Monoallelic Expression and Dominance Interactions in Anthers of Self-Incompatible *Arabidopsis lyrata*[1]

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Genetic dominance and recessiveness are most commonly addressed in the context of mutated alleles that confer aberrant phenotypes but have rarely been explained for functional variants. An opportunity to gain a mechanistic understanding of interactions between naturally occurring functional allelic variants is presented by the self-incompatibility (SI) system of crucifers. This intraspecific mating barrier, which allows the epidermal cells of the stigma to recognize and reject self-related pollen, is based on the activity of a large number of haplotypes of the *S*-locus complex. Each haplotype encodes highly divergent allelic variants of the *S*-locus receptor kinase (SRK), a transmembrane protein of the stigma epidermis that determines SI specificity in the stigma (Stein et al., 1991; Takasaki et al., 2000), and the *S*-locus Cys-rich protein (SCR), a pollen coat-localized ligand for SRK (Kachroo et al., 2001), which determines SI specificity in pollen (Schopfer et al., 1999; Takayama et al., 2000). Self-pollination is proposed to trigger an *S* haplotype-specific receptor-ligand interaction between SRK and SCR, which leads to the arrest of self-related pollen at the stigma surface (Nasrallah, 2000).

In self-incompatible crucifers, including *Brassica* species and *Arabidopsis lyrata*, a wild, self-incompatible relative of Arabidopsis, pollen SI specificity is determined by the diploid genotype of the pollen-producing parent rather than by the genotype of individual haploid pollen grains (Bateman, 1954; Thompson and Taylor, 1966; Kusaba et al., 2001; Schierup et al., 2001). Consequently, genetic interactions between *S* haplotypes occur in the specification of SI phenotype in pollen as well as in stigmas. Allelic interactions of codominance, dominance, incomplete dominance, or mutual weakening occur, and these interactions can differ in stigma and pollen, consistent with the activity of distinct determinants of SI specificity in these two tissues.

Recessiveness in pollen confers an advantage on an *S* haplotype by allowing pollen of the recessive genotype to elude the *S* haplotype-specific stigmatic surveillance mediated by SRK. In fact, "pollen-recessive" alleles attain high frequencies in populations (Uyenoyama, 2000). Elucidation of the molecular basis of *S* haplotype recessiveness in pollen is important for understanding the mechanism of SI and the evolution and maintenance of *S* haplotypes in a population. Here, we examine the genetic interaction of two *S* haplotypes of *A. lyrata* and elucidate the molecular basis of their dominant/recessive relationship in pollen.

We recently isolated the *SRK* and *SCR* genes from two *A. lyrata S* haplotypes designated *Sa* and *Sb* (Kusaba et al., 2001). Reciprocal crosses of *SaSb* to *SaSa* and *SbSb* revealed that, in the stigma, *Sa* and *Sb* exhibit a codominant interaction with "weakening" of *Sa*. In pollen, *Sa* is recessive to *Sb*, and pollen grains from *SaSb* plants exhibit *Sb* specificity. These interactions imply that *SRKa* and *SRKb* are both active in heterozygotes, although the *SRKa* allele exhibits somewhat lower activity. In contrast, the activity of *SCRa* is completely masked in *SaSb* heterozygotes.

We found significant differences in the temporal and spatial distribution of *SCRa* and *SCRb* transcripts in *Sa* and *Sb* homozygotes, respectively. On RNA gel blots (Fig. 1A) and by reverse transcriptase-PCR, *SCRa* transcripts were detected in early stage anthers, which contain a tapetum (a cell layer derived from diploid cells of the sporophyte that serves as nurse tissue for the developing haploid microspores and that degenerates before anther dehiscence), but were not detectable in late-stage anthers, which lack tapetal cells (Fig. 1A). In contrast, *SCRb* transcripts were detected at early and late stages of anther development (Fig. 1A) as previously described (Kusaba et al., 2001). In situ hybridization of *SbSb* anthers demonstrated that *SCRb* is expressed sporophytically in the tapetal cell layer and gametophytically in microspores (Fig. 2), as described for all *Brassica SCR* alleles examined to date (Schopfer et al., 1999; Schopfer and Nasrallah, 2000; Takayama et al., 2000; Shiba et al., 2001). In contrast, *SCRa*, which is the only "pollen-recessive" allele isolated to date, exhibits strict sporophytic expression (Fig. 2). Thus, functional *SCR* alleles can vary dramatically in their expression pattern, and expression of *SCR* in the tapetal cell layer is sufficient for SI. The additional gameto-

