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The different mechanisms of gametophytic self-incompatibility

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Self-incompatibility (SI) involves the recognition and rejection of self or genetically identical pollen. Gametophytic SI is probably the most widespread of the SI systems and, so far, two completely different SI mechanisms, which appear to have evolved separately, have been identified. One mechanism is the RNase system, which is found in the Solanaceae, Rosaceae and Scrophulariaceae. The other is a complex system, so far found only in the Papaveraceae, which involves the triggering of signal transduction cascade(s) that result in rapid pollen tube inhibition and cell death. Here, we present an overview of what is currently known about the mechanisms involved in controlling pollen tube inhibition in these two systems.

Keywords: self-incompatibility; pollen tube inhibition; RNase; calcium signalling; protein kinase; actin depolymerization

1. INTRODUCTION

Self-incompatibility is an extremely important genetically controlled mechanism that regulates the acceptance or rejection of pollen that lands on the stigma of the same species. It is one of the most important devices whereby higher plants prevent inbreeding, and it is thought that the huge success of the Angiosperms as a group is partly due to the evolution of SI. SI is generally controlled by a single, multiallelic, *S*-locus. In species where SI is controlled gametophytically, pollen exhibits the haploid phenotype of the *S*-allele that it carries. Pollen grains carrying *S*-alleles that are genetically identical (incompatible) to those carried by the pistil on which they land are recognized as 'self' and so are discriminated from genetically different (compatible) pollen. The incompatible pollen is selectively inhibited at a specific stage during pollination so that self-fertilization is prevented.

Gametophytic SI is probably the most widespread of the SI systems, among which are the Solanaceae, Rosaceae, Scrophulariaceae, Leguminoseae, Onagraceae, Campanulaceae, Papaveraceae and Poaceae (Franklin *et al.* 1995). Two mechanistically different single-locus gametophytic SI systems have been investigated in detail at the molecular level. The first of these is the S-RNase system originally found and extensively characterized in members of the Solanaceae (reviewed in McCubbin & Kao 2000) and subsequently also reported in the Rosaceae (Sassa *et al.* 1993) and Scrophulariaceae (Xu *et al.* 1996). The second is found in the Papaveraceae, namely, *Papaver rhoeas* L. (Lawrence *et al.* 1978).

Although it was expected that the mechanisms involved in controlling sporophytic and gametophytic SI might be different, it came as some considerable surprise when it was initially discovered that different species controlled by gametophytic SI used different mechanisms (Foote *et al.* 1994). This suggests that gametophytic SI has evolved completely independently several times. We describe the different mechanisms used by two gametophytic SI systems to achieve self-pollen inhibition.

2. S-RNase-BASED SI

In the Solanaceae and other families that possess an S-RNase-based SI system, an incompatible pollen grain alighting on the stigma germinates and begins to grow through the transmitting tract of the style. However, the growth of this incompatible pollen tube is arrested when it has reached about one-third of the way through the style. Early pioneering studies investigated the stylar proteins of different *S*-genotypes to find proteins associated with SI. Analysis of stylar proteins from *Nicotiana alata* resulted in the identification of an S-glycoprotein of *ca.* 32 kDa that exhibited genetic linkage to the *S*-locus (Anderson *et al.* 1986). Subsequent cloning and analysis of a large number of alleles of the gene encoding these proteins from different members of the Solanaceae revealed that they are highly polymorphic, exhibiting between 39% and 98% sequence identity (Anderson *et al.* 1989; Clark *et al.* 1990; Ai *et al.* 1990; Xu *et al.* 1990; Ioerger *et al.* 1990; Kheyr-Pour *et al.* 1990).

