring self-incompatible *B. napus* lines (Gowers, 1989).

Genetics of Self-Incompatibility in *Brassica*

The genetics of SI in family *Brassicaceae* were deciphered in the early 1950s by Bateman (1955) who described control by a single Mendelian locus, the S (Sterility) locus, which exists as multiple alleles or variants, each of which encodes a distinct mating specificity. In self-incompatible plants of this family, pollen will not develop on a stigma that expresses the same alleles as the pollen parent. As expected for a system in which new alleles have a reproductive advantage and therefore will increase in frequency toward equilibrium, the number of S-locus alleles is usually large, being estimated at 22 in *Iberis* (Bateman, 1955), 34 in *Raphanus* (Sampson, 1957), 30 in *B. rapa* (Nou *et al.,* 1993), and more than 50 in *Brassica oleracea* (Brace *et al.,* 1994). The various naturally occuring, classically defined S-alleles that have been described in *Brassica* have been arranged in a dominance series based on their genetic behaviour relative to other alleles in heterozygous plants (Thomson and Taylor 1966). A classical genetic analysis has grouped the *Brassica* S-alleles into two categories based on their phenotypic effect on self-incompatibility characteristics. The first group of alleles (high-activity) are placed relatively high on the dominance scale and exhibit a strong self-incompatible phenotype in which an average of 0 to 10 pollen tubes develop per self-pollinated stigma. The second group of alleles (low-activity) demonstrate a weak or leaky self-incompatible phenotypic effect in which 10 to 30 pollen tubes develop per self-pollinated stigma and they are considered to be recessive (Nasrallah *et al.,* 1991).

Molecular analysis of the S-locus region shows that this locus is a complex locus spanning many kilobases and containing several physically linked transcriptional units that cosegregate perfectly with SI phenotype (Boyes *et al.,* 1997, Casselman *et al.,* 2000). A subset of genes within the S-locus complex ("S haplotype") is highly polymorphic as expected for genes involved in recognition, and specific combinations of allelic forms of each of these genes are thought to define different SI specificities. Thus, the S-locus may be viewed as a master recognition locus that encodes the function(s) required for the stigma to distinguish self-related from self-unrelated pollen.

S-Locus Genes *SLG* and *SRK*

As stated above, several closely linked genes have been identified at the S-locus. Two of them, *SLG* (for S-locus glycoprotein; Nasrallah *et al.,* 1985) and *SRK* (for S-locus receptor kinase; Stein *et al.,* 1991), are though to be involved in the perception of self-pollen by the stigma. Both genes are highly polymorphic, and both are expressed specifically at the surface of mature stigmas. Low levels of mRNA for these two genes are also present in anthers (Sato *et al.,* 1991, Stein *et al.,* 1991), but the protein products of the genes have not been detected in this tissue (Delorme *et al.,* 1995). Mutations in *SRK* (Goring *et al.,*

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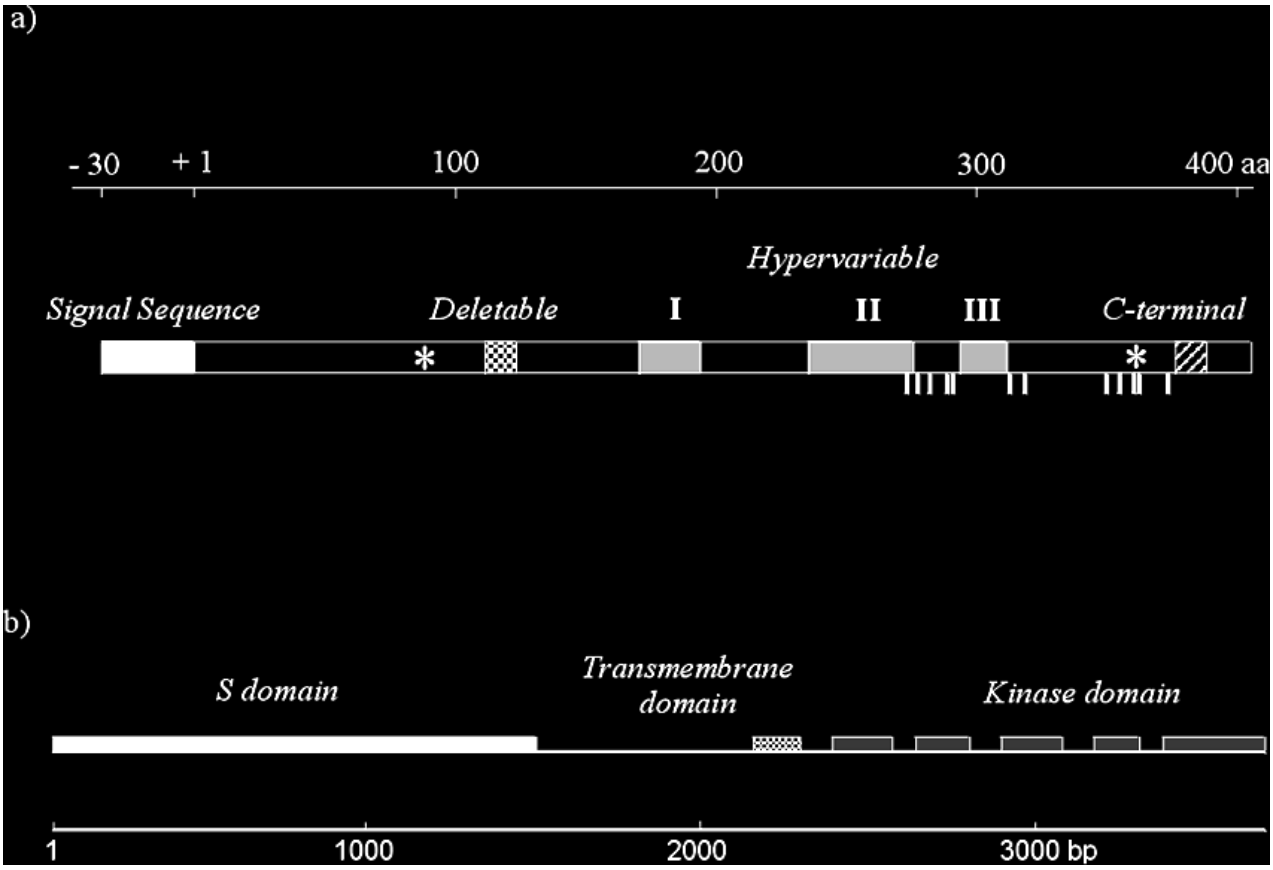

