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Self-incompatibility genes of *Brassica oleracea*: Expression, isolation, and structure

(*S* locus/transcript localization)

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ABSTRACT **We demonstrate by *in situ* hybridization the cell-type-specific expression of transcripts encoded by the self-incompatibility (*S*) locus of *Brassica oleracea*. These transcripts are not detected early in stigma development, their expression is switched on in the papillar cells of the stigma surface, and their levels increase in these cells in correlation with the acquisition by the stigma of the self-incompatibility response. By using a probe derived from the untranslated sequences at the 3′ end of *S* cDNA, an *S*-gene copy expressed in the papillar cells has been isolated from among the multiple *S*-related copies that occur in the *Brassica* genome. Structural analysis of this gene shows that it lacks introns. In light of the strict spatial and temporal regulation of *S*-gene expression in precisely the cells that constitute the barrier to self-pollination, the self-incompatibility response may be viewed as a cell–cell interaction between one pollen grain and one papillar cell.**

Self-incompatibility systems in plants operate prior to fertilization during the interaction between the male gametophyte (pollen or pollen tube) and the female sporophytic tissue of the pistil. This genetic mechanism results in the restriction of self-pollination and is thought to promote outbreeding and heterozygosity in many plant species ranging from monocots to dicots (1).

The study of genes controlling these incompatibility reactions is amenable to analysis by molecular biological techniques (2, 3). In the crucifer plant *Brassica oleracea*, cDNA clones have been isolated that encode glycoproteins involved in the self-incompatibility response (4). In this plant, self-incompatibility is under the genetic control of one locus, the *S* locus, for which a large series of natural alleles are known (5), and occurs whenever the same *S* allele is expressed in pollen and stigma. Thus, self-pollen is discriminated from genetically unrelated pollen and its normal development is arrested. At the cytological level, the incompatibility response in *Brassica* is manifested within minutes of pollination by the inhibition of pollen germination and/or pollen tube growth into the cells of the stigma surface (6). Although the genetic control of self-incompatibility and the details of the cellular interactions operating during pollen rejection have been extensively studied in various plant species, the mechanism by which self-pollen is recognized and restricted remains unknown.

RNA blot analysis of stigma *S* transcripts has established the temporal pattern of *S*-gene expression in the developing stigma and its correlation to the developmental regulation of self-incompatibility in *Brassica* (2). In self-incompatible members of this genus, immature stigmas are initially fully compatible and allow the development of self-pollen. It is only at ≈1 day prior to flower opening that stigmas acquire the capacity to discriminate between self- and nonself-pollen and thus become self-incompatible. The level of *S* transcripts and the synthesis of their protein products, the *S*-locus-specific glycoproteins (SLSGs), have been shown to increase during stigma development and to reach maximal levels simultaneously with the onset of the self-incompatibility response (2, 7).

We have used *S*-cDNA clones (cDNA clones encoding SLSG) to study the expression and structural organization of the self-incompatibility genes of *B. oleracea*. In this paper, we report on the cell-specific expression of the *S* locus in the papillar cells of the stigma. We also report on the identification and isolation, from among the multiple *S*-related sequences in the *Brassica* genome, of an *S*-gene copy expressed in these papillar cells.

MATERIALS AND METHODS

Plant Material. The *Brassica* plants used in this analysis were *B. oleracea* var. *acephala* inbred kale lines homozygous for the S_6, S_{13}, and S_{22} self-incompatibility alleles and derived from plant material initially obtained from the Gene Bank Facility at Wellesbourne, U.K., courtesy of D. J. Ockendon. The S_{14} homozygote is an inbred developed at Cornell University and belongs to the variety *capitata* (cabbage). *S*-allele homozygotes of *Brassica* are produced and maintained by self-pollination of immature compatible bud stigmas. The *Arabidopsis thaliana* material was derived from the wild-type Landsberg erecta strain.