Analysis of the S-proteins revealed that they exhibit sequence homology to the catalytic domain of two fungal RNases: Rh from *Rhizopus niveus* and T_2 from *Aspergillus oryzae* (McClure *et al.* 1989). Further studies confirmed that these proteins were indeed ribonucleases (S-RNases; McClure *et al.* 1989; Clark *et al.* 1990; Xu *et al.* 1990; Gray *et al.* 1991; Singh *et al.* 1991). Transgenic studies, demonstrating gain and loss of function together with the analysis of self-compatible mutants established that catalytically active S-RNases are crucial for the rejection of

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incompatible pollen (Lee *et al.* 1994; Murfett *et al.* 1994; Royo *et al.* 1994; Ishimizu *et al.* 1995). Thus, it is proposed that S-RNases are allele-specific cytotoxins.

Sequence comparisons of S-RNases have revealed that they are organized into five conserved regions C1–C5, although the C4 region differs in the Rosaceae (Ushijama *et al.* 1998). In addition, there are two hypervariable regions that form a continuous surface on one side of the protein. Whether S-specificity resides entirely within the hypervariable regions is debatable. Although the S_{11} allele of *Solanum chacoense* was successfully changed into a closely related S_{13} allele (Matton *et al.* 1997), hypervariable domain swap experiments involving more diverged alleles have not proved successful (Verica *et al.* 1998), but this may be due to the hybrid proteins failing to fold correctly.

The identity of the pollen S-protein remains elusive, but two suggestions as to its nature have been made. One proposal is that it is a receptor or translocator that internalizes the S-RNase molecules in an allele-specific manner (McClure *et al.* 1989, 1990). The S-RNase then degrades the pollen rRNA leading to the cessation of pollen tube growth. Recent evidence indicates that this model is unlikely to be correct, since immunocytochemical studies using mono-specific polyclonal antibody directed against S_{11}-RNase have revealed *in vivo* uptake of the S_{11}-RNase protein by S_{12} pollen tubes (Luu *et al.* 2000). These studies strongly suggest that uptake of the S-RNase is independent of *S*-genotype.

An alternative model, that takes into account the allele-independent uptake of S-RNases has been proposed (Thompson & Kirch 1992; Golz *et al.* 1999, 2000). In this case, it is suggested that the pollen *S*-gene product is a RNase inhibitor within the pollen tube. This recognizes and inhibits all S-RNases via an interaction with a low-affinity binding site except when the S-RNase is allelic. In this case, binding occurs via a high affinity, allele-specific site, which somehow prevents interaction with the low affinity site. This leaves the S-RNase fully active and able to inhibit pollen tube growth. A modified version of the inhibitor model has also been proposed (Luu *et al.* 2000). These authors envisage a binary system comprising a general RNase inhibitor and a separate pollen S-protein. It is proposed that the S-protein interacts with its cognate S-RNase and that this blocks any interaction between the latter and the general inhibitor. Thus, the S-RNase remains active and able to inhibit the pollen.

Subsequently, Luu *et al.* (2001) added a further refinement to the inhibitor model, suggesting that the pollen S-protein functions as a tetramer. This enables them to account for the behaviour of heteroallelic pollen (i.e. pollen carrying two *S*-alleles) produced by tetraploid plants or plants with a radiation-induced duplication of the *S*-locus; so-called 'pollen part' mutants. This pollen is typically SI (de Nettancourt 1977; Golz *et al.* 1999, 2000), yet based on the assumption that binding of the pollen S-protein to the S-recognition site on the S-RNase is thermodynamically favoured over any interaction with the RNase activity domain, one would predict it should be SI. However, this apparent contradiction may be explained if the pollen S-protein usually functions as a homotetramer. Interaction of products from two *S*-alleles in the heteroallelic pollen would follow a binomial distribution, resulting in a high proportion of heterotetramers. It is proposed that these would not be capable of an allele-specific interaction with the corresponding S-RNases, so S-RNase inactivation would not be blocked and pollen tube growth would continue just as if it were a normal compatible pollination. We present in figure 1 a working model for the S-RNase SI system based upon this proposal.

