

Endocytosis in elongating root cells of *Lobelia erinus*

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Summary

Endocytosis was demonstrated in elongating cortical and epidermal root cells of *Lobelia erinus* using the apoplast marker lanthanum nitrate. Lanthanum treatment produced electron-dense deposits throughout the cell wall, as well as in coated and smooth vesicles, partially coated reticula, and multivesicular bodies. This labelling pattern was observed in root cells that had been ultrarapidly frozen on a copper mirror and freeze-substituted (cryofixation) or fixed by conventional transmission electron microscope (TEM) techniques. The amount of endocytosis occurring was measured by counting the

number of vesicles μm^{-2} in root cells at various stages of development. Endocytosis occurred most in actively elongating cells, and least in mature cells, which were no longer elongating. The relationship between endocytosis and active cell wall secretion suggests that endocytosis may be acting to remove excess plasma membrane material added during exocytosis of secretory vesicles.

Key words: endocytosis, multivesicular body, membrane recycling.

Introduction

Endocytosis has been demonstrated in isolated higher plant protoplasts by the uptake of endocytic markers such as cationized ferritin (Joachim and Robinson, 1984; Tanchak *et al.* 1984, 1988). In this process, vesicles bud off from the plasma membrane, internalizing plasma membrane material as well as a portion of extracellular medium. The endocytic vesicles are believed to carry membrane material to other organelles such as the multivesicular body, the partially coated reticulum, the Golgi and the central vacuole (Tanchak and Fowke, 1987; Record and Griffing, 1988).

In contrast, endocytosis in intact plant tissues has been difficult to study, as the cell wall presents a physical barrier that prevents endocytic markers from interacting with the cell. Heavy-metal salts, such as lanthanum and lead, can cross the cell wall and act as apoplast markers, labelling the extracellular medium (Robards and Robb, 1974; Evert *et al.* 1985; Peterson *et al.* 1986). In recent studies authors, utilizing these compounds, have described the organelles involved in endocytosis (Hubner *et al.* 1985) but have not examined the role of endocytosis in higher plant cells.

It has often been speculated that endocytosis is a mechanism for plasma membrane recycling during secretion (Joachim and Robinson, 1984; Hubner *et al.* 1985; Raven, 1987; Steer, 1988). During the development of each plant cell, a phase of intense secretion occurs as the cell wall is deposited during elongation. In this study, populations of cells within the developing primary root of *Lobelia erinus* were used as examples of secretory cells (cells in zone of elongation) or non-secretory cells (mature epidermal and cortical cells). Endocytosis was demonstrated using lanthanum (La^{3+}) as a marker for the extracellular medium. The amount of endocytosis occur-

ring in each cell population was quantified in order to address the question of whether vesicular membrane recycling occurs in actively secreting higher plant cells.

In addition to conventional electron microscopy fixation, cells treated with markers for endocytosis were ultrarapidly frozen and freeze-substituted (cryofixation). This technique stops the flow of membranes in the cell within milliseconds (Jones, 1984) and is useful for the preservation of labile cytoplasmic membrane structures (McCully and Canny, 1985).

Materials and methods

Electron microscopy

Lobelia erinus seeds were donated by Buckerfield's Seed Co., Vancouver, BC. The seeds were germinated on filter paper moistened with deionized distilled water for 7 days.

The seedlings were incubated in heavy-metal salt solutions following a modification of the procedure of Hubner *et al.* (1985). The solutions used were aqueous 5 mM $\text{La}(\text{NO}_3)_3$ (JBS no. 104, supplied by JBEM, Dorval, PQ), with pH adjusted to 7.6 using 1 M NaOH or aqueous 5 mM $\text{Pb}(\text{NO}_3)_2$ (Allied Chemicals, no. 1838), pH 5.6. The roots were immersed in either of the solutions or in distilled water (control) for 1 h at room temperature. They were then fixed without rinsing, by ultrarapid freezing or by conventional chemical fixation.

For ultrarapid freezing, seedlings were mounted on moistened filter paper, which was stuck to parafilm, and glued to a soft foam pad that is backed by a steel planchette. A polished copper disc was cooled to liquid nitrogen temperature in the Reichert-Jung KF80, excess water was blotted from the filter paper and the steel planchette was attached to magnet on the MM-80 plunging arm of the apparatus. Immediately the sample was released to slam into the copper block. After freezing, the parafilm next to the filter paper becomes brittle, allowing easy separation of the samples from the foam. The filter paper containing the samples was then processed for freeze-substitution in a Reichert-Jung CS Auto.

Freeze-substitution was carried out at -80°C for 48–56 h, followed by a gradual rise in temperature of 10 deg. C h^{-1} to a final temperature of 0°C . The substitution medium was 1% OsO_4 in absolute acetone. After the final temperature was reached, the substitution medium was replaced several times with absolute acetone. The acetone then acted as the solvent for Spurr embedding.

For conventional chemical fixation, a mixture of 1.5% formaldehyde, 1% glutaraldehyde, buffered in 50 mM Pipes, pH 7.4, for 1 h was used. After rinsing in buffer for 15 min, twice, the roots were postfixed in 1% OsO_4 , buffered as above, for 2 h at room temperature, or overnight at 4°C . Dehydration was performed using a graded series of methanol solutions; propylene oxide was the solvent for the subsequent Epon resin (JBEMBED 812) infiltration and embedding.

Light microscope sections ($0.5\ \mu\text{m}$) were cut with glass knives and stained with 1% Toluidine Blue in 1% sodium borate for 45 s. For electron microscopy, silver sections (60–90 nm) were obtained using a Reichert OMU3 ultramicrotome and mounted on copper grids. The sections were poststained for 25 min in saturated uranyl acetate in 70% methanol. The sections were examined on a Zeiss EM10C.