Flower buds and flowers along a *Brassica* inflorescence represent a developmental progression from the youngest buds at the growing tip to the flowers at its base. The day of flower opening is taken as a reference point for bud development, and developmental stages are expressed in days prior to flower opening.

***In Situ* Hybridization.** *In situ* localization of transcripts was performed on frozen sections and on sections obtained from paraffin-embedded tissue. For experiments with frozen tissue, stigmas were dissected from *Brassica* buds, immediately embedded in OCT compound (Lab-Tek Division, Miles), and quickly frozen on dry ice. Eight-micrometer-thick frozen tissue sections were obtained at −18°C with a SLEE HR cryomicrotome (SLEE, London) and affixed to poly(L-lysine)-coated slides. Treatment of the sections was as described by Hafen and Levine (8). Following fixation in 4% paraformaldehyde in phosphate-buffered saline (0.07 M Na_2HPO_4/0.03 M NaH_2PO_4/1.3 M NaCl), the sections were dehydrated in a graded series of ethanol, treated with 0.2 M HCl and proteinase K to hydrolyze and digest proteins, postfixed in 4% (wt/vol) paraformaldehyde, dehydrated, and air-dried. The probe was prepared from BOS6, a recombinant pUC8 plasmid containing an S_6-cDNA insert (4). The gel-purified insert was radiolabeled with [^{3}H]dCTP by nick-translation (9), and probe size was reduced to an average length of 150 base pairs (bp) by controlled DNase treatment.

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Abbreviation: SLSG, *S*-locus-specific glycoprotein.

Hybridization was performed in the presence of 50% formamide/0.6 M NaCl/10 mM Tris·HCl, pH 7.5/2 mM EDTA/1× Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/10% dextran sulfate at 37°C for 18 hr. The slides were washed at 37°C over a period of 18–24 hr in six changes of a solution containing 50% formamide, 0.6 M NaCl, 10 mM Tris·HCl (pH 7.5), and 1 mM EDTA. After dehydration, the slides were coated with a thin film of Kodak NTB-2 nuclear track emulsion, air-dried, and exposed at 4°C for 2 weeks. Following development and fixation, the slides were stained with Giemsa, air-dried, and mounted in Permount. For experiments with paraffin sections, *Brassica* buds were fixed in 4% (wt/vol) paraformaldehyde in 0.1 M phosphate buffer (pH 7.0) for 2 hr at room temperature and dehydrated prior to embedding in paraffin. Ten-micrometer-thick sections were cut and mounted on poly(L-lysine)-coated glass slides. After removal of the paraffin with xylene, the sections were rehydrated and prepared for hybridization essentially as described (10, 11). Single-stranded ^{35}S-labeled RNA probes were synthesized from the BOS6 cDNA insert subcloned into the Bluescript plasmid (Strategene, San Diego, CA) using an *in vitro* transcription system (Stratagene). The fragment sizes of the RNA probes were adjusted to a mass average of ≈150 bases by limited alkaline hydrolysis (10). *In situ* hybridization was carried out at 45°C in 50% formamide/0.3 M NaCl/20 mM Tris·HCl, pH 8.0/1 mM EDTA/1× Denhardt's solution/10 mM dithiothreitol/10% dextran sulfate (10). An ^{35}S-labeled antimessage-strand RNA was used to localize *S* transcripts, whereas an ^{35}S-labeled message-strand RNA was used to monitor nonspecific hybridization to the tissue sections. After hybridization, the sections were treated with RNase A at 20 μg/ml and washed at 50°C in several changes of a buffer containing 50% formamide/0.3 M NaCl/10 mM Tris·HCl/10 mM sodium phosphate, pH 6.8/5 mM EDTA/1× Denhardt's solution/10 mM dithiothreitol (12). Autoradiography was performed as described by Angerer and Angerer (13). The sections were stained with 0.05% toluidine blue in 8.7 mM sodium benzoate. Sections were examined under bright-field and dark-field illumination with a Zeiss Standard 16 microscope. Under dark-field illumination, a concentration of bright grains above background indicates the formation of specific DNA·RNA hybrids.

