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Interaction between a coating-borne peptide of the *Brassica* pollen grain and stigmatic *S* (self-incompatibility)-locus-specific glycoproteins

(cell recognition/self-incompatibility)

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ABSTRACT **Methods are described for the removal of the sporophytic pollen grain coating of *Brassica oleracea* and for the isolation of coat polypeptides. The coat contains a small number of proteins ranging from 6 to 45 kDa. Many of the larger proteins are glycosylated, while all carry high positive charges resulting in pI values from 8.5 to 11. Polypeptides with pI values of 9.5, 9.0, and 8.5 possess strong esterase activity. No major differences could be detected in either pI values or molecular masses of pollen-coating polypeptides from grains carrying different sporophytically expressed *S* (self-incompatibility) alleles. Mixing pollen coat proteins with stigmatic extracts results in a conspicuous binding interaction involving female *S*-locus-specific and perhaps *S*-locus-related glycoproteins. This interaction, which is reversed by heating in the presence of SDS, results in an apparent charge shift of the female glycoprotein(s) of up to 2 pI units. The male participant in this interaction has been isolated by using a combination of fast protein liquid chromatography and reverse-phase HPLC and was shown to be a 7-kDa nonglycosylated peptide. Experiments with whole pollen cultured *in vitro* show challenge with stigmatic extracts to stimulate the release of gametophytic and sporophytic polypeptides and to result in the formation of a conspicuous interaction product, demonstrating the 7-kDa peptide to be freely available within the coating of pollen *in vivo*.**

The self-incompatibility (SI) systems of higher plants are emerging as a family of unique signaling systems evolved from mechanisms already operative in the pollen/pistil interaction (1). Thus, in *Nicotiana* and other members of the Solanaceae, self-pollen is rejected by a mechanism involving a stylar RNase (2, 3), an enzyme common to the styles of many plants and hypothesized to play a role in defense against pathogens. In most species, SI is regulated by a simple genetic system based on few loci and large numbers of alleles (4, 5); generally, if pollen and stigma carry alleles in common, the pollen/stigma interaction is disrupted. The genetics of SI in *Brassica* and other members of the Cruciferae and Compositae is not so straightforward; the pollen S phenotype is determined by the *S*-allelic constitution of the parent plant, rather than that of the haploid grain (6).

Through a series of elegant molecular studies, Nasrallah *et al.* (7) have demonstrated two types of sequence to be linked to the *S* locus of *Brassica*. One (*S* locus glycoprotein; *SLG*) encodes a 55-kDa glycoprotein (*S*-locus-specific glycoprotein; SLSG) expressed in the stigmatic papillae, and the other encodes a transmembrane kinase (*S* receptor kinase; *SRK*) expressed in the organs of both sexes. Sequence comparisons suggest that the *SRK* features a domain, which may be extracellular, with maintained homology to the *SLG*. Further, other gene families with considerable homology to the *SLG* are also present in the genome (8–10) but unlinked to the *S* locus (*S* locus related; *SLR*). Interestingly, reporter constructs driven by the *SLG* promoter are expressed in the pistil and microspores of transgenic *Nicotiana* (11), but in the stigma and anther tapetum of transgenic *Arabidopsis* and *Brassica* (12, 13), suggesting that expression is regulated according to whether SI is sporophytically or gametophytically determined. Some expression does however occur in the microspores of transgenic *Brassica*, pointing to a low level of gametophytic activity. *SLG* transcripts do not occur in the anther (9), so the promoter must drive the expression of either the *SRK* and/or an unidentified male determinant.

Complexity at a molecular level is reflected in the cell biology of the SI response in *Brassica* because, on capture, the pollen draws water and a spectrum of stigmatic molecules through the dry stigmatic cuticle via the pollen coat (14). Following self-pollinations, development is rapidly arrested by a biostatic mechanism requiring protein synthesis and glycosylation (15). The pollen coat is derived from the sporophytic tapetum (16) and the fact that the *SLG* promoter drives expression in this layer suggests that it may be the location of determinants involved in the male SI response (1, 17). We report here the removal and partial characterization of the pollen grain coating and describe an interaction between a 7-kDa coating peptide and female *S*-linked and related glycoprotein.