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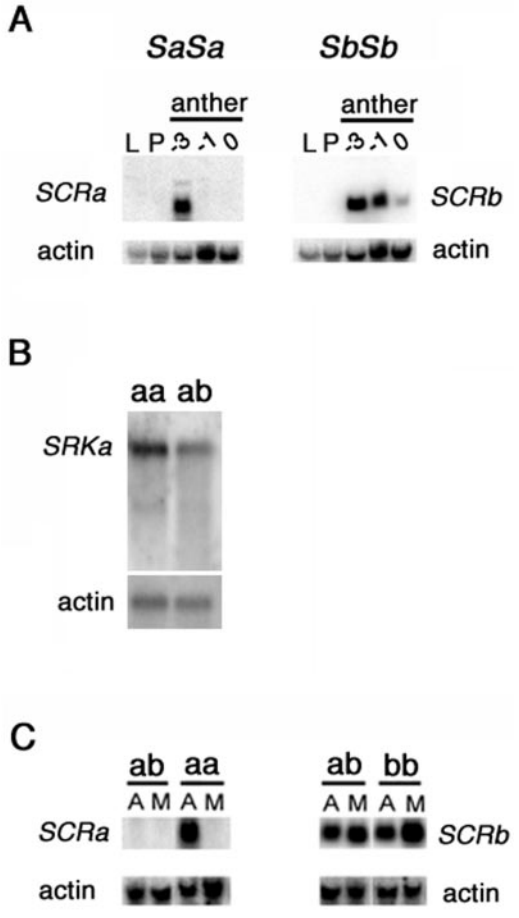

Figure 1. Differential expression of *S*-locus genes in *A. lyrata.* A, Developmental regulation of *SCRa* and *SCRb* expression in *A. lyrata* anthers. Total RNA (15 μg per lane) was isolated from *SaSa* and *SbSb* leaves (L), pistils (P), and anthers at three stages of development: −3 anthers (with intact tapetum) and −1 anther (after degeneration of the tapetum) were collected at 3 d and 1 d before flower opening, respectively. Mature anthers (containing mature pollen grains) were collected from open flowers (0). Blots were probed with *SCRa* or *SCRb* cDNAs, which, being only 35% similar, serve as allele-specific probes. Hybridization with actin served as a loading control. Hybridization signals were quantitated using a PhosphorImager and the ImageQuant program (Molecular Dynamics, Sunnyvale, CA). B, Expression of *SRKa* in *A. lyrata* stigmas. poly(A^+) RNA (2 μg per lane) was isolated from *SaSa* (aa) and *SaSb* (ab) stigmas (−1 stage) and probed with the *SRKa* ectodomain and with actin as a loading control. C, Expression of *SCRa* and *SCRb* in *SaSa* (aa), *SbSb* (bb), and *SaSb* (ab) anthers. Total RNA (15 μg per lane) was isolated from anthers (A) and microspores (M) collected from −3-stage buds. Probes are as in A.

phytic expression exhibited by *SCRb* and all known *Brassica SCR* alleles might be redundant or serve to boost SCR levels in individual pollen grains.

We examined the expression of the *SRK* and *SCR* alleles in *SaSb* heterozygotes. We found no difference in the level of *SRKA* transcripts beyond that expected from reduced gene dosage in heterozygous stigmas relative to homozygous stigmas (Fig. 1B). Thus, the weakening of *Sa* activity in heterozygous stigmas, like dominant/recessive relationships in the *Brassica* stigma (Hatakeyama et al., 2001), is not related to differences in *SRK* expression levels. It may be based on interference between receptor or ligand isoforms either in the SRK-SCR interaction or in the recruitment of downstream effectors of the SI response.

