Endoplasmic Reticulum—Dictyosome—Secretory Vesicle Associations in Pollen Tubes of *Lilium longiflorum* Thunb.[1]

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Rapid growth of pollen tubes is restricted to the tube tip. The localized deposition of wall materials depends upon the addition of membrane-bounded packets of wall material in the form of secretory vesicles (3, 5, 8, 9). The vesicle membranes contribute new plasma membrane, and the vesicle contents provide precursors for the cell wall (6). These vesicles originate from the Golgi apparatus (8, 9, 10) but other cell components may be involved. Chief among these is an extensive membranous reticulum which extends throughout the area of vesicle production and deposition. This report describes the endoplasmic reticulum of lily pollen tubes including an unusual secretory vesicle-endoplasmic reticulum association.

Materials and Methods

Anthers of *Lilium longiflorum* Thunb. var. Ace were collected from greenhouse grown plants, allowed to dry and dehisce, and stored at —70° C. Pollen was seeded on liquid medium (10% sucrose; 10 ppm boric acid). After 3 hr at room temperature, tubes from germinated grains were fixed at room temperature in a solution of 0.1 M glutaraldehyde, 0.1 M acrolein and 0.1 M sodium cacodylate buffer (pH 7.2) for 1 hr. This was followed sequentially by post-fixation with 1% osmium tetroxide in cacodylate buffer (pH 7.2) for 2 hr, and transfer to 0.1 M buffer succeeded by distilled water and saturated uranyl acetate overnight at 5° C. Specimens were again washed with distilled water, dehydrated in a graded ethanol series followed by acetone and were embedded in an epon - araldite mixture. Polymerization was carried out in a nitrogen atmosphere at 70° C for about 72 hrs.

Thin sections were cut with a diamond knife using a Porter-Blum MT-2 ultramicrotome. They were subsequently post stained with lead citrate (7) and viewed using a Philips EM/200.

The methods used provide minimal distortion of the protoplasm as observed by phase microscopy. Fixation and staining were judged adequate for the maintenance and discernment of membranous organelles. Satisfactory fixation is suggested by the preservation of the intercisternal elements of dictyosomes (See Fig. 3).

Observations and Discussion

Ultrastructural studies of the pollen tube (3, 5, 8, 9, 10) have suggested a mechanism of tip growth involving secretory vesicles produced by dictyosomes of the Golgi apparatus. In Fig. 1, the portion of the

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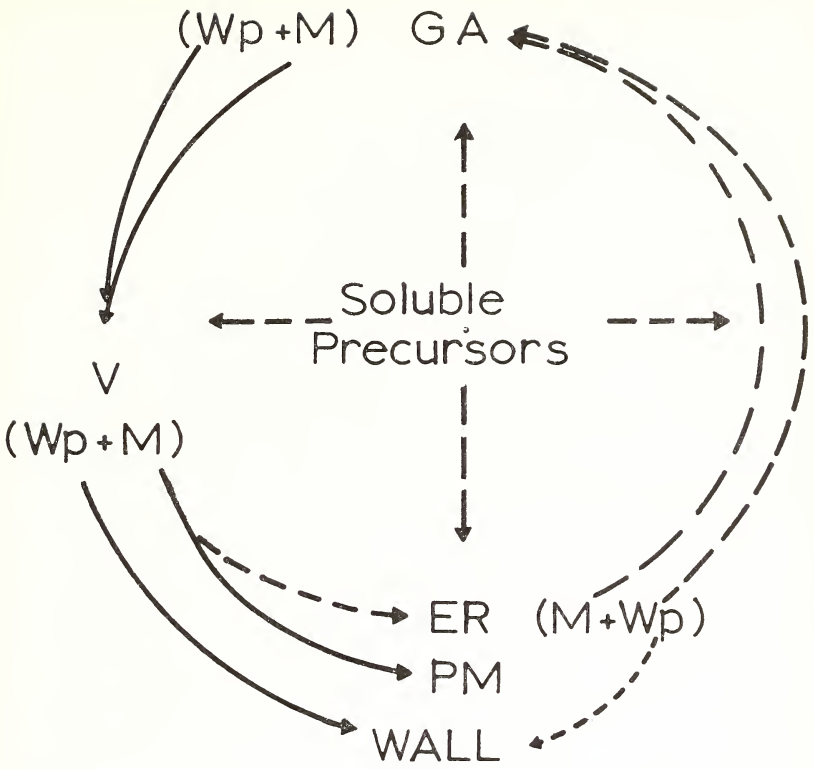

Figure 1. Cell wall matrix polysaccharides and membrane compartmentalization during pollen tube tip growth.

scheme connected by solid lines represents the contribution of membrane (M) and wall precursors (Wp) to the plasma membrane (PM) and the wall. These vesicles are assumed to migrate to the cell surface where the vesicle membranes fuse with the plasma membrane and discharge their contents into the cell wall region. Assuming that the contribution of the vesicle contents to the cell wall matrix involves no radical volume changes, it is possible to calculate the amount of membrane available for surface growth of the plasma membrane. Such calculations are shown in Table 1 and show the possibility of a remarkable stoichiometry between vesicle surface and increase in plasma membrane during steady state growth. As is true with most first approximations, the actual situation appears more complicated.