Energy dispersive X-ray microanalysis

Lanthanum-treated or control samples were chemically fixed and embedded as above. Sections of $0.5\ \mu\text{m}$ in thickness were mounted on Formvar-coated carbon-stabilized copper or beryllium grids, and examined using a Zeiss EM10C adapted for scanning transmission electron microscopy (STEM), energy dispersive X-ray analysis (EDX, Link AN 10 000). Region scans were made at $\times 20\ 000$ magnification for 200 s, with spot size 15 nm.

Quantification of endocytosis

After conventional chemical fixation, 15 individual roots, were sectioned for each treatment. From each individual root tip, three cells from each of the three developmental stages (meristem, elongating, and mature) were measured. This gave a sample size of 45 cells/developmental stage. The vesicle diameter, number of vesicles/cell, and cytoplasmic area (defined as ground cytoplasm area within plasma membrane excluding vacuoles and nuclei) were measured using the Kontron (IPS) interactively with the transmission electron microscope (TEM). The number of vesicles/cell was divided by the cytoplasmic area/cell to determine vesicles μm^{-2} ; this permits comparison between cells of different sizes. Frequency distributions were generated and non-parametric statistical tests performed (Kruskal-Wallis and Mann-Whitney U test).

Measurements of cell dimensions

Fifteen individual roots were examined and from each root at least four cells from each developmental stage were measured using a light microscope linked *via* video to the Kontron IPS. For cell wall thickness, the width of the cell wall from plasma membrane to plasma membrane was measured under the TEM, on line with the Kontron IPS.

Results

To test for endocytosis, intact seedling roots were immersed in the apoplastic tracer, lanthanum nitrate, followed by either conventional fixation or cryofixation. In the roots prepared using chemical fixation, endocytosis was compared in three different developmental stages: meristem, elongating and mature cells. Meristematic cells were defined as apical cells prior to differentiation (Fig. 1A). For the elongating (Fig. 1B) and mature (Fig. 1C) stages, epidermal cells and the two to three layers of underlying cortical cells were studied.

In ultrarapidly frozen (cryofixed) roots, only the epidermal cells of the root surface that contacted the copper block

were preserved, as expected in an uncryoprotected sample (Sitte *et al.* 1987; Plattner and Bachmann, 1982). Near the epidermal cell surface, material may be vitrified or the ice crystals formed may be extremely small due to the rapid

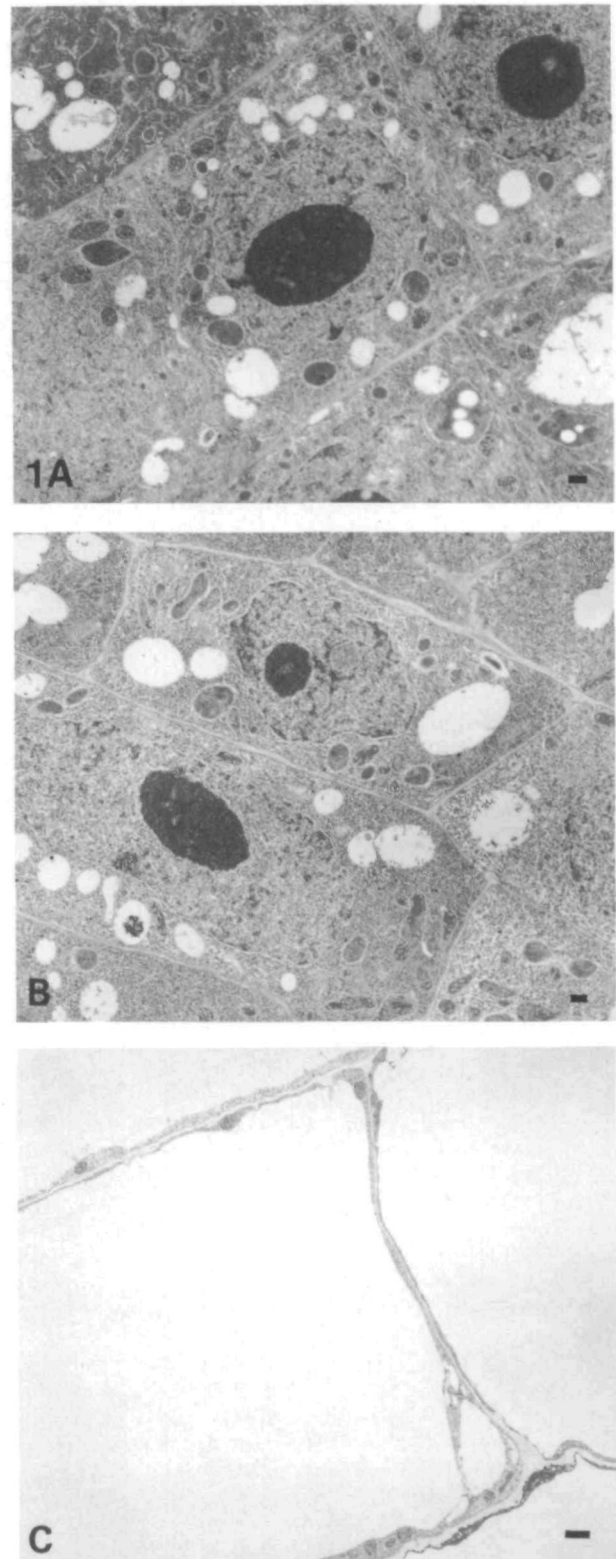


Fig. 1. Differentiating cortical and epidermal cells of *L. erinus* primary root. (A) Meristematic cells. (B) Elongating cells. (C) Mature cells. Bar, $1\ \mu\text{m}$.

