Stigma development in Nicotiana tabacum. Cell death in transgenic plants as a marker to follow cell fate at high resolution

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Abstract Pistil development was studied in transgenic tobacco plants in which the stigma is ablated by expression of a stigma-specific cytotoxic gene. These plants offer a tool to investigate the process of differentiation of the secretory zone, in that cell death caused by barnase activity provides a marker to follow cell fate at high resolution. After fusion of the carpel walls in the region most distal from the ovary, the epidermal cells begin to divide in both wild-type and stigmaless plants. Divisions in the L1 layer of the pistil are immediately followed by the morphogenetic events that lead to three different cell types: rounded-angular cells showing an equal number of anti- and periclinial divisions, cells that are more oblong forming the transition zone, and the square cells of the transmitting tissue dividing mostly anticlinally with respect to the original carpel wall. In the stigmaless plants, cell death caused by the expression of STIG I-barnase begins at stage -1 and proceeds gradually, but is always associated with round epidermal cells and with angular-rounded cells underneath them. Studies at the ultrastructural level show that cell death caused by barnase activity occurs first in solitary cells and gradually extends to groups of cells. In situ hybridizations using the STIG 1 RNA probe in wild-type pistils confirm these results. Most likely, the cells in which STIG 1 is expressed are those that have just differentiated into the secretory cell type. Our results indicate that the transition zone or neck is autonomously differentiated from the secretory zone and the transmitting tissue. Furthermore, our results indicate that in both wild-type and stigmaless pistils secretion of lipids most likely occurs through the plasmodesmata. This observation suggests that bulk transport can occur via plasmodesmata.

Key words Nicotiana tabacum · Stigma · Ablation · Development · Lipid secretion

Introduction

The female reproductive organ of angiosperms, the pistil, is composed of the stigma, style and ovary. Pistil development in Datura, a solanaceous species, was described by Satina (1944). The floral apex has three independent germ layers, designated L1, L2 and L3. Initiation of carpel primordia is determined by divisions of the cells of the innermost L3 layer in various planes. In Datura this group of cells chiefly contributes to the lower portions of the carpel wall and septae, which eventually become the major part of the ovary. The two carpel primordia fuse soon after initiation, resulting in a single ovary enclosing two locules. Continuous upward growth of the carpel wall results in the formation of the style with its two lobes. The margins of the carpel walls become connected and form the stylar canal. According to Satina, the secretory zone and the transmitting tissue in the style develop from the L1 layer, which is epidermal in origin. Differentiation of the transmitting tissue begins at the base of the stigma and progresses vertically down towards the base of the style. The cortex develops from the L2 layer. The contribution of the L3 layer in the style is limited to the formation of two vascular bundles, which differentiate from the dorsal bundles of the ovary. The results of Satina’s study do not show at what point in time the secretory zone differentiates from the stylar transmitting tissue.

The aim of this study was to perform a developmental analysis of the differentiation of the pistil of Nicotiana tabacum, another solanaceous species with a wet stigma. Stigmas are classified as “wet” when at the receptive stage the surface is covered with a sticky exudate secreted by the cells of the secretory zone (Cresti et al. 1986; Dumas 1977; Dumas et al. 1978; Heslop-Harrison and Shivanna 1977; Knox 1984; Mackenzie et al. 1990). We studied pistil development in transgenic tobacco plants in which the stigma is ablated by expression of a stigma-specific cytotoxic gene, STIG I-barnase (Goldman et al. 1994). These plants offer a tool to investigate the process of differentiation of the secretory zone. Goldman et al.
(1994) showed that STIG 1-barnase expression occurs very early during pistil development. Cell death caused by barnase activity provides a marker to follow cell fate at high resolution. Comparison of developmental stages of wild-type and stigmaless pistils was used to gain more insight into the process of pistil development and, in particular, the differentiation of the secretory zone of the stigma and the transmitting tissue in the style.

Materials and methods

Plant material

Nicotiana tabacum cv. Samsun and transgenic STIG 1-Barnase tobacco plants (Goldman et al. 1994) were grown under growth chamber conditions (day phase, 15 h at 20°C, 65% relative humidity; dark phase, 9 h at 18°C, 65% relative humidity). Flower buds at different stages of development –3, –2, –1, 1, 2, 6 and 11, (Koltunow et al. 1990) were collected from both wild-type and stigmaless tobacco plants.

