Recognizing Self in the Self-Incompatibility Response

Ram Dixit and June B. Nasrallah*

Department of Plant Biology, Cornell University, Ithaca, New York 14853

François Jacob once facetiously lamented the arrangement in the human body whereby reproduction is the only function for which an individual is equipped with only one-half of the necessary organs, thus entailing the expenditure of a substantial amount of time and energy into finding the other half (10). The spatial separation of the sexual partners (or organs) is indeed an obligatory feature of sexual reproduction, but curiously, organisms that are hermaphroditic and hence have the potential for self-fertilization are frequently seen to be indulging in the social facet of sexuality, i.e. the search for a mate.

Plants, being sessile organisms, cannot actively search for mates but have contrived a variety of genetic and nongenetic mechanisms to hinder self-pollination and promote cross-pollination. The time and energy spent on facilitating cross-pollination is amply compensated for by an increased vigor of the organism as demonstrated by Darwin (4). Self-incompatibility (SI) is an example of a genetic barrier to self-fertilization and represents the most common antiselfing mechanism among the angiosperms.

CLASSICAL VIEW OF THE SI RESPONSE

The SI response is a phenomenon that occurs after pollination has taken place and entails the recognition and selective inhibition of "self" pollen grains by cells of the pistil. Early genetic studies (for review, see 5) of the Solanaceae and Brassicaceae (crucifer) families established that specificity in the SI response is controlled by a single multi-allelic locus called the *S* locus, and that the SI response is instigated when pollen and pistil are derived from plants sharing a common *S*-locus variant. Subsequent studies in other taxa established a similar single-locus control in some, but also demonstrated control by more than one recognition locus in others (as in the Poaceae family).

Because the ultimate outcome of the SI reaction—i.e. inhibition of self-related pollen and prevention of self-fertilization—is the same in different plant families, one view proposed SI to be monophyletic in origin. Yet it was also recognized that dramatic differences occur in the SI response of different taxa. One difference, which has formed the basis of a major classification scheme for SI systems, is the mode of genetic control of the pollen SI phenotype. In sporophytic SI, the pollen phenotype is determined by the diploid *S* complement of the parent plant, whereas in gametophytic SI, the pollen phenotype is determined by the *S*-locus variant carried in its haploid genome. Even more dramatic are differences in the site of inhibition of pollen or pollen tube. For example, in crucifers, the epidermal cells of the stigma prevent hydration and further development of the incompatible pollen grains, whereas in the Solanaceae family, incompatible pollen tubes are free to make their way into the style where their growth is severely and selectively retarded. In all systems, the *S* locus was thought to encode molecules that act as *S*-allele-specific tags on pollen grain or tube and on cells of the pistil which allow the discrimination of self versus non-self. One hypothesis regarding the mechanism of self-recognition was that an inhibitory dimer is produced by homophilic binding of identical SI gene products carried by the pollen and pistil. A second hypothesis envisioned the *S* locus to be a complex locus containing distinct pollen and pistil specificity determinants (for review, see 5).

In this note, we summarize the major progress achieved during the last 2 decades in isolating genes that encode the determinants of specificity in the SI response and understanding the molecular basis of self-recognition.

MOLECULAR DIVERSITY OF SI SYSTEMS

Starting in the 1980s, molecular methods were applied to the study of SI in species exhibiting single-locus control, namely *Brassica* sp., *Nicotiana* sp. and other Solanaceous plants, and *Papaver rhoeas*. Based on the earlier success of immunochemical methods in identifying an *S*-locus-linked stigma-specific protein in *Brassica* (17), these molecular studies focused initially on the isolation of genes that were: (a) expressed specifically in the pistil at the site of pollen arrest and in correlation with the acquisition of the SI response by the developing pistil, (b) polymorphic between strains carrying different SI specificities, and (c) genetically linked to the *S* locus. These studies resulted in the isolation of the *S*-locus glycoprotein gene (16) and later the *S*-receptor kinase gene (19) in *Brassica* (a sporophytic system), the *S*-RNase gene (1) in *Nicotiana* (a gametophytic system), and the *S* glycoprotein (9) in *Papaver* (also a gametophytic system). It is significant that the *S*-locus-associated genes isolated in each of the three species were found to bear

* E-mail jbn2@cornell.edu; fax 607–255–5407.

no sequence similarity to one another, providing a first clue that SI evolved independently and probably multiple times in different lineages of the angiosperms. Thus, a major outcome of these studies was the realization that the "*S* loci" of the Brassicaceae, Solanaceaeae, and Papaveraceae families are nonhomologous loci, and that the term "SI" represents not one process but a variety of mechanistically distinct processes (see below) that are similar only in their ultimate outcome. In addition, the uniqueness of the SI system in the three families refutes any relatedness assumed by the classification of SI systems on the basis of gametophytic or sporophytic control of pollen SI phenotype.