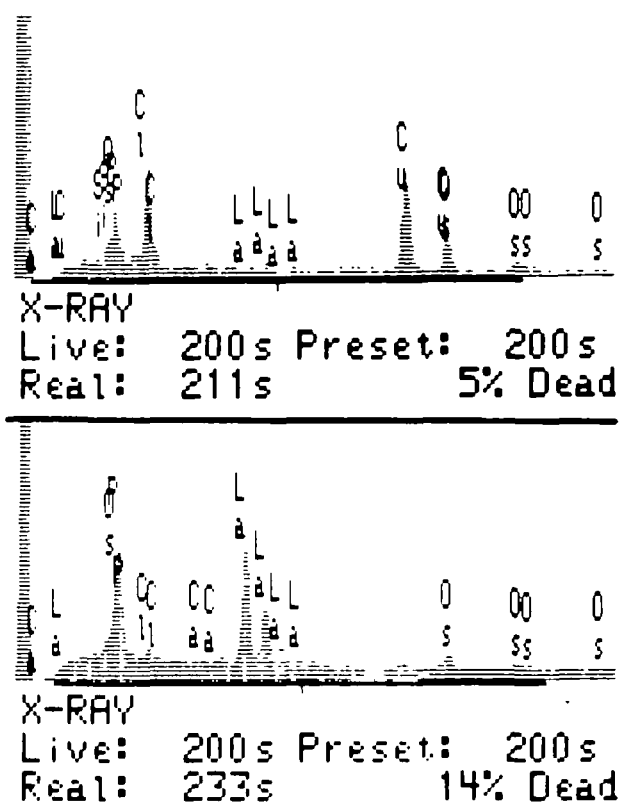


Fig. 2. X-ray spectrum collected while scanning region of cell wall of distilled water-treated control roots (top spectrum) or lanthanum-treated roots (lower spectrum). X-rays with energy levels characteristic for lanthanum can be identified (La, lanthanum; Ca, calcium; Os, osmium; Cl, chloride; Si, silica; P, phosphorus; Cu, copper).

rate of cooling. The preservation of ultrastructure in these regions was excellent.

Lanthanum-treated cells showed electron-dense crystals throughout their cell walls, although the intensity varied with wall architecture. To confirm the identity of the heavy electron-dense deposits in the tissues as lanthanum, X-ray microanalysis was performed. When the electron beam was scanned over the deposits in the cell wall, a spectrum with characteristic lanthanum peaks was collected; there were no corresponding peaks in control plants treated with distilled water rather than lanthanum (Fig. 2). Lead was tested as a possible marker for endocytosis (Hubner *et al.* 1985). Lead treatment produced electron-dense deposits throughout the ground cytoplasm, suggesting that it diffused through the plasma membrane. Thus lead was not used as a marker for endocytosis in this study.

In addition to apoplasmic labelling, lanthanum-treated cells showed electron-dense label along the cell surface, in coated pits, in membrane invaginations, and in coated and smooth vesicles of all cell types. The criterion for considering a structure to be labelled was the presence of dark deposits with density similar to deposits in the cell wall, which were identified by X-ray microanalysis as lanthanum. These dense deposits were not found in control root cells treated with distilled water instead of lanthanum. Uranyl acetate post staining was necessary to visualize coated membranes; comparisons with unstained lanthanum-treated samples provided a control for this step.

Table 1. Lanthanum-labelled vesicle diameters

Type of vesicle	Mean diameter (nm)	Standard deviation (nm)	Sample size
Coated vesicle	101	±14	129
Smooth vesicle	106	±34	1037

Table 2. Total number of coated versus smooth lanthanum-labelled vesicles found in 30 cells sampled for each stage of development

Developmental stage	Coated vesicles (cv)	Smooth vesicles (sv)	Ratio cv:sv
Meristem	20	176	1:6
Elongating	57	438	1:8
Mature	21	262	1:12

Lanthanum treatment resulted in labelled smooth and coated vesicles in cryofixed (Fig. 3A–C) as well as in chemically fixed roots (Fig. 3D–F). Coated vesicles displayed the characteristic bristles on their cytoplasmic surface and have a mean diameter of 101 nm (Fig. 3F, Table 1). Smooth vesicles, i.e. lacking a cytoplasmic coat, were also labelled (Fig. 3A–E). The mean diameter of the smooth, lanthanum-labelled vesicles was 106 nm (Table 1). There were more smooth vesicles present than coated; the ratio of smooth:coated vesicles varied between cell types (Table 2).

Multivesicular bodies, small vacuoles (0.2–0.5 μm in diameter) containing numerous vesicles in their lumina, were labelled following lanthanum treatment (Fig. 4A–D). The lanthanum label was associated with the intraluminal vesicles; this was especially apparent in the cryofixed material. Following cryofixation, vesicles appeared to be fusing or budding from the periphery of the multivesicular body into the cytoplasm (Fig. 4D).

In cryofixed material, the partially coated reticulum was also labelled with lanthanum deposits (Fig. 4E). This branching network of tubules and vesicles was continuous with small vacuoles in some cases. This organelle was not preserved in chemically fixed material.

It should be noted that the endoplasmic reticulum was labelled by lanthanum in some cells, but that this pattern was relatively infrequent. The central vacuole in these cells contained electron-dense deposits, even in distilled water-treated control samples. Thus, it was not possible to draw conclusions about the presence or absence of endocytic marker in this organelle.

