Protoplasma (1997) 200: 87–98

PROTOPLASMA

High-pressure freezing improves the ultrastructural preservation of in vivo grown lily pollen tubes

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Received March 13, 1997
Accepted July 30, 1997

Summary. We have used high-pressure freezing followed by freeze substitution (HPF/FS) to preserve in vivo grown lily pollen tubes isolated from the style. The results indicated that HPF/FS (i) allows excellent preservation of the pollen tubes, (ii) maintains in situ the stylar matrix secreted by the transmitting tract cells, and (iii) preserves the interactions that exist between pollen tubes. Particular attention has been given to the structure of the pollen tube cell wall and the zone of adhesion. The cell wall is composed of an outer fibrillar layer and an inner layer of material similar in texture and nature to the stylar matrix and that is not callose. The stylar matrix labels strongly for arabinogalactan proteins (AGPs) recognized by monoclonal antibody JIM13. The zone of adhesion between pollen tubes contains distinct matrix components that are not recognized by JIM13, and apparent cross-links between the two cell walls. This study indicates that HPF/FS can be used successfully to preserve in vivo grown pollen tubes for ultrastructural investigations as well as characterization of the interactions between pollen tubes and the stylar matrix.

Keywords: Cell wall; Callose; High-pressure freezing; Stylar matrix; Arabinogalactan proteins.

Abbreviations: AGPs arabinogalactan proteins; FS freeze substitution; HPF high-pressure freezing.

Introduction

In higher plants, the pollen tube carries sperm cells from the site of pollen deposition on the stigma to the ovary to allow fertilization. Recent developments have increased our understanding of pollen tube biology, i.e., germination, growth, and interactions with the matrix secreted by the transmitting tract cells (for a review, see Taylor and Hepler 1997). During the past 10 years, ultrastructural studies on pollen tubes have taken advantage of the improved preservation afforded by cryofixation methods (e.g., Lancelle et al. 1987, 1997; Cresti et al. 1987; Tiwari and Polito 1988; Lancelle and Hepler 1989, 1992; Derksen et al. 1995; Ueda et al. 1996). However, these studies mainly dealt with in vitro grown pollen tubes, which do not reflect the interactions between pollen tubes and the stylar matrix. Indeed, the growth rate and diameter of the in vivo and in vitro lily pollen tube are dramatically different, demonstrating that culture media do not completely replicate the physical and chemical environment of the style (Rosen 1971, Li and Linskens 1983). Nutrition, chemical guidance, and adhesion roles of the stylar matrix have been postulated to explain the differences observed between in vitro and in vivo growth (see Lord et al. 1996). It is therefore necessary to preserve the stylar matrix in situ as well as the connections between pollen tubes in order to speculate on their functional importance.

Preservation of the stylar matrix is a major problem encountered with open styles. Recently, the use of ruthenium red during chemical fixation has slightly improved retention of the stylar matrix (Jauh and Lord 1995). Another strategy that has shown good results in the preservation of extracellular matrices is the use of cryofixation (Kandasamy et al. 1991, Roy et al. 1996). In the present study, we have used high-

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pressure freezing followed by freeze substitution (HPF/FS) to preserve in vivo grown pollen tubes. HPF has proven to be beneficial for studies in plant cytology, cell development processes and phytopathology (for a review, see Kiss and Staehelin 1995). Compared to other cryofixation methods, HPF increases the depth to which samples can be frozen without ice crystal damage (Dahl and Staehelin 1989, Robards 1991) and is thereby suitable for in vivo grown lily pollen tubes isolated from the style.

Our results demonstrate that we have achieved the degree of ultrastructural preservation that is found with cryopreserved pollen tubes grown in vitro, while maintaining the connections between pollen tubes and with the secreted stylar matrix. The improved morphology allows a better understanding of the adhesion events involved in pollination. The zone of adhesion between pollen tubes contains distinct matrix components and apparent cross-links between the two pollen tube cell walls.

Material and methods

Plant material

Two self-incompatible cultivars of *Lilium longiflorum* (Thunb.) were grown in a greenhouse under ambient conditions. Cross-pollination of cv. Nellie White flowers with pollen from cv. Ace flowers was done 2 days after anthesis.

High-pressure freezing

Styles were removed from the flower 48 h after pollination and a sample was dissected and examined with decolorized aniline blue to locate pollen tubes. Corresponding stylar segments were dissected longitudinally and pollen tubes delicately removed from the style as described by Li and Linskens (1983) and cut to retain the pollen tube cell, which extends 3–5 mm from the tip. They were immediately loaded in gold sample hat holders that had been coated with a solution of 100 mg of lecithin per ml in chloroform. The holders were filled with either a solution of 15% Dextran (39,000 MW; Sigma Chemical Co., St. Louis, MO, U.S.A.), the medium used for growth of in vitro pollen tubes (Lancelle and Hepler 1992) or the style exudate. The holders were frozen in a Balzers HPM 010 high-pressure freezer (Baltec, Middleburg, CT, U.S.A.) and then quickly transferred to liquid nitrogen for storage where the two halves were separated. The time needed to excise the style, isolate the pollen tubes, load the specimen holder, and then transfer into the HPF was kept to a minimum and was usually less than 90 s.

Freeze substitution

The holders containing the samples were transferred to vials containing 1% OsO_4 in anhydrous acetone at –80 °C. Substitution was carried out at –80 °C for 36 h followed by slow warming over a period of 8 h. After several rinses in acetone, the HPF samples were teased from the holder with a needle and rinsed again in acetone. Samples were en bloc stained in 5% uranyl acetate in methanol for 2 h. After acetone rinses, all specimens were infiltrated in Epon-Araldite as described by Roland and Vian (1991).

Chemical fixation

Two chemical fixation procedures were used: (i) cut segments of the pollinated style were immersed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 2 h, or (ii) styles were longitudinally excised and fixative was added to the intact pollen tubes before cutting them into segments. Samples were rinsed in buffer and post fixed in 1% OsO_4 in buffer. After dehydration in an acetone series, samples were embedded in Spurr's resin.

Immunogold labeling

The monoclonal antibody JIM13 recognizes a carbohydrate portion of arabinogalactan proteins (AGPs; Knox et al. 1991). The specificity of the polyclonal antibody directed towards β(1→3) glucopyranose ([callose]; Euromedex, Souffelweyersheim, France) has been described by Northcote et al. (1989). Immunogold labeling was performed on ultrathin sections as described by Roy et al. (1997). Quantitative evaluations of the labeling were conducted on AGPs and callose visualization. Briefly, a series of random negatives (about 10 for each experiment) were digitized and the images were analyzed with an imaging software. Gold particles were counted and their numbers were correlated with specific areas in the population of cells. For example, the density of labeling was obtained for each negative by correlating the total number of gold particles with each of the different zones.