MATERIALS AND METHODS

Plant Material. Greenhouse-grown plants of inbred *Brassica oleracea* lines homozygous for the S_{25}, S_{29}, and S_{63} incompatibility alleles (obtained from D. J. Ockendon, Horticulture Research International, Wellesbourne, Warwickshire, U.K.) were used in all the work reported. Stigmas and pollen were collected from newly opened flowers and either extracted immediately or stored at −70°C.

Isolation of Pollen Coat Proteins. Coating was removed from pollen grains by adding 800 μl of cyclohexane to 75 mg of pollen and agitating until suspended (5 sec). After separation by centrifugation (14,000 × *g*, 20 sec), the cyclohexane fraction was applied to microscope slides upon which it rapidly vaporized. Pollen coat residue was collected and maintained at or near 0°C. To recover coating polypeptides from isolated pollen coat, amassed isolates from a total of 300 mg of pollen were resuspended in 350 μl of a 50 mM phosphate buffer (pH 7.0) with the aid of an ultrasonic cell

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Abbreviations: SI, self-incompatibility; SLG, *S* locus glycoprotein (gene); SLSG, *S*-locus-specific glycoprotein (protein); SRK, *S* receptor kinase; IP, interaction product; IEF, isoelectric focusing; SLR, *S* locus related; pcp[7], 7-kDa pollen coat protein.

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disruptor (Microson MS-50). Lipids were removed by repeated centrifugations for 20 min at 21,000 × g.

Viability Tests for Coatless Pollen. For structural investigations, pollen grains were vapor-fixed as described by Elleman and Dickinson (14). The integrity of pollen plasma membranes was assayed using the method of Heslop-Harrison and Heslop-Harrison (18). Pollen, with and without coatings, was germinated *in vitro* [medium: 10 mM Tris/20% (wt/vol) polyethylene glycol 1000/$CaCl_2 \cdot 2H_2O$ (243 mg/liter)/KNO_3 (100 mg/liter)/H_3BO_3 (10 mg/liter), pH 8.35].

Isolation of S_{25} and S_{63} Stigmatic Glycoproteins. S_{25} glycoprotein was eluted from a Mono Q anion-exchange column [fast protein liquid chromatography (FPLC) system, Pharmacia LKB] in 20 mM Tris·HCl at pH 8.0. The S_{63} glycoprotein was also eluted from the same column, but using a 20 mM ethanolamine buffer at pH 9.5. Fractions containing S glycoproteins were further purified by gel filtration using a Superdex 75 HR 10/30 gel-filtration column (Pharmacia FPLC system). Stigmas were harvested immediately prior to fractionation, homogenized in the appropriate elution buffer, and centrifuged at 14,000 × g, 4°C, to remove cell debris.

Fractionation of Pollen Coat Polypeptides by Gel Filtration. Coat proteins were isolated from pollen by using the procedure described above and precipitated by the addition of solid $(NH_4)_2SO_4$ to saturation. After a 30-min incubation on ice with occasional gentle agitation, proteins were pelleted by centrifugation for 40 min at 21,000 × g, 4°C, and resuspended in 50 mM phosphate buffer at pH 7.0. Proteins were separated in sample buffer using a Superdex 75 HR 10/30 gel-filtration column.

Purification of Coat Proteins by HPLC. Fraction 29 eluted from the Pharmacia Superdex 75 column was run on a Vydac C_4 reverse-phase column (150 × 2 mm; Hichrom, Reading, U.K.) equilibrated in 0.1% trifluoroacetic acid in 2% acetonitrile. A gradient of 2–50% acetonitrile was applied over 50 min at a flow rate of 0.2 ml/min. The major peak of activity (peak 16) was identified by interaction with the stigmatic SLG and rechromatographed on an Aquapore RP-300 column (30 × 2 mm) (Applied Biosystems) in the same solvents as before but using a much shallower gradient of 10–45% acetonitrile over 80 min.

Protein Separation and Analysis. *Isoelectric focusing (IEF).* Five percent polyacrylamide gels with a pH range of 3.5–10 were run in a Multiphor II unit (Pharmacia LKB). Gel composition and electrophoretic conditions were as described in the manufacturer's instructions.

SDS/PAGE. SDS/PAGE was carried out using the Bio-Rad Mini-Protean II dual slab system employing a discontinuous buffer system (19). Separation of low molecular weight fractions obtained following gel filtration was achieved using a Tris/Tricine buffer system (20).