The *Lobelia erinus* root tip consists of tissues at various stages of development, providing examples of cell types with different secretory activities. Unlike protoplasts, whose secretory product may diffuse into the growth medium, the intact root cell is surrounded by its secretory product: the cell wall. The cells' dimensions before (meristem) and after elongation (mature cells), and the cell wall thickness (Table 3), provide the data for calculating the volume of cell wall produced during elongation (see Appendix). *L. erinus* roots are small and the elongation phase is limited to three to four tiers of cells, which provides an ideal system for comparing actively secreting cells (meristem, elongating) with cells with lower secretory activity (mature cells).

The extent of endocytic activity in each developmental stage was measured by counting the number of lanthanum-labelled vesicles μm^{-2} (Fig. 5). The data were stan-

Table 3. Cell lengths and cell wall widths of differentiating primary root cells of *Lobelia erinus*

A. Cell length

Developmental stage	Mean (μm)	Standard deviation (μm)	Sample size
Meristem	18	± 4	44
Elongating	30	± 14	44
Mature	80	± 14	22

B. Cell wall width

Developmental stage	Mean (μm)	Standard deviation (μm)	Sample size
Meristem	0.15	± 0.3	38
Elongating	0.21	± 0.7	50
Mature	0.40	± 0.2	50

standardized for cell size in order to compare endocytosis between developmental stages consisting of cells of different sizes. The elongating cells had the highest amount of labelled vesicles μm^{-2} , while the mature cells had the lowest amount. The data did not conform to a normal distribution, therefore non-parametric statistical tests were employed. According to the Kruskal-Wallis test, the three developmental stages had significantly different amounts of endocytosis. Mann-Whitney ranking indicates significantly greater amounts of endocytosis in the elongating stage than in the mature stage.

The endomembrane structures labelled by lanthanum in these experiments are closely related to the endomembranes involved in secretion. The secretory pathway in plants is rich in polyanions (e.g. pectins), which could have been bound to lanthanum (La^{3+}) if it diffused through the

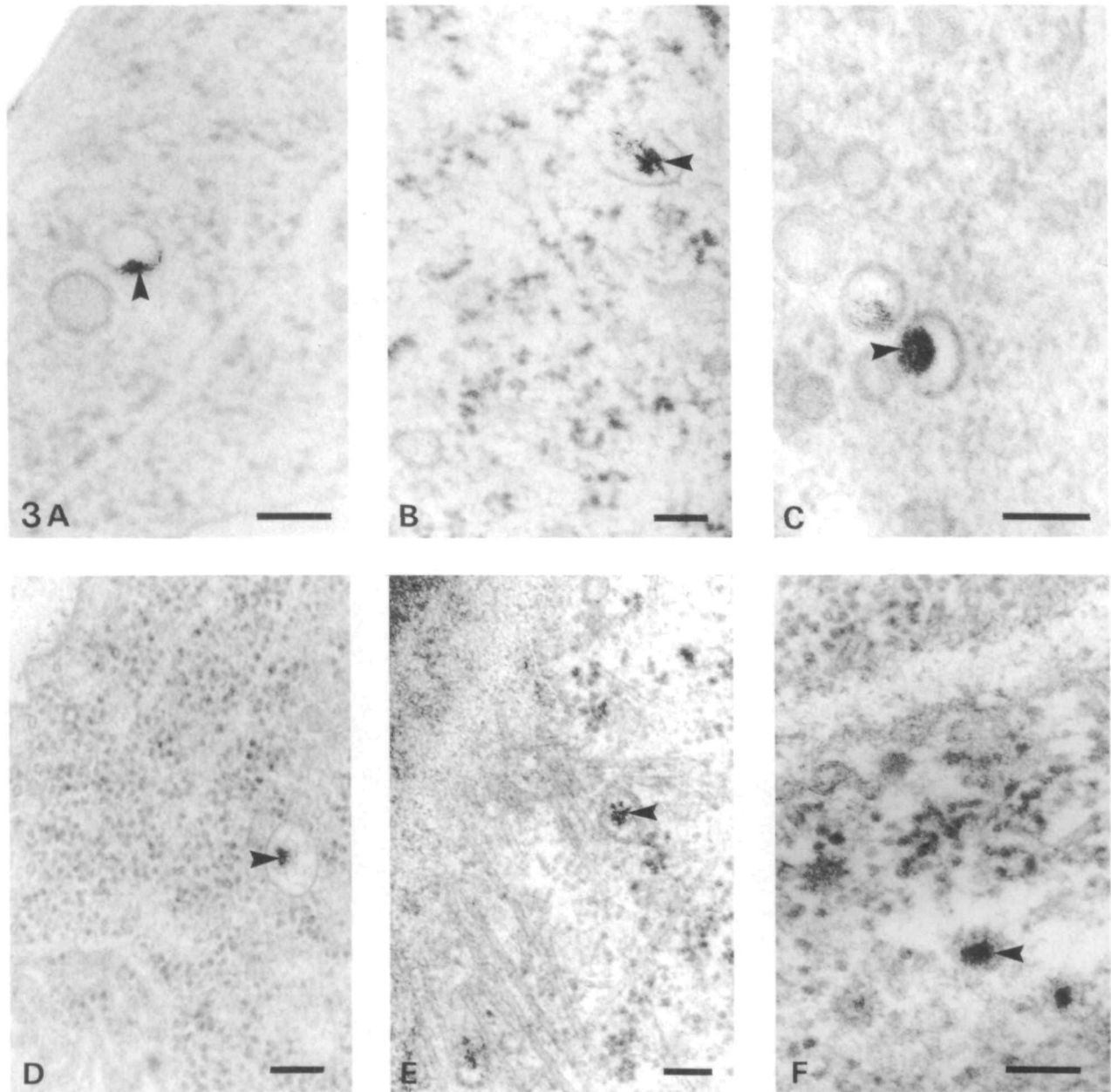


Fig. 3. Lanthanum-labelled vesicles. (A–C) Smooth vesicles, cryofixed root. (D–E) Smooth vesicles, chemical fixation. (F) Coated vesicles, chemical fixation. Electron-dense deposits seen in vesicles (arrowheads). Bar, 100 nm.