Results

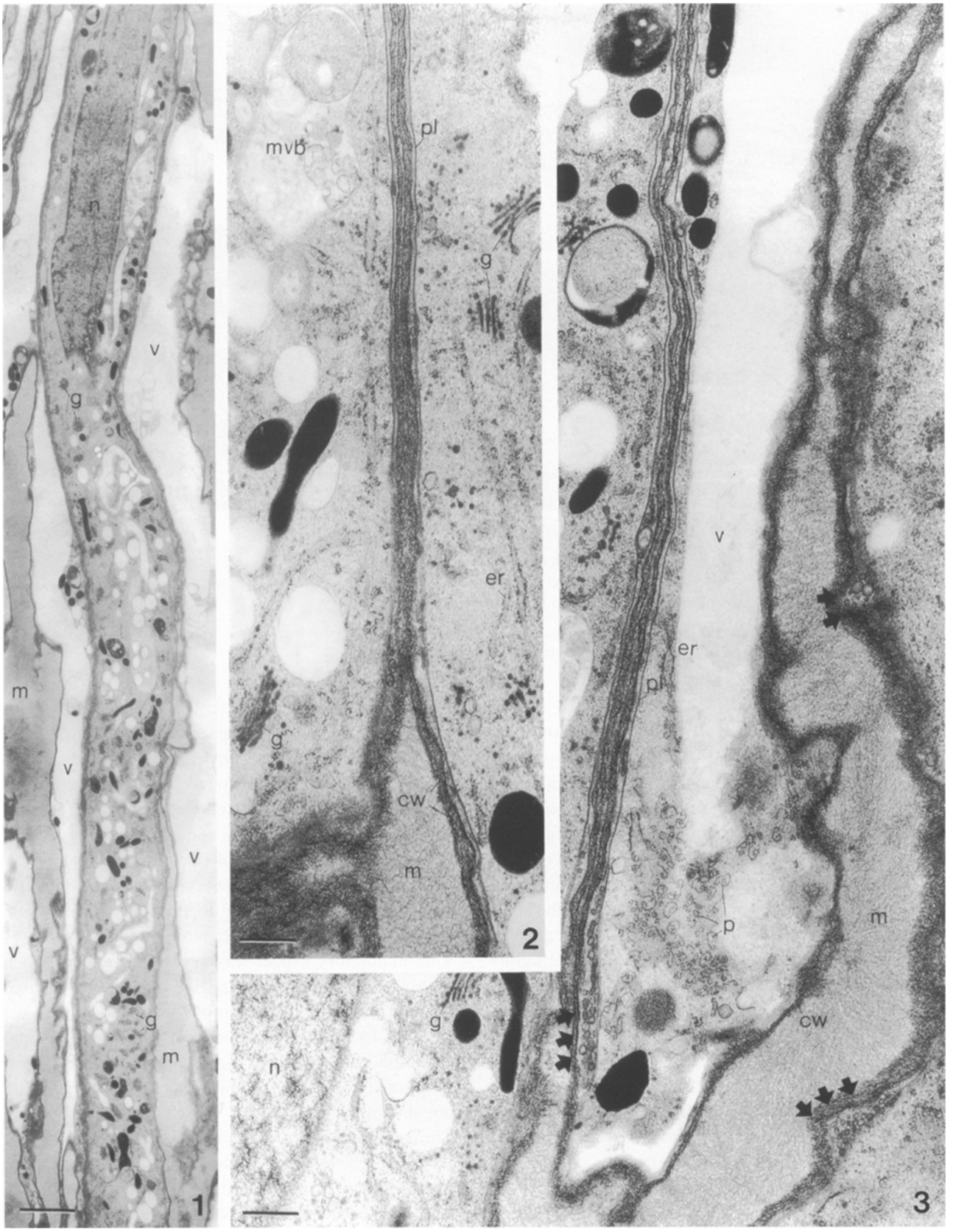
As shown in Fig. 1, HPF/FS yielded good preservation of a great length of the pollen tube cell. Pollen

Figs. 1–3. Morphology of in vivo grown lily pollen tubes preserved by HPF/FS

Fig. 1. General view. The pollen tube in the center is surrounded by a stylar matrix (*m*) that keeps a population of pollen tubes together. Spent pollen tubes are characterized by a large vacuole (*v*). No particular organization is observed in the cytoplasm of this tube cell, which is at some distance from the tip as indicated by the presence of the vegetative nucleus (*n*). Golgi apparatus (*g*), vacuoles and other organelles are homogeneously distributed within the cell. Bar: 2.5 μm

Fig. 2. Association between two pollen tube cells. The two cells have a dense cytoplasmic content with numerous organelles. Their two cell walls (*cw*) are closely appressed in one area, while they are completely separated by the stylar matrix (*m*) in another. The plasmalemma (*pl*) is smooth. The cell wall is composed of two layers. *er* Endoplasmic reticulum, *mvb* multivesicular body. Bar: 0.4 μm