Figure 1. (a) Structure of the *SLG* gene in *Brassica.* Shaded boxes represent the hypervariable regions I, II, III. Filled, stippled, and hatches boxes show signal sequence, the deletable region, and the C-terminal variable region, * indicates perfectly conserved potential N-linked glycosylation sites. Boldface bars are the 12 conserved cysteine residues (Kusaba *et al.,* 1997). (b) Structure of the *SRK* gene, shaded boxes show exons (Stein *et al.,* 1991).

1993, Nasrallah *et al.,* 1994) and loss of *SLG* expression (Toriyama *et al.,* 1991, Nasrallah *et al.,* 1992, Shiba *et al.,* 1995) have been associated with SI. Another gene at the S□locus,*SCR* (S□locus cysteine-rich protein) encodes a protein expressed only in anthers and this has been identified as the male SI determinant (Schopfer *et al.,* 1999).

SLG gene (Figure 1a) is about 1.3 kb in length and encodes a 55kDa glycoprotein secreted into the papillar cell wall. Although the deduced amino acid sequence of *SLGs* are highly polymorphic, 12 conserved cysteine residues are found in common, suggesting the importance of the structure formed by the cysteine residues for the common function of the glycoprotein. There are several potential N-linked glycosylation sites, but only two conserved sites (Kusaba *et al.,* 1997). With respect to sequence similarity, *SLG* genes have been classified into two groups: class I and class II (Chen and Nasrallah 1990). Class I *SLG* genes exhibit about 65% homology in deduced amino acid sequence to class II *SLG* genes. Expressed S-locus glycoproteins can be discriminated using monoclonal antibodies between classes (Kandasamy *et al.,* 1989). Interestingly, all of the class II S haplotypes identified thus far are pollen recessive, whereas all of the class I S haplotypes are dominant (Okazaki *et al.,* 1999). This feature is common in *Brassica* species. None of the class I genes so far reported contains an intron. In contrast, an intron at the C terminus seems to be a typical structure of observed class II *SLG* genes (Hatakeyama *et al.,* 1998). In addition at least two *SLG* II genes have been shown to produce a transcript that encodes a membrane-bound isoform (Tantikanjana *et al.,* 1993, Cabrillac *et al.,* 1999). But Hatakeyama *et al.,* (1998) demonstrated that unusual structure of SLG^2 was not the sole determinant of its pollen-recessive nature. The role played by *SLG* in the SI response started to be questioned after the characterisation of self-incompatible plants which express a very low level of SLG, and, in particular, after the discovery of naturally self-compatible haplotypes which express a high level of SLG (Gaude *et al.,* 1995). New observations suggest that *SRK,* rather than *SLG*, plays a key role in the SI reaction (see below).

The *SRK* gene (Figure 1b) encodes a membrane-associated protein. A number of pieces of evidence indicate that a functional *SRK* is required for SI response in

Brassica. The predicted SRK protein consists of a potentially glycosylated extracellular domain (S domain) that shares extensive sequence similarity with SLG (90%) within haplotypes and contains 12 cysteine residues found in all members of the S□gene family in the *Brassicaceae*. This domain is joined via a single-pass transmembrane domain to a cytoplasmic region that has sequence similarity to protein kinases. Bacterially expressed SRK kinase domains show serine/threonine autophosphorylation activity (Goring and Rothstein 1992). SRK may be related to plant proteins involved in defence against pathogens, another plant recognition system (Pastuglia *et al.,* 1997a). In the S^3 haplotype of *B. oleracea*, *SRK* has also been shown to encode a truncated, soluble form of SRK corresponding to its extracellular domain: the eSRK protein (for extracellular domain of SRK). eSRK may be generated by alternative splicing of *SRK* transcripts. This result is particularly interesting because of the identification of soluble truncated forms of receptor kinases in some animal signal transduction pathways (PDGF receptor, EGF receptor) (Giranton *et al.,* 1995).