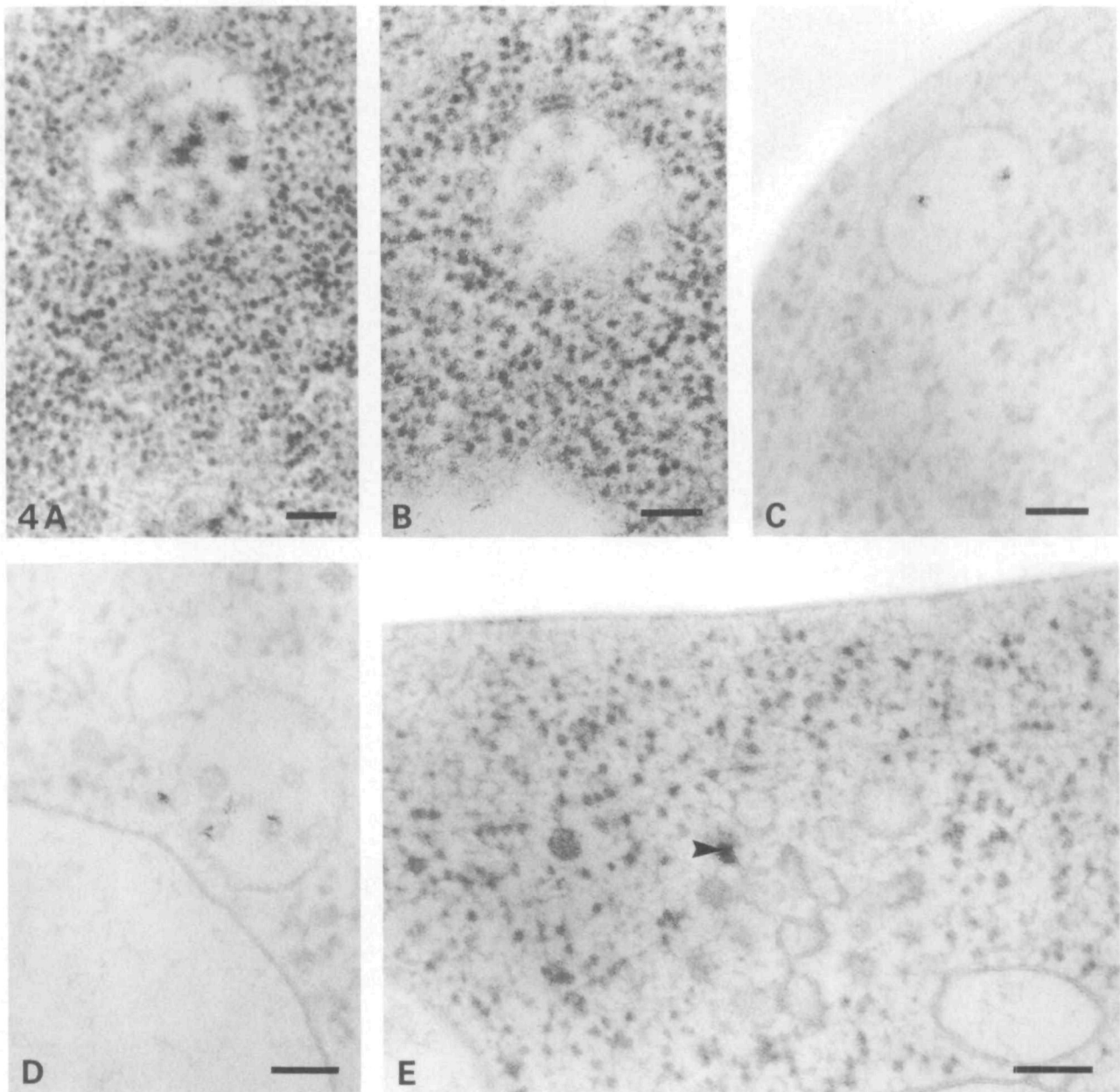


Fig. 4. (A) Multivesicular body (MVB), lanthanum-treated cell, chemical fixation. Dense deposits associate with intraluminal vesicles. (B) MVB, distilled water-treated cell, chemical fixation. No dense deposits. (C) MVB, lanthanum-treated cell, cryofixation. Note deposits near vesicles. (D) MVB with associated cytoplasmic vesicles, lanthanum-treated cell, cryofixation. (E) Partially coated reticulum, lanthanum-treated cell, cryofixation. Dense deposits found in reticulum (arrowhead). Bar, 100 nm.

membrane. As a control experiment, cryofixed roots were sectioned and stained for polysaccharides using alkaline bismuth (Fig. 6A). The distinct staining pattern obtained, i.e. staining polarity across *cis* versus *trans*-Golgi cisternae and staining of larger plasma membrane associated vesicles, can be contrasted with the staining pattern following lanthanum treatment, i.e. there was no staining of Golgi cisternae and labelling of smaller plasma membrane-associated vesicles (Fig. 6B).

To compare lanthanum-treated cells with cells with small available amounts of calcium, roots were treated with 5 mM EGTA (a calcium chelator). EGTA-treated cells had swollen endoplasmic reticulum and altered cell wall

porosity (data not shown); lanthanum-treated cells had ultrastructure similar to that of controls.