Fig. 3. Association between a pollen tube cell and a spent pollen tube. The spent pollen tube characterized by its large vacuole is flanked by two pollen tube cells. Note that the spent pollen tube still contains numerous polysomes (*p*). The arrows indicate areas where the outer layer of the cell wall is clearly detached from the plasmalemma. Bar: 0.4 μm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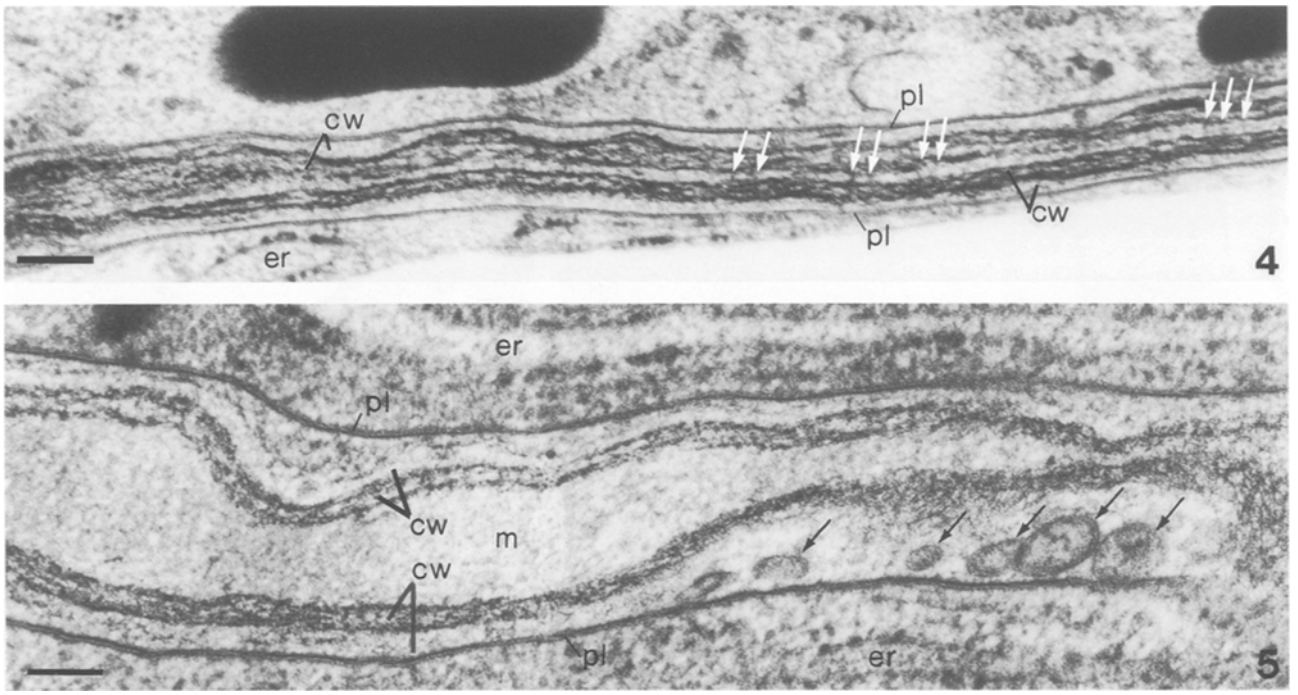


Figs. 4 and **5.** Details of the complex between pollen tube cells. *er* Endoplasmic reticulum, *pl* plasmalemma

Fig. 4. Appressed area. Two pollen tubes are separated by a fibrillar complex that is composed of the two cell walls (*cw*) of the pollen tubes and some stylar matrix components. Note the apparent links between the two cell walls (arrows). The cell walls are composed of an outer layer of dense fibrillar components and an inner layer. Bar: 0.17 μm

Fig. 5. Separated area. Note the textural similarity between the stylar matrix (*m*) and the inner layer of the cell wall. Cytoplasmic blebs surrounded by a membrane are present at the surface of the tube cell (arrows) Bar: 0.1 μm

tube cells were surrounded by spent portions of other pollen tubes, which are characterized by the presence of large vacuoles. The stylar matrix, which has been secreted by the transmitting tract cells, held the pollen tubes in compact groups. No significant differences were observed in the quality of structural preservation when the cells were immersed either in a solution of 15% Dextran, the in vitro growth medium, or the style exudate. However, even in blocks of adequately frozen material, some cells or parts of some cells contained drying artifacts which are judged so by the presence of condensed and heavily stained areas of the cytoplasm (data not shown).

Pollen tube cells (Fig. 2) adhered to one another or to spent pollen tubes (Fig. 3) through a fibrillar complex. High magnifications of the adhesion areas show that the fibrillar complex between the two plasmalemmas was composed of the two cell walls of the tube cells and what is presumably the secreted matrix of the transmitting tract cells. Even in areas of close adhesion, the two cell walls were separated from each other by a thin layer of stylar matrix components (Fig. 4). However, apparent links were evident between the cell walls (Fig. 4). These links were absent when cells were separated by an expanded layer of stylar matrix components (Fig. 5). The cell wall appeared as a multilayered structure that was composed of a dense and homogeneous outer layer of fibrillar components and an inner layer with the same texture as that of the stylar matrix surrounding the pollen tubes (Figs. 4 and 5). The dense layer of fibrillar components was wavy. This undulatory aspect was especially evident when cytoplasmic blebs were trapped within the inner layer (Figs. 2 and 5). These cytoplasmic blebs were surrounded by a clear bilayered membrane (Fig. 5) and their continuity with the plasmalemma could be seen in glancing sections through the plasmalemma (data not shown).

The general quality of ultrastructural preservation at the cellular level is illustrated in Fig. 2, 3, and 6. The plasmalemma was smooth and organelles had a smooth surface contour. Mitochondria and plastids were heavily contrasted, which makes recognition of ultrastructural details difficult. Vacuoles surrounded

