Journal of Experimental Botany, Vol. 61, No. 7, pp. 2015–2025, 2010
doi:10.1093/jxb/erp383 Advance Access publication 22 January, 2010

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

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

Michael J. Wheeler†, Sabina Vatovec and Vernonica E. Franklin-Tong*

School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

† Present address: Warwick HRI, Wellesbourne, Warwick CV35 9EF, UK.
* To whom correspondence should be addressed: E-mail: v.e.franklin-tong@bham.ac.uk

Received 5 November 2009; Revised 8 December 2009; Accepted 9 December 2009

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 Ca^{2+}-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.

Key words: Cell–cell recognition, *Papaver rhoeas*, pollen *S*-determinant, pollen tube inhibition, PrpS, receptor, self-incompatibility, self-recognition.

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

Abbreviations: ARC, Arm Repeat Containing; as-ODN, antisense oligodeoxynucleotide; ABC transporter, ATP-Binding Cassette transporter; BAK1, BRI1-Associated receptor Kinase 1; BRI1, BRassinosteroid Insensitive 1; CLV, CLAVATA; HAE, HAESA; HSL2, HAESA Like 2; IDA, Inflorescence Deficient in Abscission; MLPK, *M*-Locus Receptor Kinase; PCD, Programmed Cell Death; PrpS, *Papaver rhoeas* pollen *S*-determinant; PrsS, *Papaver rhoeas* stigma *S*-determinant; RLK, Receptor-Like Kinase; SBP, S-protein Binding Protein; SCR/SP11, *S*-locus Cysteine Rich/S-locus Protein 11; SERK1, Somatic Embryogenesis Receptor-like Kinase 1; SI, self-incompatibility; SLF, *S*-Locus F Box; SLG, *S* Locus Glycoprotein; s-ODN, sense oligodeoxynucleotide; SPH, *S*-Protein Homologue; SRK, *S*-locus Receptor Kinase; S-RNase, *S*-locus Ribonuclease.

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, controlled by the *S*- (Self-sterility or Self-incompatibility) locus, provide important mechanisms which prevent inbreeding. They allow 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 Plantaginaceae; 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/SCR 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.*, 2000). 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 (Newbigin *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*

(*PrsSn*$_1$). 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.*, 1998). 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.*, 1999). 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 rhoeas*

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. rhoeas* S_1 locus resulted in the identification of a putative open reading frame <0.5 kb from *PrsS*$_1$. 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. rhoeas* pollen *S*) (Wheeler *et al.*, 2009). As *PrpS* was identified so close to *PrsS*$_1$, sequencing around the *PrsS* gene of each haplotype was initially attempted to identify alleles of *PrpS* from plants of *S*-haplotypes S_3 and S_8. Inverse PCR was used to isolate 7 kb of sequence adjacent to *PrsS*$_3$ and 6 kb of sequence adjacent to *PrsS*$_8$. However, analysis of this did not identify any sequences homologous to *PrpS*$_1$. 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 *PrpS*$_1$, was used to identify two further alleles: *PrpS*$_3$ and *PrpS*$_8$. 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 *PrpS*$_3$ and *PrpS*$_8$ 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 *PrpS1* and *PrpS*$_8$ was examined in segregating full-sib families. This established that each *PrpS* allele was linked to its cognate *PrsS* allele; i.e. *PrpS*$_3$ co-segregated with *PrsS*$_3$, *PrpS*$_8$ co-segregated with *PrsS*$_8$, and *PrpS*$_1$ co-segregated with *PrsS*$_1$ (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 rhoeas* is no exception; the pistil proteins $PrsS_1$ and $PrsS_3$ exhibit 46% sequence divergence; $PrsS_1$ and $PrsS_8$ have 40%, and $PrsS_3$ and $PrsS_8$ have 46% divergence. Analyses of PrpS proteins show that they exhibit a similar level of polymorphism to PrsS proteins; the $PrpS_1$ and $PrpS_3$ predicted amino acid sequences are 50% divergent; $PrpS_1$ and $PrpS_8$ exhibit 40% divergence; $PrpS_3$ and $PrpS_8$ 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 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. rhoeas*, 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 S_1S_3 challenged with recombinant $PrsS_1$ and $PrsS_3$ was inhibited. When as-ODNs specifically designed to knock down only *PrpS*$_1$ were added, inhibition of 50% of pollen (assumed to be carrying S_1) was significantly alleviated (Fig. 1). No alleviation of the SI response occurred either with sense oligodeoxynucleotides (s-ODN) or with as-ODNs designed to *PrpS*$_8$. This showed allelic specificity of the alleviation. Alleviation of SI in pollen from S_3S_8 plants was also demonstrated. S_3S_8 pollen exhibited a full SI response in the presence of recombinant $PrsS_3$ and $PrsS_8$, which was alleviated in the