Discussion

The theoretical and practical problems associated with studying endocytosis in higher plant cells have been emphasized (Bradfute *et al.* 1964; Cram, 1980; Robinson, 1985; Steer, 1985; Romanenko and Kovtun, 1986). Theoretically, the turgor pressure that exists in these cells could make internalization of plasma membrane vesicles energetically unfavourable. Practically, the importance of endocytosis in intact plants has been enigmatic in the

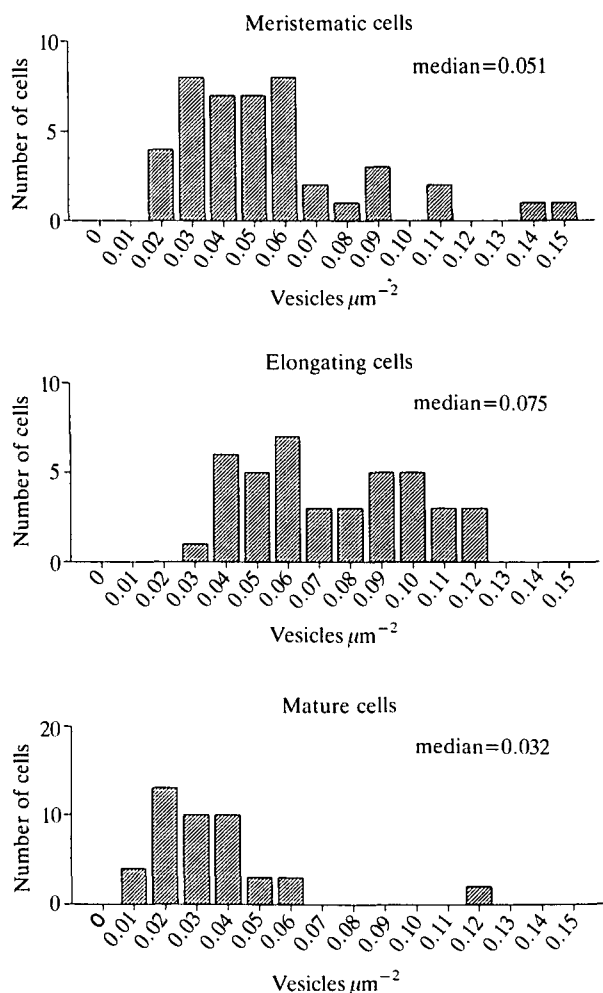


Fig. 5. Density of endocytic vesicles in different developmental stages along axis of differentiation in primary root. Meristematic cells have relatively high endocytosis; elongating cells have highest. Mature cells have lowest amount of endocytosis. Sample size for each stage was 45 cells.

absence of endocytic markers. The use of cryofixation and the apoplast tracer lanthanum have eliminated many of these problems.

Lanthanum labelling of smooth and coated vesicles indicates that a subpopulation of small (100 nm diameter) vesicles present in the cytoplasm are endocytic in nature. Small vesicles with a high surface area:volume ratio are the most effective means of transporting membrane material in relation to aqueous content. The work required to internalize a vesicle against turgor is directly dependent on the volume of the vesicle (Saxton and Breidenbach, 1988; Gradmann and Robinson, 1989).

There was a greater number of smooth vesicles than coated vesicles labelled by lanthanum following both chemical fixation and cryofixation. The presence of labelled smooth vesicles near the plasma membrane in cryofixed root cells may indicate that all endocytic vesicles need not be coated, or that removal of the protein coat occurs extremely quickly. Relatively little is known about coated vesicles from plant cells (Robinson and Depta, 1988; Coleman *et al.* 1988). It has been reported that there are two populations of coated vesicles in plant cells, smaller (60–70 nm) coated vesicles associated with the dictyosome and larger (100 nm) coated vesicles associated with the

plasma membrane (Van der Valk and Fowke, 1981; Coleman *et al.* 1988). The lanthanum-labelled vesicles found in this study were of the 100 nm size class, consistent with them having a plasma membrane origin.

The partially coated reticulum (PCR) appears to be part of the endocytic pathway in the *L. erinus* root cells. This finding adds support to the contention that the PCR is an endosome (Tanchak *et al.* 1988), rather than an extension of the *trans*-Golgi network (Hillmer *et al.* 1988). The vesicles associated with the PCR were consistently smaller than the secretory vesicles of the *trans*-Golgi face. The lack of preservation of the PCR following chemical fixation may reflect the fragmentation of this reticulum by aldehydes, in a manner analogous to the fragmentation of other cytoplasmic reticula (Mersey and McCully, 1978; Gilkey and Staehelin, 1986).

The process of endocytosis in plants is not as well characterized as that in animal cells, where the pathways of receptor-mediated and fluid-phase endocytosis have been described (Steinman *et al.* 1983; Goldstein *et al.* 1985; Gruenberg and Howell, 1989). Using a functional definition of endosome, i.e. an organelle that is labelled following exposure to endocytic marker (Helenius *et al.* 1983), the multivesicular body of the *L. erinus* root cells can be considered an endosome. Morphologically, the multivesicular bodies observed were identical to the late multivesicular endosomes in animal systems (Miller *et al.* 1986; Croze *et al.* 1989), and the multivesicular body of higher plant protoplasts (Tanchak and Fowke, 1987; Record and Griffing, 1988). It is not known if the plant multivesicular body has analogous properties to the late multivesicular endosome of animal systems, such as acting as a compartment for sorting hydrolase receptors or low pH (Kornfeld and Mellman, 1989).