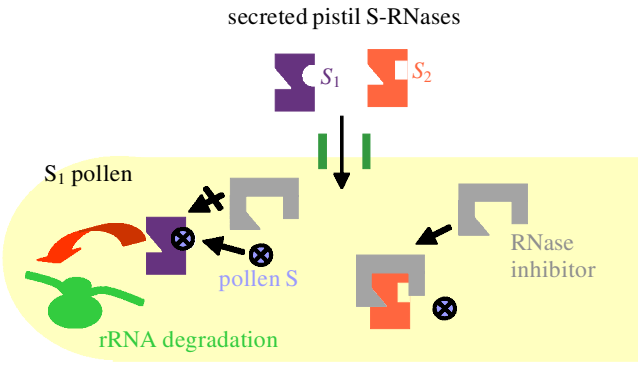

Figure 1. A model for the S-RNase mechanism of gametophytic SI found in the Solanaceae, Rosaceae and Scrophulariaceae. The figure presented here is based on the currently favoured inhibitor model, first proposed by Thompson & Kirch (1992), with the modifications recently suggested by Luu *et al.* (2001). Secreted S-RNases, S_1 (blue) and S_2 (red) are taken up, possibly as part of a protein complex (see McClure *et al.* 1989; Gray *et al.* 1991) by a growing pollen tube of genotype S_1. The S_2 S-RNase is recognized and is bound by a RNase inhibitor (grey) that inactivates the S-RNase. By contrast, the S_1 S-RNase is bound at an allele-specific binding site by the S_1 pollen protein (light blue), which is possibly a tetramer (see Luu *et al.* 2001). This interaction between the pollen S-protein and its cognate S-RNase is themodynamically favoured over the interaction with the RNase inhibitor and effectively blocks binding to the inhibitor. Hence the S_1 S-RNase remains active and degrades the pollen tube rRNA, resulting in the arrest of pollen tube growth approximately one-third of the way down the style.

Although isolation and biochemical characterization of the pollen component(s) have yet to be confirmed, two possible candidates have been identified. Yeast two-hybrid studies using an S-RNase from *Petunia hybrida* as bait has resulted in the isolation of PhSBP1 (Sims & Ordanic 2001). This protein contains a RING-HC finger domain at the C-terminus, suggesting that it may function as an E3 ubiquitin ligase, which targets protein substrates for degradation via the ubiquitin pathway (Joazeiro & Weissmann 2000). However, since PhSBP1 does not exhibit allelic variation or haplotype-specific interaction with S-RNases, the protein cannot be the pollen *S*-gene product *per se*, but may be the general RNase inhibitor proposed in the 'binary' version of the inhibitor model described in figure 1. The second candidate, a novel F-box gene, was identified as a pollen-expressed, *S*-linked gene on a bacterial artificial chromosome carrying the S_2 locus from *Antirrhinum hispanicum* (Lai *et al.* 2002). In common with the RING domain proteins, F-box proteins are known to promote ubiquitin-mediated protein degradation (Craig & Tyers 1999).

Attempts have recently been made to identify other components involved in the SI response in the Solanaceae. McClure *et al.* (1999) used differential screening of an

N. alata style cDNA library with style RNA from *N. alata* and the closely related, but self-compatible species *N. plumbaginifolia*. An *N. alata* specific clone designated HT was identified and found to encode a 101 residue asparagine-rich protein of unknown function. Transgenic experiments revealed that HT is essential in the SI response as *N. plumbaginifolia* × *N. alata* $S_{c10}S_{c10}$ hybrids transformed with an antisense construct of HT lose the ability to reject S_{c10} pollen. HT is also required for SI in *Lycopersicon* (Kondo *et al.* 2002). There is now increasing evidence to suggest that *in planta* S-RNases comprise a large complex, interacting with a range of pistil-transmitting tract proteins, and it is speculated that these complexes may aid the uptake of the S-RNases by the pollen tube (McClure *et al.* 2000; Cruz-Garcia *et al.* 2003).

In conclusion, despite extensive efforts by several groups to identify the pollen *S*-gene in the Solanaceae and other species with an S-RNase SI system, its identity remains to be resolved, although current evidence now favours the inhibitor model (figure 1). Although isolation of the pollen *S*-gene remains a major goal, studies by McClure and colleagues have identified other aspects of this system that require further investigation. As mentioned above, their studies have revealed that one or more factors other than the products of the *S*-locus are required for a functional SI system. In addition, they have obtained evidence that reveals a role for S-RNases in interspecific SI (Murfett *et al.* 1996).