Light microscopy

Stigmas were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for 2 h at room temperature followed by post-fixation in 1% osmium tetroxide in the same buffer. Tissues were dehydrated in ethanol and propylene oxide and embedded in Spurr’s resin. Sections of 1 µm were stained for light microscopy (LM) by standard procedures: (1) toluidine blue for cytoplasmic staining (0.1% in 1% borax) and (2) Sudan black B for lipids. The sections were pre-treated with 70% ethanol for 1–2 min and stained in a freshly filtered solution of Sudan black B at 60°C for 1 h (0.4 g Sudan black B in 70% ethanol for 12 h at 37°C). Sections were rinsed with 70% ethanol, Coomassie brilliant blue (0.2% in 60% acetate, 3:1 v/v) and acid fuchsin (1% in water) were used for staining of total proteins. Fresh 100 µm vibratome sections were stained with auramine O (0.01% in 0.05 M Tris-HCl buffer at pH 7.2) for cutin.

Electron microscopy

Stigmas were fixed and embedded for electron microscopy (EM) as described for LM. Thin sections were post-stained with uranyl acetate and lead citrate according to standard procedures and viewed with a JEOL JEM 100 CX II. The proportion of peri- and anticlinal division planes was investigated by analysing EM micrographs and defined in relation to the outer surface of the original carpel wall. The mitotic index includes cells in both karyo- and cytotkinesis. Detection of acid phosphatase and non-specific esterases was carried out as described by Schou and Mattson (1985).

Scanning electron microscopy

Stigmas were fixed for scanning electron microscopy (SEM) in 1% osmium tetroxide for 2 h at room temperature, dehydrated in a water-ethanol series and critical-point dried. The material was mounted on stubs and coated by sputtering with gold. The samples were examined with a JEOL JSM-T300.

In situ hybridizations

The STIG 1 cDNA was cloned in both sense and antisense orientation in pBluescript SK+ (Stratagene), and RNA probes were transcribed using T7 RNA polymerase and digoxigenin-labelled UTP (Boehringer, Mannheim). Plant tissues were fixed with 4% paraformaldehyde, 0.25% glutaraldehyde in 0.01 M sodium phosphate buffer at pH 7.2 for 4 h under vacuum, dehydrated via a graded ethanol series and embedded in Paraplast (Klinipath, Duiven, The Netherlands). Sections 7 µm thick were dried on polylysine-coated slides at 37°C overnight, deparaffinized with xylene and rehydrated via a graded ethanol series. Pre-hybridization treatment, hybridization and washes were performed as described by Scheres et al. (1990) except that DTT was omitted in the washing solutions. Digoxigenin detection was performed according to de Almeida-Engler et al. (1994) using Boehringer block in PBT at pH 7.5 and a 1:3000 dilution of the anti-DIG-alkaline phosphatase conjugate.

Results

Structural changes during development

Scanning electron microscopy observations

Pistil development was followed with scanning electron microscopy (SEM) in wild-type and stigmaless flower buds from stages –3 to 11. At stage –3 the carpel primordia are already fused, showing the two lobes of the stigma. Epidermal cells start to differentiate in papillae along the fusion plane of the carpels at stage –2, then gradually increase in number over the stigmatic surface, but do not develop on the outer edges of the lobes. No differences were observed between wild-type and stigmaless pistils at these stages of development (data not shown).

At stages –1/1 the first differences occur. On the stigmaless pistils the cells start to degenerate and acquire a shrunken appearance, whereas on the wild-type pistils the papillae continue to elongate (Fig. 1A–D). This process proceeds, and at stages 10–11 no papillae remain on the stigmaless pistils, leaving an almost flat surface (Fig. 1E, F). In the fusion plane, in the groove region, some pores can be seen but no droplets of exudate are present on the surface (Fig. 1F). In wild-type stigmas, papillae size increases further and at stage 10 papillae are covered with small droplets of exudate (Fig. 1G, H). The wild-type stigma has a spherical, convex shape (Fig. 1C, G). In contrast, the stigmaless pistil becomes concave and flattened at the extremity of the fusion plane (Fig. 1A). This feature becomes more extreme at maturation (Fig. 1E).