presence of as-ODNs designed to knock down $PrpS_8$, but not by either s-$PrpS_8$-ODNs or as-$PrpS_1$-ODNs (Wheeler *et al.*, 2009). Thus, the PrpS as-ODNs alleviate the SI response, demonstrating that PrpS plays a functional a 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).

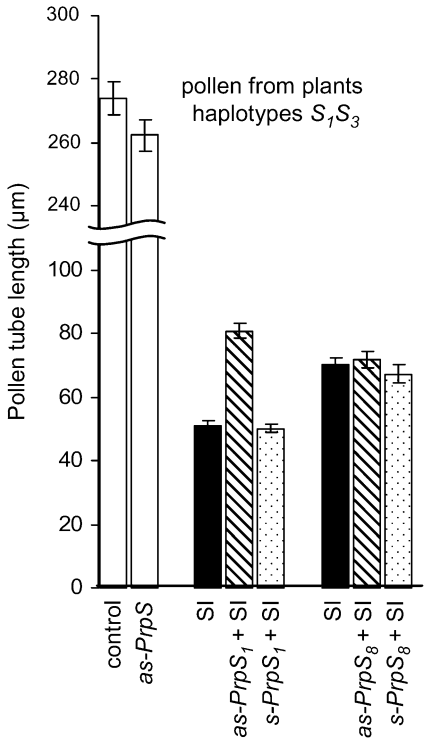

Fig. 1. PrpS function demonstrated by use of anti-sense oligodeoxynucleotides. Pollen of *S*-haplotypes S_1S_3 was challenged with recombinant $PrsS_1$. This produced an incompatible response in half of the pollen (haplotype S_1) which was quantified by measuring pollen tube lengths. Addition of antisense oligodeoxynucleotide designed to the $PrpS_1$ open reading frame (*as-PrpS*$_1$) alleviated the SI response and sense oligodeoxynucleotide (*s-PrpS*$_1$) had no alleviating effect. Neither antisense or sense oligodeoxynucleotide designed to $PrpS_8$ sequence (*as-PrpS*$_8$ and *s-PrpS*$_8$) 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.

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 $PrsS_1$ to peptides corresponding to the extracellular domain of $PrpS_1$ was detected (Wheeler *et al.*, 2009). Furthermore, this appears to be an *S*-specific interaction, as pistil $PrsS_1$ did not bind to corresponding peptides for the extracellular domain of $PrpS_8$ (Fig. 3). In addition to the Western-ligand blotting data, artificial peptides based on the predicted extracellular region of $PrpS_1$, 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

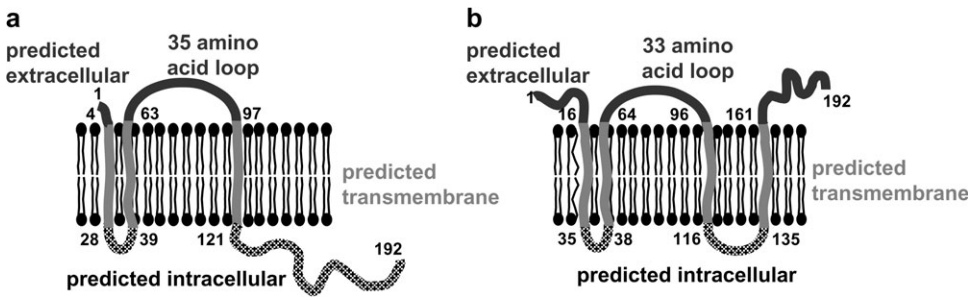

Fig. 2. Possible structural topologies of $PrpS_1$. Several different programmes were used to predict the topology of PrpS proteins. Two possible predictions of $PrpS_1$ topology are shown: (a) TMHMM (Krogh et al., 2001) predicts three transmembrane spanning domains and (b) PredictProtein (Rost et al., 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 $PrpS_1$.