In contrast, the *SCR* alleles were differentially regulated in heterozygotes. *SCRb* transcripts were detected in *SaSb* anthers (Fig. 1C) and were localized to tapetum and microspores as in *Sb* homozygotes (Fig. 2). However, *SCRa* transcripts were drastically reduced in heterozygotes relative to *Sa* homozygotes (Figs. 1C and 2), with average reductions of approximately 80-fold and 30-fold estimated from long ex-

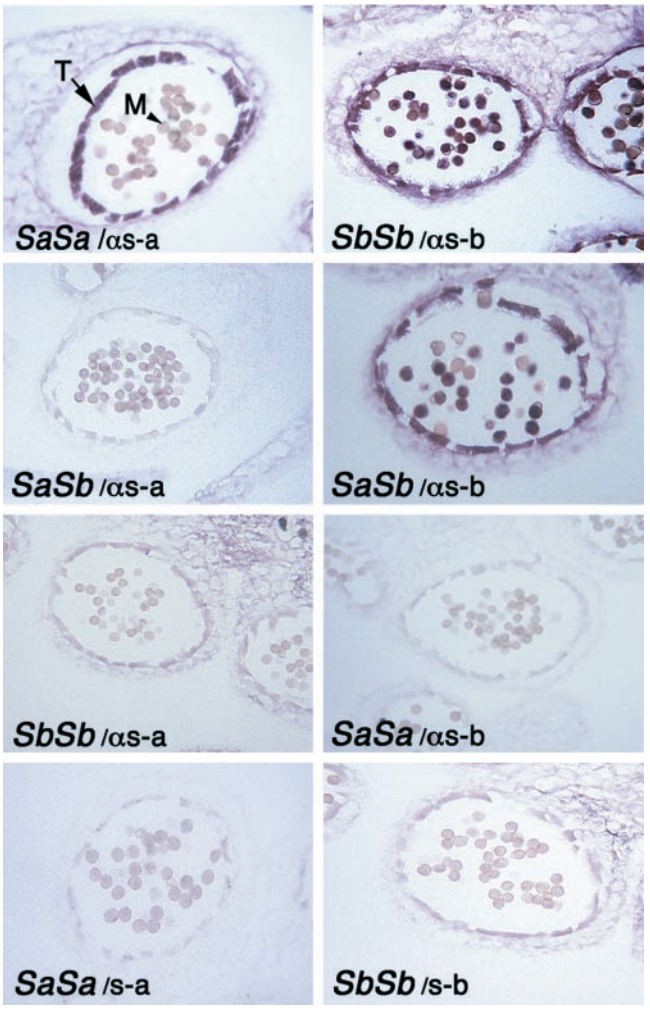

Figure 2. In situ localization of *SCRa* and *SCRb* transcripts. Paraffin-embedded sections were prepared from *SaSa*, *SbSb*, and *SaSb* −3-stage anthers and hybridized with dioxigenin-labeled RNA probes transcribed in vitro essentially according to protocols at http://www.Arabidopsis.org/cshl-course. The probes were: αs-a, antisense *SCRa*; αs-b, antisense *SCRb*. Negative controls: Sense *SCRa* (s-a) and sense *SCRb* (s-b) RNA probes. T, Tapetum; M, microspores. Some microspores in *SaSb* anthers did not hybridize with the αs-b probe, in keeping with the expected segregation of *Sa* and *Sb* microspores. Magnification, 450×.

posures of RNA gel blots and quantitative reverse transcriptase-PCR, respectively. In contrast, *SCRb* transcripts were reduced by only approximately 10% in heterozygotes relative to *Sb* homozygotes. Importantly, comparison of eight *SaSa* and eight *SaSb* plants generated by forced selfing of an *SaSb* plant in which *SCRa* was "silent" showed that *SCRa* was expressed in *SaSa* progeny and "silenced" in their *SaSb* sibs. Thus, the low-expression state of *SCRa* is not heritable and is probably not due to an unlinked modifier gene influencing *SCRa* transcription or the stability of its transcripts.