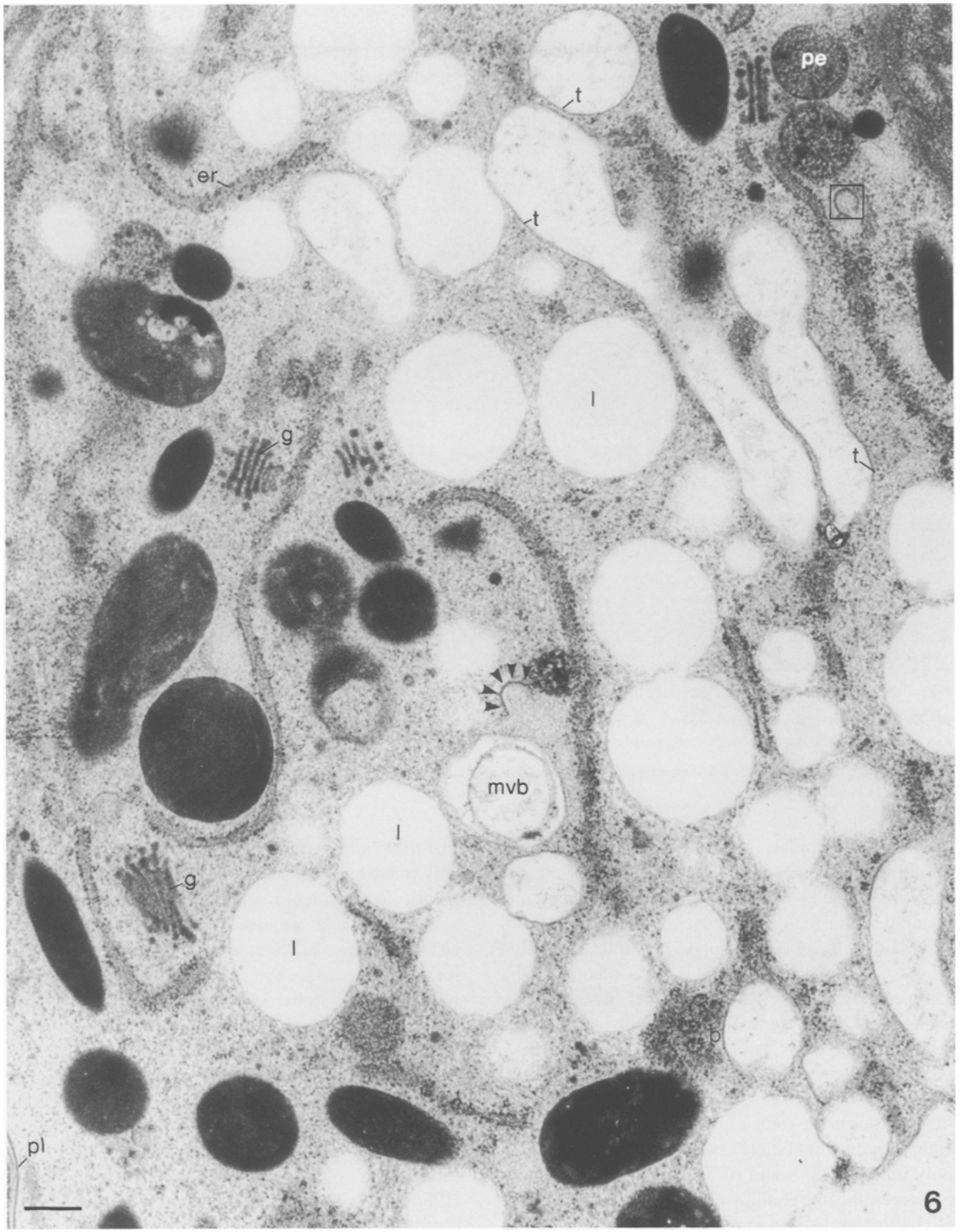
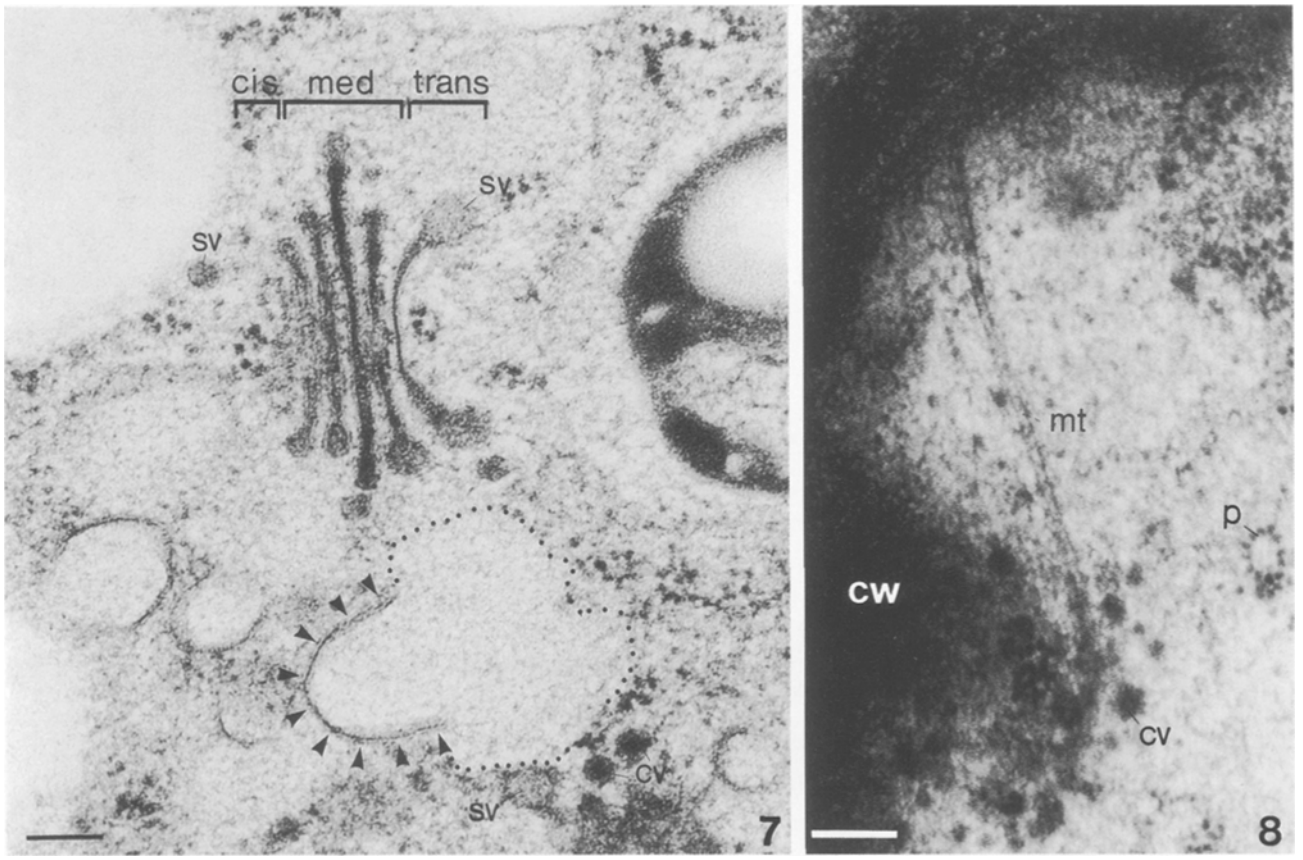


Fig. 6. Components of the pollen tube. Note that some large vesicles are disrupted (arrowheads) whereas others are not (within the box). Bar: 0.3 μm. *er* Endoplasmic reticulum, *g* Golgi apparatus, *l* area where lipid droplet existed, *mvb* multivesicular body, *pe* peroxysome, *pl* plasmalemma, *t* tonoplast