Other S-Locus-Linked Genes

To date, several S-linked genes have been identified in the region downstream of the *SLG* genes (~15). Molecular studies aimed at identifying pollen ligand (S locus linked and anther expressed gene) have resulted in the identification of several candidate genes. Boyes and Nasrallah (1995) have described a gene, designed *SLA* (S-locus anther), with anther-specific expression for the S^2 haplotype of *B. oleracea. SLA* is transcribed from two promoters to produce two complementary anther-specific transcripts, one spliced and the other unspliced that accumulate in an antiparallel manner in developing microspores and anthers. The sequence of the spliced transcript showed the presence of two open reading frames that predict proteins of 10 and 7.5 kDa. Neither transcript was produced in a self-compatible *B. napus* strain carrying an S^2-like haplotype, indicating that the *SLA* gene in this strain is nonfunctional. This result initially suggested that *SLA* played an essential role in the SI response, but Pastuglia *et al.,* (1997b) have found a mutant *SLA* allele, interrupted by a large insert resembling a retrotransposon in both self-incompatibility and self-compatibility lines of *B. oleracea.* These data indicate that a functional *SLA* gene is not required for the SI response in *Brassica.*

In the *B. napus* W1 line two S-linked genes *SLL1* and *SLL2* (for S□locus-linked genes 1 and 2, respectively) are located downstream of *SLG* gene (Yu *et al.,* 1996). The *SLL1* gene is S-locus specific, whereas the *SLL2* gene is not only present at the S-locus but is also present in other parts of the genomes in *Brassica* ssp lines. Expression of the *SLL1* gene is only detectable in anthers of self-incompatible plants and is developmentally regulated during anther development, whereas the *SLL2* gene is expressed in anthers and stigmas in both self-incompatible and self-compatible plants, with the highest levels of expression occurring in stigmas. Although *SLL1* and *SLL2* are linked to the S-locus region, it is not clear whether these genes function in self-incompatibility or serve some other cellular roles in pollen-pistil functions. The S-linked *ClpP* (protease homologue) gene also seems unlikely to function in the SI reaction because of its expression pattern (Letham and Nasrallah 1998).

Recently, three chromosomal "S" regions, the 910 and A14 S□haplotypes of *B. napus* (Cui *et al.,* 1999) and the S^9 haplotype of *B. rapa* (Suzuki *et al.,* 1999), have been completely sequenced and characterized. In addition to the previously described *SLG, SRK, SLL1, SLL2,* and *ClpP* genes, these studies have identified ~10 additional genes (Figure 2). Comparison of the gene organisations of the 910 and A14 haplotypes revealed a few striking structural features. Interestingly, the region between the *SLG-A14* and *SRK-A14* (~32 kb) is filled with retroelements and, in comparsion to the 910 haplotype, the distance is only 6 kb, and no gene/ORF was found in this sequence. According to Boyes *et al.,* (1997), Cui *et al.,* (1999) suggested that chromosomal rearrangements, accumulation of transposons, and haplotype-unique genes all have contributed to the heteromorphism in the S□locus, helping to suppress recombination and maintaining the gene pair as a genetic unit.

Suzuki *et al.* (1999) have demonstrated that four of the new *SP* genes (S-locus protein *SP5*, *SP6*, *SP8* and *SP11*) located at S□locus of the S^9 haplotype appear to be expressed specifically in reproductive organs. Therefore, the gene cluster around the S-locus, in addition to controlling SI, may be involved in developmental processes and/or cell-cell interactions in the reproductive organs. In tomato, the genes controlling floral trains have been mapped near the S-locus. Although the tomato S-locus is not homologous to the *Brassica* in the evolutionary sense, this suggests a possible gene complex participating in reproductive function (Bernacchi and Tanksley 1997).

An interesting finding in the study of Suzuki *et al.,* (1999) is that predicted mature protein of *SP11* gene is the same protein as male determinant (pollen ligant) of the SI (Figure 2). Schopfer *et al.,* (1999) have published this protein, which they named S□locus cysteine-rich protein (SCR), soon after the publication of Suzuki *et al.,* (1999) (see below).

***SLG* and *SRK* Related Genes**

Many different genes structurally related to *SLG* and *SRK* form a complex multigene family, expressed in the sexual and/or vegetative tissues of the *Brassicaceae* and more distantly related botanical families. This suggests that they take part in ubiquitous recognition mechanisms (Elleman and Dickinson, 1994). These genes that exhibit sequence similarity to the *SLG* genes, but that are genetically unlinked to the S-locus, are designated S-locus-related (*SLR*) genes. Three members of this family, denoted *SLR1*, *SLR2*, and *SLR3,* have been shown to be expressed (Lalonde *et al.* 1989, Boyes *et al.* 1991, Cock *et al.,* 1995). Genes *SLR1* and *SLR2* are linked to each other but unlinked (like *SLR3*) to S-locus thus they do not participate directly in the recognition of self and nonself in self-incompatibility. The *SLR1* is expressed in the stigma and secretes the corresponding protein into the cell walls of mature stigmatic papillae just like *SLG*. Using of FISH (florescence *in situ* hybridization) in *B. napus* have documented that *SLG* and

