REVIEW PAPER

The pollen S-determinant in *Papaver*: comparisons with known plant receptors and protein ligand partners

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Abstract

Cell–cell communication is vital to multicellular organisms and much of it is controlled by the interactions of secreted protein ligands (or other molecules) with cell surface receptors. In plants, receptor–ligand interactions are known to control phenomena as diverse as floral abscission, shoot apical meristem maintenance, wound response, and self-incompatibility (SI). SI, in which ‘self’ (incompatible) pollen is rejected, is a classic cell–cell recognition system. Genetic control of SI is maintained by an S-locus, in which male (pollen) and female (pistil) S-determinants are encoded. In *Papaver rhoeas*, PrsS proteins encoded by the pistil S-determinant interact with incompatible pollen to effect inhibition of pollen growth via a Ca²⁺-dependent signalling network, resulting in programmed cell death of ‘self’ pollen. Recent studies are described here that identified and characterized the pollen S-determinant of SI in *P. rhoeas*. Cloning of three alleles of a highly polymorphic pollen-expressed gene, *PrpS*, which is linked to pistil-expressed *PrsS* revealed that *PrpS* encodes a novel ~20 kDa transmembrane protein. Use of antisense oligodeoxynucleotides provided data showing that *PrpS* functions in SI and is the pollen S-determinant. Identification of *PrpS* represents a milestone in the SI field. The nature of *PrpS* suggests that it belongs to a novel class of ‘receptor’ proteins. This opens up new questions about plant ‘receptor’–ligand pairs, and *PrpS*-PrsS have been examined in the light of what is known about other receptors and their protein–ligand pairs in plants.


Introduction

The ability to discriminate between self and non-self is important to all multicellular organisms. This is known as allorecognition, and enables organisms to construct defence systems to protect themselves from exogenous attack. Allorecognition is integral to the function of the animal immune response (Hughes, 2002), vegetative incompatibility in fungi (Glass et al., 2000), the plant hypersensitive response (Dangl and Jones, 2001), fusion histocompatibility in lower animals (Scofield et al., 1982; De Tomaso et al., 2005; Nyholm et al., 2006) and self-incompatibility (SI) in flowering plants (Takayama and Isogai, 2005; Franklin-Tong, 2008). Allorecognition systems rely upon loci with multiple alleles and high levels of polymorphism. These are maintained by negative frequency dependent selection, whereby new (and therefore rare) alleles within a population have higher fitness, and thus become more numerous. The relative fitness of new alleles decreases with their increasing frequency within the population, so when equilibrium is reached, the frequency of each allele is constant.
reached, high numbers of alleles are maintained within a population (Wright, 1948). Parallels between various non-analogous recognition systems were recognized, and their importance appreciated, long before the molecular basis of these systems was elucidated (Burnet, 1971; Nasrallah, 2002, 2005; De Tomaso et al., 2005). The evolution of these systems and the co-evolution of both components such that they maintain interaction while diversifying from parent alleles, are of considerable interest. While the origin and maintenance of these systems may appear similar, there are also key differences. For example, SI uses ‘self’ recognition, while the animal immune response and plant pathogen response recognize ‘non-self’. Here we examine what is known about perception of ‘self’ in SI, focusing on new data relating to the *Papaver* pollen *S*-determinant, and make comparisons with other recognition systems that utilize classic receptor–ligand type interactions.

**Self-incompatibility (SI)**

Many plants are hermaphrodites, a strategy which increases the chances of an individual plant having progeny. Because of this, it is possible that an individual plant will self-fertilize. To avoid self-fertilization and concomitant problems with inbreeding depression, many plants have evolved self-incompatibility (SI). These genetically controlled systems with inbreeding depression, many plants have evolved to fertilize. To avoid self-fertilization and concomitant problems caused by this, it is possible that an individual plant will self-fertilize. Pollen and pistil tissues. Crucially, when two cognate allelic determinants interact, they should trigger an SI response, thus allowing discrimination between ‘self’ (incompatible) pollen, which is rejected, and ‘non-self’ (compatible) pollen, which is allowed to fertilize.

There are key criteria which apply to all SI systems without exception. They must have two core components: a male (pollen) *S*-determinant and a female (pistil) *S*-determinant. The two *S*-determinants for cognate recognition need to be physically linked at the *S*-locus. They must be inherited together otherwise the SI system would not be maintained. Pollen and pistil *S*-determinants are also expected to have co-evolved. SI systems depend upon the *S*-determinant genes being multi-allelic (with as many as 60 alleles at a single locus). Thus, another criterion is that the *S*-determinant alleles must be highly polymorphic, as this allows for discrimination and recognition specificity. Because these genes function in such a specific manner, it is expected (and it has been found without exception) that they display tissue-specific and developmental expression in pollen and pistil tissues. Crucially, when two cognate allelic gene products interact, they should trigger an SI response, resulting in the inhibition of incompatible pollen.