Isolation of Plant DNA and Southern Analysis. Plant DNA was extracted from nuclei isolated from leaf tissue by the procedure of Bingham *et al.* (14) and isolated by CsCl/EtdBr centrifugation. Restriction fragments were fractionated on 0.9% agarose gels and blotted (15) to a GeneScreen*Plus* membrane (New England Nuclear). Blots were prehybridized and hybridized at 65°C in 10% dextran sulfate (wt/vol)/330 mM sodium phosphate, pH 7.0/10 mM EDTA/5% (wt/vol) $NaDodSO_4$/salmon testes DNA at 100 μg/ml. Probes were labeled with ^{32}P by nick-translation. Two different probes were used: (*i*) a purified S_{13}-cDNA fragment lacking *Nco* I sites and extending from the *Pst* I site to the 3′ end of the cDNA (Fig. 5); (*ii*) a probe derived from the 3′ untranslated region of the S_{13}-cDNA insert (3′ probe in Fig. 5). The membranes were washed in 0.3 M NaCl/40 mM Tris·HCl, pH 7.8/2 mM EDTA/0.5% $NaDodSO_4$ at 65°C and exposed to Kodak XAR-5 film at −70°C with an intensifying screen.

Construction of Genomic Libraries, Isolation, and Analysis of Recombinant Clones. One hundred micrograms of plant DNA was partially digested with *Sau*3A, and the fragments were size-fractionated by centrifugation on salt gradients (16). Genomic libraries were constructed by ligating 10- to 20-kilobase (kb) fragments to EMBL4 DNA digested to completion with *Bam*HI. The ligated DNA was packaged *in vitro* using Gigapack extracts (Stratagene). The phage was plated without prior amplification on *Escherichia coli* host strain Q359. Plaques were hybridized with the purified *S*-cDNA insert derived from the S_6 homozygous genotype and labeled with ^{32}P by nick-translation. Hybridizations and washing conditions were as described (17). Positively hybridizing phage were purified, and their DNA was isolated according to the method of Thomas and Davis (18). Single- and double-restriction digests of the DNA were performed, and restriction sites were mapped by electrophoresis in 0.7% agarose gels and Southern analysis with nick-translated S_6 cDNA. For sequence analysis, fragments showing homology to the probe were subcloned into pUC13, and more detailed maps were obtained. Smaller fragments were then subcloned into the M13 vectors mp18 and mp19 (19). Sequence analysis of both strands was carried out by the dideoxynucleotide chain-termination method (20) using a synthetic 17-mer (New England Biolabs) as primer.

RESULTS

***S* Transcripts Are Localized to the Papillar Cells of the Stigma Surface.** The *Brassica* stigma is a heterogeneous structure consisting of different types of cells with basically different functions (Fig. 1*A*). Exposed at the stigma surface are ≈3000 papillae. Underlying this crown of papillar cells is a body of rounded basal stigma cells. These lead to the transmitting tissue of the style through which the pollen tube grows as it makes its way to the ovary. Analysis of proteins and tran-

