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Tubular and Filamentous Structures in Pollen Tubes: Possible Involvement as Guide Elements in Protoplasmic Streaming and Vectorial Migration of Secretory Vesicles

Werner W. Franke, Werner Herth, William J. VanDerWoude, and D. J. Morré

Department of Cell Biology, Institute of Biology II, University of Freiburg i. Br., FRG, and Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana, U.S.A.

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Summary. An ultrastructural study of the pollen tubes of *Lilium* and *Clivia* has demonstrated three different classes of longitudinal structures which could influence patterns of protoplasmic streaming and/or serve as "guide elements" in the vectorial migration of secretory vesicles: (a), cortical and noncortical microtubules; (b), microfilaments; and (c), subcortical tubules and cisternae of the endoplasmic reticulum ("subsurface cisternae"). Morphological details of these structures are described. Colchicine concentrations which lead to the complete disappearance of the microtubules affect neither germination of the pollen nor cytoplasmic streaming and tip growth of the elongating pollen tubes. Tip growth is initially uninhibited by cycloheximide, and cytoplasmic streaming is insensitive to this inhibitor. However, both of these processes are sensitive to cytochalasin B and vinblastine. Our results suggest that neither microtubules nor subsurface cisternae are essential for cytoplasmic streaming and directional secretion of cell surface materials in the pollen tube but would be consistent with an involvement of microfilamentous structures in these processes. Additionally, the possible importance of the lateral cross-link elements interconnecting all three types of structures is discussed.

Introduction

A function for microtubules and microfilaments as "guide elements" for vectorial subcellular movements is indicated not only for mitotic chromosomes (for review, see Luykx, 1970) but also for translocations of endocytotic and exocytotic vesicles (Newcomb, 1969; Kamiya, 1971). Microtubules appear to be involved in vesicle translocations in the melanophores of *Fundulus* (Bikle *et al.*, 1966), neurons (Schmitt, 1968; Kreutzberg, 1969; Dahlström, 1969; Smith, 1970; Smith *et al.*, 1970), cnidoblast formation in *Hydra* (Lentz, 1965), rhabdite formation in *Planaria* (Lentz, 1967), insulin secretion in pancreatic beta cells (Malaisse-Lagae *et al.*, 1971), cell plate formation in cultured cells of the

endosperm of *Haemanthus katherinae* (Hepler and Jackson, 1968), cell wall deposition in secondary thickening during xylem formation in higher plants (Pickett-Heaps, 1968; Maitra and De, 1971; Northcote, 1971), directed migration of cytoplasmic components in the coenocytic marine alga *Caulerpa prolifera* (Sabnis and Jacobs, 1967) and in vesicle movements during mitosis (McIntosh *et al.*, 1969). Additionally, microtubules have been discussed in regard to localization of wall deposition and during secondary thickenings of xylem elements in higher plants (Pickett-Heaps, 1968; Maitra and De, 1971; Northcote, 1971). Microfilaments appear to be involved not only with the flow of vesicles, e. g. in neurons (Schmitt, 1968), but also with protoplasmic streaming, e. g. slime molds (e. g. Komnick *et al.*, 1970; Wooley, 1970), in amoeba (Weihing and Korn, 1969; Pollard and Korn, 1971), *Nitella* (Nagai and Rebhun, 1966), *Chara* (Pickett-Heaps, 1967), *Avena* coleoptiles (O'Brien and Thimann, 1967) and cultured nerve cells (Yamada *et al.*, 1971, see there for further references).

