GAMETOPHYTIC SELF-INCOMPATIBILITY
(Newbigin et al., 1993; Matton et al., 1994; Dodds et al., 1997)

Most widespread throughout most plant families.
Most work in *Solanaceae* (one locus)
Gramineae (two loci–S and Z, independent loci)

How it works?
One locus–if pollen same genotype as female parent, then incompatible reaction
i.e., pollen must have a genotype distinct from either allele in female parent.
Pollen expresses the S allele-specific product and rejected by the stigma if that carries the same allele.

In grasses, 2 loci:

i. Give more breeding efficiency because many more possible genotypes, and therefore, higher cross-compatibility.
ii. Pollen needs one allele different *at either locus* from that present in female parent to become cross compatible.
iii. Assume maternal plant is S1S2-Z1Z2. A paternal plant S1S2-Z1Z3 will produce pollen of genotypes S1Z1 and S2Z1 that are incompatible; and S1Z3 and S2Z3 that are compatible

Model system #1:
Nicotiana alata (A. Clarke)
Petunia
Solanum

Pollen only expresses the single S allele present in its haploid genome

Phenotype:

a. Incompatible and compatible pollen germinate.
b. Incompatible pollen tubes grow more slowly than compatible pollen tubes.
c. As the incompatible pollen tube grows, its wall thickens, with callose (a polysaccharide) deposition at the tip–resulting in the tips swelling and bursting.

S-locus contains one known gene (S = Sterility)
(Lee et al., 1994; Murfett et al., 1994)

a. Encodes an S-RNase–a stylar extracellular glycoprotein with ribonuclease activity.
b. High similarity to RNaseT2 from *Aspergillus oryzae*.
c. Present in stigma, style, and restricted area of ovary, but expressed at *high levels in the style.*
d. There are high levels of this gene product in self-incompatible species, whereas very low levels in self-compatible species.
f. Generalized features:

a. *5 conserved domains*, including two of which contain histidine residues essential for ribonuclease activity.
b. 2 hypervariable domains, alleles vary in these regions.
c. Evolutionary perspective: are old and arose before speciation of the *Solanaceae.*

g. RFLPs are known among the different S alleles.
h. Other genes may also be present at the S locus (e.g., the pollen component of SI)

S-RNase activity is
a) *necessary* for rejection of incompatible pollen (i.e., SI) and,
b) *sufficient* to determine the incompatibilty phenotype of style.

Experimental Proof (Lee et al., 1994)
Petunia inflata
Transformation experiments were carried out with antisense constructs.

1). Transformed an antisense S3-RNase gene construct into a plant with genotype S2S3.
  a) Some transformed plants had reduced S3-RNase levels, as expected–these accepted S3 pollen.

  b) Other transformed plants had reduced S3 and S2-RNase–these plants accepted both S2 and S3 pollen.

2). Transformed a sense S3-RNase construct into plant with genotype S1S2.
  These plants expressed the S3-RNase and rejected S3 pollen.

3). From this and other studies, the level of incompatibility is related to the level of expression of the stylar S-RNase. Transgenic plants with low expression of a novel S-RNase due to anti-sense RNA expression had correspondingly low levels of incompatibility–i.e., high self seed set.

Ribonuclease activity of S-RNase is important
(Huang et al., 1994)
1. a. Transformed S3 plants with a construct with the second histidine residue replaced by an asparagines residue in S3. This protein showed no enzyme activity. Transformed S3 plants with modified S3 showed no S3-RNase activities.
   b. Protein accumulated to wild-type levels, but plants lacked RNase activity.
   c. Plants accepted S3 pollen.

(Royo et al., 1994)
2. a. The sequences of S-alleles from self-compatible (SC) and self-incompatible (SI) *Lycopersicon peruvianum* were determined.
   b. The SC phenotype has a nonfunctional S-allele.

c. In the SC plants, the S-RNase protein accumulates in style but lacks activity due to spontaneous mutation of the first histidine residue necessary for RNase activity to asparagines rsidue.

(Matton et al., 1997)

3. Hypervariable regions possibly code for allele-specificity
   a. A plant S12S14 was transformed with an S11 S-RNase–it rejected S11 pollen
   b. S11 and S13 are most similar S alleles with distinct phenotypes (recognition specificity). They differ by only 10 amino acids, four in the hypervariable region.
   c. A chimeric S11 that has the S13 hypervariable (HV) region now rejected S13 pollen.
   d. Suggests the presence of <u>two domains in the protein</u>: 1) recognition and 2) RNase activity. HV region determines the allele-specificity (recognition).

<u>Mode of action</u>

Stylar S-RNases act as allele-specific cytotoxins and inhibit growth of only incompatible pollen tubes by degrading their rRNA.