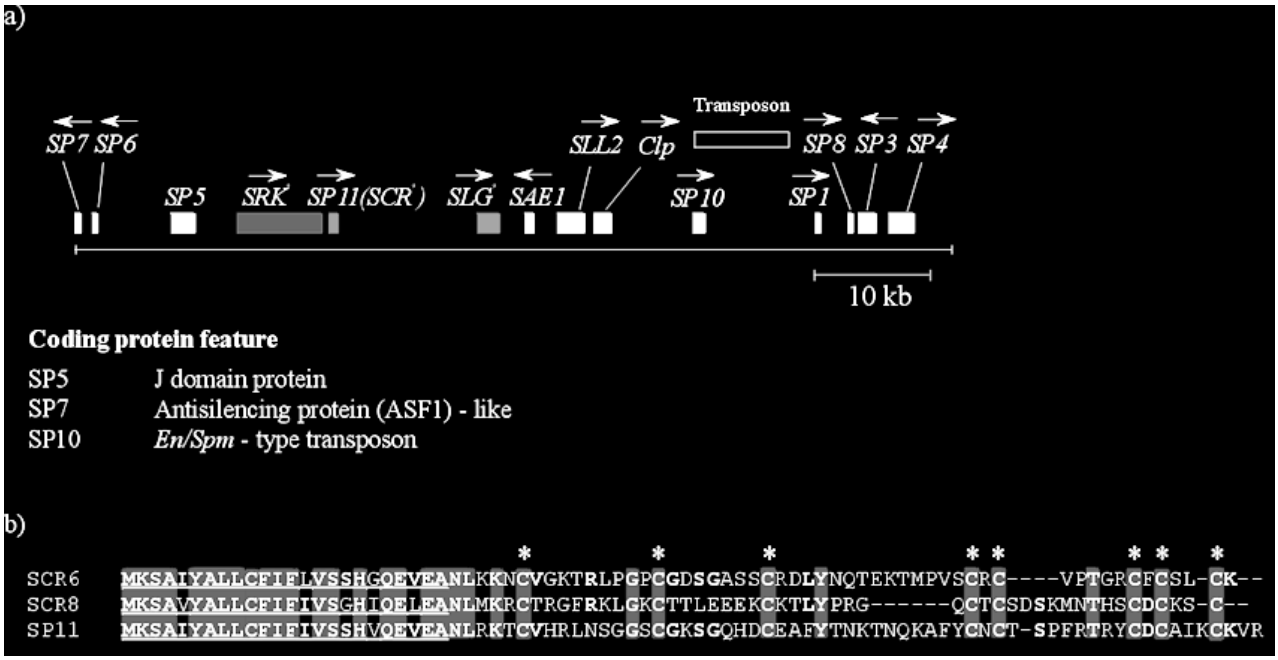

Figure 2. (a) Gene map of the 76 kb genomic fragment of the S^9 haplotype of *Brassica rapa.* Filled boxes denote locations of the 14 genes and horizontal arrows above the genes indicate the direction of transcription. The open box represents a transposon-like sequence (Suzuki *et al.,* 1999). (b) Amino acid sequence alignment of the predicted SCR^6, SCR^8 and SP11 proteins. The putative signal peptides are underlined. Bold letters mark amino acids that are identical in at least two sequences. Conserved amino acids are shaded. The eight conserved cysteine residues are indicated by asterisks.

SLR1 genes are located on different chromosomes and the *SLR1* genes were detected at homologous positions on both the *oleracea*- and *rapa*-type chromosomes (Kamisugi *et al.,* 1998). Transgenic plants with an antisense *SLR1* gene exhibited normal self-incompatibility despite having no detectable *SLR1* protein (Franklin *et al.,* 1996). However, Luu *et al.,* (1999) has demonstrated that antisense *SLR1* plants, with reduced levels of the SLR1 glycoprotein, display significantly altered kinetics of pollen adhesion. Consequently, *SLR1* (and *SLGs*) seem to be involved primarily in pollen-stigma adhesion.

SLR2 is also expressed in the stigma and shows a high degree of sequence similarly to *SLG* isolated from pollen-recessive haplotypes (Tantikanjana *et al.,* 1996; Kusaba *et al.,* 1997). *SLR3* appears to belong to a sub-family of S-related genes that are expressed with different patterns in vegetative and floral organs (Cock *et al.,* 1995). This is a novel pattern of expression compared with that of *SLG, SRK, SLR1* and *SLR2*, which are expressed specifically in stigmas and anthers.

PCP Proteins and the Male Determinant of Self-Incompatibility

Because neither the SLG nor the SRK proteins have been identified in anthers, the current model for SI in *Brassica* ssp. involves the presence of a protein receptor (ligand) on the pollen that is recognised by an SLG-SRK complex in stigma and that leads to pollen rejection. It was suggested to be a small molecule of the PCP (pollen coat protein) family located in the pollen coating (Stephenson *et al.* 1997). Doughty *et al.,* (1993, 1998) identified a small pollen coat protein PCP-A1, (for PCP*,* class A*,* 1), that binds with high affinity to SLGs (and SLR1) irrespective of S□genotype PCP-A1 is characterized by the presence of a structurally important motif consisting of eight cysteine residues shared by the plant defensins. They reported the cloning of *PCP-A1* from *Brassica oleracea* and demonstrated that it is unlinked to the S-locus. But recombinant analysis of the *B. rapa* S^8 haplotype has shown that the male SI determinant is contained in a 65-kb chromosomal segment encompassing *SLG* and *SRK* (Boyes *et al.,* 1997). *In situ* localization of *PCP-A1* transcripts revealed that they accumulate specifically in pollen at the late binucleate/ trinucleate stage of development rather than in the tapetum, which previously was taken to be the principal source of the pollen coat. In subsequent studies with self-compatible *B. napus,* proteins similar but not identical to PCP-A1 were found in the pollen coat that bound an SLG and SLR1 (Hiscock *et al.,* 1995, Dickinson *et al.,* 1998). Takayama *et al.,* (2000 b) have identified two proteins (SLR1-BP1 and SLR1-BP2) which are members of the PCP-A family. Kinetic analysis showed that these proteins specifically bound SLR1 with high affinity. Another PCP (PCP1) has also been identified in *B. oleracea* that although closely related to PCP-A1, lacks the ability to bind stigmatic S□proteins (Stancev *et al.,* 1996). Recently, as stated above, the *SCR* (or *SP11*) gene has been identified as the male determinant of SI. SCRs represent a new class of small, secreted, cysteine-rich proteins, distinguishable from members of the