For many years it has been a major goal of research into SI to identify the two core SI components: the pollen and pistil *S*-determinants. Knowing the identity of the genes and proteins responsible for this interaction will provide us with a better understanding of the basis for this cell-cell recognition system, mechanisms involved in the prevention of ‘selfing’, and how SI systems have evolved. From analysis of the gene sequences identified as pollen and pistil *S*-determinants in a range of different species it is clear that SI has evolved independently several times (Allen and Hiscock, 2008). Three SI systems have been particularly well characterized at a molecular level. These comprise the *Brassica* SI system, which has sporophytic control; the S-RNase system which is gametophytically determined and found in three families: the Solanaceae, Rosaceae, and Plantaginaeaceae; and the *Papaver* system, also determined by the genotype of the gametophyte. A good review of the genetics, genes identified as the *S*-determinants and the mechanisms thought to be involved in inhibition of incompatible pollen can be found in Takayama and Isogai (2005). More recent information can be found in Franklin-Tong (2008). Studying SI in *Brassica* and *Papaver* has led to insights into the ligands and receptors involved in this interaction and knowledge about some of the downstream intracellular signalling events and mechanisms involved in the regulation of pollen tube growth. The S-RNase SI system utilizes a different type of interaction.

**SI in Brassica**

In *Brassica* SI, the pistil *S*-determinant was identified as a member of a gene family that included an *S*-Linked Glycoprotein, *SLG* (Nasrallah et al., 1985). This led to the identification of *SRK* (*S*-Receptor Kinase), a receptor kinase gene that is linked to the *S*-locus (Stein et al., 1991). Its role in SI was demonstrated using transformation, although this was not straightforward due to problems caused by co-suppression (Takasaki, 2000). *SRK* belongs to the large family of plant receptor-like kinases (RLK). Many different genes encoding RLKs have been identified and analysed (Morillo and Tax, 2006). The identification of *SRK* as the pistil *S*-determinant provided a clear model for the operation of *Brassica* SI, namely, that SI triggered a classic receptor-mediated signalling cascade.

The pollen *S*-determinant was identified by sequencing around the *S*-locus (Schopfer et al., 1999; Suzuki et al., 1999). This identified *SCR/SP11*, and its function in SI as the pollen *S*-determinant was confirmed by transformation experiments (Schopfer et al., 1999). The protein encoded by *SCR/SP11* is a small (~9 kDa), cysteine-rich, secreted protein, which is a member of the defensin superfamily. The identity of *SCR/SP11* as a small secreted protein suggested it might function as a ligand that interacted with *SRK*. Evidence for physical interaction between cognate *SRK* and *SP11* has been shown (Kachroo et al., 2001; Takayama et al., 2001). Interaction results in transphosphorylation and dimerization of the kinase domain of *SRK*, leading to activation of elements of a signalling cascade (Giranton et al., 2000). Thus in *Brassica* SI, a signalling ligand, *SCR/SP11*, derived from the pollen coat, is perceived at the surface of the stigmatic papilla cells.

Other components have been shown to be involved in downstream events in SI in *Brassica*. A protein named ARC1 (Arm Repeat Containing) was identified as interacting with the kinase domain of *SRK* in a phosphorylation-dependent manner (Stone et al., 2003). ARC1 has an E3 ubiquitin ligase activity, which provides further clues to downstream events
in *Brassica* SI. Another kinase, MLPK (*M*-Locus Protein Kinase) has been shown to play a role in SI downstream of SRK (Murase et al., 2004). This is a novel cytoplasmic serine/threonine protein kinase anchored to the plasma membrane of papilla cells. Data suggest that it interacts with SRK to transduce SI signalling (Kakita et al., 2007). Thioredoxin-h-like proteins have also been shown to interact with SRK (Bower et al., 1996; Cabrillac et al., 2001). Thus, there is considerable amount of information about signalling components interacting with SRK to mediate SI in *Brassica*. However, the mechanism by which this signal feeds back to result in incompatible pollen rejection is currently unknown. Most recently, transgenic studies have demonstrated involvement of Exo70A1, a putative component of the exocyst complex, in regulating SI in *B. napus* (Samuel et al., 2009). The exocyst complex is known to regulate polarized secretion, so brings new information to models about *Brassica* SI. Other studies have focused on how cellular alteration is triggered by SI which we cannot cover here.