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 protochordate allorecognition 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?

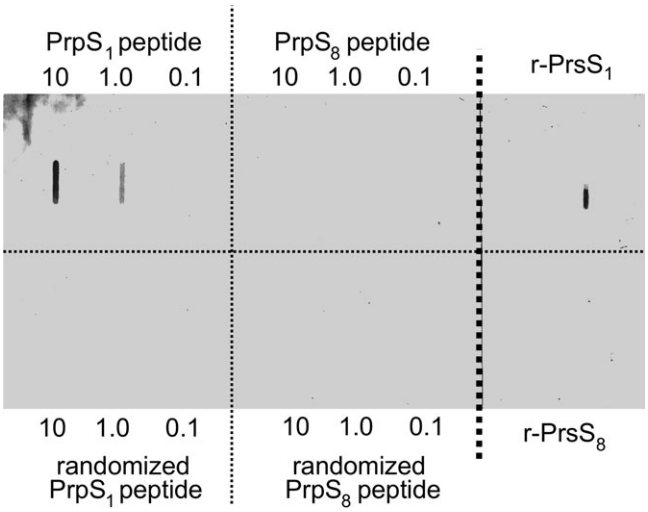

Fig. 3. Demonstration of interaction between PrpS and PrsS. The *S*-specific interaction between recombinant $PrsS_1$ and the 15-mer residues within the predicted extracellular domain of $PrpS_1$ along with its randomized control peptide was tested using a western-ligand blot assay. A dilution series 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 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 to PrpS 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.

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 (Lease and Walker, 2006), that are thought likely to act as ligands. As with putative receptor 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

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

Although plant genomes encode many thousand of plasma membrane-bound proteins and hundreds of secreted proteins very few interacting pairs of receptors with their corresponding protein-ligands have been identified. This table lists those cognate pairs identified so far.

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

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),

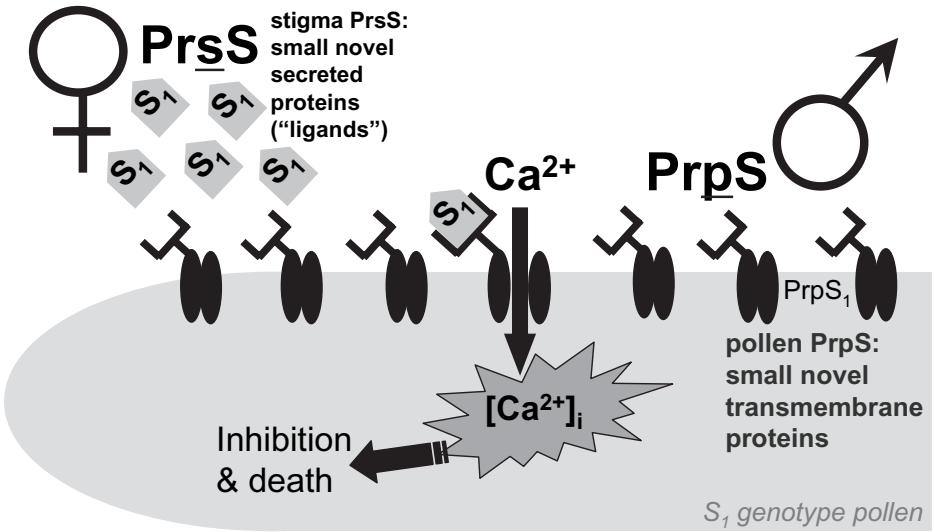

Fig. 4. A possible model for operation of PrpS-PrsS interaction. PrsS is a small novel secreted protein that is proposed to act as a signalling ligand, interacting with PrpS in an *S*-specific manner. PrpS is a small novel transmembrane protein that is proposed to act as a ''receptor'' for PrsS proteins. When PrsS interacts with PrpS (only in an incompatible situation, when both *S*-alleles match; in this case S_1), 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 $PrpS_1$; 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.