Figs. 7 and **8.** Some ultrastructural features of the pollen tube

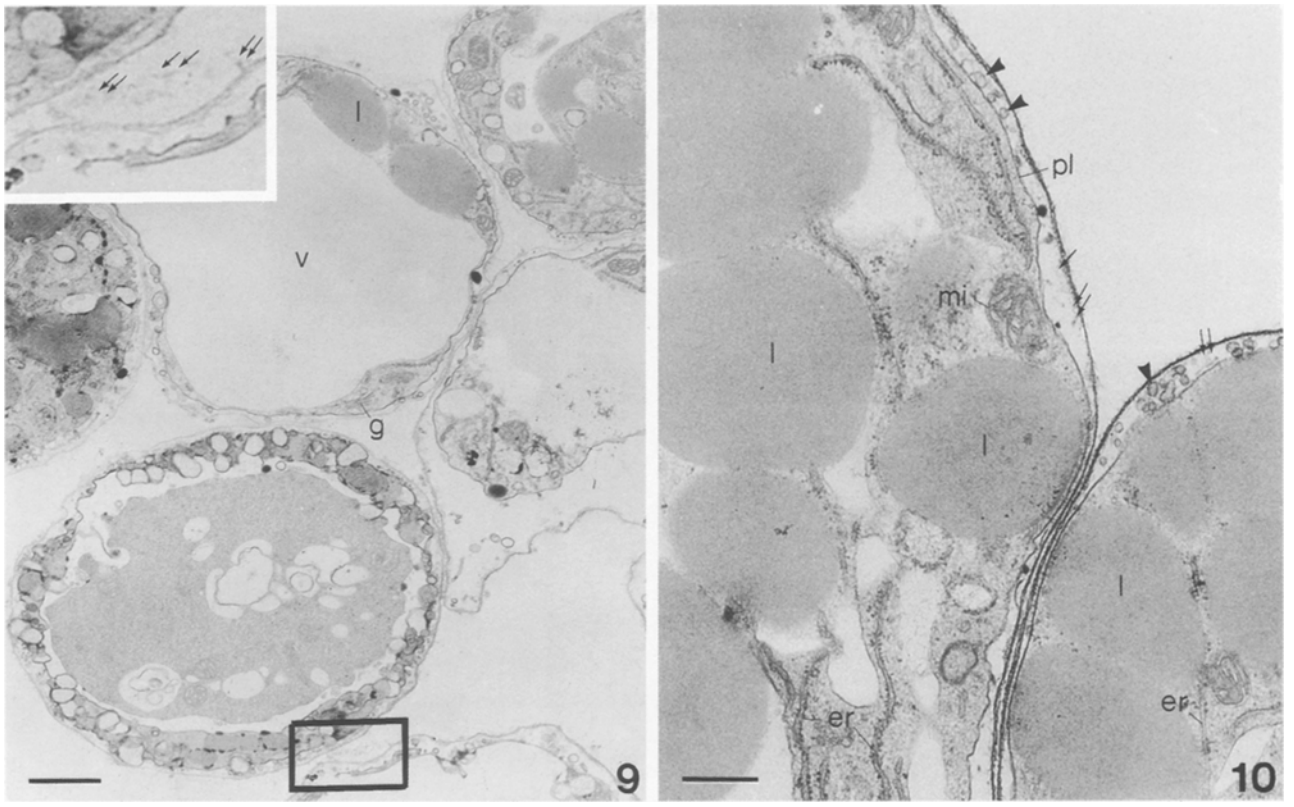
Fig. 7. Dictyosomes show a clear cis-to-trans polarity. They are composed of 5 to 8 stacks. Arrowheads outline the membrane of a large vesicle that is disrupted. The granular content has spread out (dotted line). Bar: 0.13 μm. *cv* Coated vesicle, *sv* secretory vesicle

Fig. 8. A tangential section shows a well preserved microtubule (*mt*). Coated vesicles (*cv*) are seen in the vicinity of the plasmalemma. Bar: 0.2 μm. *cw* Cell wall, *p* polysome

by a clear tonoplast appeared fully intact. Large vesicles with a size and texture different from the secretory vesicles (Fig. 7) were often disrupted and resulted in an irregular granular content partly bounded by a membrane (Figs. 6 and 7). Similar disruption of the vesicles has been observed in other HPF/FS samples (Hyde et al. 1991; Roy pers. obs.). Clear round areas that were not surrounded by a membrane represent lipid droplets in which the lipids may have been extracted during freeze substitution (Fig. 6, compare with Fig. 11). Cells showed a dense population of ribosomes, some of which were attached to the endoplasmic reticulum (Figs. 2 and 6) or assembled in polysomes (Figs. 3 and 6). Cells showed many dictyosomes, the morphology of which was well defined. No particular distribution of the Golgi apparatus was distinguished within the tube cell (Fig. 1). "Multivesicular bodies" were seen along the length of the tube cell (Figs. 2 and 6).

The morphology of the Golgi apparatus was remarkably consistent in the tube cell. A distinct cis-to-trans polarity was evident in all the dictyosomes observed and Golgi stacks were usually composed of 5 to 8 cisternae (Fig. 7). Secretory vesicles usually surrounded the dictyosomes and trans-Golgi networks were not always observed. Coated vesicles appeared associated with the Golgi apparatus (Fig. 7) but they were also found in the cortical cytoplasm (Fig. 8). Microtubules were intact and straight (Fig. 8), but microfilaments could not be convincingly demonstrated.

The ultrastructure of pollen tubes prepared by chemical fixation (Figs. 9 and 10) differed from that of HPF/FS (Figs. 2, 3, and 6) in several significant aspects. The most striking difference is in the low abundance of a stylar matrix in chemically fixed samples (Fig. 9). Only a relatively small amount was visible attached as remnant patches to the surface of the



Figs. 9 and **10.** Ultrastructural aspects of in vivo grown lily pollen tubes prepared by chemical fixation

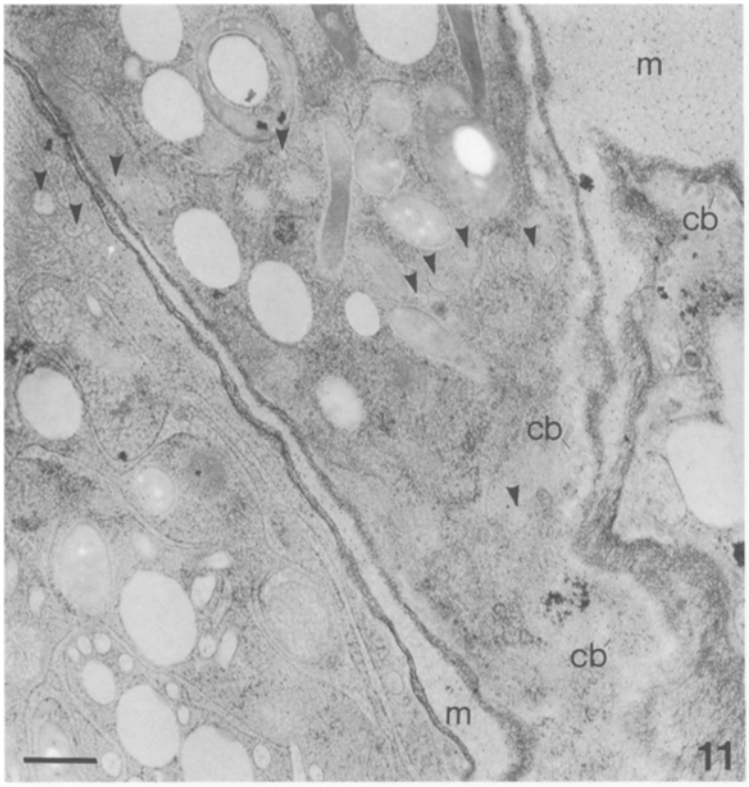
Fig. 9. General view. Transverse sections of pollen tubes. Although the stylar matrix is not preserved, pollen tubes are still adhering. *g* Golgi apparatus, *l* lipid droplets, *v* vacuoles. Bar: 0.9 μm. **Inset** Enlargement of the boxed area. Remnants of the stylar matrix are visible as patches on the surface of the pollen tube (arrows)