### The S-RNase SI system

The S-RNase system of SI, exhibited by the Solanaceae, Rosaceae, and Plantaginaceae, was named because the female S-determinant is a ribonuclease (Anderson et al., 1986; Murfett et al., 1994). This identification provided a clear potential mechanism for the inhibition of incompatible pollen in this system; the S-RNase is proposed to act as a cytotoxin. Thus, data indicate that this important self-recognition system does not utilize a classic receptor–ligand type interaction. It has been shown that S-RNases enter pollen tubes, but in a non-discriminatory manner (Luu et al., 2008). Although rRNA degradation occurs in incompatible pollen tubes, it is difficult to know whether this is the direct cause of pollen rejection or a secondary effect of SI. The male S-determinant of this system encodes an F-box protein and was named *SLF* (*S*-locus F box) (Lai et al., 2002; Sijacic et al., 2004). Evidence that *SLF* encodes the pollen S-determinant was provided using transformation (Qiao et al., 2004; Sijacic et al., 2004). Subsequently, S-linked F-box genes have been identified in several different species, including *Prunus dulcis* (Ushijima et al., 2003) and *P. mume* (Entani et al., 2003). *SLF* genes have a high degree of sequence polymorphism, but because this is a large gene family and there are so many of them in the vicinity of the *S*-locus, identifying which is the pollen S-determinant is complicated (see Sassa et al., 2009, for a recent review). As mentioned earlier, it is expected that pollen and pistil S-determinants evolve together, as gene pairs. However, sequence analyses suggest that the *S-RNase* genes do not appear to have always co-evolved with adjacent *SLF* genes (Newbiggin et al., 2008). This raises the question of whether they interact in a simple lock-and-key manner as expected, or whether there is some other way in which they operate.

Identifying the F-box proteins as male S-determinants provided a potential mechanism for the operation of this SI system. F-box proteins function as part of the E3 ubiquitin–ligase complex, which targets proteins for degradation (Qiao et al., 2004; Hua and Kao, 2006). This has led to the model of non-self S-RNase degradation whereby S-RNases are ubiquitinated using some sort of (unknown) discriminatory system (see Hua et al., 2008, for a recent review). However, the story is not simple, as in some SI systems, notably the Solanaceae, other non-S-linked pistil proteins, such as HT-B and 120K, are taken up by the pollen tube. This provides another (not necessarily mutually exclusive) model for the operation of SI, involving compartmentalization of S-RNases in compatible pollen to restrict S-RNase cytotoxicity, with HT-B stability regulating the breakdown of vacuolar compartments and the release of S-RNases (Goldraij et al., 2006). Thus, this SI system reveals a very different basis and mechanism for achieving ‘self’ recognition and rejection.

### SI in *Papaver*

In *Papaver rhoeas* (the field poppy), considerable effort has focused on attempting to establish the function of the SI-specific events triggered in incompatible pollen. There is a well-integrated and co-ordinated Ca\(^{2+}\)-dependent signalling response in incompatible pollen, resulting in the rapid inhibition of pollen tube growth, involving major alterations to the actin (Geitmann et al., 2000; Snowman et al., 2002; Thomas et al., 2006) and microtubule (Poulter et al., 2008) cytoskeletons, culminating in programmed cell death (Bosch and Franklin-Tong, 2007; Thomas and Franklin-Tong, 2004). We will not review key data on the intracellular signalling events or nature of cell death in this SI system here. For that, the reader is referred to recent reviews (Franklin-Tong, 2007; Bosch and Franklin-Tong, 2008; Bosch et al., 2008). Much effort has also been spent attempting to establish the nature of the pollen S-determinant. Here the focus is on data relating to the male and female S-determinants, which are responsible for the initial cell-cell recognition event in *Papaver* SI. Recent evidence identifying PrpS as the pollen S-determinant represents a milestone, and fills a major gap in our knowledge of this SI system.

### The female S-determinant in *Papaver rhoeas*

The pistil S determinant for *P. rhoeas* was identified some time ago, using N-terminal amino acid sequencing of pistil proteins segregating with *S*-haplotypes, separated on isoelectric focusing gels (Foote et al., 1994; Walker et al., 1996; Kurup et al., 1998). Until recently, the pistil-S determinant in *P. rhoeas* was simply described as ‘S’ gene’ and/or ‘S’ protein’, however, the recent discovery of the pollen S-determinant necessitated renaming the pistil S-determinant *PrsS* (for *Papaver rhoeas* stigma S determinant) in order to provide a clearer nomenclature (Wheeler et al., 2009).