THE PISTOL (SIC) IS THE LAST LINE OF DEFENSE FOR THE FLOWER

In the Solanaceae family, as well as the Scrophulariaceae and the Rosaceae families, which also have an *S*-RNase gene associated with their *S* loci, SI is proposed to be based on the cytotoxic effect of the S-RNase on self-related pollen tubes. In *Nicotiana* and *Petunia* sp., the activity of the S-RNases has been shown to be necessary for the SI response and transformation experiments have demonstrated that the S-RNase represents the pistil component of the SI response (13, 15). The S-RNase is secreted into the transmitting tissue and is proposed to enter the pollen tube and degrade its cellular RNA (for review, see 7). The pollen SI factor in this system is proposed to be an *S*-allele-specific transporter or an intracellular *S*-allele-specific inhibitor of the S-RNase (7).

In the case of *Papaver*, the stigma-specific S glycoprotein is a small protein capable of *S*-allele-specific inhibition of pollen tube development in an in vitro pollen germination assay (12), presumably by interacting with a pollen-derived *S*-allele-specific receptor. This interaction would presumably result in the variety of responses observed within incompatible pollen tubes upon exposure to S glycoprotein, including protein phosphorylation, elevation in cytosolic Ca^{2+} levels, and changes in the actin cytoskeleton, all of which are known to result in inhibition of pollen tube growth (11).

In *Brassica*, self-recognition is based on the activity of the S-receptor protein kinase (SRK), which is expressed specifically in the stigma epidermis. Of the three SI systems investigated so far, the *Brassica* system is unique in that the biochemical events that are precipitated by self-recognition and that result in the inhibition of self pollen occur in cells of the pistil rather than in the pollen or pollen tube. The requirement for SRK in SI was known for some time from the analysis of self-fertile plants that occur spontaneously or are generated by a transgenic approach. However, it was only recently that a transgenic approach succeeded at modifying SI specificity in stigmas (3, 21). These transgenic experiments demonstrated that SRK is the determinant of SI specificity in the stigma, and that the cell wall-localized S-locus glycoprotein, which shares a high degree of sequence similarity with the ectodomain of SRK, enhances the strength of the SI response (21), possibly by facilitating post-translational maturation and accumulation of the SRK receptor (6).

THE POLLEN DETERMINANT OF SPECIFICITY IN THE SI RESPONSE

In comparison with the pistil, pollen has suffered from a general lack of understanding through the ages. Before the experimental demonstration of plant sexuality by Camerarius, the involvement of the ovary in seed production was never in doubt; however, pollen had yet to be perceived as male sexual structures. Pollen was instead perceived to represent a type of floral excrement or to function in the service of bees that deliberately acquired pollen to achieve better stability in flight. Our understanding of the pollen component of the SI response has suffered a similar lapse in understanding.

Subsequent to the isolation of the pistil components of SI in *Brassica*, the Solanaceae family, and *Papaver*, work focused on determining if these genes also functioned in pollen, as predicted by the dimer hypothesis of *S*-gene action. When it became obvious that they did not, a variety of approaches were used to identify the pollen determinant of SI, including differential cloning strategies as well as development of bioassays for pollen proteins (20). It was ultimately direct cloning of the *S* locus that resulted in a major breakthrough and the isolation of the pollen SI specificity gene in *Brassica* (18). Sequence analysis and transcriptional mapping of a chromosomal segment spanning the *SLG* and *SRK* genes led to the identification of *SCR* (*S*-locus Cys rich), a gene that is exclusively expressed in the anthers and exhibits *S*-genotype-associated polymorphism. It is most significant that analysis of a loss-of-function mutant strain and gain-of-function transgenic plants proved that *SCR* is both necessary and sufficient for determining SI specificty in pollen (18). It is expected that the small (<8 kD) hydrophilic SCR protein is secreted and incorporated into the pollen coat. Thus, the stigma and pollen SI specificity molecules of crucifers are located at the surfaces of the interacting cells as was predicted by Bateman in the 1950s based on the rapidity of the SI response in this family.

The *S*-locus-encoded molecules provide specificity for the SI response, but the culmination of the response, i.e. inhibition of self pollen, must involve the participation of other proteins. In *Brassica,* the current working model for the SI response envisages that SCR would bind to SRK in an *S*-genotype-specific manner. This results in the activation within the stigma epidermal cell of a signal transduction cascade, the endpoint(s) of which are the immediate

cause of self-pollen arrest (18; Fig. 1). Details of this SRK-mediated signal cascade are poorly understood although some elements are beginning to emerge.