3. SI IN *PAPAVER*

A gametophytic system of SI control is also found in the field poppy, *Papaver rhoeas* L.. However, study at the molecular and biochemical level has revealed that the stigmatic *S*-gene and the mechanisms involved in pollen inhibition are completely different from that of the Solanaceae. This may be due, at least in part, to differences in the physiology of this SI system, since, in contrast to the Solanaceae, inhibition of incompatible pollen occurs on the stigmatic papillae and initial arrest of pollen growth is rapid, occurring within minutes.

(a) *Stigmatic S-proteins and a postulated pollen receptor*

The stigmatic S-proteins are small (*ca*. 15 kDa) extracellular signalling molecules. An estimated 66 *S*-alleles are predicted to exist in this species (Lane & Lawrence 1993). Several alleles (S_1, S_3, S_8 and Sn_1 from *P. nudicaule*) of the stigmatic *S*-gene have been cloned (Foote *et al.* 1994; Walker *et al.* 1996; Kurup *et al.* 1998). Although the exact basis of allelic specificity remains to be elucidated, site-directed mutagenesis of the S_1 protein has established that certain residues located in hydrophilic surface loops are crucial for the recognition of S_1 pollen (Kakeda *et al.* 1998; Jordan *et al.* 1999).

Recently, an extensive family of SPHs has been identified in *Arabidopsis* (Ride *et al.* 1999) and tomato (Testa *et al.* 2002). To date, 81 members of the *Arabidopsis SPH* gene family have been identified. They are predicted to encode small (*ca*. 15 kDa) secreted proteins that are remarkably similar in structure to the poppy S-proteins. Since *Arabidopsis* is self-compatible, they cannot be involved in SI; nevertheless, it seems likely that they are involved in some other aspect of cell–cell signalling, possibly during development (Ride *et al.* 1999; F. C. H. Franklin, V. E. Franklin-Tong and J. P. Ride, unpublished data).

The stigmatic S-proteins interact with the pollen *S*-gene product that is believed to be a plasma membrane receptor. The nature of the pollen receptor is unclear. One candidate, an SBP, has been identified that specifically binds the S-proteins (Hearn *et al.* 1996). SBP is a pollen plasma membrane glycoprotein of 70–120 kDa. Binding to the S-protein is at least partly dependent on the glycan moiety of SBP. *In vitro* studies indicate that SBP specifically binds S-proteins but suggest this is not allele-specific. Hence, SBP may be an accessory receptor rather than the pollen S-receptor itself. However, as analysis of S-protein mutants has revealed that all mutants that exhibit reduced ability to inhibit incompatible pollen are also reduced in SBP binding activity (Jordan *et al.* 1999), it is conceivable that SBP is the pollen S-receptor. Resolution of this issue awaits cloning of SBP and/or the S-receptor.

(b) *A Ca^{2+}-based signalling cascade is triggered in incompatible pollen*

It is well established, through Ca^{2+}-imaging studies, that rapid increases in $[Ca^{2+}]_i$ are stimulated in a pollen tube encountering incompatible stigmatic S-proteins (Franklin-Tong *et al.* 1993, 1995, 1997). It is therefore proposed that the SI response in *Papaver* is a receptor-mediated response, with the stigmatic S-protein acting as a signal molecule that interacts with the postulated pollen S-receptor, triggering an intracellular signalling cascade(s) in incompatible pollen tubes that results in the rapid inhibition of pollen tube tip growth. Evidence that it was the stigmatic S-proteins alone that were sufficient to elicit this response came from use of recombinant S-proteins, which showed that they acted as signal molecules (Franklin-Tong *et al.* 1995). These increases in $[Ca^{2+}]_i$ are thought to initiate the SI signalling cascade, resulting in a complex set of events that ultimately lead to death of the incompatible pollen.