Movements thought to be mediated by microtubules are usually characterized by "saltatory motion" (for review, see Freed and Lebowitz, 1970 and Tilney, 1971). Cytoplasmic streaming, on the other hand, appears to involve microfilaments (Wessels *et al.*, 1971; Kamiya, 1971; see, however, Sabnis and Jacobs, 1967). Since transport of exocytotic vesicles has been shown to be a common mechanism for surface growth of plant cells (Dashek and Rosen, 1966; Northcote and Pickett-Heaps, 1966; Mollenhauer and Morré, 1966; Mühlethaler, 1967; Brown *et al.*, 1970; Van Der Woude *et al.*, 1971; Morré and VanDerWoude, 1972) it is important to determine the nature of the elements responsible for directing the vectorial flow of materials, particularly in those examples in which the extrusion of wall material is not uniform around the cell but is restricted to special sites, e. g., fungal hyphae, root hairs and pollen tubes (c. f. Grove *et al.*, 1970). The present study compares microtubules, microfilaments and endoplasmic reticulum as potential guide elements for oriented tip growth of pollen tubes.

Materials and Methods

Culture of Pollen Tubes

Anthers of *Lilium longiflorum* Thunb. var. Ace were collected from open flowers of greenhouse grown plants and were air dried for 8–10 h followed by 4 h at —20° C. Pollen was separated from anthers by shaking at —20° C. on a No. 50 sieve and then stored at —65° C. One to two mg of pollen grains were germinated for 2 h at 25° C on the surface of 2 ml of a 10% sucrose, 10 p.p.m. boric acid solution in each of a number of 4 cm covered block glass vessels.

Anthers of *Clivia miniata* were collected from open flowers of room grown plants and the fresh pollen was germinated as above without any drying or storage of pollen grains.

Electron Microscopy

Fixation and washes were performed using a multiple suction funnel device made by Hölzel-Technik, Dorfen, Germany, The specimen suspensions were placed on supporting millipore filters, and the specific suspension media were removed by suction. Fixations were performed at 0° C, 25° C or 38° C for 30 min in a solution containing 2% glutaraldehyde and 0.05 M sodium cacodylate buffer (pH 7.2) with or without 3 mM $CaCl_2$. The fixed specimens were thoroughly washed with ice-cold buffer, fixed for 2 h in 2% osmium tetroxide (same buffer), washed in distilled water, then placed in 1% aqueous uranyl acetate for 12 h, dehydrated in a graded ethanol series, transferred to propylene oxide, propylene oxide: Epon (1:1) and finally embedded in Epon. Thin sections were cut using a Reichert OmU2 Ultramicrotome. Sections were poststained with lead citrate and uranyl acetate and examined with the Siemens Elmiskop 1A.

Treatment with Inhibitors

Ungerminated pollen grains as well as growing pollen tubes (2 h after germination) were treated with the following inhibitors by adding the appropriate amount of stock solution (1–100 microliters) to the sucrose-boric acid culture medium. The effects on germination and pollen tube growth were followed with the light microscope in vivo and in the electron microscope after fixation.

Colchicine: from 10^{-8} to 2×10^{-4} M.

Vinblastine sulfate: from 5 to 20 µg/ml.

Cytochalasin B: stock solution 1 mg/ml of DMSO, final concentrations in the culture medium 0.03 to 30 µg/ml, DMSO 1%.

Cycloheximide: from 10^{-3} to 10^{-5} M.

Cytochalasin B was obtained from Imperial Chemical Industries Ltd., Cheshire, England. All other drugs were obtained from SERVA Feinbiochemica, Heidelberg, Germany.