1. Initial research indicated that pollen may not synthesize rRNA, and relies on a fixed amount of previously synthesized rRNA, so its degradation would stop growth.
2. Recent studies indicate that pollen does synthesize some rRNA, so if this model is true, then degradation must be faster than synthesis.
3. Support for "2":
   a. Transfer of an arrested pollen tube to a compatible stigma allows growth to resume, indicating that tube could replace degraded rRNA.
   b. A low level of S-RNase may allow incompatible pollen to successfully fertilize. A high rate of S-RNase activity is necessary for a SI reaction, may be because pollen can replenish the degraded rRNA.

<u>Possible mechanisms</u>

How to explain the selective degradation of only incompatible rRNA?

(Golz et al. 1999)

1. Model 1–Receptor model: only the specific S-RNase that degrades incompatible rRNA is taken up by the pollen tube.

2. Model 2–Inhibitor model: all S-RNases are taken up, but ones not involved in incompatibility are inactivated/degraded.

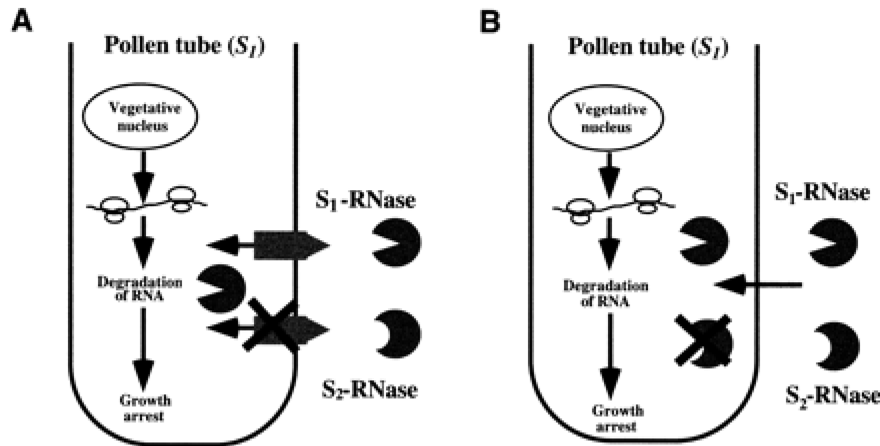

Figure 1. Two models of events involved in inhibiting the growth of an S_1 pollen tube in an S_1S_2 style. (A) The receptor model; (B) the inhibitor model (from Golz et al. 1999).

Model 2 is most likely correct:

Luu et al. 2000

1. Luu et al. (2000) showed by applying immunocytochemical labeling technique that S-RNase accumulates in cytoplasm of all pollen-tube haplotypes.
2. 15 amino acid (aa) peptide representing the hypervariable region of the S11-RNase was used to raise polyclonal antibody. This antibody recognizes only S11 –RNAse but not S13-RNase (differ by 3 aa).
3. Pollen tube growth arrested between upper 1/3rd and ½ of the styles with 48 h following self-pollination. Therefore, 18 h following pollination studied for accumulation of S11-RNase in a S11S13 genotype.
4. Pollen tubes were labeled in the cytoplasm (Figure 1 b and c). Most labeling was observed in pollen tube cytoplasm (60 fold over background, and over 5 folds over extracellular matrix).
5. S11-S13 genotype was pollinated by pollens from a S12S12 genotype and accumulation of S11-RNase was monitored. Again labeling reactions were more abundant in the compatible pollen cytoplasm (Figure 2a)-comparable to that in in the incompatible pollen tube cytoplasm (Figure 1 b and c).
6. No labeling was seen in the compatible S11S13 pollen tubes in S12S14 styles (Figure 2c). Here no S11-RNase and no antibody reaction.
7. Conclusion: S-RNase is taken up equally in both compatible and incompatible pollen tubes. Model 2 is correct.