Fig. 10. Adhesion zone. Patches of fibrillar material are visible beneath the cell wall outer layer (arrows). Plasmalemma (*pl*) and organelle membranes undulate but lipid droplets (*l*) are well preserved. Note the presence of cytoplasmic blebs (arrowheads). *er* Endoplasmic reticulum, *mi* mitochondria. Bar: 0.5 μm

pollen tube (Fig. 9 inset). Patches of fibrillar material were also visible beneath the outer cell wall layer (Fig. 10). Chemically fixed samples had wavy membranes (Fig. 10). However, lipid droplets were left intact and were easily recognizable within the tube cell (Figs. 9 and 10).

In order to characterize the material that surrounds the tube cell, we used antibodies directed towards AGPs (JIM13; Knox et al. 1991) and callose (Northcote et al. 1989). The stylar matrix was homogeneously and densely labeled with JIM13 (Figs. 11 and 12). The inner layer of the cell wall, which may or may not contain cytoplasmic blebs, was also labeled with JIM13 confirming its similarity with the stylar matrix (Fig. 12). Conversely, the outer fibrillar layer of the cell wall was less stained with gold particles (Figs. 11 and 13). The vicinity of the plasmalemma (Fig. 12) as well as the cytoplasmic blebs (Figs. 11–13) were decorated with gold particles. Within the cytoplasm, gold particles could be seen in the trans-Golgi network (Fig. 12) and vesicles (Figs. 11 and 12). Preincubation of JIM13 monoclonal antibody with a solution of 100 mM arabinose resulted in no labeling (data not shown). Quantitation of gold particles confirmed the electron microscopic observation (Fig. 15). A high concentration of gold dots was found in the matrix (159.82 ± 3.93 gold particles/μm^2), the inner cell wall (128.40 ± 6.93 gold particles/μm^2) and along the plasmalemma (131.18 ± 5.64 gold particles/μm^2).

Callose labeling was heterogeneously distributed along the pollen tube cell wall. Some cells had a continuous labeling (Fig. 14) whereas other cells showed staining only in discrete areas. Gold particles were predominantly seen in the interface between the two layers of the cell wall (Fig. 14). A weak labeling was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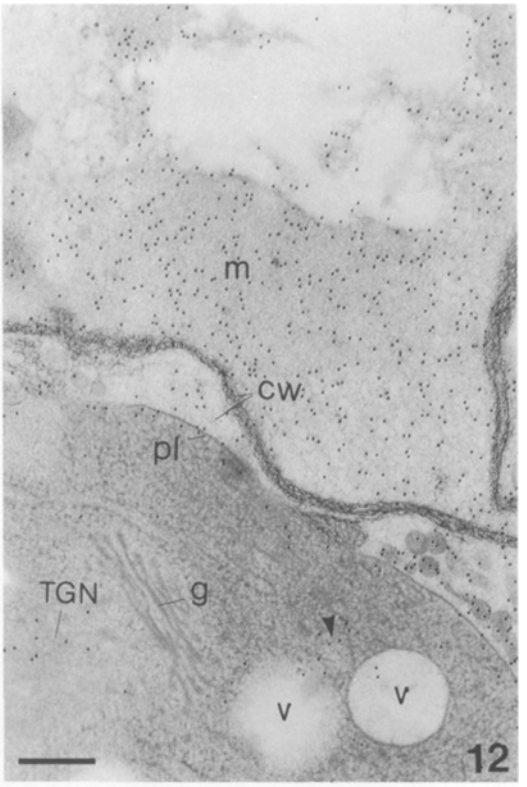
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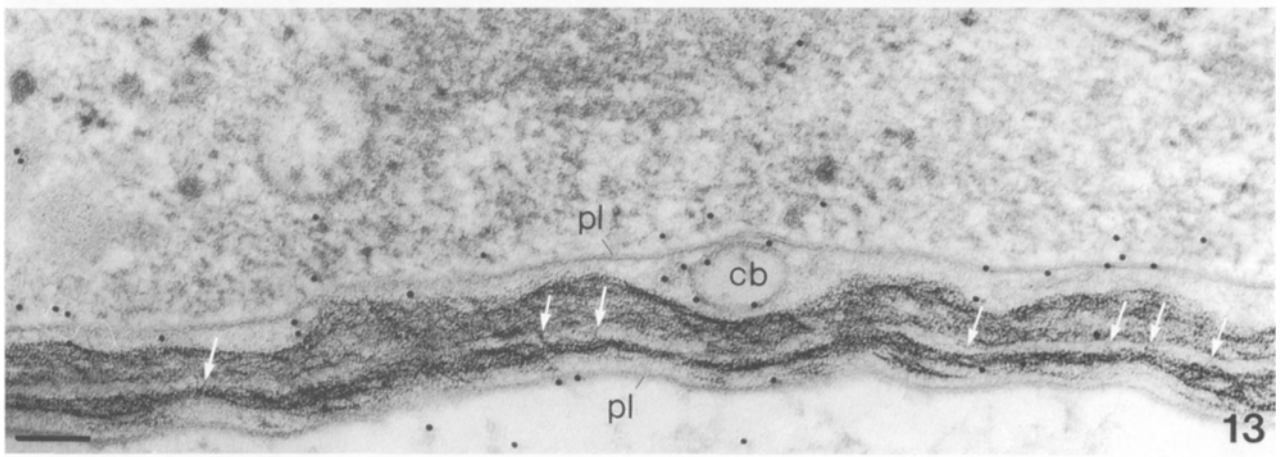
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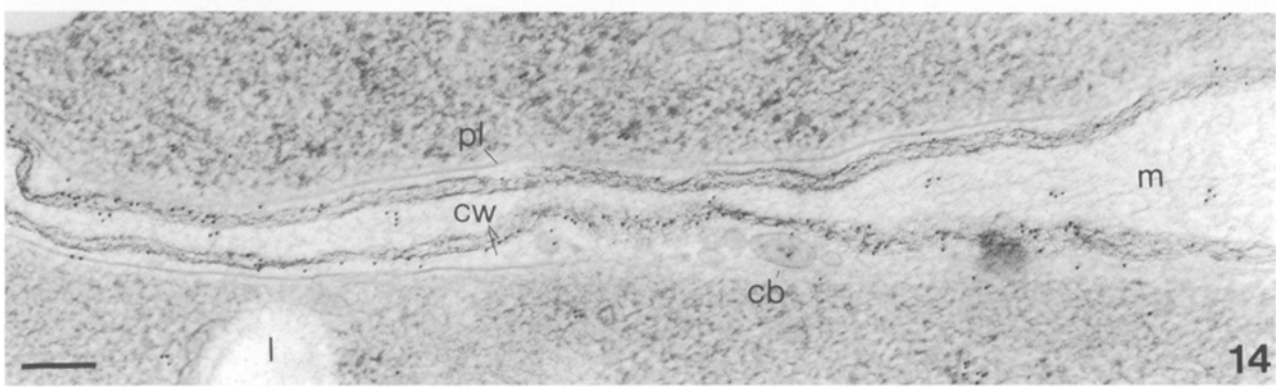
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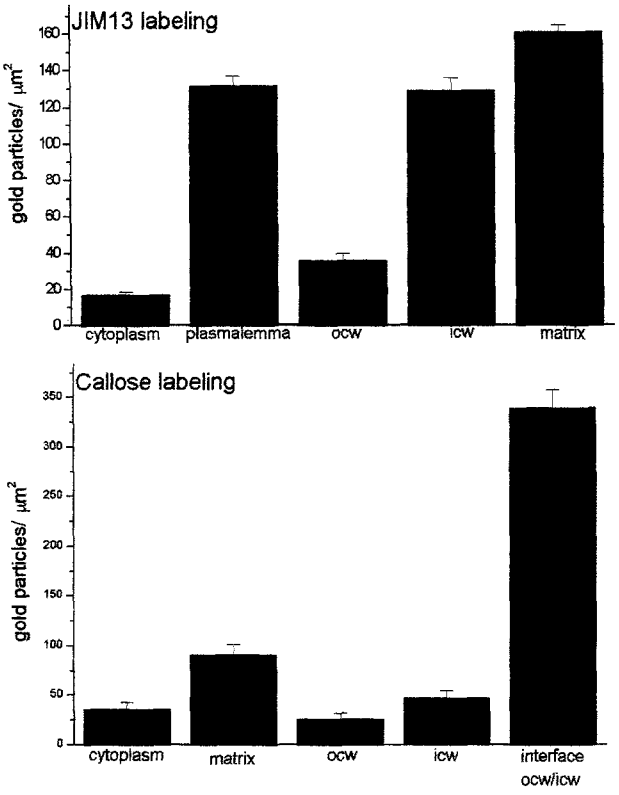