The PrsS protein is a small (~15 kDa) protein secreted by the stigmatic papilla cells (Foote et al., 1994). There is sequence information for four *PrsS* alleles in *P. rhoeas* (*PrsS_1*, *PrsS_3*, *PrsS_7* and *PrsS_8*) and one from *P. nudicaule*.
(PrsSn1). The primary amino acid sequence of the proteins encoded by PrsS is very polymorphic (40–46% divergence between alleles; Walker et al., 1996), but they all have a highly conserved predicted secondary structure comprising several β-strands separated by hydrophilic loops. No obvious hypervariable regions exist, but site-directed mutagenesis revealed that sites in hydrophilic loops 2 and 6 are essential for biological activity (Kakeda et al., 1999). Although, when they were first identified, PrsS proteins had no clear homologues in the databases, they have since been found to be members of a large, plant-specific protein family named SPH (S-Protein Homologue) (Ride et al., 1998). In Arabidopsis thaliana the SPH family has 84 members. Most of the SPH proteins are floral-expressed, but at least two (SPH1 and SPH74) are expressed in developing leaves and appear to be involved at the interface of leaf development and plant defence responses (MJ Wheeler, EM Bell, EB Holub, J Ride, VE Franklin-Tong, FCH Franklin, unpublished data).

As the Papaver female S-determinant, PrsS has a signal peptide and is secreted, this suggested that it may act as a signalling ligand. Considerable experimental data provided good evidence that this was the case. Recombinant PrsS proteins can be expressed in E. coli and they retain their biological activity. Use of an in vitro bioassay (Franklin-Tong et al., 1988) established that PrsS interacts with pollen in an S-specific manner and that incompatible interactions trigger a rapid increase in cytosolic free Ca\(^{2+}\) in incompatible pollen tubes (Franklin-Tong et al., 1993).

Subsequent studies showed that Ca\(^{2+}\) influx is stimulated by SI (Franklin-Tong et al., 2002) and that interactions between PrsS and incompatible pollen activate downstream signalling events resulting in programmed cell death (Franklin-Tong, 2007; Bosch and Franklin-Tong, 2008; Bosch et al., 2008). Thus, it was expected that the male S-determinant was likely to be a membrane-bound receptor that interacted with PrsS.

The male S-determinant in Papaver rhoesas

The pollen S-determinant was recently identified (Wheeler et al., 2009). As both male and female S-determinants must be encoded by the S-locus, the strategy used for the identification of pollen-S was by using sequence analysis of the S-locus adjacent to the female S-determinant. Sequencing of a cosmid clone containing the P. rhoesas S1 locus resulted in the identification of a putative open reading frame <0.5 kb from PrsS1. RT-PCR revealed that PrpS was transcribed in pollen and that the level of transcript increased during anther development (Wheeler et al., 2009). As this pollen-expressed gene was proximal to the pistil S-determinant, it was investigated further as a candidate for pollen S and designated PrpS (P. rhoesas pollen S) (Wheeler et al., 2009). As PrpS was identified so close to PrsS1, sequencing around the PrsS gene of each haplotype was initially attempted to identify alleles of PrpS from plants of S-haplotypes S1 and S8. Inverse PCR was used to isolate 7 kb of sequence adjacent to PrsS1 and 6 kb of sequence adjacent to PrsS8. However, analysis of this did not identify any sequences homologous to PrpS1. This is not altogether surprising, given the variable size of S-loci in other species (Cui et al., 1999). An alternative approach, using a combination of 3′- and 5′-RACE-PCR and RT-PCR employing degenerate primers designed to PrpS1, was used to identify two further alleles: PrpS3 and PrpS8. Southern blotting established that PrpS was a single copy gene, so providing confidence that these are alleles of a single gene, rather than other members of a multi-gene family.

The open reading frames of both PrpS1 and PrpS8 encode a predicted protein of ~20 kDa. Using PCR with oligonucleotide primers designed to the open reading frame on all three PrpS alleles, the segregation of PrpSI and PrpS8 was examined in segregating full-sib families. This established that each PrpS allele was linked to its cognate PrsS allele; i.e. PrpS1 co-segregated with PrsS1, PrpS8 was co-segregated with PrsS8, and PrpS1 co-segregated with PrsS1 (Wheeler et al., 2009). Thus, all three alleles of PrpS are at the S-locus.