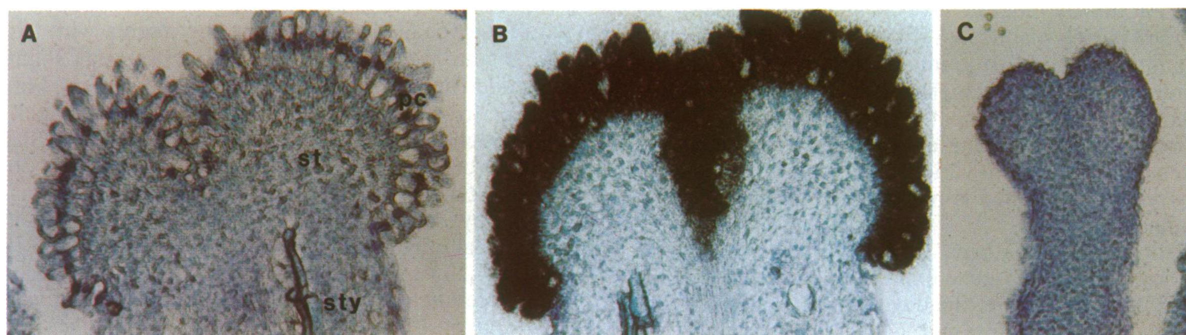

FIG. 1. Cell type and temporal specificity of *S*-gene expression in the *Brassica* stigma. Buds were fixed and embedded in paraffin, and longitudinal paraffin sections through the stigma and upper part of the style were hybridized *in situ* with ^{35}S-labeled single-stranded RNA probes as outlined. After a 2-day exposure, the sections were developed and stained with toluidine blue and examined by bright-field microscopy at a magnification of ×63. (Final magnification, ×30.) (*A*) Control hybridization to a stigma at 1 day prior to flower opening with a message-strand RNA probe. The absence of silver grains shows the low-background signal obtained. The elongated papillar cells (pc), the underlying stigma cells (st), and a portion of the style (sty) are shown. (*B*) Hybridization to a stigma at 1 day prior to flower opening with an antimessage-strand RNA probe. The black grains over the papillar cells represent specific RNA·RNA hybrids. (*C*) Hybridization to a stigma at 5 days prior to flower opening with an antimessage-mRNA probe. Note the absence of hybridization signal over the papillar cell initials of the stigma surface.

scripts prepared from whole stigmas cannot therefore provide information on the cellular localization of S transcripts.

To determine which cell type in the mature self-incompatible stigma expresses S mRNA, we performed *in situ* hybridization experiments to ^{35}S-labeled RNA probes transcribed from S_6-cDNA sequences. Longitudinal paraffin sections through the stigma and upper portion of the style were obtained (Fig. 1) and processed (see *Materials and Methods*). The micrograph in Fig. 1*B* indicates that the ^{35}S-labeled antimessage-strand RNA hybridized intensely with RNA in the papillar cells of the mature stigma. The specificity of the hybridization was demonstrated by the absence of signal in the ^{35}S-labeled message-strand RNA control (Fig. 1*A*). Our results thus demonstrate that S transcripts are exclusively localized in the cytoplasm of the elongated surface papillar cells and not in the underlying cells of the stigma and style.

We also attempted to correlate the intensity of hybridization signal in papillar cells at different stages of development with the developmental regulation of self-incompatibility in the stigma. Tissue sections were obtained from compatible and incompatible stigmas belonging to one inflorescence, affixed to the same slide, and thus subjected to identical hybridization conditions. As shown in Fig. 1*C*, an immature stigma at ≈5 days prior to flower opening shows no detectable hybridization signal with the ^{35}S-labeled antimessage-strand RNA probe. The subsequent increase in S transcripts to the high levels detected in the fully developed self-incompatible stigma (Fig. 1*B*) was shown by hybridization to ^{3}H-labeled S-cDNA probes. Tritiated probes allow for quantitation of silver grains, and comparison of grain counts indicated that the density of the autoradiographic signal in the papillar cells of a self-compatible stigma at 3 days prior to flower opening (Fig. 2 *B* and *C*) was lower (by a factor of 10) than in the cells of a fully mature self-incompatible stigma at 1 day prior to flower opening (Fig. 2*D*).