Fig. 15 a, b. Quantitative analysis of labeling. **a** JIM13 labeling. Matrix, plasmalemma, and inner cell wall (*icw*) show dense labeling. Gold particles on the inner cell wall were counted whether cytoplasmic blebs were present or not. **b** Callose labeling. Most of the label appears over the interface that includes part of the inner (*icw*) and the outer cell wall (*ocw*)

seen within the stylar matrix and the cytoplasm. We are uncertain if the labeling seen in the cytoplasm is specific or represents a low background. Callose has been specifically described in the cell wall (Northcote et al. 1989, Balestrini et al. 1994, Samuels et al. 1995, Roy et al. 1997) but recently some labeling associated with Golgi-derived vesicles has been illustrated in pollen tubes (Hasegawa et al. 1996). The control with preincubated antibody with a solution of laminarin showed no staining (data not shown). Only areas that show a continuous labeling were quantified in order to decipher the distribution of the labeling. The outer cell wall and inner cell wall were poorly decorated compared to the interface including part of the two layers; respectively 24.94 ± 7.14 gold particles/μm^2 and 46.60 ± 7.73 gold particles/μm^2 were found in the outer and inner cell wall whereas 338.05 ± 18.81 gold particles/μm^2 were quantified in the area that includes the interface.

Discussion

HPF/FS has allowed us to preserve a high percentage of in vivo grown pollen tube cells, which meet the ultrastructural criteria of well-frozen material and sustain comparison with in vitro grown pollen tubes preserved by other ultra-rapid freezing techniques. These criteria are: smooth appearance of membranes and turgid organelles (Lancelle et al. 1986, Cresti et al. 1987, Lancelle and Hepler 1992, Derksen et al. 1995); dense cytoplasmic matrix (Lancelle and Hepler 1992); and improved preservation of the Golgi apparatus (Derksen et al. 1995), coated vesicles (Derksen et al. 1995), and the cytoskeleton (Lancelle et al. 1987). However, microfilaments were not convincingly observed in our preparation, which confirms previous studies using HPF (Ding et al. 1992). The significant advantage of HPF/FS compared to other ultra-rapid cryofixation methods is that we have been able to obtain a high yield of well preserved material. Plunge-freezing the samples in liquid propane, a technique used successfully for in vitro grown pollen tubes (Lancelle et al. 1987, Lancelle and Hep-

Figs. 11–14. Immunolabeling of the pollen tube cell wall and the stylar matrix

Figs. 11–13. Localization of AGPs. JIM 13 labeling

Fig. 11. Labeling is mainly detected outside of the cell. Within the cell, gold particles are associated with large vesicles (arrowheads). *cb* Cytoplasmic blebs, *m* stylar matrix. Bar: 0.6 µm

Fig. 12. Labeling is detected along the plasmalemma (*pl*), on the stylar matrix (*m*), on the cell wall inner layer, which may or may not contain cytoplasmic blebs, but not much on the outer layer. Within the cytoplasm, a vesicle (arrowhead), the trans-Golgi network (*TGN*), and the vacuoles (*v*) are labeled. *g* Golgi apparatus, *cw* cell wall. Bar: 0.30 µm

Fig. 13. AGPs are present on the membrane that surrounds the cytoplasmic bleb (*cb*), but not in the zone of adhesion where the links are present (arrows). Bar: 0.12 µm

Fig. 14. Localization of callose. Anti-β(1→3) glucopyranose labeling. Callose is distributed on the interface between the two layers of the cell wall. Note that the labeling is less dense in the upper cell. Gold particles are also associated with the stylar matrix (*m*). Bar: 0.17 µm. *l* Area where lipid droplet existed

ler 1992), gave very poor preservation of the in vivo grown tube cells (data not shown). This is due to the thickness of the sample and the resulting slow rates of cooling leading to ice crystal damage. With HPF, the high pressure hinders crystallization, which permits slow cooling without distorting ice crystals.

