SELF REJECTION DURING POLLEN-PISTIL INTERACTIONS

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Plants being sessile by nature have developed sophisticated mechanisms to assist in their selection of mating partners. During a compatible pollination event, pollen grains that have landed on the stigmatic surface at the top of the pistil will adhere to the stigmatic cells, hydrate, germinate and form pollen tubes. The actively growing pollen tubes are often capable of penetrating the stigmatic cell walls to grow down through the style and finally reach the ovary to deliver sperm for fertilization. In self-incompatible pollen-pistil interactions, many of these steps may be blocked following the recognition of self-pollen. As a result, this response prevents inbreeding and promotes out-crossing between unrelated individual plants (reviewed in Lord and Russell, 2002).

In general, plant self-incompatibility can be classified into two categories, homomorphic and heteromorphic. These terms describe the flower morphology, where homomorphic plants produce identical flowers, and the determination of self-incompatibility or compatibility is predominantly regulated at the genetic level. In contrast, heteromorphic flowers can differ in the lengths of the pistil styles and the stamen filaments (e.g. flowers with short styles and long filaments versus flowers with long styles and short filaments). The molecular mechanisms underlying heteromorphy have remained largely elusive, and most of what is known to date about self-incompatibility has been uncovered through studies on homomorphic systems. In these systems, self-incompatibility has been found to be regulated by at least two tightly-linked genes at what was traditionally known as the *S* locus. Each *S* gene has multiple alleles, and self-pollen rejection occurs whenever there is a match in the *S* alleles between the male and female tissues. The self-incompatibility system can also be either sporophytic or gametophytic in nature. In a sporophytic system (e.g. *Brassicaceae*), the identity of the pollen grain is determined by the parental genotype where the pollen *S* products are thought to be produced by the surrounding parental tissue and deposited on the pollen during development. In contrast, pollen grain identity in gametophytic systems (e.g. *Solanaceae*, *Papaveraceae*) is determined by its own haploid genotype where the developing pollen synthesizes its own pollen *S* product (reviewed in Stone and Goring, 2001).

In recent years, exciting progress has been made in unravelling the molecular basis of self-incompatibility in three different systems. Interestingly, it is becoming clear that these systems have evolved very different mechanisms to reject pollen, though one system has now been identified in three different families. The mustard family (*Brassicaceae*), which includes crops such as canola, radish, broccoli and cauliflower, utilizes a ligand-receptor kinase for pollen rejection (reviewed in Hiscock and McInnis, 2003). In the field poppy (*Papaveraceae*), a process called programmed cell death has been found to cause self-pollen rejection (reviewed in Franklin-Tong and Franklin, 2003). Finally, the third system involves an *S* RNase which degrades self-pollen RNA, and has

been found in the potato (*Solanaceae*), rose (*Rosaceae*), snapdragon (*Scrophulariaceae*), and bellflower (*Campanulaceae*) families (reviewed in Kao and Tsukamoto, 2004).

In *Brassica* self-incompatibility, it was initially thought that a single gene product encoded by the *S* locus determined the self-incompatibility response, but further research indicated this was not true. Still referred to as the "*S* locus", this region is comprised of three tightly linked genes encoding the *S* locus glycoprotein (SLG), the *S* receptor kinase (SRK), and the *S* locus cysteine-rich /*S* locus protein 11(SCR/SP11). When a self-pollen grain lands on the top of the pistil, the SCR/SP11 ligand present on the pollen surface binds to SRK. SRK is found in the plasma membrane of the stigmatic cells (pistil), and passage of SCR/SP11 through the stigmatic cell wall is thought to be assisted by SLG, also localized to this cell wall. Following binding of SCR/SP11, SRK becomes activated in the pistil (reviewed in Hiscock and McInnis, 2003). Interestingly, a second kinase has been recently identified to also be involved in the *Brassica* self-incompatibility, the *M* locus protein kinase (MLPK). MLPK may form a complex with SRK to continue the signalling in the pistil (Murase *et al.*, 2004). Ultimately, this signalling pathway will lead to self-pollen rejection by the pistil. The mechanism by which this occurs is thought to lie with the next protein in the pathway, ARC1. ARC1 is an E3 ubiquitin ligase which promotes the degradation of proteins by the proteasome. Therefore, ARC1 is thought to cause the degradation of pistil proteins which would normally be required to promote compatible pollinations. As a result, germination and pollen tube growth are blocked in rejected self-pollen grains (Stone *et al.*, 2003; Goring & Walker, 2004).

The *Papaveraceae* self-incompatibility system also involves signalling pathways which are, however, quite different from the *Brassica* system. A small ligand-like *S* protein has been found to be secreted by stigmatic cells at the top of the pistil. The stigmatic *S* protein is thought to bind to an unidentified pollen *S* receptor to initiate signalling inside the pollen. Several signalling events have been observed including a rapid increase in Ca^{2+} levels, protein phosphorylation, and depolymerization of the actin cytoskeleton resulting in growth arrest of the self-pollen (reviewed in Franklin-Tong and Franklin, 2003). Recently, programmed cell death has also been identified as the definitive contributor in this rejection response. Key features of programmed cell death including nuclear DNA fragmentation, leakage of cytochrome *c* from the mitochondria, and cleavage of poly (ADP-ribose) polymerase were all observed during self-pollination in *Papaver*. The result of programmed cell death is an irreversible rejection of the self-incompatible pollen (Thomas and Franklin-Tong, 2004).

The *S* RNase-based self-incompatibility was first identified in the *Solanaceae* and more recently in the *Rosaceae*, *Scrophulariaceae*, and *Campanulaceae*. The *S* RNase is found in the style of the pistil where the rejection response occurs to block self-pollen tube growth. The *S* RNase is proposed to enter the pollen tube, though the mechanism of entry is unknown, and inhibit pollen tube growth by the degradation of pollen RNA (reviewed in Kao and Tsukamoto, 2004). Consistent with this, pollen rRNA was found to be

degraded following self-incompatible pollinations (McClure *et al.*, 1990). Based on several pieces of evidence, the mechanism of action for the *S* RNase was explained by the inhibitor model which proposed that all *S* RNases were taken up into the pollen tube, and that pollen *S* inhibitors prevented non-self *S* RNases from functioning (reviewed in Kao and Tsukamoto, 2004). The recent identification of the pollen *S* protein as the *S* locus F-box (SLF) protein fits nicely with this model (Sijacic *et al.*, 2004). F-boxes are members of the larger protein complexes, the SCF complexes, which are also involved in targeting proteins for degradation by the proteasome. One can speculate that SLF may fit into the inhibitor model by mediating the degradation of all non-self *S* RNases, and therefore allow the continued growth of compatible pollen tubes. Following a self-incompatible pollination, an allelic match between SLF and *S* RNase would somehow prevent the degradation of *S* RNase, and pollen tube growth would be arrested by the degradation of the pollen RNA.

Though a large amount of information is now known about each of the different self-incompatibility systems, many pieces of the puzzles are still missing. All three known molecular mechanisms which plants have adopted to prevent inbreeding differ greatly with the only commonality between the *S* RNase and *Brassica* SRK systems being the employment of ubiquitin-mediated protein degradation. Recent findings have furthered our understanding of these systems, but it will be exciting to follow how these stories continue to unfold, and to see what new systems will be uncovered as other self-incompatible plant families are studied.

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