Light microscopy and electron microscopy observations

At stage –2 no differences were observed between the stigmaless and wild-type pistils by light microscopy.

Fig. 1A–H  SEM micrographs of stigmaless and wild-type tobacco pistils. A Stigmaless pistil at stage 1. ×400. B Detail of the surface in the groove region showing degenerating papillae. ×1750. C Wild-type stigma stage 1. ×400. D Detail of the surface in the groove region showing developing papillae. ×1750. E Stigmaless pistil at stage 11. ×200. F Detail of the surface in the groove region showing some pores and a flat surface. ×1750. G Wild-type stigma at stage 11. ×140. H Detail of the surface in the groove region showing papillae. Stigmatic surface and papillae with droplets of exudate (ex). ×1750
The carpel walls are fused only at the top of the style and the stylar canal is open and gradually closes from the stigma towards the ovary (Fig. 2B).

The epidermis of the carpel walls consists of more-or-less rectangular cells, developing into papillae in the centre of the stigma. Interestingly, the epidermal cells at the centre of the stigma show both anticlinal and periclinal division planes, whereas epidermal cells at the outer edges, along the parenchyma, divide only anticlinally (Fig. 2C).

At the uppermost region of the fusion plane, immediately underneath the epidermis, a zone of angular-rounded cells can be distinguished (Fig. 2A, B, D). These cells show some vacuoles and divide both peri- and anticlinally. Cells below this zone show a more oblong shape, denser cytoplasm and relatively more anticlinal divisions than cells of the original carpel wall along the fusion plane. These cells constitute the transition to the transmitting tissue (Fig. 2A, B, D). The transmitting tissue cells in the stylar canal are square-to-oblong, showing dense cytoplasm and predominantly anticlinal division planes. The changes in cell shape are gradual. The parenchyma consists of large square-to-rectangular cells with large vacuoles, and they divide both peri- and anticlinally. The transition from parenchyma to the adjacent tissues is gradual. Within the parenchyma the vascular bundles start to develop on both sides.

At stage −1 differentiation in cell shape and content becomes more pronounced (Fig 2E, F) and, as with SEM, the first differences between wild-type and stigmaless pistils become visible. In contrast to the wild-type, the outgrowth of the papillae in stigmaless pistils starts to cease (Fig. 2E, F). In both wild-type and stigmaless pistils, deposition of a precipitate in the vacuoles of the papillae was observed. The vacuolisation in the epidermal layer and in the angular-rounded cells underneath the epidermis proceeds. However, in the stigmaless pistils some of these cells show heavily stained cytoplasm (Fig. 2E). Electron micrographs revealed that these cells have degenerated (Fig. 5A), whereas in wild-type pistils no degeneration occurs (Figs. 2F, 5C). At this stage, in situ hybridization on sections of wild-type pistils shows expression of STIG1 in solitary cells; they have the appearance of dead cells caused by expression of the STIG1-barnase in stigmaless pistils (Figs. 2E, 3A).

In stigmaless pistils the arrest of papillae development is obvious at stage 1 (Fig. 2G). This observation is in agreement with the results of the SEM images (Fig. 1B). During development the tissues expand due to cell division (Fig. 2G,H). However, under the epidermal layer in the stigmaless pistils, the vacuolated, angular-rounded cells progressively die, preventing expansion of this area (Fig. 2H). Continuing degeneration occurs in small clusters of cells (Fig. 5A). Also, in situ hybridization on wild-type pistils shows an increase in the number of epidermal and angular-rounded cells in which STIG1 is expressed (Fig. 3B). However, the number of cells in which STIG1 is expressed in the wild-type is greater than the number of cells that die in the stigmaless pistils (Figs. 2G, 3B).