Nondenaturing native PAGE. Acidic nondenaturing gels (12% polyacrylamide resolving gel with a 4% stacking gel) were cast and run using the same apparatus as for SDS/PAGE gels. The buffer system used was based on that described by Goodenough and Owen (21), and gels were run at 200 V constant voltage for 1 hr; polarity was reversed because separation was toward the cathode.

Electroblotting. Electroblotting was carried out in the Bio-Rad Mini Trans-Blot electrophoresis transfer cell using the method of Towbin *et al.* (22).

Staining of SDS/PAGE, native PAGE, and IEF gels. For Coomassie staining, gels were fixed and stained in 30% (vol/vol) methanol, 10% (vol/vol) acetic acid, and 0.1% (wt/vol) Coomassie R-250 for 1–3 hr, depending on gel thickness. Destaining was carried out with repeated washes of 25% methanol/10% acetic acid. Silver staining was carried out using the Protostain silver stain system (National Diagnostics, Manville, NJ). However, prior to staining, IEF gels were first treated as follows: gels were fixed in 30% (vol/vol) isopropyl alcohol/10% (wt/vol) trichloroacetic acid/3.5% (wt/vol) 5-sulfosalicylic acid for 1 hr and transferred to 30% isopropyl alcohol/12% trichloroacetic acid; the solution was changed at regular intervals over a 2-hr period.

Glycoprotein detection (Con A affinity blotting). Prior to Con A binding, proteins were identified by incubating the blots in 1% acetic acid containing 0.5% (wt/vol) Ponceau S. Blots were destained in 1% acetic acid for 20 min followed by several washes in TBS-Tween 20 [10 mM Tris·HCl, pH 7.4/0.14 M NaCl/0.1% (vol/vol) Tween]. Glycoproteins binding Con A were identified following the methods of Faye and Chrispeels (23).

Radioiodination of Pollen Coat Proteins. Reverse-phase HPLC-purified 7-kDa pollen coat protein (pcp^7) was labeled with ^{125}I (≈0.85 μCi/μg; 1 Ci = 37 GBq; obtained from Amersham) by using the Iodogen method (24).

Interaction Between Stigmatic Extracts and Pollen Coat Proteins. Stigmatic and pollen coat proteins were isolated as described in the previous sections, and interactions were carried out in 18-μl volumes for between 20 and 60 min at room temperature. Interaction mixes were then analyzed using PAGE and IEF. Interactions involving radioiodinated peptide were also analyzed using IEF. However, dissociation between coating peptide and stigmatic molecules occurs at the pI of the hybrid molecule, and for this reason these gels were not run to equilibrium but stopped after half the normal running time. Autoradiographs were prepared from these gels using Kodak XAR-5 film and an exposure time of 20 min at −70°C.

Protein Emission from Pollen in Aqueous Solutions and "*in Vivo*" Interaction Between Whole Pollen and Stigmatic Polypeptides. Ten milligrams of pollen was suspended in 100 μl of 0.3 M mannitol and gently agitated every 5 min over the two incubation times (15 and 30 min). After incubation, the pollen was removed by filtration, and the supernatant was analyzed by IEF at pH 3.5–10. For interaction experiments between stigmatic extracts and secreted proteins, 65 S_{25} stigmas were first homogenized in 100 μl of the mannitol solution; cell debris was removed by centrifugation (total stigmatic protein concentration, 15 mg/ml). Ten milligrams of pollen was then added, and the mixture was treated as above.

RESULTS

Pollen Coating Removal Using Solvent Washes. Cyclohexane washes resulted in complete removal of the coating with no detectable degradation of the constituent polypeptides and little deleterious effect on the pollen (see below). Approximately 1500 μg of coating polypeptide could be extracted from 300 mg of fresh pollen—far more than is extracted using aqueous media. Extractions in germination or other osmotically balanced media produce a wide spectrum of proteins in which those of gametophytic origin predominate.

After treatment, pollen was examined by scanning and transmission electron microscopy. The exine surface was revealed to be free of coating, whereas cytoplasm of the grains, fixed by dry methods, which prevent pollen hydration (14), appeared to be unaffected by solvent treatment. Coating-free grains both germinated and produced tubes *in vitro*, but at low levels; 15–20% of the grains generated tube initials and 10% formed elongated tubes. However, the fluorescein diacetate test for viability (18) indicated >90% of the grain protoplasts to be intact and to contain functional esterases.