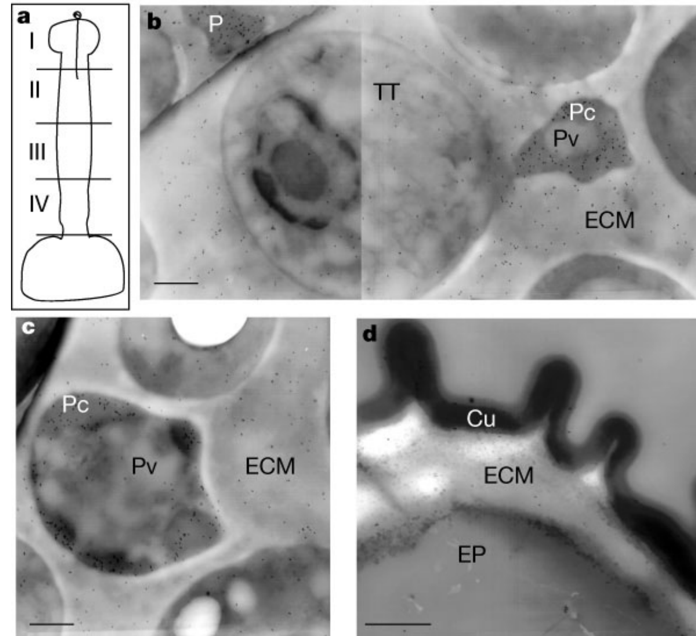

Figure 1 S_{11}-RNase entry into incompatible pollen tubes. **a**, Self-pollinated styles of an $S_{11}S_{13}$ plant were fixed 18 hours post-pollination, sectioned in region II, and stained sequentially with the anti-S_{11} antibody and a 20-nm gold-labelled secondary antibody. **b**, **c**, The apical tips of all growing pollen tubes (P) are labelled to a greater extent than either the transmitting tissue cells (TT) or the extracellular matrix (ECM) separating the cells. Label inside pollen tubes appears associated with the cytoplasm (Pc) rather than the vacuolar spaces (Pv), unlike the labelling found in transmitting tissue cells. **d**, Only background labelling is present in the cytoplasm, the surrounding cell wall or the cuticle (Cu) of epidermal cells (EP), which do not express S-RNases. All scale bars are 1 μm.

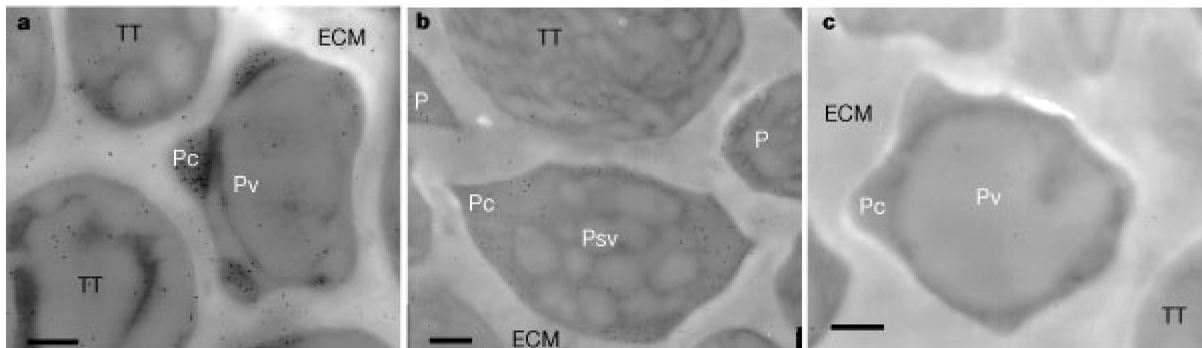

Figure 2 S_{11}-RNase entry into compatible pollen tubes. Pollinated styles were treated as described in Fig. 1. **a**, A fully compatible cross of $S_{11}S_{13}$ styles pollinated with S_{12} pollen. **b**, A semi-compatible cross of S_{11} S_{13} plants pollinated with S_{13} and S_{14} pollen. The region observed is close to the middle of the style and is deduced to contain only fast-growing compatible S_{14} pollen tubes. The several electron-lucent regions correspond to secretory vesicles (Psv). **c**, A fully compatible cross of $S_{12}S_{14}$ styles pollinated with S_{11} and S_{13} pollen, showing the background labelling of the pollen tubes in the absence of an S_{11}-RNase. Cells are labelled as in Fig. 1 and all scale bars are 1 μm_1-RNase entry into compatible pollen tubes.

Progress towards identifying the male determinant-possible inhibitor protein

Golz et al. 2001

1. In order to determine the male determinant Newbigin group in Australia developed some mutants in *Nicotiana alata* by irradiating with gama rays. These mutations affect the self-incompatibility phenotype of the pollen but not that of the style. These are called pollen-part mutants (PPM).
2. Gama irradiation causes deletion, chromosomal aberrations such as translocation, inversion.
3. Golz et al. 2001 reported that the PPMs were frequently associated with the duplication of S3 alleles. S3S6 was mutagenized.
4. In their mutant analyses they used three S-linked genes: two pollen-expressed genes, 48A and 167A and a leaf-expressed gene CP100 as probes in DNA-gel blot analysis.
5. Use of these flanking marker genes allowed them to discover duplications in the entire S3 allele or part of the S3 allele not including the S3-Rnase gene.
6. Thus, S3 and S6 alleles now can be in the same gamete and a "competitive interaction" between two different S alleles results break down of compatibility.
7. The absence of pollen S deletions indicate the necessity of pollen S factor for pollen viability – authors interpreted perhaps the factor is an RNase inhibitor, essential to protect against S-RNase.
8. Loss of self-incompatibility following polyploidation was reported earlier.
   One problem in mapping the genes in the S locus is the low rates of recombination. Development of these PPM mutants especially with a small duplication is expected to expedite the mapping and identification of the male determinants.