As mentioned earlier, a high level of allelic sequence polymorphism is a well-documented feature of S-locus proteins (Ioerger, 1990; Charlesworth, 1995; Kohn, 2008). Papaver rhoesas is no exception; the pistil proteins PrsS1 and PrsS3 exhibit 46% sequence divergence; PrsS1 and PrsS8 have 40%, and PrsS3 and PrsS8 have 46% divergence. Analyses of PrpS proteins show that they exhibit a similar level of polymorphism to PrsS proteins; the PrpS1 and PrpS3 predicted amino acid sequences are 50% divergent; PrpS1 and PrpS8 exhibit 40% divergence; PrpS3 and PrpS8 are 47% divergent (Wheeler et al., 2009). There are two conserved regions within PrpS proteins. One overlaps with part of a predicted extracellular domain; the other comprises part of a hydrophobic region around the centre of the protein.

Demonstrating PrpS function

Although PrpS had the criteria expected of a pollen S-determinant, it was necessary to establish that it had the correct biological function of a role in S-specific inhibition of pollen tube growth. The hypothesis was that knockdown of PrpS would result in alleviation of pollen tube inhibition in an S-specific manner. As stable transformations are not possible in P. rhoesas, the in vitro SI bioassay (Foote et al., 1994) was used, in combination with antisense oligodeoxynucleotide (as-ODN) treatment of pollen tubes (de Graaf et al., 2006). Pollen grown in the presence of ‘self’ recombinant PrsS proteins resulted in the cessation of pollen tube growth; thus, all pollen from plants of S-haplotypes S1–S3 challenged with recombinant PrsS1 and PrsS3 was inhibited. When as-ODNs specifically designed to knock down only PrpS1 were added, inhibition of 50% of pollen (assumed to be carrying S1) was significantly alleviated (Fig. 1). No alleviation of the SI response occurred either with sense oligodeoxynucleotides (s-ODN) or with as-ODNs designed to PrpS8. This showed allelic specificity of the alleviation. Alleviation of SI in pollen from S3S8 plants was also demonstrated. S3S8 pollen exhibited a full SI response in the presence of recombinant PrsS3 and PrsS8, which was alleviated in the
presence of as-ODNs designed to knock down PrpS8, but not by either s-PrpS8-ODNs or as-PrpS1-ODNs (Wheeler et al., 2009). Thus, the PrpS as-ODNs alleviate the SI response, demonstrating that PrpS plays a functional role in Papaver SI, and that it acts in an S-specific manner. This provided firm evidence that PrpS is the pollen S-determinant (Wheeler et al., 2009).

**What is PrpS?**

Sequence information predicts that PrpS encodes a highly hydrophobic protein with several predicted transmembrane passes; the exact topology is not yet known; Fig. 2 shows two potential secondary structure predictions for PrpS. Experimental data provided evidence that PrpS is a transmembrane protein. Western blotting detected PrpS in pollen membrane-enriched extracts, and immunolocalization demonstrated localization at the pollen tube plasma membrane (Poulter, 2009; Wheeler et al., 2009); GFP-fusions confirm this localization (BHJ de Graaf et al., unpublished data). There is some evidence for endocytosis and/or recycling, as PrpS is also detected internally (Poulter, 2009); endocytosis and recycling of animal receptor proteins is common (Maxfield and McGraw, 2004).

Notably, regardless of its precise topology, all structural predictions suggest PrpS has an extracellular domain of around 35 amino acids. It was postulated that this region might be involved in binding to PrsS, and this was confirmed experimentally. Using a Western-ligand blot approach, binding of PrsS1 to peptides corresponding to the extracellular domain of PrpS1 was detected (Wheeler et al., 2009). Furthermore, this appears to be an S-specific interaction, as pistil PrsS1 did not bind to corresponding peptides for the extracellular domain of PrpS8 (Fig. 3). In addition to the Western-ligand blotting data, artificial peptides based on the predicted extracellular region of PrpS1, when added to the in vitro SI bioassay, alleviated SI-induced incompatible pollen inhibition (Wheeler et al., 2009). This provides further evidence for S-haplotype specific interactions between PrsS and PrpS. Thus, PrpS has plasma membrane localization and functions in SI-mediated pollen inhibition.