The Recovery of Pollen-Coating Polypeptides from Solvent Washes. The removal of cyclohexane by evaporation, followed by sonication of the residue in aqueous solution, gave good yields of coat polypeptides. However, the procedure could have had a deleterious effect on extracted polypeptides, so stigmatic proteins were exposed to identical treatments. Neither alterations to M_r nor to pI values were noted.

The Polypeptides Present in the Coating of *B. oleracea*. While the majority of the polypeptides run as a single band on acidic native PAGE, a family of smaller subsidiary proteins is also present. A more complex pattern emerges with SDS/PAGE (see Fig. 1, lane a); a number of major polypeptides are present having molecular masses of 30–45 kDa, with other major bands running at 20 kDa and 17 kDa, and a second group of proteins runs close to the front. This latter group of bands with molecular masses of 6–14 kDa was further characterized with PAGE, FPLC, and HPLC (see below). The most abundant of these solvent-extracted coat polypeptides can be detected in diffusate from intact pollen grains incubated in osmotically balanced aqueous medium (25), but at very low levels. Interestingly, a major coat polypeptide of 37 kDa was revealed as being composed of a glycosylated and nonglycosylated fraction while other polypeptides of 44, 35, and 32 kDa were also shown to be glycosylated (see Fig. 1, lane b). A 15-kDa polypeptide is also glycosylated; however, this could only be resolved on blots following electrophoresis using the system described by Schägger and von Jagow (20). The low molecular mass peptides (6–10 kDa) are not significantly glycosylated.

Pollen coating extract was also analyzed using IEF (see Fig. 1, lane c). In all the genotypes studied, coat polypeptides run in two main groups, one between pI 8.5 and 9 and another, far more highly charged between pI 9.5 and 11.

FPLC on Superdex 75 gave good separation of coat polypeptides on the basis of molecular mass. However, analysis using IEF revealed most fractions to contain several proteins, often of different pI values.

Coat polypeptides were run on SDS/PAGE, native gels, and IEF from a number of lines of *B. oleracea* homozygous for different *S* alleles. While minor differences could be observed both in molecular mass and pI of polypeptides, no major S-specific bands were identified.

The Enzymes of the Pollen Coat. Blotting of pollen and pollen coat proteins from IEF followed by staining for esterase revealed strong activity associated with at least four polypeptides localized in the pollen coat (data not shown). Three of these have pI values between 8.65 and 9.0, while the other focuses at 9.5. No significant differences in enzymic activity could be detected between the *S* genotypes used in this study. It is clear from comparison of lanes containing coat polypeptides with total pollen homogenate that the majority of pollen esterase activity is localized in the coat. Acidic native PAGE of pollen coat proteins stained for esterase (data not shown) indicates activity to be associated with polypeptides of 30–40 kDa. Acidic native PAGE of pollen coat proteins stained for acid phosphatase detected activity in two polypeptides between 60 and 80 kDa. Again no differences in activity could be detected between *S* genotypes.

The Behavior of Male and Female Polypeptides in Mixtures. Mixture of coat polypeptides with crude stigmatic extracts induced a striking interaction. After IEF, the glycoprotein band regarded as representing the SLSG in our gels decreased in intensity with the generation of a new band at a higher pI (see Fig. 2). Despite careful inspection of the stigmatic protein spectrum following an interaction, no evidence could be found of any other female polypeptides being affected. While SLSGs are identifiable by their segregation with *S* alleles, SLRs are not, and it is possible that if SLSGs and SLRs run close to each other in IEF and PAGE gels the interactions also involve this latter group of glycoproteins.