The rapid loss of the oscillating apical high $[Ca^{2+}]_i$ that is typical of all tip-growing cells, which accompanies the increases in the shank of the pollen tube, coincides with the inhibition of pollen tube tip growth. This loss of high apical $[Ca^{2+}]_i$ is likely to play a part in the initial inhibition of pollen tube growth, as low levels of apical $[Ca^{2+}]_i$ are associated with loss of growth. It could be speculated that vesicle fusion, which occurs specifically at the tip and requires high $[Ca^{2+}]_i$, might be rapidly inhibited in the SI response, and thus be responsible for pollen tube inhibition. The localization of the increases in $[Ca^{2+}]_i$ stimulated by the SI response in the 'shank' of the pollen tube (Franklin-Tong *et al.* 1993, 1995, 1997) was surprising. Although inhibition of pollen tube growth may be mediated by inositol trisphosphate ($Ins[1,4,5]P_3$)-induced Ca^{2+} release (Franklin-Tong *et al.* 1996), there is no conclusive evidence for any involvement of an inositide signalling pathway in the SI response (Straatman *et al.* 2001). However, there is now good evidence for a role for extracellular Ca^{2+} in the SI response, and that influx of extracellular Ca^{2+} occurs at the 'shank' of the pollen tube rather than at the tip (Franklin-Tong *et al.* 2002). Downstream of the initial Ca^{2+} signals, there is evidence of sev-

eral pollen components that are altered upon challenge with incompatible S-proteins in an S-specific manner. We briefly describe the identification and preliminary characterization of some of these components.

(c) *p26: a phosphorylated inorganic pyrophosphatase*

It is thought that one of the earliest downstream targets of the SI response is a CDPK, which is considered to be responsible for the rapid increase in phosphorylation of p26, a cytosolic pollen protein (Rudd *et al.* 1996). We have recently cloned p26 using a PCR probe designed from the amino acid sequence from p26 (Rudd & Franklin-Tong 2003; J. J. Rudd, B. de Graaf, E. M. Bell, M. Patel, F. C. H. Franklin and V. E. Franklin-Tong, unpublished data). Analysis of the p26 sequence revealed that it shares between 80–90% amino acid sequence identity to plant soluble inorganic pyrophosphatases. This activity has been confirmed by biochemical assays on the recombinant p26 protein. Preliminary data indicate that under conditions of raised $[Ca^{2+}]$, when p26 is phosphorylated, its pyrophosphatase activity is reduced (Rudd & Franklin-Tong 2003; J. J. Rudd, B. de Graaf, E. M. Bell, M. Patel, F. C. H. Franklin and V. E. Franklin-Tong, unpublished data). This suggests that p26 activity is almost certainly altered in the SI response.

These data form the basis of a theory about the contribution of p26 to incompatible pollen tube inhibition. Soluble inorganic pyrophosphatases often drive cellular biosynthetic reactions (Cooperman *et al.* 1992) and play an important part in generating both ATP and the biopolymers required for making new membranes and cell walls. It is proposed that since the phosphorylation of p26 during the SI response is likely to result in a reduction in its pyrophosphatase activity, this would almost certainly result in the depletion of biopolymers, such as long-chain carbohydrates and proteins, which contribute to pollen tube membranes and cell walls. This would result in the inhibition of tip growth. Although this proposal needs to be tested, it provides a feasible mechanism whereby pollen could be inhibited by the SI response. Since phosphorylation is generally reversible, it is likely that this may represent a temporary measure to ensure relatively rapid arrest of tip growth.