Having established that PrpS is the poppy pollen S-determinant, comprising a transmembrane protein, and that its predicted extracellular loop region interacts with PrsS, an

![Fig. 1. PrpS function demonstrated by use of anti-sense oligodeoxynucleotides. Pollen of S-haplotypes S1S3 was challenged with recombinant PrsS1. This produced an incompatible response in half of the pollen (haplotype S1) which was quantified by measuring pollen tube lengths. Addition of antisense oligodeoxynucleotide designed to the PrpS1 open reading frame (as-PrpS1) alleviated the SI response and sense oligodeoxynucleotide (s-PrpS1) had no alleviating effect. Neither antisense or sense oligodeoxynucleotide designed to PrpS8 sequence (as-PrpS8 and s-PrpS8) had any effect on SI, thereby demonstrating that the alleviation of inhibition was S-specific. Fifty pollen tubes were measured in three independent experiments (150 in total); error bars indicate s.e.m.](image)

![Fig. 2. Possible structural topologies of PrpS1. Several different programmes were used to predict the topology of PrpS proteins. Two possible predictions of PrpS1 topology are shown: (a) TMHMM (Krogh et al., 2001) predicts three transmembrane spanning domains and (b) PredictProtein (Rost et al., 2004) predicts four transmembrane domains. A consistent feature of the predictions is a ~35 amino acid extracellular loop (indicated in dark grey). Functional studies have demonstrated that this region is involved in the interaction between PrpS and PrsS and so is likely to be the region of PrpS in which S-allelic specificity resides. The numbers indicate the amino acid residue for PrpS1.](image)
obvious question is: what is PrpS? PrpS is a novel gene, encoding a protein with no immediately identifiable function. Extensive searches of databases have failed to identify orthologues of PrpS genes in other genomes. Interestingly, the gene fester, which was recently identified as a protochorperate allrecognition receptor (Nyholm et al., 2006) has similarities to PrpS, although they share no homology. Both encode small transmembrane proteins with no strong homology to any other proteins in the database, and no other recognizable motifs. Thus, here is another example of a cell–cell recognition determinant which is completely novel. This will no doubt open up further debate about the origins and evolution of self/non-self recognition systems in general. It has been assumed for many years that the Papaver pollen S-determinant would be some sort of receptor, as interaction of PrsS with incompatible pollen resulted in the triggering of an intracellular signalling network. It certainly is one-half of an interacting cognate pair, comprising PrsS-PrpS, and has an unequivocal biological function, specifying self-recognition. However, does it conform to current ideas about plant transmembrane receptors?

**Known plant protein–ligand/receptor pairs**

Plant genomes have thousands of transmembrane proteins located at the plasma membrane (Schwacke et al., 2003). However, few of these proteins have known functions. Despite the lack of data regarding their function, many plasma membrane-located proteins are likely to act as receptors. Some receptors are involved in the perception of non-protein signals; the best characterized of these include receptors that perceive ethylene (Chang et al., 1993), abscisic acid (Pandey et al., 2009), and brassinosteroids (Wang et al., 2001). However a small, but growing, number of receptors are known to respond to endogenous protein ligands. Plant genomes encode many hundreds of secreted proteins very little is known about the functions of these putative protein ligands, and only a handful of known protein–ligand/receptor pairs are known (Matsubayashi, 2003); see Table 1.

In all known plant protein–ligand/receptor interacting pairs identified to date, the receptor involved is a receptor kinase (Table 1). The Brassica SI pollen S-ligand (SCR/SP11) and its cognate receptor (SRK), which is a serine/threonine receptor kinase belongs to this class/group. How this particular pair operates was described briefly earlier. Perhaps the best-known example of a plant protein–ligand/receptor pair is CLV(CLAVATA)1/CLV3. Interaction of a processed peptide derived from CLV3 (Fletcher et al., 1999; Ogawa et al., 2008) with the receptor-like kinase CLV1 in a complex with another membrane-bound protein CLV2, initiates a signalling network involved in regulating the ability of the plant shoot to exhibit both determinate development.

**Table 1.** Receptor-protein ligand pairs identified to date in plants

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Receptor</th>
<th>Type of receptor</th>
<th>System under control of interacting pair</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLV3</td>
<td>CLV1/CLV2</td>
<td>Leucine Rich Repeat</td>
<td>Control of shoot apical meristem size in <em>Arabidopsis thaliana</em></td>
<td>Ogawa et al., 2008</td>
</tr>
<tr>
<td>SCR/SP11</td>
<td>SRK</td>
<td>Receptor-like kinase</td>
<td>Self incompatibility in <em>Brassica</em> spp.</td>
<td>Kachroo et al., 2001; Takayama et al., 2001</td>
</tr>
<tr>
<td>IDA</td>
<td>HAE/HSL2</td>
<td>LRR-RLK</td>
<td>Control of floral abscission in <em>A. thaliana</em></td>
<td>Cho et al., 2008; Stervink et al., 2008</td>
</tr>
<tr>
<td>PSK</td>
<td>PSKR1</td>
<td>LRR-RLK</td>
<td>Cellular differentiation</td>
<td>Matsubayashi and Sakagami, 2000</td>
</tr>
<tr>
<td>systemin</td>
<td>SR160</td>
<td>LRR-RLK</td>
<td>Mediation of the systemic wound response in tomato</td>
<td>Scheer and Ryan, 2002</td>
</tr>
<tr>
<td>PrsS</td>
<td>PrpS</td>
<td>Novel, unknown</td>
<td>Self incompatibility in <em>Papaver rhoes</em></td>
<td>Wheeler et al., 2009</td>
</tr>
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</table>