Acknowledgements

Work in the laboratory of VE Franklin-Tong is funded by the Biotechnology and Biological Sciences Research Council (BBSRC). MJ Wheeler is also funded by the BBSRC and S Vatovec is funded by a BBSRC studentship. We are indebted to the many people involved in generating data towards identification of PrpS and contributing to our research effort over the years.

References

Aker J, de Vries SC. 2008. Plasma membrane receptor complexes. *Plant Physiology* **147,** 1560–1564.

Allen AM, Hiscock SJ. 2008. Evolution and phylogeny of self-incompatibility systems in Angiosperms. In: Franklin-Tong VE, ed. *Self-incompatibility in flowering plants*. Berlin Heidelberg: Springer-Verlag, 73–102.

Anderson MA, Cornish EC, Mau S-L, *et al*. 1986. Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana alata*. *Nature* **321,** 38–44.

Bosch M, Franklin-Tong VE. 2007. Temporal and spatial activation of caspase-like enzymes induced by self-incompatibility in *Papaver* pollen. *Proceedings of the National Academy of Sciences, USA* **104,** 18327–18332.

Bosch M, Franklin-Tong VE. 2008. Self-incompatibility in *Papaver*: signalling to trigger PCD in incompatible pollen. *Journal of Experimental Botany* **59,** 481–490.

Bosch M, Poulter NS, Vatovec S, Franklin-Tong VE. 2008. Initiation of programmed cell death in self-incompatibility: role for cytoskeleton modifications and several caspase-like activities. *Molecular Plant* **1,** 879–887.

Bower MS, Matias DD, Fernandes-Carvalho E, Mazzurco M, Gu T, Rothstein Steven J, Goring DR. 1996. Two members of the Thioredoxin-h family interact with the kinase domain of a *Brassica S* locus receptor kinase. *The Plant Cell* **8,** 1641–1650.

Burnet FM. 1971. 'Self-recognition' in colonial marine forms and flowering plants in relation to the evolution of immunity. *Nature* **232,** 230–235.

Butenko MA, Patterson SE, Grini PE, Stenvik GE, Amundsen SS, Mandal A, Aalen RB. 2003. Inflorescence deficient in abscission controls floral organ abscission in Arabidopsis and identifies a novel family of putative ligands in plants. *The Plant Cell* **15,** 2296–2307.

Cabrillac D, Cock JM, Dumas C, Gaude T. 2001. The *S*-locus receptor kinase is inhibited by thioredoxins and activated by pollen coat proteins. *Nature* **410,** 220–223.

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM. 1993. Arabidopsis ethylene-response gene *ETR1*: similarity of product to two-component regulators. *Science* **262,** 539–544.

Charlesworth D. 1995. Multi-allelic self-incompatibility polymorphisms in plants. *Bioessays* **17,** 31–38.

Chinchilla D, Shan L, He P, de Vries S, Kemmerling B. 2009. One for all: the receptor-associated kinase BAK1. *Trends in Plant Science* **14,** 535–541.

Cho SK, Larue CT, Chevalier D, Wang H, Jinn TL, Zhang S, Walker JC. 2008. Regulation of floral organ abscission in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **105,** 15629–15634.

Clark SE. 2001. Cell signalling at the shoot meristem. *Nature Reviews Molecular and Cell Biology* **2,** 276–284.

Cui Y, Brugiere N, Jackman L, Bi YM, Rothstein SJ. 1999. Structural and transcriptional comparative analysis of the *S* locus regions in two self-incompatible *Brassica napus* lines. *The Plant Cell* **11,** 2217–2231.