(d) *A putative MAPK is activated in the SI response*

Another pollen component that displays altered activity stimulated by the SI response is a putative MAPK, p56. In-gel kinase assays have allowed the identification of the increased activation of a 56 kDa putative MAPK activity in incompatible pollen undergoing the SI response (Rudd & Franklin-Tong 2003; J. J. Rudd, K. Osman, S. Whitaker, F. C. H. Franklin and V. E. Franklin-Tong, in preparation). Several pieces of biochemical evidence suggest that p56 is a putative MAPK homologue. For example, p56 can be immunoprecipitated by TEY antibody that is specific for activated MAPKs and it is sensitive to the MAPK inhibitor apigenin. Furthermore, data also indicate that p56 activation occurs downstream of Ca^{2+} increases. However, at present there are no data that indicate what might be the role of this signalling cascade, or what the ultimate target of the MAPK signal is. One hint as to the possible role of the activation of this component comes from studies of stress responses in plants, in particular, the hypersensitive response to pathogens, as these involve the activation of MAPKs (see Innes (2001) for a recent review). At a simplistic level, the SI response might be thought of as a stress response, and this might provide some clues as to its function in the SI response. As SI-stimulated p56 activation peaks several minutes after initial inhibition of pollen tube growth (Rudd & Franklin-Tong 2003), p56 is not likely to play a direct part in early inhibition events. This suggests another function, perhaps in later, downstream events that may be involved in making the initial inhibition of growth irreversible. One possible suggestion is that p56 may feed into a PCD signalling cascade, since data have recently emerged suggesting a role for MAPK activation in the induction of PCD in plants (Yang *et al.* 2001). This topic will be addressed in §§ 3f and 4.

(e) *The actin cytoskeleton is a target for the SI signals*

Evidence that the SI response targets the pollen F-actin cytoskeleton has recently been obtained. In incompatible pollen tubes, very rapid and dramatic changes in the organization of the F-actin cytoskeleton have been observed. Other distinctive alterations involve the formation of punctate actin foci, which continues for several hours after the initial stimulation (Geitmann *et al.* 2000; Snowman *et al.* 2002). Although there are few examples of rearrangement of the actin cytoskeleton in response to specific stimuli in plant cells to date, the cytoskeleton is a major target of signals (Nick 1999; Staiger 2000). Furthermore, it has recently been established that a rapid, large and sustained depolymerization of F-actin was stimulated as a consequence of an incompatible response (Snowman *et al.* 2002). Within 5 min after SI induction, F-actin concentrations were less than 50% that of the controls, and they remained low at 1 h after SI induction. Since there is good evidence that increases in $[Ca^{2+}]_i$ in pollen tubes can stimulate actin depolymerization, this suggests that the SI-induced $[Ca^{2+}]_i$ increases may target signalling to the actin cytoskeleton. An interesting difference between the timing of the actin depolymerization in pollen grains and pollen tubes suggests that it is polar tip growth that is targeted (Snowman *et al.* 2002).

(f) *Evidence for PCD triggered by SI in pollen*

There is preliminary evidence that suggests that a PCD signalling cascade is triggered in incompatible pollen. The F-actin studies indicate this, since apoptosis in some animal cell types can trigger sustained F-actin depolymerization (Korichneva & Hämmerling 1999; Rao *et al.* 1999), and virtually the only examples of this pattern of depolymerization arise from cells undergoing apoptosis. This suggests that PCD may be triggered by SI in incompatible pollen. Interestingly, F-actin depolymerization may stimulate apoptosis in some cell lines (Janmey 1998; Korichneva & Hämmerling 1999; Rao *et al.* 1999). Data that are consistent with the idea of PCD come from evidence of S-specific nuclear DNA fragmentation, which is a hallmark feature of PCD, in incompatible pollen tubes (Jordan *et al.* 2000). Furthermore, artificially increasing $[Ca^{2+}]_i$ in pollen tubes also results in DNA fragmentation