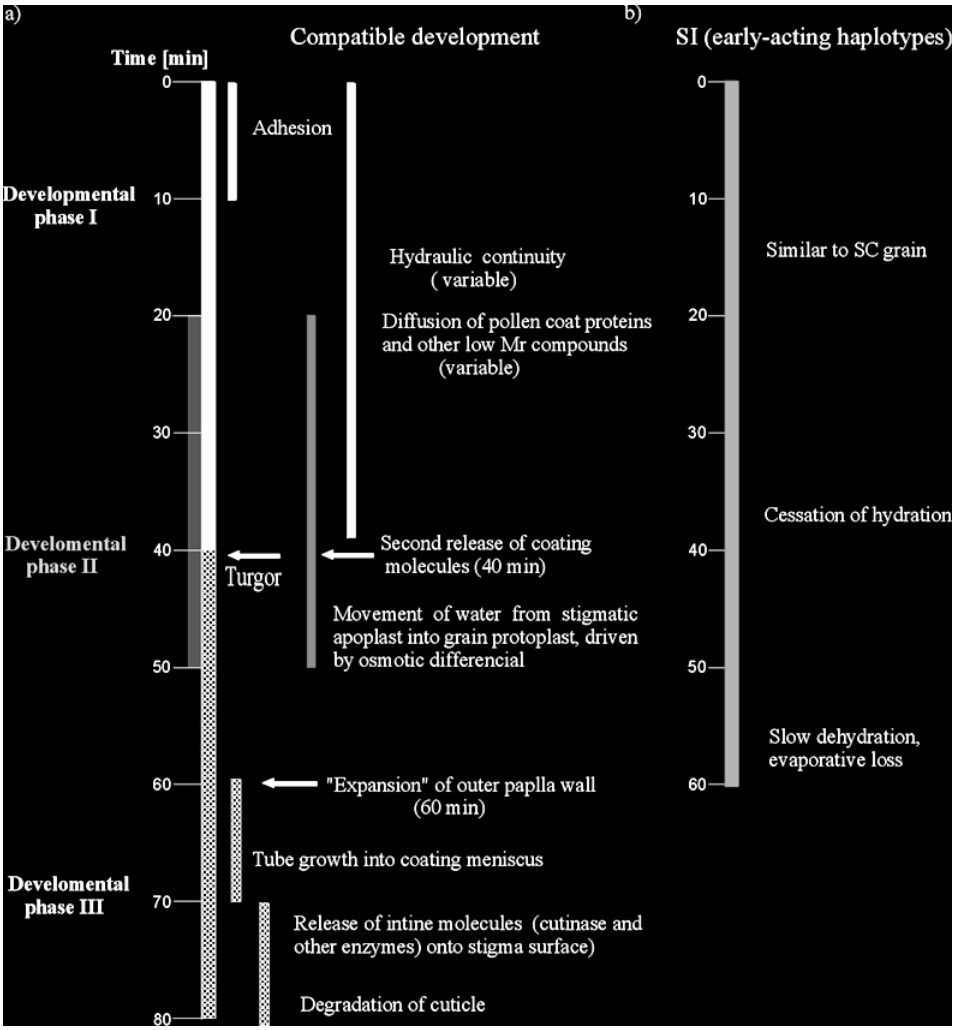

Figure 3. Pollen development on stigma surface in a) self-compatible and b) self-incompatible *Brassica oleracea* (according to Dickinson *et al.,* 1995).

PCP family. The *SCR* gene is tightly linked to the *SLG/SRK* pair and shows an anther specific developmentally regulated expression profile. The evidence that SCR is the pollen determinant of SI was obtained by two different approaches. First, *Brassica* plants transformed with the *SCR* gene of a new S□haplotype were found to acquire the new S□haplotype on the pollen side, but not on the stigma side (Schopfer *et al.,* 1999). Second, recombinant SCR protein produced in *Escherichia coli* was used in a pollination assay to show that it could induce the SI response in the stigma (Takayama *et al.,* 2000 a). Comparisons of the deduced amino acid sequence of SCR proteins from 21 S□haplotypes revealed a high degree of polymorphism (Watanabe *et al.,* 2000).