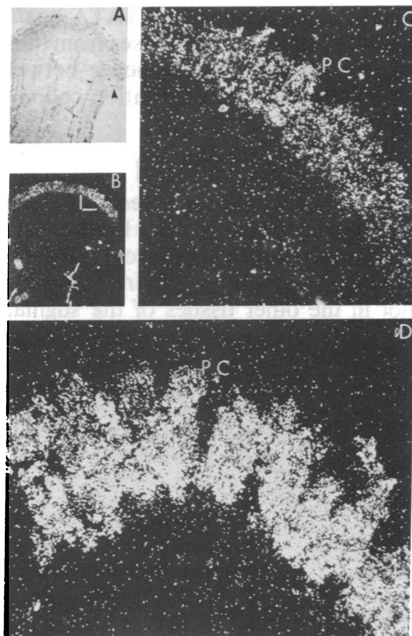

FIG. 2. S-gene expression in the developing *Brassica* stigma. (*A*–*C*) Section from a stigma at ≈3 days prior to flower opening. (*D*) Section from a stigma at 1 day prior to flower opening. (*A*) Bright-field view of a section through stigma and style, with arrowhead delineating the crown of papillar cells. (*B* and *C*) Dark-field view of the section in *A*. In *B*, the arrow points to the layer of papillar cells. The white lines delineate a sector that is shown under higher magnification in *C* to allow the visualization of individual papillar cells (PC) measuring approximately 25 μm × 100 μm. Note the concentration of white grains over the layer of papillar cells and the absence of hybridization from the rest of the stigma and the style. The white areas in the style are due to light reflection by vascular bundles. (*D*) Section through a mature stigma showing a dozen fully enlarged papillar cells measuring approximately 30 μm × 125 μm. Note the increase in grain density in these incompatible papillar cells when compared to the compatible cells in *C*. (*A* and *B*, ×10; *C* and *D*, ×50.)

Multiple Copies with Varying Degrees of Homology to the S cDNA Occur in the *Brassica* Genome. The S transcripts expressed in the papillar cells of the stigma appear to constitute a homogeneous population. This conclusion is based on the analysis of stigma S cDNAs isolated by hybridization to a purified S_6-cDNA insert probe (2) from each of three *Brassica* lines homozygous for the S_6, S_{13}, and S_{14} alleles. For each of these genotypes, several independently isolated S cDNAs were analyzed. Sequence information obtained for 350 bp from each of the 3′ and 5′ ends of these cDNAs has demonstrated their identity (data not shown). It appears therefore that the major species of stigma S transcripts are encoded by one gene.

However, isolation of this gene from a genomic library is complicated by the occurrence of other related sequences in the *Brassica* genome (2). An estimate of the number of these sequences was obtained by Southern analysis of leaf DNA digested with *Nco* I, an enzyme that lacks CXG in its recognition sequence and is therefore insensitive to the high level of methylation of plant DNA. Fig. 3 shows that multiple bands are obtained when the S_{22}, S_{13}, and S_{14} homozygotes are analyzed with a nick-translated S_{13}-cDNA fragment lacking *Nco* I sites. Some 11 fragments with varying degrees of intensity appear, suggesting that the *Brassica* genome contains an approximately equivalent number of DNA sequences related to the SLSG structural gene. The complex restriction patterns are representative of all *Brassica* homozygotes analyzed so far and also of other genera of the crucifer family as illustrated for *A. thaliana* (Landsberg) in Fig. 3.

Due to this complex organization, it has not been possible to directly identify and isolate the self-incompatibility gene copy expressed in the stigma. In fact, when genomic libraries

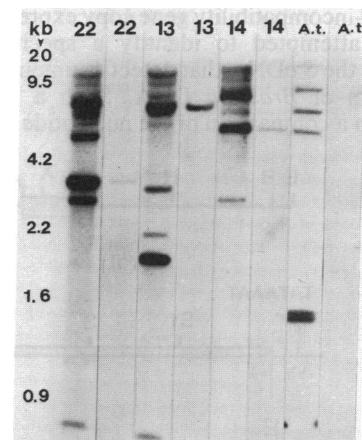

FIG. 3. Southern analysis of S-homologous sequences in *Brassica* and *Arabidopsis*. The pairs of lanes labeled 22, 13, and 14 contain DNA from *B. oleracea* plants homozygous for these alleles. The DNA in lanes labeled A.t. was from *A. thaliana* (Landsberg). Ten micrograms of nuclear DNA was digested to completion with *Nco* I and the fragments were electrophoresed in a 0.9% agarose gel. The positions of λ DNA molecular weight markers are shown. In each pair of lanes, the left lane shows the complex hybridization pattern obtained with the S_{13}-cDNA insert probe, and the right lane represents hybridization of the same filter with the gene-specific probe derived from the 3′ untranslated region of the S_{13} cDNA. Note that this gene-specific probe detects a single band in the *Brassica* homozygotes but not in *Arabidopsis*.