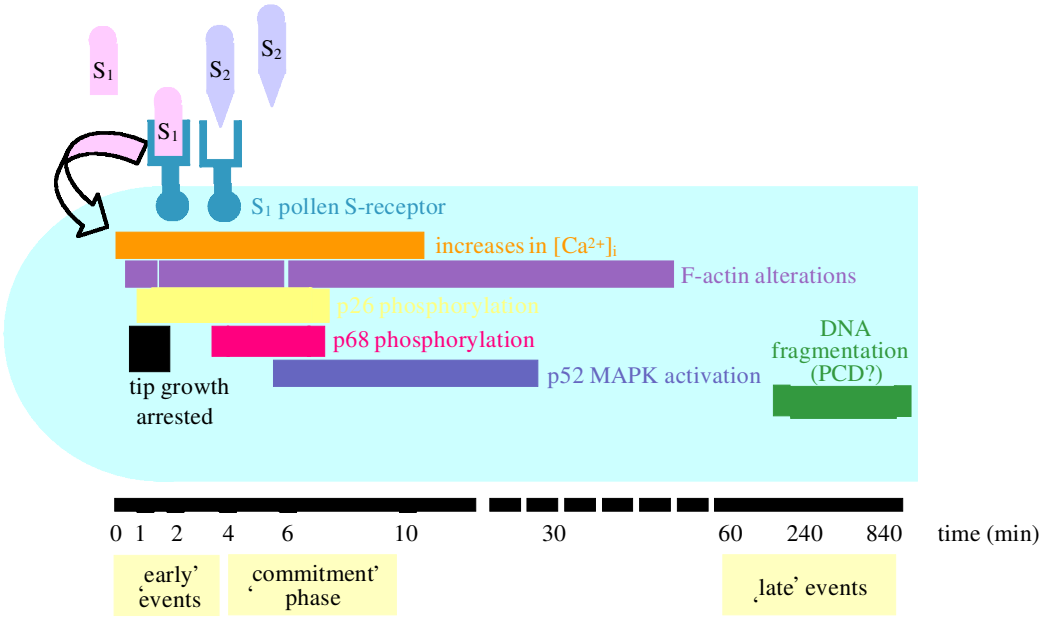

Figure 2. A model for the gametopytic SI system in *Papaver rhoeas*, with the signalling cascade and targets presented as a time-scale of events. Secreted stigmatic S-proteins (indicated in pink and mauve) interact with the pollen S-receptor. In the diagram, the S-receptor is depicted as a single entity, but may be a receptor complex with SBP (see § 3a for details). An allele-specific interaction, such as binding S_1 protein to S_1 pollen, results in an immediate increase of cytosolic free Ca^{2+} within the pollen. A series of events are then triggered in the incompatible pollen. Within 1 min, a series of 'early' events occur. There is a dissipation of the apical $[Ca^{2+}]_i$ gradient that is required for continued pollen growth, and the activation of a CDPK. The CDPK phosphorylates p26, a soluble inorganic pyrophosphatase. *In vitro* studies suggest that hyperphosphorylation of p26 reduces its catalytic activity such that the biosynthetic capability of the pollen is decreased, thereby limiting or inhibiting growth. After *ca*. 240 s, phosphorylation of another protein, p68, occurs via a Ca^{2+}-independent cascade. At present, the role of p68 is unknown. Dramatic reorganization of the pollen actin cytoskeleton becomes apparent within 1–2 min, together with extensive depolymerization of F-actin. At 5 min, increased activation of p56 MAPK is detected. This activity peaks at 10 min, and remains high for at least 30 min. After a period of *ca*. 10 min, the pollen has passed through a 'commitment' phase and is now destined to die. Until this point, the SI reaction can be reversed (at least *in vitro*) by washing off the incompatible S-protein. Actual cell death takes several more hours, and the pollen nuclear DNA undergoes fragmentation, suggesting the involvement of a PCD pathway. Preliminary data suggest that a caspase-like activity is triggered quite early in the response.

(Jordan *et al.* 2000). Since there is good evidence for both DNA fragmentation and Ca^{2+} signalling being involved in the induction of PCD in plant cells (Greenberg *et al.* 1994; Levine *et al.* 1997; Pennell & Lamb 1997), it suggests that the Ca^{2+} signals induced by SI might also trigger PCD in pollen. Although these data suggest that PCD might be induced in incompatible pollen, this is one of the later events of PCD, since it is only detectable several hours after SI induction.