The SI Recognition Mechanism in *Brassica*

It was accepted that each *SLG* gene shows higher similarity to the *SRK* S□domain of the same haplotype than to any other *SLG* allele (Stein *et al.* 1991). It had been thought that an *SLG* would be more closely related in its sequence to the *SRK* of the same haplotype than to any other *SLG* because *SLG* and *SRK* of the same haplotype would have the same S specificity. During the last decade many *SLGs* and *SRKs* sequences became available for analysis. Some comparisons between *SLG* and *SRK* of the same S haplotypes showed considerable differences in the three hypervariable regions (see Figure 1), which are thought to be involved in the determination of S□specifity (Kusaba *et al.* 1997). Kusaba *et al.* (2000) have demonstrated that the amino acid sequence of the S□domain of *SRK* is highly conserved between S□haplotypes with the same recognition specificity suggesting that the S□domain is important for recognition. Whereas *SLG* varies much more than *SRK* between different lines that share the same self-recognition specificity. However, it has been found that the deduced amino acid sequences of the same *SLGs* from different haplotypes (S^{23} and S^{29} of *B. oleracea,* S^{46} and S^{8} of *B. rapa*) show extremely high similarity. In both cases, the S□domains of *SRKs* of these haplotypes were less similar. The S□domain of SRK^{8} and SRK^{46} exhibit only 85.6% similarity in amino acid sequence and the hypervariable

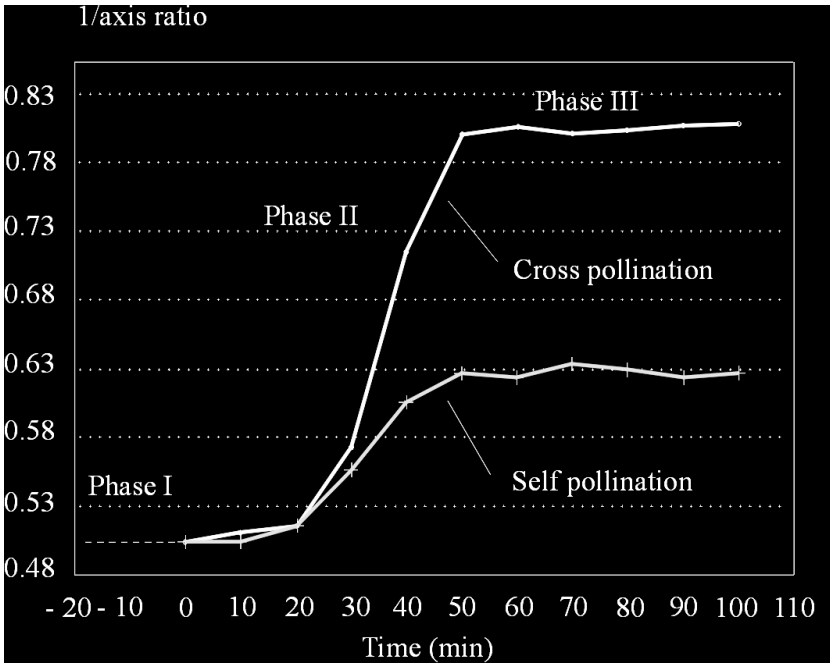

Figure 4. Hydration of self and cross pollen as represented by change in grain axis ratio with time (Elleman and Dickinson 1994).

regions of *SRK*[23] had a number of differences from those of *SRK*[29]. Although, the S□domain of *SRK*[7] from *B. oleracea* also exhibited very high similarity to *SRK*[46] from *B. rapa*, the hypervariable regions of these *SRKs* differed by two amino acid residues in the hypervariable region II (Kusaba and Nishio 1999). Moreover, Cabrillac *et al.,* (1999) have observed that S^{15} haplotype has two distinct *SLG* genes. This implies that the two *SLG* genes are redundant or that they are not required for recognition in SI.

Recently, Takasaki *et al.,* (2000) have confirmed that the *SRK*, but not *SLG* determines S□haplotype specificity in the pistil. They examined the function of *SRK* by introducing an *SRK* gene of S^{28}□haplotype (class I) into plants homozygous for a class II S^{60}□haplotype. Using this way they minimize the problem of co-suppression encountered in all the previous transformation experiments. Obtained *SRK*[28] transgene plants rejected pollen of S^{28} haplotype. The role of the *SLG* gene in the SI reaction remains unclear. Nasrallah *et al.,* (1992) demonstrated that the *scf-1* mutation, which reduces expression of *SLG* but not *SRK,* causes a self-compatible phenotype in *B. rapa.* But Gaude *et al.,* (1995) pointed out that there is also reduced expression of *SLR1* and *SLR2*. It became clear that *SLG* was not involved in S□haplotype specificity, however Takasaki *et al.,* (2000) have shown that, although *SRK* alone is sufficient for the pistil S□haplotype specificity, the self-incompatibility response is strongest if *SLG* of the same S□haplotype is also present.

Current Model of SI Reaction

By analogy with mammalian intercellular signalling mechanisms the SLG and SRK are envisaged as constituting a receptor complex. In the case of self-pollination, SCR protein (pollen ligand) is bound to SRK, resulting in activation of the receptor and initiation of a signal transduction cascade that ultimately leads to pollen inhibition (Kao and McCubbin 2000). It is possible that an SLG has a role in the binding of its cognate SRK with the pollen ligand by forming a complex with the S□domain of the SRK and facilitating the process of the recognition reaction (Takasaki *et al.,* 2000; Figure 5). The SI reaction is localized to the site of contact because in mixed pollinations of a papillar cell with incompatible and compatible pollen grains, the latter are not arrested (Sarker *et al.,* 1988). Some important data for the study of SI have been provided by investigation of pollen hydration.