Dangl JL, Jones JDG. 2001. Plant pathogens and integrated defence responses to infection. *Nature* **411,** 826–833.

de Graaf BH, Rudd JJ, Wheeler MJ, Perry RM, Bell EM, Osman K, Franklin FC, Franklin-Tong VE. 2006. Self-incompatibility in *Papaver* targets soluble inorganic pyrophosphatases in pollen. *Nature* **444,** 490–493.

De Smet I, Voss U, Jurgens G, Beeckman T. 2009. Receptor-like kinases shape the plant. *Nature Cell Biology* **11,** 1166–1173.

De Tomaso AW, Nyholm SV, Palmeri KJ, Ishizuka KJ, Ludington WB, Mitchel K, Weissman IL. 2005. Isolation and characterization of a protochordate histocompatibility locus. *Nature* **438,** 454–459.

Entani T, Iwano M, Shiba H, Che FS, Isogai A, Takayama S. 2003. Comparative analysis of the self-incompatibility (S-) locus region of *Prunus mume*: identification of a pollen-expressed F-box gene with allelic diversity. *Genes to Cells* **8,** 203–213.

Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM. 1999. Signaling of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems. *Science* **283,** 1911–1914.

Foote HC, Ride JP, Franklin-Tong VE, Walker EA, Lawrence MJ, Franklin FC. 1994. Cloning and expression of a distinctive class of self-incompatibility (*S*) gene from *Papaver rhoeas* L. *Proceedings of the National Academy of Sciences, USA* **91,** 2265–2269.

Franklin-Tong VE. 2007. Inhibiting self-pollen: self-incompatibility in *Papaver* involves integration of several signaling events. *Journal of Integrative Plant Biology* **49,** 1219–1226.

Franklin-Tong VE. 2008. *Self-incompatibility in flowering plants: evolution, diversity, and mechanisms*. Berlin, Heidelberg: Springer-Verlag.

Franklin-Tong VE, Holdaway-Clarke TL, Straatman KR, Kunkel JG, Hepler PK. 2002. Involvement of extracellular calcium influx in the self-incompatibility response of *Papaver rhoeas*. *The Plant Journal* **29,** 333–345.

Franklin-Tong VE, Lawrence MJ, Franklin FCH. 1988. Self-incompatibility in *Papaver rhoeas*. 1. Characterization of the stigmatic component. *Heredity* **61,** 286–286.

Franklin-Tong VE, Ride JP, Read ND, Trewavas AJ, Franklin FCH. 1993. The self-incompatibility response in *Papaver rhoeas* is mediated by cytosolic-free calcium. *The Plant Journal* **4,** 163–177.

Geitmann A, Snowman BN, Emons AMC, Franklin-Tong VE. 2000. Alterations in the actin cytoskeleton of pollen tubes are induced by the self-incompatibility reaction in *Papaver rhoeas*. *The Plant Cell* **12,** 1239–1251.

Giranton JL, Dumas C, Cock JM, Gaude T. 2000. The integral membrane *S*-locus receptor kinase of *Brassica* has serine/threonine kinase activity in a membranous environment and spontaneously forms oligomers in planta. *Proceedings of the National Academy of Sciences, USA* **97,** 3759–3764.

Glass NL, Jacobson DJ, Shiu PK. 2000. The genetics of hyphal fusion and vegetative incompatibility in filamentous ascomycete fungi. *Annual Review of Genetics* **34,** 165–186.

Goldraij A, Kondo K, Lee CB, Hancock CN, Sivaguru M, Vazquez-Santana S, Kim S, Phillips TE, Cruz-Garcia F, McClure B. 2006. Compartmentalization of S-RNase and HT-B degradation in self-incompatible *Nicotiana*. *Nature* **439,** 805–810.

Hearn MJ, Franklin FCH, Ride JP. 1996. Identification of a membrane glycoprotein in pollen of *Papaver rhoeas* which binds stigmatic self-incompatibility (S-) proteins. *The Plant Journal* **9,** 467–475.

Hua Z, Fields A, Kao TH. 2008. Biochemical models for S-RNase-based self-incompatibility. *Molecular Plant* **1,** 575–585.