Associated with the regions of vesicle formation and deposition is an extensive membranous reticulum (Fig. 2) which extends throughout the tube cytoplasm. It is a complex system of anastomosing tubules and cisternae and is identified here as endoplasmic reticulum (ER). In the apical 5 to 10 μ of the tube the ER is without associated ribosomes (Figs. 3, 4). Behind the tip, the ER is largely agranular but limited regions have associated ribosomes. Granular endoplasmic reticulum is

TABLE 1.

Calculated Rate of Vesicle Production and Cell Surface (Membrane and Cell Wall) Increase for Elongating Pollen Tubes.

Rate of tube elongation*	12μ/min
Tube diameter*	16μ
Wall matrix thickness*	.05μ
Vesicle diameter*	.30μ
Vesicle volume	.014μ^3
Vesicle surface	.28μ^2
Increase in wall volume	30μ^3/min
Vesicle production	2150/min
Vesicle membrane production	600μ^2/min
Increase in plasma membrane	600μ^2/min

* Direct measurements. Remamining entries are calculated from these measurements.

most frequently encountered in mature regions of the tube. The bulk of the ribosomes and polyribosomal configurations in the area of vesicle formation and deposition are found free in the cytoplasm (Figs. 3, 4). Ribosomes are present throughout the tube cytoplasm and, in contrast to a previous report (9), extend to the extreme tube tip. The lumen of the ER has a fibrous appearance which suggests a nonproteinaceous accumulated product.

Two types of membrane associations implicate ER as part of the endomembrane system involved in cell wall deposition. Transition elements (4) of the ER are closely associated with dictyosomes (Fig. 3) and with the masses of accumulated product in the tube tip (Fig. 4, 5). The ER - product associations are characteristic and frequently occur in the regions of vesicle fusion (Fig. 4). Higher magnification (Fig. 5) shows that these masses of product are partly enveloped by ER (double arrows) and partly surrounded by the single membranes (single arrows) characteristic of secretory vesicles. In the regions adjacent to ER there is no evidence of any single limiting membrane. Instead, the outer surface of the ER cisterna (black double arrows) bounds the accumulated product. Where portions of membranes appear in cross section, they are well stained immediately adjacent to the regions of product accumulation (Figs. 4, 5). Associations of ER with amorphous material appear elsewhere in the cytoplasm without evidence of a contributory association with secretory vesicles. Such an accumulation in Fig. 3 is adjacent to a dictyosome. The contents of these regions appear similar to those of the dictyosome-derived vesicles but the characteristic single limiting membrane is absent.

One explanation of these observations is that the ER secretes polysaccharides directly into the cytoplasm. The polysaccharides might originate from the fibrous material contained in the ER lumina. A chemical transformation as simple as methylation of free carboxyl groups during secretion could account for the differences in staining. These materials might then combine with the contents of Golgi apparatus-