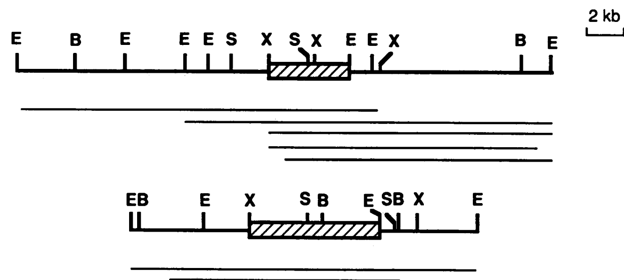

FIG. 4. Restriction maps of two S-homologous regions cloned from the S_6 homozygote. Boxes denote the extent of homology to S_6 cDNA. Thin lines below the maps represent the regions contained in the individual λ clones analyzed. The clones were mapped for the indicated restriction enzymes by single and double digestions. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; S, *Sal* I; St, *Sst* I; *X*, *Xho* I.

are screened with S-cDNA probes, a number of different S-homologous sequences are isolated. Two different genomic libraries were constructed in the bacteriophage λ vector EMBL4 from DNA of S_6 and S_{13} homozygous plants and screened with a purified S_6-cDNA insert probe. The screening of 10^6 recombinants in the S_6 library yielded a total of 77 hybridizing phage, whereas 22 clones were isolated after screening 5×10^5 plaques from the S_{13} library. Overlapping clones were identified and sorted into groups based on their restriction maps. Ten regions, all showing homology to the S cDNA but differing in their restriction maps and encompassing some 200 kb of DNA, have been defined in this way (J.B.N. and M.E.N., unpublished data). The structures of two such regions are shown in Fig. 4 to illustrate the divergence observed in restriction maps. As suggested by the varying levels of hybridization signal observed following Southern analysis of *Brassica* DNA (Fig. 3), the different S-related copies derived from the same S genotype also show varying degrees of homology to S cDNA. For example, sequence analysis of the S-related fragments diagramed in Fig. 4 has shown 84% and 65% overall homology to the S_6 cDNA.

The Transcribed Self-Incompatibility Gene Is Intronless. To isolate the self-incompatibility gene copy expressed in stigma papillae, we attempted to identify a specific restriction fragment from the S cDNA that detects a unique sequence on Southern blots of *Brassica* DNA. Such a fragment was identified from a comparison of the nucleotide sequence of S cDNA with that of cloned genomic S sequences not encoding this cDNA. As is the case for many gene families, DNA sequences corresponding to the coding regions are far more conserved than the 5′ and 3′ untranslated regions. Accordingly, a fragment derived from the 3′ untranslated region of S_{13} cDNA hybridizes with only one restriction fragment in different *Brassica* homozygotes. This 3′ probe is shown in Fig. 3 to hybridize to an ≈3.5-kb, ≈8-kb, and ≈6.5-kb restriction fragment in the S_{22}, S_{13}, and S_{14} homozygotes, respectively. The *Arabidopsis* genome, on the other hand, shows no hybridization to this gene-specific probe.

The 3′ probe was used in hybridization experiments to the already isolated genomic clones. None of the S_6 genomic clones and only one S_{13} clone hybridized with this probe. The identity of this positively hybridizing S_{13} genomic sequence as encoding the stigma S_{13} cDNA was verified by restriction mapping and nucleotide sequence analysis. The most striking feature of this gene is demonstrated by the alignment of the restriction map (Fig. 5) and nucleotide sequence (not shown) of the genomic sequence with that of its cDNA. The nucleotide sequence of the protein-coding region of the gene predicts an uninterrupted protein sequence identical to that predicted from the nucleotide sequence of the previously reported S_{13} cDNA (4).