**Fig. 3.** Demonstration of interaction between PrpS and PrsS. The S-specific interaction between recombinant PrsS$_1$ and the 15-mer residues within the predicted extracellular domain of PrpS$_1$ along with its randomized control peptide was tested using a western-ligand blot assay. A dilution series of 15-mer PrpS peptides (in μg, as indicated) corresponding to part of the 35 amino acid predicted extracellular domain together with randomized peptides were bound to the PVDF membrane. Recombinant PrsS$_1$ proteins were also bound to demonstrate S-specificity of the antiserum. The PVDF membrane was then incubated with recombinant PrsS$_1$ protein and binding of PrsS$_1$ to PrpS$_1$ was detected using PrsS$_1$ antiserum. S-specific binding of PrsS$_1$ to PrpS$_1$ and not to PrpS$_8$ was observed. No binding was detected between PrsS$_1$ and the randomized control peptide.
and indeterminate growth (Clark, 2001). Another plant protein-ligand/receptor pair is IDA/HAE. IDA (inflorescence deficient in abscission) encodes a secreted protein ligand involved in regulating floral abscission (Butenko et al., 2003). HAE (HAESA) and HSL2 (HAESA-like 2) are receptor kinase-encoding genes epistatic to IDA and thought likely to encode the interacting partners (Cho et al., 2008; Stenvik et al., 2008). Thus, the canonical plant receptor involved in the perception of secreted protein ligands is a receptor kinase (see Chinchilla et al., 2009; De Smet et al., 2009, for recent reviews on this topic).

Crucially, the *Papaver* PrpS amino acid sequence contains no kinase or other known catalytic domains, so PrpS is clearly not a receptor kinase. Thus, PrpS-PrsS stands alone amongst those transmembrane receptors currently identified and characterized as perceiving endogenous protein ligands. Knowing this, is it correct to call PrpS a ‘receptor’? It all depends on what is meant by this terminology. It clearly is not a ‘classic’ defined/identified receptor. It is proposed that PrpS acts as a novel class of ‘receptor’ that interacts with PrsS in a very specific manner and triggers an intracellular signalling network resulting in a specific biological response. The fact that PrpS cannot be assigned to a specific family of plant membrane proteins is not altogether surprising. Although 18% (4589 genes) of all *Arabidopsis* genes encode proteins with two or more transmembrane domains, of these over three thousand have no known function, nor do they belong to families with known function (Ward, 2001). This indicates that there are likely to be other classes of transmembrane proteins with ‘receptor’-like function that have so far not been characterized. Thus, it is thought that PrpS falls into this challenging category.

**How does PrpS elicit an SI response?**

How then, is perception of ‘self’, mediated by interaction of PrpS with PrsS, transduced into a downstream signalling network culminating in the rejection and death of incompatible pollen? There are two possibilities. Firstly, PrpS may act as a part of a complex representing the component vital for perception of PrsS and specificity of the interaction, but not directly transducing the signal. Secondly, in a simpler scenario, PrpS would be both the perceptive apparatus and the transducer of the SI signal.

In the first scenario, PrpS could be part of a receptor complex with a catalytic domain encoded by another gene that is not linked to the S-locus. There are many examples of ligand perception by heterodimeric receptor complexes in animal biology (Wells and de Vos, 1996; Huber et al., 2003) and it is becoming apparent that multi-protein complexes are important in signal perception in plants. For example, the perception of the protein ligand CLV3 requires two plasma membrane-bound proteins, CLV1 and CLV2. SRK in *Brassica* operates as a homodimer as it is dependent on transactivation by another SRK molecule (Giranton et al., 2000); it also interacts with MLPK (Murase et al., 2004). Although not involving a protein ligand, brassinosteroid perception is carried out by a complex of plasma membrane-bound receptor kinases (BRI1, BAK1, and SERK1) as well as several cytoplasmic components in hetero-oligomeric complexes (Aker and de Vries, 2008; Chinchilla et al., 2009). Thus, PrpS may require the presence of other plasma membrane proteins to initiate signal transduction following interaction with PrsS. Another pollen protein, SBP (S-protein Binding Protein) that binds to PrsS, was previously identified (Hearn et al., 1996),