For successful pollen tube growth and fertilization grain must rehydrate on the stigma. This requires stigmatic water to flow from the papillae through the pollen coat into the grains. In the *Brassicaceae*, the connection between the pollen and stigma in established by the formation of an appressorium-like structure from pollen coat material (Kandasamy *et al.,* 1995). Water flows into the grains via this connection. Ruiter *et al.,* (1997) has characterized oleosins, which allowed transport of water from the stigma to the pollen across the pollen coat. In the presence of SI haplotypes, hydration is arrested in the second phase, before the pollen becomes fully turgid (Figures 3 and 4). The second phase of hydration lasts barely 10 min and involves the rapid transfer of water to the grain until it achieves turgor (Zuberi and Dickinson 1985, Elleman and Dickinson 1994). Therefore, water transfer from stigmatic papillar cells to pollen is a one of the checkpoints at which the outcome of pollen-stigma interactions in the crucifer family is determined. By analysis of one genetic modifier of self-incompatibility, the spontaneous *mod* mutation of *B. rapa,* it has been shown that the MOD protein (aquaporin-like protein) probably forms a channel in the plasma membrane and transports water (Ikeda *et al.* 1997).

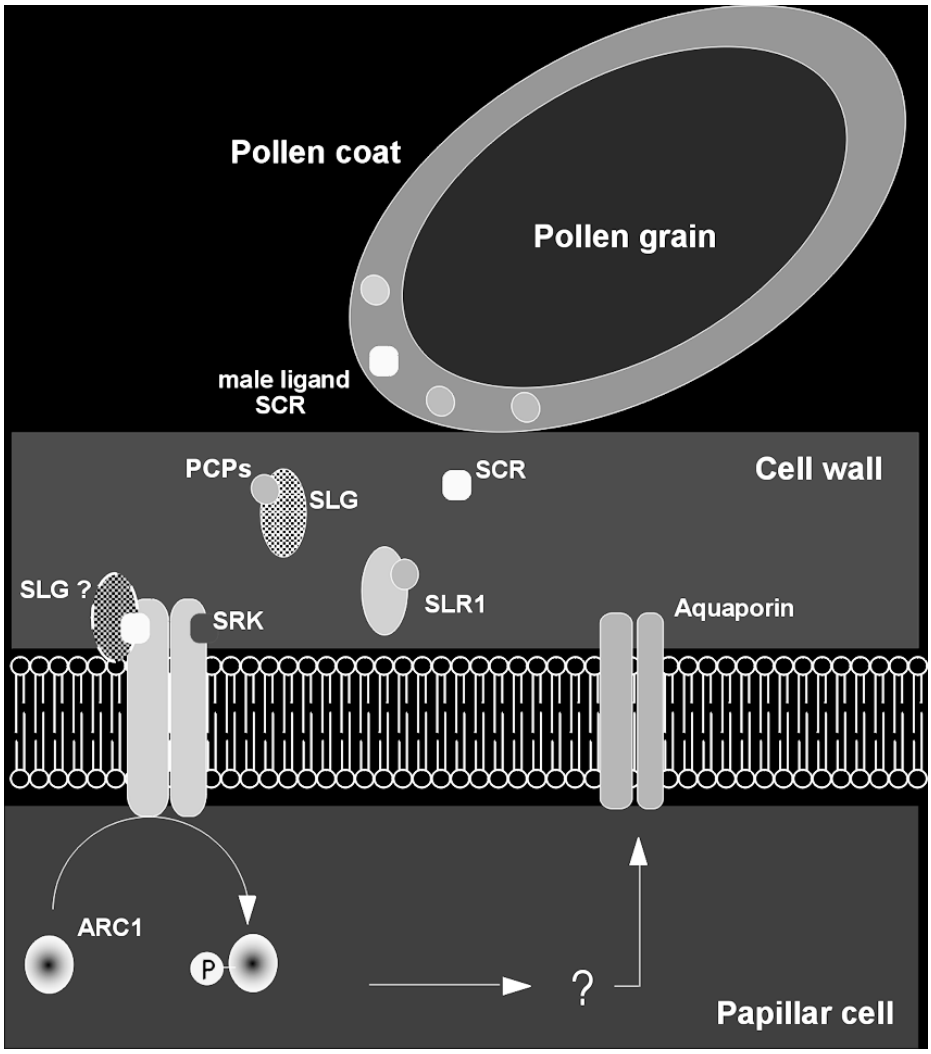

Figure 5. A model depicting the hypothetical interaction between SLG, SRK, SLR1, and pollen ligand SCR at the surface of a stigma epidermal cell. When a pollen grain alights on the papilla surface, the pollen coat, containing pollen coat proteins (PCPs – circles) and pollen ligand SCR (squares), flows to the cell wall. Arrows represents a signal transduction pathway initiated by activation of SRK and acting on its putative target, a membrane protein related to water-transporting aquaporins.