Role of aspargine-rich protein for S-allele-specific incompatibility:

Nicotiana and tomato

SI = self-incompatible; SC = self compatible

1. Some compatible species were evolved from self-incompatible species. For example, tomato became self-compatible from self-incompatible ancestors through breakdown of the incompatibility system.
2. Unilateral self-incompatibility: Incompatibility between species is seen in only one direction. *Lycopersicon peruvianum* (SI) x *L. escullentum* (SC) is an incompatible one, while the reciprocal cross is a compatible one. Likewise, *Nicotiana alata* (SI) x *N. plumbaginifolia* (SC) show reciprocal differences.
3. We have seen that expression of S-RNase leads to a functional gametophytic self-incompatibility in Petunia, Nicotiana and Solanum (Lee et al. 1994; Matton et al. 1997).
4. Breakdown of the self-incompatibility resulted from the mutation in genes including the S-locus. Expression of S-RNase fail to recover the lost self-incompatibility in self-compatible species.

(Murfett et al. 1996)

a. Transformed *N. plumbaginifolia* and other Nicotiana species with S_{A2} and S_{C10} RNase from *N. alata.*
b. Transformed *N. abacum* and *N.glutinosa* were able to restore the S RNase-dependent self-incompatibility (resulted loss of unilateral cross incompatibility).
c. However, expression of S Rnases from *N. alata* failed to confer self-incompatibility in *N. plumbaginifolia.*
d. F_1 of transgenic *N. plumbaginifolia* and *N. alata* conferred incompatibility against *N. plumbaginifolia* pollen. This suggests that a factor in addition to S RNases is missing in *N. plumbaginifolia.*
e. Therefore, an additional factor is necessary in addition to S RNases for restoring the self-incompatibility in species in which the system is broken.

(McClure et al. 1999)

f. An asparagines-rich gene (*HT*), transcribed highly in the stylar tissues, was cloned. This gene is expressed in *N. alata,* but not in *N. plumbaginifolia.*
g. Expression pattern of the gene is comparable to that of a S RNase gene.
h. Antisense expression of HT in transgenic *N. plumbaginifolia* (S_{C10}*) x N. Alata* (S_{C10} S_{C10}) fail to show the S_{C10} pollen rejection.
i. HT-protein is essential for pollen rejection.

(Kondo et al. 2002)

j. Tomato is self compatible. *L. peruvianum* produces S RNases and is self-incompatible.
k. No evidence for S RNase activity was observed for the stylar tissues in tomato.
l. Expression of the *L. peruvianum* S_6-RNase in tomato fail to show inter-specific pollen (S_6) rejection.
m. Cloned the HT gene from tomato by applying a PCR approach (primers were based on the sequences of the *Nicotiana* gene).
n. Two HT genes in tomato; transcript of *LeHT-A* was detected to a significant level in tomato style, whereas, most likely *LeHT-B* is a non-functional gene (no detectable transcripts).
o. Sequence analysis showed that none of these genes should produce functional proteins.
p. Transgenic experiments will be necessary to show if any of the genes (wild type, say from *L. peruvianum*) are required for the restoration of the pollen rejection in transgenic plants carrying a functional S RNase.

Conclusions: Factors in addition to S RNases are involved in the gametophytic self-incompatibility.

Model System #2:

Papaver rhoes, field poppy (Matton et al. 1994; Foote et al. 1994)

S-locus product

1. Small glycoproteins expressed only on stigma.
2. Purified the stigmatic glycoprotein by using a in vitro bioassay (pollen tube growth). Can inactivate pollen with the same specificity *in vitro.* Acts in an allele-specific manner.
3. Sequenced the purified protein and designed primers based on the peptide sequences.
4. PCR amplify the gene from the genomic DNA.
5. E. coli expressed protein is active.
6. Therefore, glycosyl residues in the glycoprotein is not an essential part from recognition.
7. Apparently unrelated to S-RNases (or to SLG/SRK in *Brassicacea*).

Mechanism

1. A pollen receptor possibly binds the glycoprotein, initiating a signal transduction pathway
2. Alterations in calcium levels and phosphoinositide signaling occur in incompatible pollen tubes (Franklin-Tong et al., 1996, 2002)
3. Protein phosphorylation is altered in incompatible tubes.
4. Leads to gene activation and arrest of tube growth.