A less marked interaction takes place with isolated SLSG; under these circumstances, the interaction product (IP) often becomes distributed over a wider pI range. When FPLC-fractionated pollen coat polypeptides were added to female molecules, the most active fractions contained peptides of 6–9 kDa (see Fig. 3). Further separation of the "active" FPLC fractions on reverse-phase HPLC and testing of the fractions resulting has revealed that a peptide of 7000 Da (pcp^7) is responsible for inducing the charge shift in the female glycoprotein (see Fig. 4). Interactions involving radioiodinated pcp^7 first gave confusing results, indicating that the peptide is not associated with the IP, although it must clearly have been responsible for the interaction. Subsequent experiments revealed that the IP dissociates once it reaches

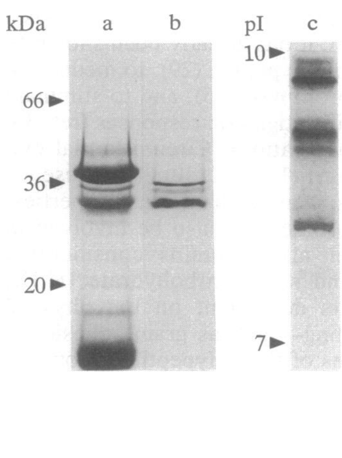

FIG. 1. Molecular mass, glycosylation, and charge characteristics of coat proteins isolated from pollen-carrying S_{63} alleles. Lane a, Coomassie-stained SDS/PAGE; lane b, Con A affinity blot; lane c, silver-stained IEF gel.

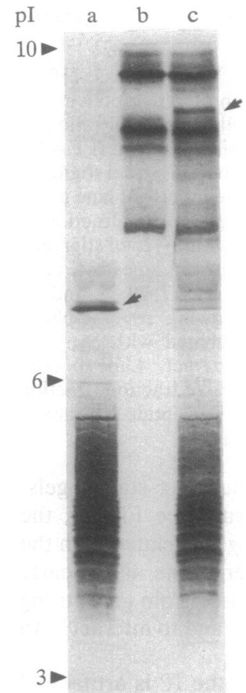

FIG. 2. Silver-stained IEF gel of total stigmatic extract incubated *in vitro* with total pollen coat proteins. Lane a, S_{25} total stigmatic extract (control). The arrow indicates SLSG. Lane b, S_{63} pollen coat proteins (control). Lane c, S_{25} total stigmatic extract incubated for 15 min with S_{63} pollen coat proteins. The arrow indicates IP.

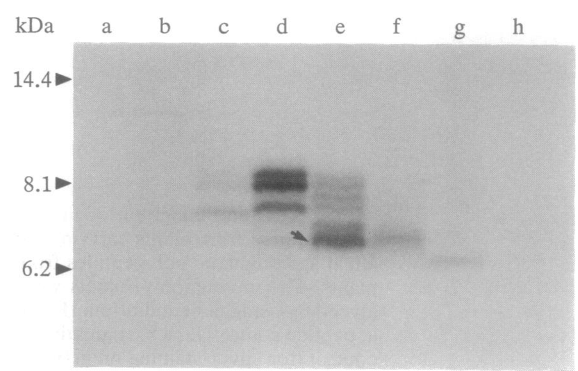

FIG. 3. Coomassie-stained SDS/PAGE gel (20) of FPLC gel-filtration-purified S_{63} pollen coat peptides. Lanes a–h, fractions 25–32. Lane e corresponds to the fraction with maximum activity against SLSGs. The major constituent of this fraction is the pcp^7 (arrow).

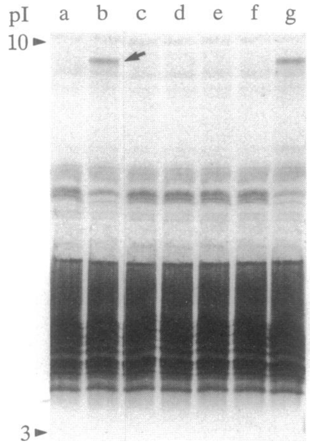

FIG. 4. **Reverse-phase HPLC purification of active pollen coat fraction following gel filtration on FPLC. A silver-stained IEF gel is shown. Lane a, S_{25} total stigmatic homogenate (control); lane g, active FPLC pollen coat fraction 29 incubated with S_{25} total stigmatic extract (control); lanes b–f, principal peaks (16, 17, 20, 22, and 25) eluted following reverse-phase HPLC incubated with S_{25} total stigmatic extract. Lane b contains the active fraction (fraction 16), the 7-kDa peptide. The arrow indicates IP.**

its pI, resulting in the smears seen in some silver-stained gels. When nonequilibrium IEF gels were run (see Fig. 5), the radiolabeled peptide was unambiguously associated with the migrating IP. Interestingly, these experiments also clearly demonstrate pcp^7, which is normally eluted from gels during fixation and staining, to have a pI of ≈10 and to interact with only one class of stigmatic molecule.