Why is expression of the *SCRa* allele suppressed in *SaSb* heterozygotes? This effect might be due to direct interference from the *SCRb* allele or from other sequences within the *Sb* haplotype, or it might result from *SCRa*-specific properties. Several eukaryotic genes exhibit monoallelic expression, with selection of the expressed allele occurring either stochastically, according to parental origin (genomic imprinting), or based on allele-inherent characteristics (Rothenburg et al., 2001). Furthermore, severe down-regulation or silencing has been documented for a number of eukaryotic genes in the form of transgene effects (Kooter et al., 1999) and other trans-sensing phenomena, such as paramutation in maize (*Zea mays*) and transvection in *Drosophila melanogaster* (Tartof and Henikoff, 1991). Many of these examples are associated with increased DNA methylation (Martienssen and Colot, 2001) or with RNA degradation effected by aberrant small (21–25 nt) interfering RNAs (Hamilton and Baulcombe, 1999; Mallory et al., 2001; Matzke et al., 2001a). We compared the DNA of leaves and anthers of *SaSa* and *SaSb* plants by methylation-sensitive restriction enzyme digestion and by genomic bisulfite sequencing (Clark et al., 1994). We detected no consistent differences between *SaSa* and *SaSb* plants in the methylation state of *SCRa* within the two exons and one intron of the gene and within approximately 500 bp of sequence 5′ of the initiating codon. We also failed to detect *SCRa* degradation products or small *SCRa*-related RNA species in small RNA-enriched fractions isolated from *SaSb* anthers at two stages of development. Nevertheless, neither phenomenon can be categorically ruled out, because modifications restricted to tapetal cells, which constitute only a small proportion of anther cells, might not be detected by current methods.

An alternative explanation for the differential expression of *SCRa* and *SCRb* in homozygotes and heterozygotes is suggested by the approximately 65% sequence divergence of *SCRa* and *SCRb* and by the extensive structural heteromorphism that distinguishes the *Sa* and *Sb* haplotypes (Kusaba et al., 2001), two features that are likely to interfere with chromosome pairing. It is possible that expression of the *SCRa* allele, but not that of the *SCRb* allele, is dependent on homolog pairing. Such dependence has been described for some eukaryotic genes (Aramayo and Metzenberg, 1996; Goldsborough and Kornberg, 1996; Matzke et al., 2001b), with expression being affected even by transient pairing of homologous chromosomes in some cases (LaSalle and Lalande, 1996). Interestingly, chromosome pairing has been described in tapetal cells (Aragon-Alcaide et al., 1997). The possibility that *SCRa* is expressed only in *Sa* homozygotes (*S*-locus homozygotes can occur naturally in the case of recessive alleles) or in heterozygous combinations that allow *S* haplotype pairing is at least consistent with the absence of *SCRa* transcripts in haploid microspores. However, further analysis of *SCRa* expression in the presence of different *S* haplotypes is required to test this hypothesis.

Irrespective of the underlying mechanism(s) for gene silencing, the recessive/dominant interaction exhibited by the *SCRa* and *SCRb* alleles in pollen is explained by the severe down-regulation of the recessive *SCRa* allele in the tapetum of *SaSb* heterozygotes, which, together with the lack of *SCRa* expression in microspores, results effectively in monoallelic expression of the dominant *SCRb* allele. The observed reduction in SCR concentration of approximately 30-fold or more results in loss of the corresponding SI specificity in pollen because too few SCR molecules are delivered to the stigma surface by any individual pollen grain for SRK activation to occur. We propose that this unusual feature of allelic differences in the temporal and spatial pattern of *SCR* gene expression, as well as allele-specific differences in susceptibility to silencing, may explain many, if not all, cases of dominant/recessive interactions and mutual weakening of *S* haplotypes in the pollen of crucifers.

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