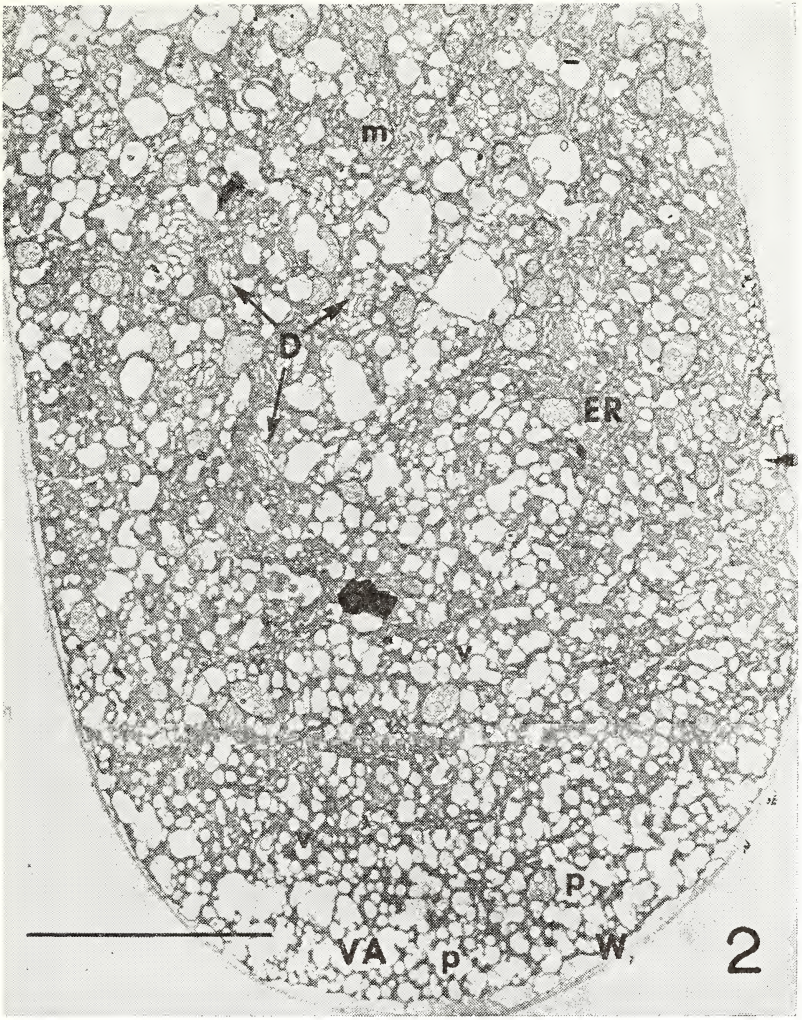

Figure 2. Electron micrograph of the apical 20μ of a *Lilium longiforum* pollen tube tip showing the spatial relationships among the extensive system of endoplasmic reticulum (ER); dictyosomes (D); secretory vesicles (v); the region of vesicle cacumulation (VA) with accumulated product (p); mitochrondria (m) and cell wall (W). Bar = 5 μ.

derived vesicles to contribute materials directly to the cell wall (dotted arrow of Fig. 1).

A limited protein synthetic capacity for pollen tube ER should not be dismissed especially for those regions with associated ribosomes. However, the pollen tube is not known to synthesize large quantities of proteins for export and abundant free polyribosomes are available for synthesis of cytoplasmic proteins. The chief function of the pollen tube is one of cell wall deposition. According to Brewbaker and Kwack (2),

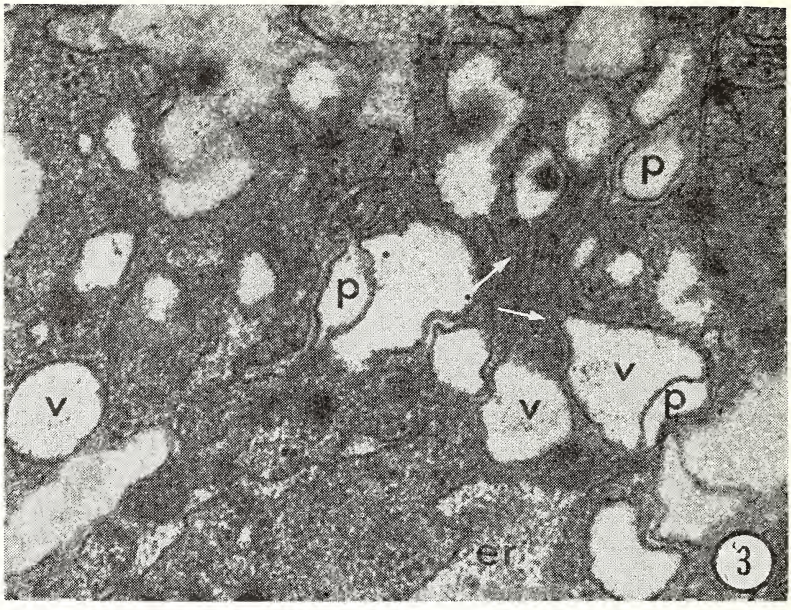

Figure 3. A portion of the cytoplasm approximately 30μ from the tube tip showing the association of endoplasmic reticulum (er) and a dictyosome (D). Intercisternal elements (i) of the dictyosome are found at the arrows. The contents of dictyosome-drived vesicles (v) differ in staining properties from those of the ER lumina but are similar to those of the presumed product accumulations bordered by ER (p). × 63,000.

rapid tube elongation is a process of cell wall synthesis with little increase in cytoplasm. Based on information from other cell types (6), it appears that the Golgi apparatus segregates products of synthesis for secretion and is endowed with only limited synthetic capacity. Thus, for future studies, we wish to consider the pollen tube ER as a potential site of polysaccharide biosynthesis. Materials would then be transferred either to dictyosomes for packaging into secretion vesicles (dashed arrows of Fig. 1) or secreted directly into the cytoplasm. Again a product transformation would account for staining differences. There is cytochemical evidence for methyl esterification of free carboxyl groups at the level of the secretory vesicle in lily pollen tubes (3).