The possibility that the generation of the IP is artifactual was investigated using a *semi vivo* assay (see *Materials and Methods*). A strong IP was again (see Fig. 6) formed, identical with that generated following the interaction *in vitro*. Further, this challenge by female polypeptides stimulated a greater release of pollen proteins than was normally emitted in osmotically balanced medium. No IP was formed in controls where coatless pollen was challenged with stigmatic molecules, but, again, an increased level of protein was secreted.

Generally, coating polypeptides interacted with stigmatic proteins under all circumstances and independently of the *S* alleles carried by the pollen; however, the strength of the interaction varied between certain crosses, and there were isolated instances of differences between self- and cross-pollinations involving the same stigmatic genotype.

DISCUSSION

Polypeptides of the *Brassica* Pollen Grain Coating. Cyclohexane rinsing removes the coating from pollen leaving the gametophytic protoplast intact. Gillessen and Brandtjes (26) have also convincingly demonstrated the viability of pollen protoplasts following coating removal with covalent solvents. Other methods reportedly isolate pollen coatings but may also extract gametophytic proteins; for example, Evans *et al.* (27) describe extraction of pollen coat with acetone, but the treatment employed was such that the protoplast plasma membrane would have been destroyed, an interpretation supported by the very different protein spectrum produced by this work. Comparatively few proteins were recovered when isolated coating was sonicated. This is surprising since the coat is derived from the secretory tapetal tissue (17) and indicates either that many proteins are lost during disintegration of the tissue or that those detected are present in unusually large amounts.

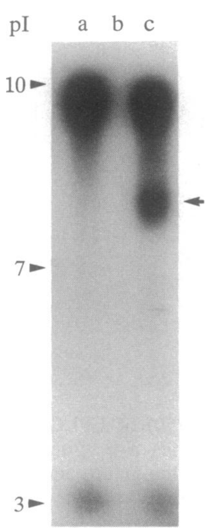

FIG. 5. Autoradiograph of interaction between pcp^7 from plants carrying the S_{29} allele and stigmatic polypeptides from S_{25} plants. The interaction products were analyzed by using nonequilibrium IEF. Lane a, peptide control; lane b, stigmatic extract control (for silver staining on gel); lane c, interaction. The gel pI gradient is shown in the left margin, but in this nonequilibrium run the polypeptides will not necessarily be at their pI values. The migrating IP (arrow) was also visible on the gel after silver staining (data not shown).

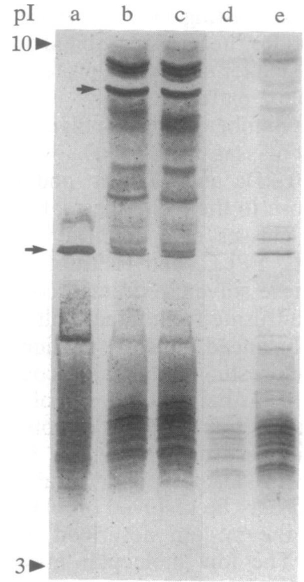

FIG. 6. Silver-stained IEF gel of proteins from intact pollen grains incubated *in vitro* with S_{25} total stigmatic extracts. Lane a, S_{25} total stigmatic extract (control). The arrow indicates SLSG. Lane b, S_{25} total stigmatic extract incubated *in vitro* for 15 min with intact S_{63} pollen grains. The arrow indicates IP. Lane c, same as for lane b, but incubation extended to 30 min; lane d, proteins emitted from intact S_{63} pollen grains following a 15-min incubation; lane e, same as for lane d, but the incubation extended to 30 min.

Representatives of each molecular mass group of coat proteins fall into both charge groups, indicating that the lower molecular mass peptides may be degradation products of those in the higher molecular mass grouping. Few coat proteins have been identified positively, but a number of the higher molecular mass grouping are active esterases, and preliminary studies suggest that these enzymes interact with molecules of the stigmatic pellicle (S. Hiscock, personal communication).