The secretory cell type used in this study was an elongating root cell, in the process of differentiation to mature epidermal and cortical cells. Polysaccharide cell wall components must be added to maintain wall thickness as the cell elongates (Dauwalder and Whaley, 1982; Dixon and Northcote, 1985). About 80% of the primary cell wall consists of cell wall matrix, i.e. pectins, hemicelluloses and proteins, derived from the exocytosis of Golgi vesicles (McNeil *et al.* 1984; Varner and Lin, 1989). By measuring the cell length and cell wall width before and after cellular elongation, it is possible to calculate the surface area of plasma membrane that must be added onto the cell surface by the exocytosis of Golgi-derived vesicles. When these calculations are performed for elongating primary root cells of *L. erinus*, the surface area predicted to result from secretory activity is found to be about eight times greater than the surface area observed in the mature, fully elongated cell (see Appendix). This calculation agrees well with similar computations (Steer, 1985; Raven, 1987), and suggests that membrane recycling occurs in these cells.

The correlation between high endocytosis and high secretory activity may indicate that endocytosis is at least one of the mechanisms for controlling cell surface area during secretion. Phospholipid transfer proteins could also play a role in membrane recycling (Steer, 1985; Staehelin and Chapman, 1987), but the physiological importance of phospholipid transfer proteins is still obscure (Helmkamp, 1986). The quantitative data in this study agree with qualitative reports that there are more coated vesicles in growing plant cells than in non-growing cells (Emons and Traas, 1986; Coleman *et al.* 1988). Endocytosis and exocytosis are believed to be linked in yeast, where secretory

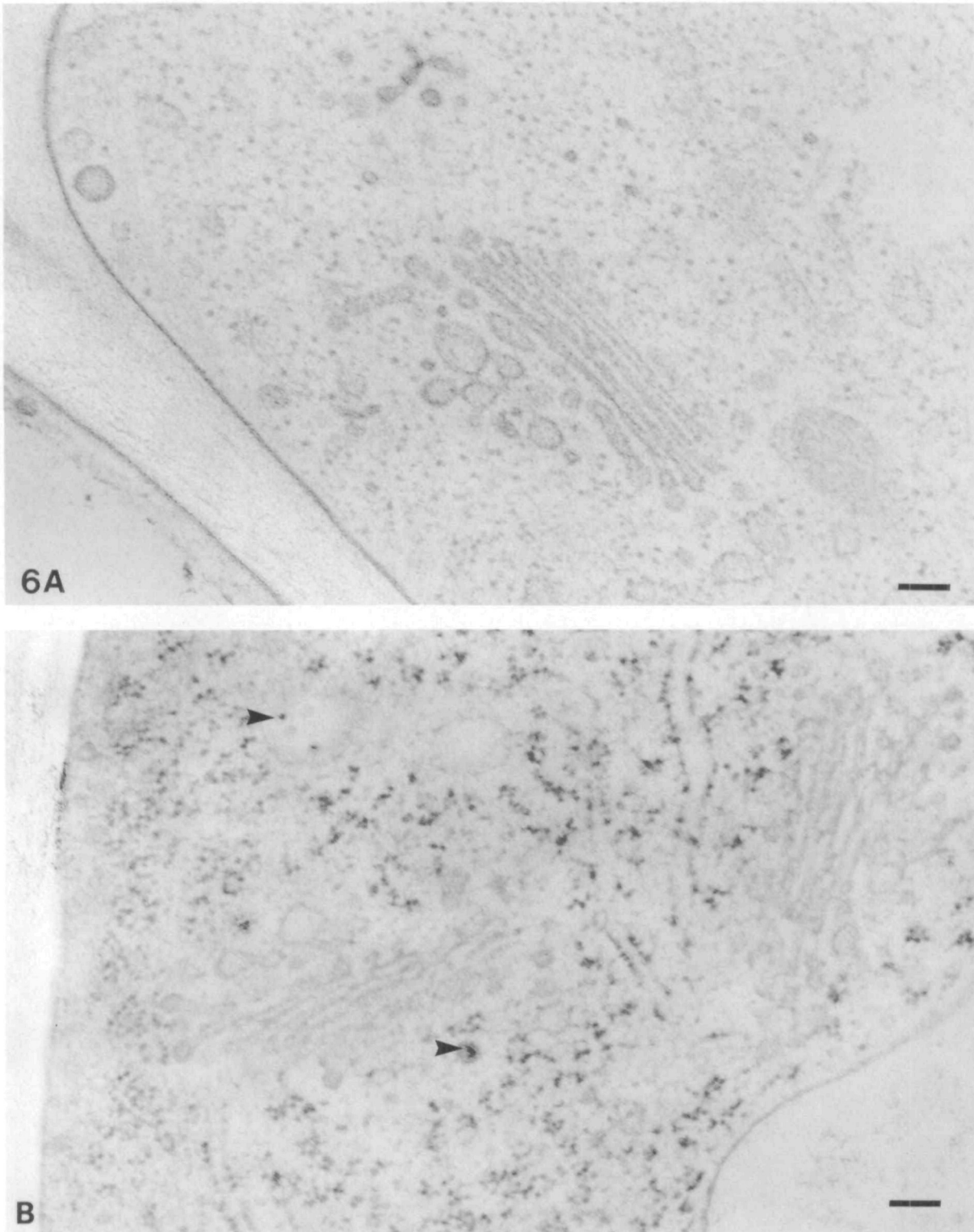


Fig. 6. (A) Control distilled water-treated root cell, cryofixation. Cell structure containing polysaccharides stained using alkaline bismuth. *Cis-trans*-Golgi cisternae polarity, secretory vesicle. (B) Lanthanum-treated cell, cryofixation. No Golgi cisternae labelling; deposits in small cytoplasmic vesicles, MVB. Bar, 0.5 μm .

mutants are deficient in endocytosis (Riezman, 1985). Vesicular membrane recycling has been demonstrated in an alga, *Boergesenia* (O'Neil and LaClaire, 1988).