The first indication of degeneration in stigmaless pistils is not clotting, but a more transparent appearance of the cytoplasm. Strikingly, this is observed only in recently divided cells (Fig. 5A). However, only one of two
daughter cells shows the dissolution of the cytoplasm (Fig. 5B); the other cell remains unaffected. The degeneration proceeds gradually, and, at stage 2, two strings of dead cells can be seen extending from the top of the pistil toward the transmitting tissue (Fig. 4A). Only the dead cells proximal to the parenchyma are compressed, whereas cells in the central part still maintain a rounded shape. At this stage the stylar canal is closed and forms a continuous tract of stigma and transmitting tissue, connecting to the ovary. In both wild-type and stigmaless pistils the parenchyma cells continue dividing and elongating, with larger cells at the outer edge and smaller cells adjacent to the transmitting tissue.

At stages 6–11 in wild-type pistils the bi-lobed shape and the secretory zone are clearly defined (Fig. 4D). In contrast, the shape of the stigmaless pistil is clearly changed, with the two lobes pointed inwards (Fig. 4C). The epidermal cells of the inner sides of the lobes have degenerated. Interestingly, degeneration of these cells occurs only in the region where, at younger stages, anti- and periclinal divisions occurred (Fig. 2C). Between the lobes, all of the dead cells are compressed; only few living cells remain visible (Fig. 4C, E). At stage 11 the exudate is released on the surface of the stigma in the wild-type (Fig. 4F), whereas no exudate is secreted on the surface of the stigmaless pistil (Fig. 4E).

Staining with auramine O was positive in both wild-type and stigmaless pistils, indicating the presence of cutin on the surface of the stigma (data not shown).

At completion of development the stigmaless pistil is approximately 5 mm shorter than the wild-type. Measurement of the elongated cells of the transmitting tissue in both wild-type and stigmaless pistils showed a reduction in average cell length of about 15% in the latter (data not shown).

Division planes

Since stigmatic and transmitting tissue both derive from the same L1 cell layer (Satina 1944), we studied the orientation of cell divisions which may influence the morphogenesis of these tissues. From here on we refer to the vacuolated, angular-rounded cells in both stigmaless and wild-type pistils as the secretory zone. In the secretory zone and in cells of the transmitting tissue the mitotic index and the proportion of anti- and periclinal division planes was investigated by EM. In stigmaless and in wild-type pistils the mitotic index in both cell types is about 5% at stages –2 and –1, decreasing to about 1% at stages 1 and 2. At stage 6, cells no longer divide, and only cell elongation occurs.

At stage –2 there are no differences in the proportions of anti- and periclinal division planes in the secretory zone of wild-type and stigmaless pistils. At this stage we find approximately 45% anticlinal and 55% periclinal division planes. At stage –1, the proportions of anti- and periclinal division planes in the secretory zone of wild-type pistils are the same as at stage –2. However, in stigmaless pistils, anticlinal divisions increase to 74% and periclinal divisions decrease to 26%. This difference probably can be accounted for by cell death. In succeeding stages these these proportions persists (Table 1).

Between stages –2 and 2, in both wild-type and stigmaless pistils, division of cells of the transmitting tissue is primarily (approximately 85%) anticlinal.

Cytoplasmic changes during development

The production and release of exudate is the main function of the secretory zone in wet stigmas (Goldman et al. 1994). Therefore, understanding this process may help us follow differentiation of the secretory zone. The exudate is mainly composed of lipids, proteins and sugars (Cresti et al. 1986). Since plastids are involved in lipid and starch biosynthesis, we searched for possible differences between plastids of wild-type and stigmaless pistils. At stage –2 there is no starch visible in the plastids of different wild-type pistil tissues. In contrast, there is a strong accumulation of starch in the plastids of the transmitting tissue in stigmaless pistils at this stage. Lower starch levels occur in all other cell types, including cells of the parenchyma adjacent to the secretory zone and transmitting tissue (Fig. 5A, B). The first synthesis of starch in plastids of wild-type pistils is observed in cells of the transmitting tissue at stage 2. Finally, at stage 6, starch accumulation is visible to a lesser extent in the cells of the transition zone, and in parenchyma cells along the secretory zone and transmitting tissue (data not shown). At stage 11 starch content in the plastids of wild-type pistils no longer differs from that in stigmaless pistils.