Hua Z, Kao TH. 2006. Identification and characterization of components of a putative petunia *S*-locus F-box-containing E3 ligase complex involved in S-RNase-based self-incompatibility. *The Plant Cell* **18,** 2531–2553.

Huber AB, Kolodkin AL, Ginty DD, Cloutier JF. 2003. Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. *Annual Review of Neuroscience* **26,** 509–563.

Hughes AL. 2002. Natural selection and the diversification of vertebrate immune effectors. *Immunology Review* **190,** 161–168.

Ioerger TR, Clark AG, Kao T-H. 1990. Polymorphism at the self-incompatibility locus in Solanaceae predates speciation. *Proceedings of the National Academy of Sciences, USA* **87,** 9732–9735.

Kachroo A, Schopfer CR, Nasrallah ME, Nasrallah JB. 2001. Allele-specific receptor–ligand interactions in *Brassica* self-incompatibility. *Science* **293,** 1824–1826.

Kakeda K, Jordan ND, Conner A, Ride JP, Franklin-Tong VE, Franklin FC. 1998. Identification of residues in a hydrophilic loop of the *Papaver rhoeas* S protein that play a crucial role in recognition of incompatible pollen. *The Plant Cell* **10,** 1723–1732.

Kakita M, Murase K, Iwano M, Matsumoto T, Watanabe M, Shiba H, Isogai A, Takayama S. 2007. Two distinct forms of

M-locus protein kinase localize to the plasma membrane and interact directly with *S*-locus receptor kinase to transduce self-incompatibility signaling in *Brassica rapa*. *The Plant Cell* **19,** 3961–3973.

Kohn JR. 2008. What genealogies of *S*-alleles tell us. In: Franklin-Tong VE, ed. *Self-incompatibility in flowering plants: evolution, diversity, and mechanisms*. Berlin, Heidelberg: Springer-Verlag, 103–121.

Krogh A, Larsson B, von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *Journal of Molecular Biology* **305,** 567–580.

Kurup S, Ride JP, Jordan N, Fletcher G, Franklin-Tong VE, Franklin FCH. 1998. Identification and cloning of related self-incompatibility *S*-genes in *Papaver rhoeas* and *Papaver nudicaule*. *Sexual Plant Reproduction* **11,** 192–198.

Lai Z, Ma WS, Han B, Liang LZ, Zhang YS, Hong GF, Xue YB. 2002. An F-box gene linked to the self-incompatibility (*S*) locus of *Antirrhinum* is expressed specifically in pollen and tapetum. *Plant Molecular Biology* **50,** 29–42.

Lease KA, Walker JC. 2006. The Arabidopsis unannotated secreted peptide database, a resource for plant peptidomics. *Plant Physiology* **142,** 831–838.

Luu D-T, Qin X, Morse D, Cappadocia M. 2000. S-RNase uptake by compatible pollen tubes in gametophytic self-incompatibility. *Nature* **407,** 649–651.

Matsubayashi Y. 2003. Ligand-receptor pairs in plant peptide signaling. *Journal of Cell Science* **116,** 3863–3870.

Matsubayashi Y, Sakagami Y. 2000. 120- and 160-kDa receptors for endogenous mitogenic peptide, phytosulfokine-alpha, in rice plasma membranes. *Journal of Biological Chemistry* **275,** 15520–15525.

Maxfield FR, McGraw TE. 2004. Endocytic recycling. *Nature Reviews Molecular Cell Biology* **5,** 121–132.

Morillo SA, Tax FE. 2006. Functional analysis of receptor-like kinases in monocots and dicots. *Current Opinion in Plant Biology* **9,** 460–469.

Murase K, Shiba H, Iwano M, Che FS, Watanabe M, Isogai A, Takayama S. 2004. A membrane-anchored protein kinase involved in *Brassica* self-incompatibility signaling. *Science* **303,** 1516–1519.

Murfett J, Atherton TL, Mou B, Gasser CS, McClure BA. 1994. S-RNase expressed in transgenic *Nicotiana* causes *S*-allele-specific pollen rejection. *Nature* **367,** 563–566.

Nasrallah JB. 2002. Recognition and rejection of self in plant reproduction. *Science* **296,** 305–308.