Although DNA fragmentation should theoretically involve a caspase-3-like activity, no caspase homologues have been identified in plants (Arabidopsis Genome Initiative 2000; Lam *et al.* 2001). Despite this, and somewhat surprisingly, there is evidence for a caspase-like activity being activated in plant cells during the hypersensitive response to pathogens (D'Silva *et al.* 1998; Richael *et al.* 2001). Preliminary evidence indicates that the caspase inhibitor Ac-DEVD-CHO blocks the DNA fragmentation normally found in incompatible pollen tubes (S. Thomas, N. D. Jordan, P. Tiwari, H. Ali and V. E. Franklin-Tong, unpublished data). This suggests that SI-induced DNA fragmentation could involve a caspase-like/DEVDase activity. Furthermore, it also suggests that targets for this activity are present in *Papaver* pollen. As this treatment overcame the inhibition of incompatible pollen tubes (S. Thomas, N. D. Jordan, P. Tiwari, H. Ali and V. E. Franklin-Tong, unpublished data), the activation of this caspase-like activity must occur quite early in the signalling cascade, and suggests that it plays an active part in the SI response. Together, these data strongly implicate PCD in being involved in SI in *Papaver*.

4. DISCUSSION: A MODEL FOR SI IN *PAPAVER RHOEAS*

We present a working model for SI in *P. rhoeas*, based on current data described here, in figure 2. We propose that stigmatic S-proteins act as signalling molecules, interacting with at least one receptor molecule on the surface of pollen tubes. SBP has previously been proposed to act potentially as an accessory receptor to an *S*-allele-specific receptor (Hearn *et al.* 1996). However, SBP may be the pollen S-receptor, though we have no firm evidence for this. An interaction between the pollen S-receptor and its matching stigmatic S-protein stimulates an incompatible reaction. This triggers an intracellular signalling cascade(s) involving Ca^{2+} acting as a second messenger (Franklin-Tong *et al.* 1993). A transient increase in cytosolic $[Ca^{2+}]_i$ involving Ca^{2+} influx is generated, and is rapidly followed by inhibition of pollen tube growth within 1–2 min (Franklin-Tong *et al.* 1993, 1997, 2002).

Three events occur very soon (probably within 1 min) after the initial SI signal. The first is the loss of the apical gradient of $[Ca^{2+}]_i$. Since this is thought to be required for vesicle fusion, this could be responsible for arrest of pollen tube growth. The second event is the rapid phosphorylation of p26 via a CDPK (Rudd *et al.* 1996). As p26 has homology to an inorganic pyrophosphatase, it is highly likely to contribute to an arrest (perhaps temporary) of biosynthesis of cell membrane and wall material essential for growth. The third event is the reorganization (Geitmann *et al.* 2000) and depolymerization of F-actin (Snowman *et al.* 2002). Since only small amounts of actin depolymerization are sufficient to perturb tip growth, this actin response could also contribute to the rapid arrest of growth. However, there are other events that are triggered in incompatible pollen after growth is inhibited. A putative MAPK (p56) is activated. Since it is activated after pollen tube arrest has been achieved, it suggests another function. It has been speculated that further events may perhaps be required to make the inhibition irreversible. A PCD-like mechanism appears to be triggered quite early in the SI response, and the MAPK could feed into this. This complex signalling cascade appears to operate to ensure that incompatible pollen does not start to grow again.

In general terms, two issues of particular importance require resolution. First, as mentioned in § 3a, the nature of S-receptor remains to be determined. Second, it is clear that the mechanism of inhibition of incompatible pollen is highly complex. Substantial progress has been made towards defining aspects of the signal transduction pathway that mediates the SI response and several associated cellular events. Nevertheless, it still remains to be established how these events interrelate and are regulated to bring about the inhibition of incompatible pollen.

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GLOSSARY

$[Ca^{2+}]$: cytosolic free Ca^{2+}
CDPK: calcium-dependent protein kinase
MAPK: mitogen-activated protein kinase
PCD: programmed cell death
PhSBP1: *Petunia hybrida* S-RNase binding protein 1
SI: self-incompatibility
SBP: S-protein binding protein
SPH: S-protein homologue