Table 1 Proportion of anti- and periclinal division planes in the vacuolated, angular-rounded cells, defined as the secretory zone, at different developmental stages of wild-type and stigmaless pistils. Division planes are defined in relation to the outer surface of the original carpel wall

<table>
<thead>
<tr>
<th>Stage</th>
<th>Wild type</th>
<th>Stigmaless</th>
</tr>
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<tbody>
<tr>
<td>–2</td>
<td>44%</td>
<td>48%</td>
</tr>
<tr>
<td>–1</td>
<td>49%</td>
<td>74%</td>
</tr>
<tr>
<td>1</td>
<td>57%</td>
<td>74%</td>
</tr>
<tr>
<td>2</td>
<td>55%</td>
<td>67%</td>
</tr>
</tbody>
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Fig. 4A–F LM median sections of stigmaless and wild-type tobacco pistils at different developmental stages stained with toluidine blue. ×90. A Stigmaless pistil at stage 2 showing two strings of dead cells (arrowheads). Parenchyma cells adjacent to the transmitting tissue are smaller than those at the outer edge. B Wild-type pistil at stage 2. C Stigmaless pistil stage 6. The two lobes are pointed inward. Epidermal cells of the lobe have degenerated (between arrowheads). D Wild-type pistil stage 6. E Stigmaless pistil stage 11. There is no secretion of exudate. F Wild-type pistil at stage 11. Secretion of exudate on the surface of the stigma (ex). VB vascular bundle.
Taken together, these results indicate that, in tobacco, synthesis of starch occurs mostly in the cells of the st lyr transmitting tissue.

Production and secretion of lipids during pistil development in wild-type and stigmaless plants were determined by staining with Sudan black B. As early as stage –2 small lipid droplets are detectable in both wild-type and stigmaless pistils. The lipid droplets accumulate mainly in the transition zone (Fig. 2A, B) between the vacuolated, angular-rounded cells underneath the epidermis and the oblong cells giving rise to the transmitting tissue. Furthermore, lipids are also present in the cells at the boundary with the parenchyma cells of the cortex. Towards the transmitting tissue, the number of lipid droplets decreases in both types of pistil. In general, fewer lipid droplets accumulate in stigmaless pistils (data not shown).

Differences between wild-type and stigmaless pistils become more clear at stage 1 (Fig. 5E, F). In wild-type pistils, lipid content strongly increases in the differentiating secretory zone and gradually decreases towards the transmitting tissue (Fig. 5F) and along the parenchyma. Lipid droplets accumulate in the cytoplasm, often at the tips and bases of the cells, and are secreted into the intercellular spaces (Fig. 5C, D). In stigmaless pistils, few lipid droplets are present in the cytoplasm, mainly in the cells of the transition zone (Fig. 5E), but they are not secreted. Finally, at stage 11, lipids are secreted into the intercellular spaces at the transition zone (Fig. 6A). Sections for LM stained with Sudan black B confirm this observation (data not shown). Remarkably, plasmodesmata in this zone seem to contain lipids (Fig. 6B).

During maturation of the wild-type pistil, at stages 6–11, the region of the secretory zone greatly enlarges. In general, electron micrographs show that lipid droplets in the cells accumulate in the neighbourhood of plasmodesmata and are surrounded by an electron-dense material (Fig. 6C), which Cresti et al. (1986) described as polysaccharides. Occasionally, some small droplets or strands of lipids are observed in the plasmodesmata. They seem to fuse in the middle lamellae, causing the walls to separate.

Discussion

Structural changes during development

Stigma-specific cell death causes large-scale developmental changes in the morphology of the pistil. SEM observations show gradual degeneration of the papillae on the surface of the pistil, and pistil shape changes from convex to concave as the lobes become folded inward. This shape resembles that in transgenic *Nicotiana tabacum* plants in which pistil cells of epidermal origin were ablated (Thornsness et al. 1991).