In the region of vesicle fusion, a transfer of membranous material from secretory vesicles to ER might accompany any scheme for polysaccharide transport. Any excess membrane would be absorbed by

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Figure 5. The product (p) accumulation of Fig. 3 at higher magnification. Bounding membrane surfaces derived from ER (double arrows) are adjacent to the accumulated product (black arrows) and the cytoplasm (white arrows). The ER lumen is bounded by these paired membranes. The single membrane similar to that bordering secretory vesicles (single arrow) may have resulted from fusion of a secretory vesicle with the mass of accumulate product. × 79,000.

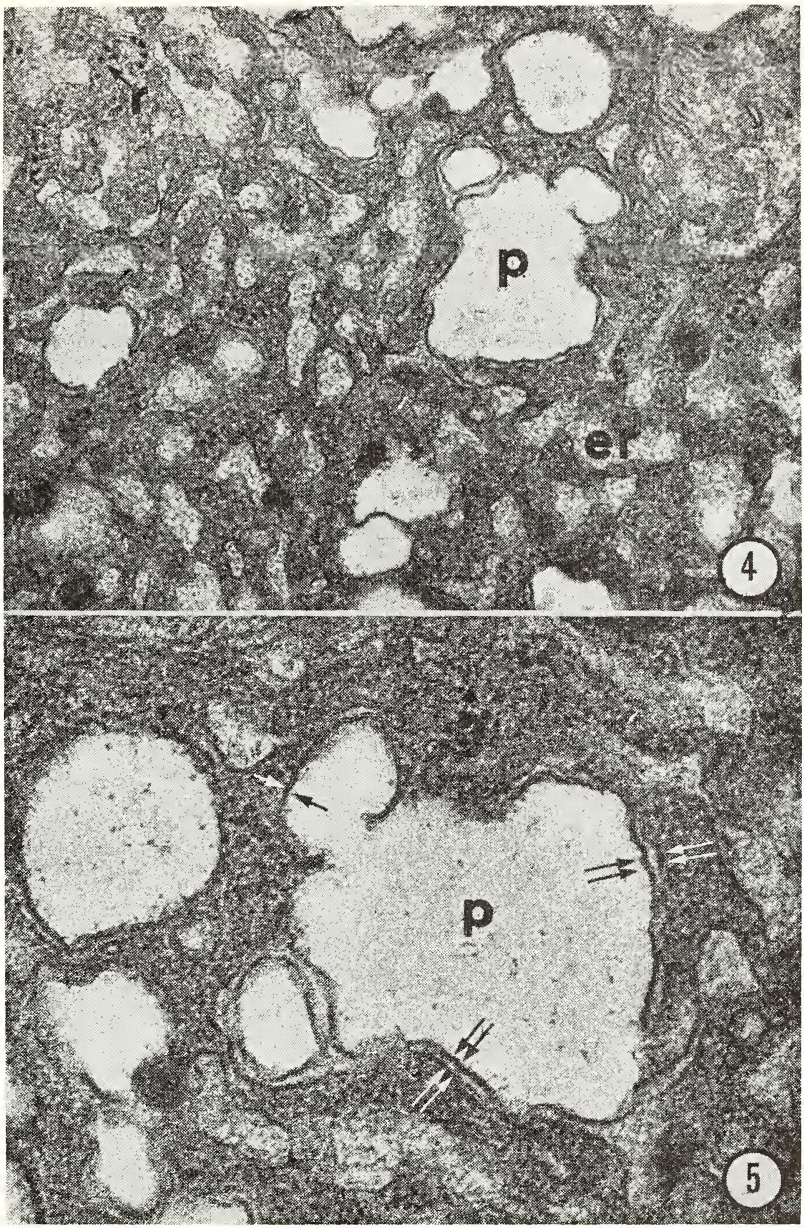

Figure 4. A portion of the cytoplasm containing extensive agranular endoplasmic reticulum approximately 7 T from the tube tip showing a product (p) accumulation bordered bby ER on the left and bottom and by a presumed secretory vesicle membrane on the top right. Ribosomes (r) often in polyribonsomal configurations do not appear to be associated with membranes. × 41,000.

the ER and ultimately transferred back to the dictyosome via a membrane belt (1). The calculations presented in Table 1 suggest that such a contribution would be limited but the possibility of membrane transfer via ER cannot be excluded.

Summary

Cell wall deposition in pollen tubes of *Lilium longiflorum* involves dictyosomes of the Golgi apparatus and associated secretory vesicles. Closely associated with these cell components is an extensive system of largely agranular endoplasmic reticulum consisting of anastomosing tubules and cisternae. In the region of vesicle accumulation and elsewhere in the tube cytoplasm, accumulations of secretory product are found to be enveloped by profiles of endoplasmic reticulum. In the region of vesicle fusion, single membrane-bounded secretory vesicles appear to fuse with these masses of product. These observations are consistent with a role of the endoplasmic reticulum in cell wall deposition.

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