Nasrallah JB. 2005. Recognition and rejection of self in plant self-incompatibility: comparisons to animal histocompatibility. *Trends in Immunology* **26,** 412–418.

Nasrallah JBK, Kao T-H, Goldberg ML, Nasrallah ME. 1985. A cDNA clone encoding an *S*-locus-specific glycoprotein from *Brassica oleracea*. *Nature* **318,** 263–267.

Newbigin E, Paape T, Kohn JR. 2008. RNase-based self-incompatibility: puzzled by pollen *S*. *The Plant Cell* **20,** 2286–2292.

Nyholm SV, Passegue E, Ludington WB, Voskoboynik A, Mitchel K, Weissman IL, De Tomaso AW. 2006. *fester*, a candidate allorecognition receptor from a primitive chordate. *Immunity* **25,** 163–173.

Ogawa M, Shinohara H, Sakagami Y, Matsubayashi Y. 2008. *Arabidopsis* CLV3 peptide directly binds CLV1 ectodomain. *Science* **319,** 294.

Pandey S, Nelson DC, Assmann SM. 2009. Two novel GPCR-type G proteins are abscisic acid receptors in *Arabidopsis*. *Cell* **136,** 136–148.

Poulter NS. 2009. *Investigating the role of the cytoskeleton and signalling in the self-incompatibility response of Papaver rhoeas.* PhD thesis, University of Birmingham.

Poulter NS, Vatovec S, Franklin-Tong VE. 2008. Microtubules are a target for self-incompatibility signaling in *Papaver* pollen. *Plant Physiology* **146,** 1358–1367.

Qiao H, Wang F, Zhao L, Zhou J, Lai Z, Zhang Y, Robbins TP, Xue Y. 2004. The F-box protein AhSLF-S2 controls the pollen function of S-RNase-based self-incompatibility. *The Plant Cell* **16,** 2307–2322.

Ride JP, Davies EM, Franklin FC, Marshall DF. 1999. Analysis of *Arabidopsis* genome sequence reveals a large new gene family in plants. *Plant Molecular Biology* **39,** 927–932.

Rost B, Yachdav G, Liu J. 2004. The PredictProtein server. *Nucleic Acids Research* **32,** W321–W326.

Samuel MA, Chong YT, Haasen KE, Aldea-Brydges MG, Stone SL, Goring DR. 2009. Cellular pathways regulating responses to compatible and self-incompatible pollen in *Brassica* and *Arabidopsis* stigmas intersect at Exo70A1, a putative component of the exocyst complex. *The Plant Cell* tpc.109.069740.

Sassa H, Kakui H, Minamikawa M. 2009. Pollen-expressed F-box gene family and mechanism of S-RNase-based gametophytic self-incompatibility (GSI) in Rosaceae. *Sexual Plant Reproduction* doi 10.1007/s00497-009-0111-6.

Scheer JM, Ryan Jr. CA. 2002. The systemin receptor SR160 from *Lycopersicon peruvianum* is a member of the LRR receptor kinase family. *Proceedings of the National Academy of Sciences, USA* **99,** 9585–9590.

Schopfer CR, Nasrallah ME, Nasrallah JB. 1999. The male determinant of self-incompatibility in *Brassica*. *Science* **286,** 1697–1700.

Schwacke R, Schneider A, van der Graaff E, Fischer K, Catoni E, Desimone M, Frommer WB, Flugge UI, Kunze R. 2003. ARAMEMNON, a novel database for *Arabidopsis* integral membrane proteins. *Plant Physiology* **131,** 16–26.

Scofield VL, Schlumpberger JM, West LA, Weissman IL. 1982. Protochordate allorecognition is controlled by a MHC-like gene system. *Nature* **295,** 499–502.

Šijačič P, Wang X, Skirpan AL, Wang Y, Dowd PE, McCubbin AG, Huang S, Kao TH. 2004. Identification of the pollen determinant of S-RNase-mediated self-incompatibility. *Nature* **429,** 302–305.