Previous observations on cryofixed pollen tubes were made on less than 2 h old in vitro grown pollen tubes. Comparisons with our 48 h in vivo grown pollen tubes are thereby difficult to establish. Our preparation method, which involves loading of the specimen in the HPF holders, does not allow us to clearly position the tip and encounters with tube tips in ultra-thin sections are rare. However, a few features of the general cytoplasm can be compared. For example, the organization of the cytoplasm into the slow and fast lanes found in in vitro grown pollen tubes (Lancelle and Hepler 1992) was not observed here (Fig. 1). The cell is surrounded by numerous cytoplasmic blebs. It is not likely that they result from the effects of high pressure as they are also observed in cells that have been chemically fixed. Removal of the pollen tubes from the style may account for such blebs, although chemical fixation of the pollen tubes within the styles results in similar structures (data not shown). Examination of published micrographs (e.g., Dickinson et al. 1982, Meikle et al. 1991) indicate that the cytoplasmic blebs can be described in in vivo grown pollen tubes in a wide range of species but are strikingly absent in images of in vitro grown pollen tubes. The functional significance of these blebs is unknown. Pollen tubes that have been kept in situ and prepared by cryofixation are required to clearly elucidate the presence of these cytoplasmic blebs.

In addition to the excellent ultrastructural preservation, using HPF/FS to cryofix in vivo grown pollen tubes has allowed us to (i) maintain the stylar matrix that surrounds the pollen tubes and (ii) preserve the interactions that exist between pollen tubes in vivo, which are absent in in vitro grown pollen tubes.

Cryofixation is known to preserve labile components of the cell wall (Howard and O'Donnell 1987, Kandasamy et al. 1991, Kiss and McDonald 1993) or extracellular polysaccharides that surround the cells (Roy et al. 1996). So far, the best results for preserving the secreted matrix that surrounds pollen tubes have been obtained with chemically fixed solid styles (Bell and Hicks 1976, Cresti et al. 1976, Anderson et al. 1987, Sanders et al. 1990), and the aggregated aspect that is often described is probably an artifact due to chemical fixation. Loss of the secreted matrix of the transmitting tract cells is a common feature associated with hollow styles prepared by chemical fixation (Rosen 1971, Dickinson et al. 1982, Jauh and Lord 1996). This is also clearly evident in our own study where chemically fixed pollen tubes are not surrounded by a stylar matrix (Figs. 9 and 10).

The material that is similar to the stylar matrix and composes the cell wall inner layer in HPF/FS samples is absent in chemically fixed samples. We are uncertain as to whether this cell wall architecture is a unique feature in mature pollen tubes grown in vivo or if this appearance is an artifact both of HPF/FS and chemical fixation. Such a structural organization of the cell wall is not seen in the young in vitro grown pollen tubes that are plunge frozen in liquid propane (Lancelle and Hepler 1992). In in vivo grown pollen tubes, the inner layer is composed of JIM13 reactive components, which reinforces its similarity with the stylar matrix. In chemically fixed *Nicotiana* samples, Meikle et al. (1991) described the inner layer as electron-lucent and rich in callose. In our study, the inner layer is electron-dense, and callose, which decorates the interface between the two layers of the cell wall, does not account for it. It is possible to imagine that a continuum between the inner wall layer and the stylar matrix operates and that the outer wall layer acts as a semi-permeable barrier through which exchanges can occur. It is worth raising the question as to whether the structural organization of the cell wall reflects a unique property of the pollen tube that intensively interacts with the stylar secretion. For example, Lind et al. (1996) have shown that a glycoprotein specific to the style passes through the pollen tube cell wall before being taken up by the cytoplasm. However, we cannot rule out the possibility that handling the pollen tubes before fixation may have artifactually generated this exchange.

Adhesion between pollen tubes seems to be provided by the cell wall–stylar matrix continuum. In an earlier study, it was proposed that this adhesion is via calcium cross-linking between unesterified pectin chains of the two pollen tube cell walls or via the interaction of the secreted stylar matrix with components of the pollen tube cell wall (Jauh and Lord 1996). The improved preservation provided by HPF/FS results in a clear picture of the pollen-tube cell wall–stylar matrix complex. Although closely appressed, the cell walls of two distinct pollen tubes are not attached per se, i.e., a continuous layer occurring between two cells as can be illustrated by the middle lamella in plant tissues. Rather, distinct matrix components are

present between the two cell walls and cross-links are sometimes apparent. These observations suggest an adhesion function of the stylar matrix. It has been postulated that the molecules of the stylar matrix involved in the adhesion between pollen tubes are AGPs due to their adhesive nature (Cheung 1995, Lord et al. 1996). However, JIM13 labeling is not visualized in the adhesion zone between pollen tubes (Fig. 13), which indicates that other components of the stylar matrix are involved or that AGPs in this specific location are not recognized by JIM13. It is also relevant to note that the cross-links seen between closely adhering pollen tubes disappear when the stylar matrix filled gap enlarges between them. This may open the pathway for the progression of other pollen tubes. Whether this defined route results from a simple mechanical pressure or from active removal of the "glue" between pollen tubes is still not clear.

In conclusion, we have shown that the HPF/FS method allows high-resolution structural analysis of in vivo grown pollen tubes within the matrix secreted by the transmitting tract cells of the style.

Acknowledgments

JIM13 antibody was a generous gift from Paul Knox. This work was supported by NSF grant MCB-93-04953 and MCB-96-01087 to PKH and NSF grant IBN 92-06577 and a "Cytonet" grant to EML. The Central Microscopy Facility at University of Massachusetts is supported by NSF grant BBS-87-14235.

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Figs. 4 and **5.** Details of the complex between pollen tube cells. *er* Endoplasmic reticulum, *pl* plasmalemma