![Fig. 4](image-url) **Fig. 4.** A possible model for operation of PrpS-PrsS interaction. PrsS is a small novel secreted protein that is proposed to act as a signalling ligand, interacting with PrpS in an S-specific manner. PrpS is a small novel transmembrane protein that is proposed to act as a “receptor” for PrsS proteins. When PrsS interacts with PrpS (only in an incompatible situation, when both S-alleles match; in this case S1), an intracellular signalling network is triggered within the incompatible pollen, resulting in inhibition of pollen tube growth and programmed cell death. As PrpS is a novel protein, it is currently unclear how it operates. However, as an S-specific interaction is known to take place, the most parsimonious explanation is that PrpS itself is the S-specific “receptor”. One possible mode of action is that it acts as an ion channel (see text for details).
but it is not known how it relates to PrpS or if it interacts with PrpS. Thus, whether PrpS forms complexes and whether this is required for function is something to be examined in the future.

The second possibility, that PrpS provides both the perceptive apparatus and the means to transduce the signal, requires further analysis of PrpS. As mentioned earlier, PrpS shows no homology to other proteins currently in the databases. However, there are some very weak matches with other transmembrane proteins from plants, animals, and prokaryotes. The closest homology is to a prokaryotic ABC transporter which has 38% identity over approximately half the length of PrpS; other transmembrane proteins have even lower levels of sequence homology. While the presence of multiple transmembrane domains in PrpS inevitably results in weak homology to other membrane bound proteins, many of the proteins with very weak homology are transporters, actively involved in moving molecules across membranes. A ‘topological homologue’ of PrpS has recently been identified as a Drosophila protein, Flower, which is involved in presynaptic vesicle endocytosis (Yao et al., 2009). The Flower protein has been shown to function as a Ca\(^{2+}\)-permeable channel. Although both Flower and PrpS are novel proteins with no obvious homologues in the database, and they share very little primary sequence homology, there are several features that make Flower look superficially similar to PrpS. Both PrpS and Flower have similar topological predictions. Interestingly, Flower has a single glutamic acid residue in a proposed transmembrane domain. Voltage-gated calcium channels have an acidic amino acid (either glutamic acid or aspartic acid) in the transmembrane domains that form a pore; generally four acidic amino acids are critical for forming a pore. Flower makes a homo-multimeric complex which could form this pore. Examination of PrpS sequences reveal that it has three aspartic acids and three glutamic acids conserved across all three PrpS proteins; several are close to the edges of putative predicted transmembrane domains. These are good candidates for pore/channel selectivity generating amino acid residues.

This exciting finding provides a clear basis to build a testable hypothesis and model for PrpS function. The proposed model is that PrpS acts as ‘receptor’ to allow S-specific recognition, but might also form a channel (Fig. 4). This channel might operate as a homo-multimer, or as part of a channel complex. This is especially relevant as it has previously been established that SI triggers increases in cytosolic free Ca\(^{2+}\) (Franklin-Tong et al., 1993) and Ca\(^{2+}\) influx in incompatible pollen (Franklin-Tong et al., 2002). Moreover, preliminary data indicate that SI interactions in incompatible pollen tubes trigger both a Ca\(^{2+}\) and K\(^+\)-permeable channel conductance (J Wu, S Wang, SJ Publicover, Y Gu, VE Franklin-Tong, unpublished data). Thus, the possibility that PrpS, a novel protein with no obvious function, forms a channel that is likely also to play the role of ‘receptor’ using its extracellular loop region by binding PrsS proteins in an S-specific manner, is a tantalizing proposition, and presents an exciting challenge for future studies.

In summary, the identification of PrpS as the pollen S-determinant in *P. rhoeas* is a milestone. The next key step will be to establish its molecular function. If PrpS does display channel activity, this will provide a completely new type of model for a ‘receptor–ligand’ system in plant cells. Longer term, studies of PrsS-PrpS interactions will hopefully also provide a better understanding of how the SI signal is perceived, how this receptor/protein ligand-type interaction operates at a molecular level, how it transduces signals resulting in pollen rejection. As the PrpS-PrsS receptor–ligand pair represents one of only a handful of known protein–ligand/receptor pairs in plants, it is hoped that these studies will contribute to knowledge about the broader field of plant receptor–ligand interactions and signal transduction in general.

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