Upon pollination, the PCR protein (male determinant) must move, with other PCP proteins, into the stigmatic cell wall through the microchannels. This diffusion must occur at an early stage post-pollination (Figure 3), for once hydratation of the pollen grain has commenced, the rate of water flow through the microchannels suggests that diffusive movement into the stigma is unlikely (Dickinson 1995). PCPs are abundant in the pollen cell wall and contact with abundant SLG and SLR1 proteins in the stigma cell wall. SLG, which is freely diffusible within the cell wall, could bind PCR at the papillar cell wall-pollen interface for presentation at the membrane and would thus act as an extracellular regulator of ligand access to the signaling receptor (Figure 5). PCR protein is presumably bound to amino acid residues within the extracellular domain of SRK. In the classical model the SI response in initiated by ligand-dependent dimerization of SRK (Nasrallah *et al.,* 1994). Giranton *et al.,* (1999) have analysed the enzymatic properties of recombinant SRK and they suggest that signal transduction during SI response is mediated by modification of a pre-existing SRK oligomeric complex rather than by ligand-dependent dimerization of SRK molecules. Similar conclusions have been drawn in animals from the study of TGF-ß signal transduction via its serine/threonine kinase receptors (Wrana *et al.,* 1994). SRK/ligand interaction could allow the recruitment of cytoplasmic targets following a conformational change of the SRK kinase domain (Giranton *et al.,* 1999).

Little is understood about the molecular mechanisms in the SRK-mediated signal transduction pathway. Treatment of pistils with okadaic acid has led to a breakdown in *Brassica* self-incompatibility, suggesting that type 1 or type 2A phosphatases are involved in this pathway (Rundle *et al.,* 1993). In a search for components of the *Brassica* self-incompatibility signaling cascade, several proteins have been found to bind to the SRK kinase domain. Two members of the thioredoxin-h family interact specifically with the SRK kinase domain, however the interaction does not seem to be phosphorylation dependent (Bower *et al.,* 1996). Another protein that has been shown to interact with the SRK kinase domain is the kinase-associated protein phosphatase (KAPP; Braun *et al.,* 1997). Subsequent analysis revealed that KAPP binds to a number of different plant receptor kinases, suggesting that it may

play a more general regulatory role. Gu *et al.,* (1998) has isolated a plant gene called *ARC1* (Arm Repeat Containing) which encodes a protein that is specifically phosphorylated by the kinase domain from different SRK. In addition *ARC1* mRNA has been detected only in the stigma, where the self-incompatibility pathway would be occurring. Recently, Stone *et al.,* (1999) have provided definitive evidence that *ARC1* is required for SI response. A homology search of the amino acid sequence revealed the presence of arm repeats in the C-terminal region of ARC1. The arm repeats have been found to participate in protein interaction (Su *et al.,* 1993). The N-terminal half of ARC1 may represent another binding domain to interact with the next step of the signaling pathway.

At the end of this signaling pathway there is probably the aquaporin-like MOD protein. The activation of SRK on self-pollination would result in MOD activation and an increase in the flow of water into the papillar cell away from pollen, thus preventing adequate rates of pollen hydration (Ikeda *et al.,* 1997).

Other Control Mechanisms in SI

The observation of Dearnaley *et al.,* (1997) that raised humidity overcomes the SI hydration block and pollen growth is blocked at a latter stage is one of several pieces of evidence that self-incompatibility in *Brassica* has numerous checkpoints (Heslop-Harrison 1975). Dearnaley *et al.,* (1999) suggest that the involvement of the aquaporin-like gene in self-incompatibility in *Brassica* species may only operate at low relative humidities and that other control mechanisms function in other environmental conditions. Despite equivalent hydration frequencies in two pollen-stigma interactions used, the germination frequency of SI pollen is considerably lower than the compatible interaction. Since it is unlikely that SI pollen fails to hydrate fully under these conditions of high relative humidity, blockage of incompatible pollen germination supports the existence of a papilla-derived germination inhibitor as previously suggested. Blocking incompatible pollen tubes from penetrating papillae may be next step in this process. Further checkpoints could include the inhibition of tube growth in style similar to the gametophytic self-incompatibility system (Dzelzkalns *et al.,*1992). This assumption was recently supported by the observation of Stone *et al.,* (1999) that transgenic plants with *ARC1*-antisense gene are affected mainly at the early stages of the self-incompatibility response (adhesion, hydration and germination). Another unknown component of the signaling pathway must act later to block the infiltration of the pollen tube into the stigmatic papillar cell wall.

The role of Ca^{2+} in *Brassica* remains unclear although concentrations of these ions are critical for pollen tube growth. It is well documented that Ca^{2+} plays an important role in many plant signal transduction pathways. Franklin-Tong *et al.,* (1995) have shown that the SI response of *Papaver* pollen tubes is mediated by a rise in cytosolic Ca^{2+}. Dearnaley *et al.,* (1997) have found that both compatible and diverse incompatible pollinations in *Brassica napus* cause transient peaks in $[Ca^{2+}]_i$, but at varying frequencies.

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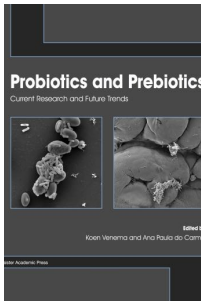

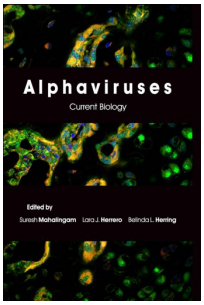

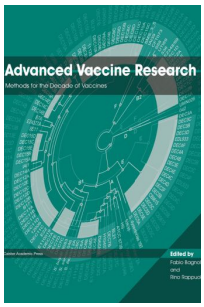

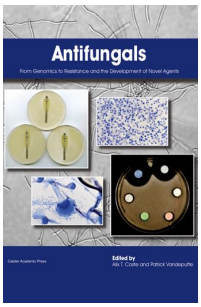

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