For membrane recycling to occur, the internalized membrane must make its way back to the Golgi, to package the

cell wall material that is being secreted. Because vesicles associated with the Golgi were not labelled in this study, a direct fusion between endocytic vesicles and Golgi does not appear likely. The labelling pattern observed in these cells is similar to that found in studies of animal systems where

horseradish peroxidase was used as a marker for endocytosis. It appeared only in the endosomal and lysosomal compartments, but not in the Golgi (Farquhar, 1981; deChastellier *et al.* 1987; Storrie and Pool, 1984). Vesicular membrane recycling could still occur indirectly: endocytic vesicles would fuse with the multivesicular body or partially coated reticulum, then small shuttle vesicles would carry membrane from the multivesicular body to the Golgi. The formation of tubules or vesicles has been proposed as a mechanism for extruding the aqueous contents of the endosome, thus permitting concentration of membrane components (Geuze *et al.* 1984; Rome, 1985). It is possible that the lanthanum would be left in the aqueous center of the multivesicular body at this stage. Only the first step of this proposed route for membrane recycling, from plasma membrane to multivesicular body or partially coated reticulum, was demonstrated in this study; the subsequent steps are speculative at this time.

In contrast to the results for maize root cap, lanthanum, not lead, seems to be the superior marker for endocytosis in primary root cells of *L. erinus*. Lanthanum did not appear to cross the plasma membrane; this is consistent with earlier findings (Revel and Karnovsky, 1967; Taylor and Hall, 1979). In previous studies where lanthanum was reported in the cytoplasm, incubations of up to 15 h in high concentrations of lanthanum were used (Van Steveninck *et al.* 1976). In the present study, short time exposure (1 h) and relatively low concentrations of lanthanum were utilized. Lanthanum may displace calcium ions (Martin and Richardson, 1979), blocking calcium channels and producing decreased intracellular calcium levels. The net effect of low intracellular calcium would be inhibition of exocytosis, which could lead to a decrease in endocytosis (Steer, 1988). If lanthanum is acting in this way, it may lead to an underestimate of the amount of endocytosis. The abundance of microtubules in the cells and EGTA controls suggest that calcium levels in the cells were not badly disrupted.

There are many unanswered questions in the area of endocytosis in higher plants regarding possible receptors and ligands, cytoskeletal connections, properties of plant endosomes, and the consequences of turgor on this process.

Appendix

Calculation of area of plasma membrane added to cell surface area during secretion of cell wall volume; comparison with cell surface area observed. Example of *Lobelia erinus* elongating root cells suggests that excess membrane material was added to the plasma membrane.

V = volume of the cell wall

$$V = 4(\text{cell length} \times \text{cell width} \times \text{wall thickness}) + 2(\text{cell width} \times \text{cell width} \times \text{wall thickness}).$$

For meristematic cells:

$$V = 4(18 \mu\text{m} \times 18 \mu\text{m} \times 0.15 \mu\text{m}) + 2(18 \mu\text{m} \times 18 \mu\text{m} \times 0.15 \mu\text{m}) = 292 \mu\text{m}^3.$$

For mature cells:

$$V = 4(80 \mu\text{m} \times 18 \mu\text{m} \times 0.40 \mu\text{m}) + 2(18 \mu\text{m} \times 18 \mu\text{m} \times 0.40 \mu\text{m}) = 2563 \mu\text{m}^3.$$

$$V = \text{total volume of wall added during elongation: } 2563 - 292 = 2272 \mu\text{m}^3.$$

20% of cell wall volume consists of cellulose that is added by plasma membrane-bound enzymes; 80% of cell wall volume is secreted by Golgi-derived vesicles fusing with the plasma membrane. Volume of cell wall matrix (pectins, hemicellulose, proteins):

$$V = 0.8 \times 2272 = 1817 \mu\text{m}^3.$$

Each cell contributes half the wall thickness, the volume of wall matrix secreted by each cell during its differentiation:

$$V = 1817 \times 0.5 = 909 \mu\text{m}^3.$$

Assuming secretion *via* spherical Golgi-derived vesicles of 120 nm diameter (radius = 60 nm = 0.06 μm) (Staehelein and Chapman, 1987), and assuming the volume of matrix in the secretory vesicles and the cell wall matrix are of similar density (Bowles and Northcote, 1976; Steer, 1985), the volume of one secretory vesicle can be calculated (r is radius):

$$V_{\text{ves}} = 4/3 \times \pi \times r^3 = 4/3 \times \pi \times (0.060 \mu\text{m})^3 = 0.000904 \mu\text{m}^3 / \text{vesicle}.$$

From the volume of the cell wall divided by the volume of one secretory vesicle, the number of vesicles required to produce the cell wall thickness observed can be calculated: $909 \mu\text{m}^3 / 0.000904 \mu\text{m}^3 = 1\,005\,133$ vesicles per cell.

The surface area (A) of one vesicle:

$$A = 4 \times \pi \times r^2 = 4 \times \pi \times (0.060 \mu\text{m})^2 = 0.045 \mu\text{m}^2.$$

The total surface area predicted (A_p) to be added by the vesicles during elongation is the number of vesicles/cell multiplied by the area of one vesicle:

$$A_p = 1\,005\,133 \times 0.045 \mu\text{m}^2 = 45\,231 \mu\text{m}^2.$$

This can be compared with the surface area observed in the mature root cell (A_o):

$$A_o = 4 \times 80 \times 18 \mu\text{m} = 5760 \mu\text{m}^2$$

$$A_p / A_o = 45\,231 / 5760 = 7.9.$$

Therefore, the surface area of plasma membrane that is expected to be added by vesicles during elongation is about eight times the surface area of the plasma membrane observed in the mature cell.

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