This transcribed gene has the signals commonly found in the flanking regions of eukaryotic genes (Fig. 5). As is the case with several plant genes, more than one poly(A) signal is found 3′ to the TAG stop codon. The sequence analysis of several S_{13} cDNA clones has indicated that the 3′-most AATAAA signal is used. A TATAAAT sequence with perfect fit to the eukaryotic consensus "TATA box" is located 5′ to the coding region starting at 55 bases from the translation initiation site. Interestingly, the sequence between this putative TATA and the translation initiation codon consists of a tract of 45 consecutive purines.

DISCUSSION

By using *in situ* hybridization for detection at the single-cell level of S transcripts in the stigma, we have demonstrated the cell-type-specific expression of self-incompatibility sequences. S transcripts are expressed exclusively in the papillar cells of the stigma but not in the other tissues of the stigma, style, and ovary. In addition, the increase in hybridization signal intensity observed during stigma development correlates with the increase in S-transcript levels and SLSG synthesis observed as

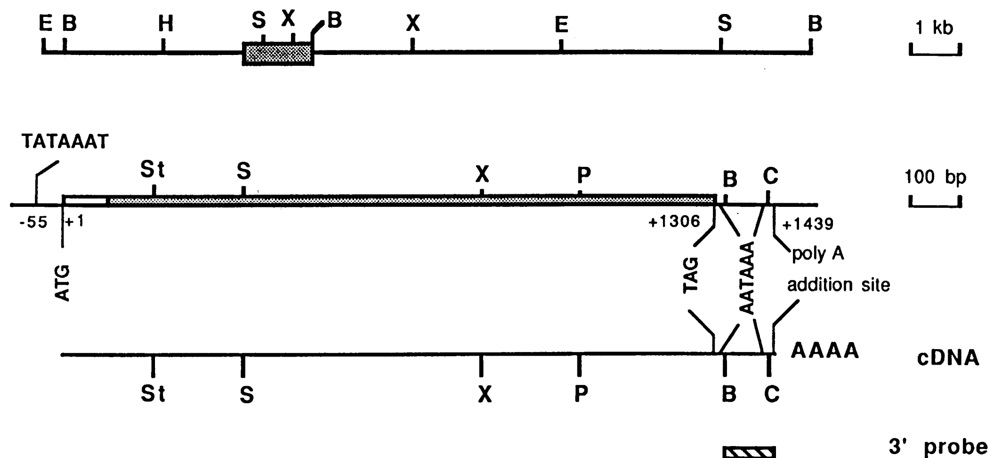

FIG. 5. Structure of the transcribed *Brassica S* gene showing its uninterrupted alignment with its cDNA. The upper line shows the restriction map of the phage insert containing the transcribed gene with its protein-coding region indicated by the box. Below, the protein-coding region and its flanking sequences are aligned with the S_{13}-cDNA map. The white area of the box delineates the signal peptide sequence. The ATG initiation codon (+1), the TAG termination codon (+1306), and the poly(A) addition site (+1439) are shown. Putative TATA (−55) and poly(A) signals (+1322 and +1410) are indicated. The ATG initiation codon is not indicated on the cDNA map since the longest S_{13}-cDNA isolated extended only into the second codon. B, *Bam*HI; C, *Cla* I; *E*, *Eco*RI; P, *Pst* I; S, *Sal* I; St, *Sst* I; X, *Xho* I.

the stigma matures and acquires the self-incompatibility response (2, 7). Interestingly, stigmas at the earliest developmental stage analyzed lacked detectable *S* transcripts in the initials that develop to form the mature elongated papillar cells.