THE *S* LOCUS AS A COMPLEX MULTIGENIC LOCUS

A major finding of the molecular analysis of SI in *Brassica,* and one that is likely to hold true for SI in other taxa with single-locus control, has been that the genetic behavior of the *S* locus as a simple Mendelian locus masks a complex and rearranged physical structure. To reflect this complexity, the "*S* alleles" of classical SI genetics are now referred to as "*S* haplotypes." The *Brassica S* haplotyes that have been mapped to date can vary significantly in overall physical size and in the relative orientations and positioning of their genes (2, 22). In this, the *Brassica S* locus is similar to other recognition loci, such as the mating type loci of *Chlamydamonas reinhardtii* and fungi, where structural heteromorphism is known to affect the frequency of recombination and contribute to the maintenance of recognition genes in a tightly linked genetic unit.

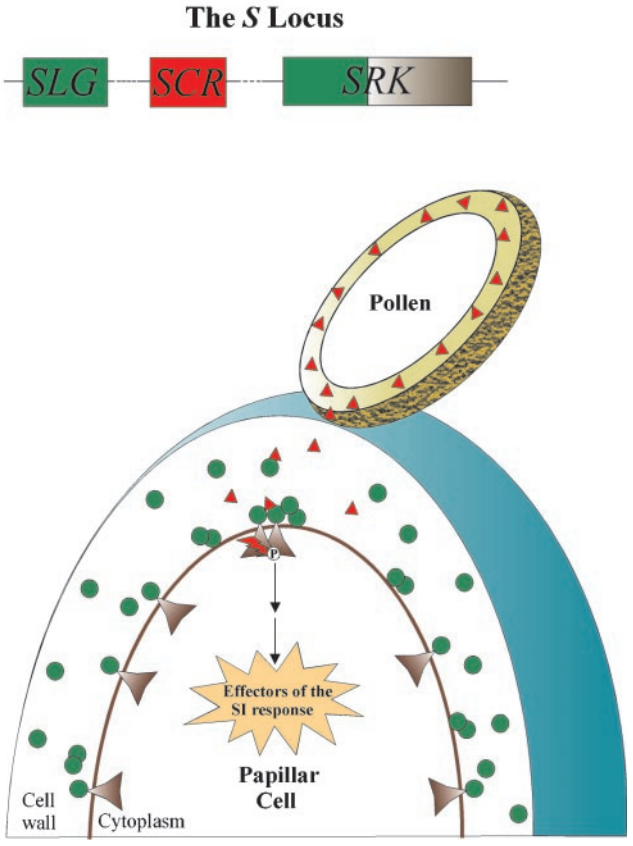

Figure 1. Recognition of self pollen in *Brassica*. The diagram shows the three genes of the *S*-locus complex (introns are not shown for simplicity) and the interactions proposed for the *S*-locus-encoded recognition molecules at the pollen-stigma interface. The genes and the corresponding protein products are color coded.

Another feature shared by plant SI systems and other recognition systems is the extensive polymorphisms of their genes. *S* locus genes have attained some of the highest levels of allelic polymorphism known for any locus, consistent with the expectation that *S* haplotypes are subject to diversifying selection. This is especially true for the small SCR protein, allelic forms of which exhibit strict conservation of only eight Cys residues and one Gly residue (18, 22). A challenge for the future is to understand how "matched" allelic polymorphisms in the pistil and pollen determinants of SI, and thus new SI specificities, are generated.

THE ROAD AHEAD

Although substantial progress has been made in the study of SI, much remains to be done. In the Solanaceae family and *Papaver,* the search for the male component is still a topic of hot pursuit. In *Brassica*, the identification of the pollen identity factor (SCR) is sure to cause a spurt of activity toward deciphering the nature of the SRK-SCR interaction and subsequent SRK activation. In all three systems, the particular domains or residues in the *S*-locus genes that are responsible for the unique SI specificity of each variant remain to be elucidated, although some progress on this issue has been made in *P. rhoeas* (12) and *Solanum* sp. (14).

Of great interest, but also more difficult to tackle, are questions relating to the origin of SI and *S*-locus genes. It now appears that the SI recognition genes were recruited from genes that are expressed in a variety of plant organs and presumably perform functions unrelated to pollination. For example, the *Brassica SRK* gene is the prototype of a class of receptor-like protein kinases that occurs in dicots and monocots, and *SCR* is likely to also be one member of a family of ligands. Functional analysis of these gene families is obviously critical for understanding the relationship of the SI self-recognition genes with gene sets that control plant growth and development or regulate the plant's defense response. Furthermore, with the identification of SCR, the putative ligand for SRK, the *Brassica* SRK-SCR system becomes one of only two receptor-ligand pairs known in plants. The other is CLV1, a member of the Leu-rich repeat class of receptor-like protein kinases, members of which function in plant development or defense, and its putative ligand, the small CLV3 peptide (8). The identification of these genes promises to usher in a productive era in the study of peptide signaling in plants, the regulation of receptor activity and ligand availability, and mechanisms of receptor activation. In this arena, the natural polymorphism of the *Brassica* SI genes should provide a unique resource for investigating the range of changes that can sustain a productive receptor-ligand interaction and for understanding the still obscure selection mecha-

nisms that direct the coevolution of receptor-ligand pairs.

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