Snowman BN, Kovar DR, Shevchenko G, Franklin-Tong VE, Staiger CJ. 2002. Signal-mediated depolymerization of actin in pollen during the self-incompatibility response. *The Plant Cell* **14,** 2613–2626.

Stein JC, Howlett B, Boyes DC, Nasrallah ME, Nasrallah JB. 1991. Molecular cloning of a putative receptor protein kinase gene

encoded at the self-incompatibility locus of *Brassica oleracea*. *Proceedings of the National Academy of Sciences, USA* **88,** 8816–8820.

Stenvik GE, Butenko MA, Aalen RB. 2008. Identification of a putative receptor-ligand pair controlling cell separation in plants. *Plant Signal Behaviour* **3,** 1109–1110.

Stone SL, Anderson EM, Mullen RT, Goring DR. 2003. ARC1 is an E3 ubiquitin ligase and promotes the ubiquitination of proteins during the rejection of self-incompatible *Brassica* pollen. *The Plant Cell* **15,** 885–898.

Suzuki G, Kai N, Hirose T, Fukui K, Nishio T, Takayama S, Isogai A, Watanabe M, Hinata K. 1999. Genomic organization of the S locus: identification and characterization of genes in SLG/SRK region of S(9) haplotype of *Brassica campestris* (syn. *rapa*). *Genetics* **153,** 391–400.

Takasaki T. 2000. The *S* receptor kinase determines self-incompatibility in *Brassica* stigma. *Nature* **403,** 913–916.

Takayama S, Isogai A. 2005. Self-incompatibility in plants. *Annual Review of Plant Biology* **56,** 467–489.

Takayama S, Shimosato H, Shiba H, Funato M, Che FS, Watanabe M, Iwano M, Isogai A. 2001. Direct ligand–receptor complex interaction controls *Brassica* self-incompatibility. *Nature* **413,** 534–538.

Thomas SG, Franklin-Tong VE. 2004. Self-incompatibility triggers programmed cell death in *Papaver* pollen. *Nature* **429,** 305–309.

Thomas SG, Huang S, Li S, Staiger CJ, Franklin-Tong VE. 2006. Actin depolymerization is sufficient to induce programmed cell death in self-incompatible pollen. *Journal of Cell Biology* **174,** 221–229.

Ushijima K, Sassa H, Dandekar AM, Gradziel TM, Tao R, Hirano H. 2003. Structural and transcriptional analysis of the self-incompatibility locus of almond: identification of a pollen-expressed F-box gene with haplotype-specific polymorphism. *The Plant Cell* **15,** 771–781.

Walker EA, Ride JP, Kurup S, Franklin-Tong VE, Lawrence MJ, Franklin FC. 1996. Molecular analysis of two functional homologues of the S3 allele of the *Papaver rhoeas* self-incompatibility gene isolated from different populations. *Plant Molecular Biology* **30,** 983–994.

Wang ZY, Seto H, Fujioka S, Yoshida S, Chory J. 2001. BRI1 is a critical component of a plasma-membrane receptor for plant steroids. *Nature* **410,** 380–383.

Ward JM. 2001. Identification of novel families of membrane proteins from the model plant *Arabidopsis thaliana*. *Bioinformatics* **17,** 560–563.

Wells JA, de Vos AM. 1996. Hematopoietic receptor complexes. *Annual Review of Biochemistry* **65,** 609–634.

Wheeler MJ, de Graaf BH, Hadjiosif N, Perry RM, Poulter NS, Osman K, Vatovec S, Harper A, Franklin FC, Franklin-Tong VE. 2009. Identification of the pollen self-incompatibility determinant in *Papaver rhoeas*. *Nature* **459,** 992–995.

Wright S. 1948. On the roles of directed and random changes in gene frequency in the genetics of populations. *Evolution* **2,** 279–294.

Yao CK, Lin YQ, Ly CV, Ohyama T, Haueter CM, Moiseenkova-Bell VY, Wensel TG, Bellen HJ. 2009. A synaptic vesicle-associated Ca^{2+}channel promotes endocytosis and couples exocytosis to endocytosis. *Cell* **138,** 947–960.