Detailed developmental analysis of the changes in stigmaless pistils, compared to the wild type, was conducted by LM and EM studies. At stage –2, after the carpel walls elongate and fuse in the most distal region from the ovary, the epidermal cells begin to divide in both wild-type and stigmaless plants. Divisions of the L1 layer of the pistil are immediately followed by the morphogenetic events that lead to three different cell types: rounded-angular cells showing an equal number of anticlinal and periclinal divisions, cells that are more oblong forming the transition zone and the square cells of the transmitting tissue dividing mostly anticlinally with respect to the original carpel wall. At this stage in both stigmaless and wild-type pistils, cell divisions in the secretory zone are 45% anticlinal and 55% periclinal, and in the transmitting tissue 85% anticlinal and 15% periclinal. We believe that this difference is associated with the first events of differentiation.

Fig. 5A–D EM micrographs of the zone with vacuolated, angular-rounded cells, defined as the secretory zone, in stigmaless and wild-type tobacco pistils at stage 1. A Stigmaless pistil with degenerated dead cells. Plastids contain starch. Note a more transparent appearance of the cytoplasm in only one of the two recently divided daughter cells (+). ×3000. B Detail of the two daughter cells, with dissolution of the cytoplasm in the left cell. The cytoplasm of the right cell is unaffected. ×20 000. C Wild-type pistil showing accumulation of lipid droplets. The plastids contain no starch. ×3000. D Secreted lipid droplets in the intercellular spaces near to the plasmalemma (Fig. 6). ×25100. E–F LM median sections of stigmaless and wild-type tobacco pistils stained with Sudan black B to distinguish lipids in angular-rounded cells (+), transition zone (■) and transmitting tissue (TT). ×1025. E Stigmaless pistil. Few lipid droplets are present in the angular-rounded cells (+) and transmitting tissue (TT). They increase in the transition zone (■). F Wild-type pistil showing a strong accumulation of lipid droplets in the angular-rounded cells (+). Lipid droplets are present in the transition zone (■), fewer in the transmitting tissue (TT). Interstitial space: L, lipids, N nucleus, P plastids, V vacuole, W cell wall
The occurrence of three different cell types from one single layer may be explained by the fact that cell fate is determined by cell position during pistil development, in a manner similar to that in the root meristem (van den Berg et al. 1995; Laskowski et al. 1995; Schiefelbein 1994). Later, differences in cell morphogenesis may be determined by the activity of specific genes. Our results show that, in stigmasless pistils, cell death caused by the expression of STIG 1-barnase begins at stage -1 and proceeds gradually, but is always associated with round epidermal cells and with angular-rounded cells underneath them. By in situ hybridizations on wild-type pistils, the expression of STIG 1 is observed in the same cell types. Most likely the first cells in which STIG 1 is expressed are those that have just differentiated into the secretory cell type.

An interesting observation we made is that cells of the epidermis, which are located nearest to the carpel fusion plane, can also divide periclinal. Similar observations were reported by Kadej et al. (1985) in Lycopersicon esculentum. Remarkably, in stigmasless pistils, only these cells die, whereas cell death never occurs in the region where cells divide only anticlinally. Furthermore, in the secretory zone cell death seems to occur only in periclinally dividing cells. This results in a decrease of the number of periclinal divisions in this region, causing a significant difference in the ratios of anticlinal and periclinal divisions in stigmasless and wild-type pistils (Table 1).

Our studies at the ultrastructural level show that cell death caused by barnase activity occurs first in solitary cells and gradually extends to groups of cells. It remains unclear why, after cell division, at first only one of the two daughter cells dies. Possibly the transacting factors that activate STIG 1 are unevenly distributed in the cell, and thus may be present in only one of the two cells after cell division.

In stigmasless pistils at stage 1 the number of cells that degenerate is lower than the number of cells expressing STIG 1 in wild-type. This could be explained by the fact that degenerated cells fail in cell-cell communication.

Another interesting phenomenon in the stigmasless pistil is that compression of dead cells begins proximal to the parenchyma and later extends to the cells in the central part. At the same time, the shape of the pistil changes as the two lobes point inward. This may result from mechanical pressure exercised by the parenchymous tissue: the outermost parenchyma cells are more elongated then those adjacent to the secretory zone and transmitting tissue. In wild-type pistils this mechanical pressure may play a role in the release of products secreted in the intercellular spaces on the stigmatic surface.