The Interaction Between Coat and Stigmatic Polypeptides. The reaction of the pcp^7 with stigmatic polypeptides to form the IP is both interesting and unexpected. While the molecular basis of the interaction requires further study, the radioiodination data confirms the pcp^7 to be present in the IP and to bind to no other class of stigmatic molecule. Likewise, when the IP is excised from the gel and dissociated in SDS (J.D. and H.D., unpublished data), PAGE analysis shows SLSGs (and perhaps SLRs)—known to be involved in SI (28)—to be principal constituents. pcp^7 is not normally detectable in the IP, owing both to dissociation from the IP (see above) and its elution from the gel during processing.

The Role of Coating Polypeptides in Pollination and SI in *Brassica*. The pollen grain coating in *Brassica* clearly plays a number of roles in pollination; it has already been shown to adhere the pollen to the stigmatic papilla (29), to mediate in the hydration of the pollen protoplast (15), and to stimulate a number of pollination-specific stigmatic responses (ref. 36; C. Elleman, personal communication). Circumstantial evidence also points to a role in SI (1, 7, 17, 30). The presence of relatively few polypeptides, many with unusual properties, in the coating suggests that some may also be involved in these processes. This layer also contains considerable amounts of lipid (26, 31) and some carbohydrate (H.D., unpublished data), so events dependent on the physical properties of the whole coating—such as grain adhesion—cannot be considered in terms of the polypeptides alone.

Hydration of the grain protoplast (32) must involve the passage of stigmatic water to the pollen plasma membrane via a hydrophilic pathway established through the stigmatic cuticle and the pollen coating. While there is circumstantial evidence that the cuticle is permeable to water (H.D., unpublished results), the route taken by water through the coating is unknown. However, the abundance of the 30- to 40-kDa polypeptides suggests that they are distributed throughout the coat, and since many are glycosylated, that these proteins would constitute a domain with high affinity for water extending across the coat, creating an effective hydration pathway. The stimulation of pollen protein emission by female molecules is potentially significant, especially since it also occurs when coat-less pollen is challenged. This response must involve the release of protoplast or intine held polypeptides.

Findings reported here strongly indicate that when molecules of the SLSG/SLR family interact with coating polypeptides, the SLSGs, and perhaps SLR, bind to the pcp^7, resulting in a marked alteration in net charge. For this reason, any hypothesis involving the SLSGs/SLRs in physiological activity must now be considered in terms of the IP. Female *S*-allele specificity is known to be carried with the SLSG (33), and it is important to determine whether the peptide interaction is affected by the *S* allele carried. Certainly the sporophytic origin of pcp^7 makes it a good candidate for the male SI determinant, but unequivocal evidence for S specificity has proved hard to find. Further, a role for the interaction in SI is called into question by the formation of a clear IP in self-compatible *Brassica napus* (S. Hiscock, personal communication). However, *B. napus* is an allotetraploid formed from *B. oleracea* and *Brassica campestris*, so the presence of SI-related molecules might be anticipated.

The discovery that the *S* locus also contains a transmembrane kinase (34) is significant and, although the locations of the SRK gene product in pollen and stigmas are unreported, they must be central to any hypothesis to explain SI. Thus, on the basis of evidence presently available, pollination seemingly involves a complex dialogue between pollen and stigma, commencing with superficial interactions, which establish hydraulic continuity and render the male and female plasma membranes accessible to signals. The precise nature of these signals and their consequences remain largely unknown, but there is accumulating evidence (7) that SLSG, presumably in the form of the IP, is capable of regulating pollen development according to the *S* alleles present. SLSG may thus act as a developmental inhibitor, operating through an SRK-encoded kinase at either the pollen or stigmatic plasma membrane. Until pcp^7s have been sequenced for a number of alleles, we must remain ignorant as to whether they carry *S*-locus specificity or simply act as a nonspecific cofactor.

Most interestingly, the discovery of sequence homology between the SLG and genes putatively encoding a class of transmembrane kinases was not made in *Brassica*, but in *Zea* by Walker and Zhang (35). These authors proposed that the kinase in *Zea*, which is expressed throughout the plant, is a component of a hitherto undescribed system of intercellular communication. The ubiquitous nature of this class of kinase and its situation at the cell surface suggests that, in *Zea* at least, it could play a role in defense against pathogens. Thus, as has been the case with the stylar RNases of the Solanaceae (2), strong selective pressure for the evolution of outbreeding systems in species with dry stigmas may also have resulted in the adaptation of an already extant system, perhaps involved in pathogen defense, to identify and reject self-pollen.

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