Significantly, our observations are in keeping with the surface inhibition of self-pollen in *Brassica* (6, 21) and with the implied role of SLSG in pollen recognition. In a compatible pollination, pollen adheres to the papillar cell surface and germinates to produce a pollen tube that penetrates the papillar wall. Incompatible pollen grains, on the other hand, either fail to germinate or produce pollen tubes that fail to invade the papillar cells. The stigma papillae thus constitute the cellular barrier to self-pollen development. They are structurally and functionally specialized for the capture and recognition of pollen grains, with a major fraction of their synthetic capacity devoted to the production of *S* transcripts and SLSGs.

Although several *S*-related gene copies have been enumerated in the *Brassica* genome, the papillar cell *S* transcripts appear to be the products of a single gene. This conclusion is based on two lines of evidence. (*i*) Only one class of *S* cDNAs, all identical to one another, has been recovered from any one homozygous genotype, indicating the existence of one predominant class of transcripts. (*ii*) It is clear that some of the other *S*-related copies cannot be functional genes since they contain a number of out-of-frame deletions and insertions (unpublished).

We have shown that the self-incompatibility gene transcribed in the papillar cells can be identified from among the other *S*-related copies by specific hybridization to a probe derived from the 3′ untranslated sequence of S_{13} cDNA. As expected, the unique restriction fragment corresponding to this gene gives a strong hybridization signal with S_{13} cDNA. Hybridization of the S_{13}-derived 3′ probe to unique restriction fragments in DNA from the S_{22} and S_{14} homozygotes (Fig. 3) demonstrates that in each of these closely related genotypes a sequence corresponding to the gene copy transcribed in the S_{13} homozygote is present. The *S*-allele-associated polymorphism exhibited by these unique fragments (Fig. 3) further indicates that the transcribed gene is derived from the *S* locus. It is interesting to note in this context that, although the linkage at the *S* locus of at least some of the *S*-related copies has been established genetically (2), none of the EMBL4 genomic clones that we have recovered contains more than one *S*-homologous sequence. The *S*-related copies must therefore be separated by stretches of flanking DNA longer than can be accommodated by the EMBL4 vector used in our study. The self-incompatibility region, although behaving as a unique genetic locus, thus appears to have a complex organization and might potentially span at least 200 kb of DNA if all gene copies map to the *S*-locus region.

More extensive sequence divergence is indicated by the lack of hybridization of the 3′ probe to *Arabidopsis* DNA. The divergence of the *Arabidopsis* *S*-related sequences from their *Brassica* counterparts is also illustrated by the fact that ≈10 times more *Arabidopsis* genome equivalents were required to obtain the hybridization signal visualized in Fig. 3. It is nevertheless interesting that *S*-related sequences are detected in *Arabidopsis* and that several copies have been conserved in its simpler genome. Members of this genus are self-fertilizing, and expression of *S*-related sequences might imply for their products a function other than inhibition of self-pollen, such as rejection of pollen from unrelated species (22). However, we have not determined if these sequences are transcribed in *Arabidopsis*, and it remains possible that they represent nonfunctional remnants of an *S*-gene system. It has in fact been shown in *Brassica* (23, 24) that self-compatibility can arise by the occurrence of mutations at unlinked modifier loci that alter the expression of the *S* locus. The question of *Arabidopsis* sequences as inactive remnant loci can now be addressed to determine if, as suggested by Whitehouse (25), self-incompatibility is the ancestral trait.

In *Brassica*, the transcribed self-incompatibility gene has some interesting features. It is uninterrupted by introns, and the placement of the putative TATA box predicts a short untranslated 5′ leader. Although the untranslated leaders of most plant genes analyzed to date are substantially longer, leaders as short as 9 bp have been reported (26). More striking is the observation that the 5′ leader is exclusively composed of purines. However, in the absence of sequence information for other transcribed self-incompatibility genes or other similarly regulated genes, it is difficult to assess the significance of this purine tract. Further investigation is necessary to determine the involvement of this tract and of upstream sequences in the high-level, cell-type-specific and developmentally regulated expression of this gene in the stigma.

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