Cytoplasmic changes during development

One method of studying cell differentiation is to follow the cytoplasmic changes at the ultrastructural level in the different cell types.

At stage -2, in wild-type and to a lesser extent in stigmasless pistils, small lipid droplets are detectable mainly in the transition zone. At stage 1 in wild-type pistils, a strong increase in lipid production in the differentiating secretory zone occurs and lipids are secreted into the intercellular spaces. In stigmasless pistils, the lipid content in the transition zone remains low until, finally, at stage 11 these lipids are secreted.

Plastids are involved in lipid biosynthesis. At stage -2 the plastids in the cells of the secretory zone, transition zone and transmitting tissue of wild-type pistils do not accumulate starch. In contrast, in stigmasless pistils at the same stage starch is visible in the plastids of all these tissues. These results suggest that alterations during development of the secretory zone are reflected in changes in the biosynthetic processes which occur in the plastids. The synthesis of both fatty acids and starch occurs within the plastids and may utilize carbon precursors from the cytosol. According to Kang et al. (1994), some of those precursors may be used in both pathways. Differentiation of the secretory zone seems to influence the production of starch and fatty acids in the adjacent tissues.

The transition zone has been described in Petunia by Herrero and Dickinson (1979) and named the “neck”, which is defined as part of the transmitting tissue. In Petunia the neck produces mostly esterases, while acid phosphatase and peroxidases are distributed uniformly in the secretory zone, neck and transmitting tissue. Our results show that in tobacco the transition zone is functionally distinct from the secretory zone and the transmitting tissue, as the activity of acid phosphatase is limited to the neck cells. Furthermore, the neck cells in both wild-type and stigmasless pistils produce and secrete lipids, which is not characteristic of the transmitting tissue. Therefore, we consider that this transition zone is not part of the transmitting tissue. These cells never die in stigmasless pistils. Taken together, we conclude that, in tobacco, the transition zone or neck is autonomously differentiated from the secretory zone and the transmitting tissue.

Secretion of lipids

Production and secretion of exudate is the main function of the secretory zone, and fatty acids are the major com-
ponent of the exudate in the pistil (Atkinson et al. 1993; Cresti et al. 1986; Kandasamy et al. 1987). According to previous investigations, the secretion of lipophilic materials in Nicotiana (Cresti et al. 1986; Kandasamy et al. 1987) is closely related to the occurrence of a tubular type of smooth endoplasmic reticulum. No observations were made about the mode of transfer, though an eccrine mode or non-visible form of secretion has been suggested (Cresti et al. 1986; Dumas et al. 1977; Heslop-Harrison 1983; Sedgley and Blesing 1983). Our results show that in both wild-type and stigmaless pistils secretion of lipids most likely occurs through the plasmodesmata. This observation suggests that bulk transport can occur via plasmodesmata. Secretion via plasmodesmata is supported by the fact that acid phosphatase activity, detected as deposition of lead sulphide, is localized only in the plasmodesmata and in the middle lamella. Acid phosphatase activity in the plasmodesmata was also described by Franceschi and Lucas (1982) in Chara. According to the molecular sieve theory, macromolecules are trapped between the plasma-lamella and the wall and are prevented from being secreted (Trevithik et al. 1960). Instead, the middle lamella, which is composed of pectins, could serve as a pathway for secretion into the intercellular spaces. Taken together, our data indicate that secretion of lipids and acid phosphatase begins through the plasmodesmata, reaches the middle lamella and, finally, the intercellular spaces. It is noteworthy that recent findings indicate that the plasmodesmata canal can dilate to allow macromolecular transport (reviewed by Epel 1994; Lucas et al. 1993). However, esterases, which are smaller molecules, seem to be secreted in a different way, since the activity of esterases is homogeneously dispersed throughout the wall.

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References

Dumas C (1977) Lipochemistry of the proagamic stage of a self-incompatible species: neutral lipids and fatty acids of the secretory stigma during its glandular activity, and of the solid style, the ovary and the anther on Forsythia intermedia Zab. Planta 137: 177–184
Goldman MHS, Goldberg RB, Mariani C (1994) Female sterile tobacco plants are produced by stigma-specific cell ablation. EMBO J 13: 2976–2984