Glycerol Extraction of Pollen Tubes

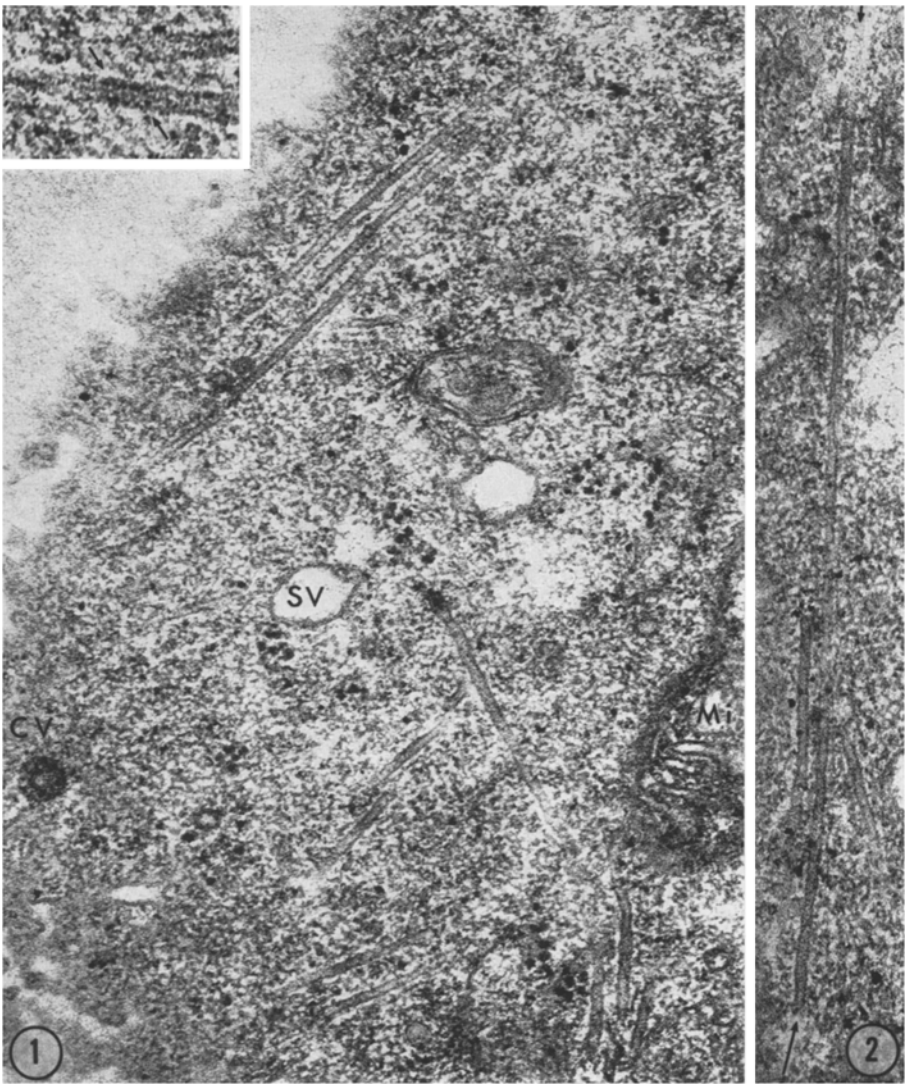
Pollen was germinated for 2 h in the sucrose medium. After low speed centrifugation, the pelleted cells were extracted for 10 min at room temperature using 40% glycerol (0.005 M EDTA, 0.12 M KCl in 0.01 M phosphate buffer, pH 7.8; according to Hoffman-Berling, 1954, and Simard-Duquesne and Coullard, 1962). The extraction was continued after changing the solution once at 4° C overnight. Fixation for electron microscopy was as described above.

Results

In the growing pollen tube three classes of longitudinal structures occur: microtubules, microfilaments, and cisternae or tubules of the endoplasmic reticulum.

A. Microtubules and Related Structures

In contrast to the existing literature which is negative with respect to the occurence of microtubules in the pollen tube (c. f. for reviews see, e. g., Larson, 1965; Gullvåg, 1966; Rosen, 1968; Crang and Miles, 1969) we found that microtubules are present in the growing pollen tube with



Figs. 1 and 2. Survey electron micrograph of the cortical zone of the growing pollen tube of *Lilium longiflorum* as seen in grazing sections which are acute (Fig. 1) and parallel (Fig. 2) to the tube axis. Note the many microtubules in this zone which do not exhibit a strong orientation but sometimes are oriented axially (Fig. 2). The inset shows the helical pitch of the microtubular substructure (arrows). The microtubule in Fig. 2 is recognized as being continuous in between the arrows. *Mi* mitochondrion; *W* cell wall; *sv* smooth vesicle. Fig. 1, ×65000; Fig. 2, ×70000; inset in Fig. 1, ×145000

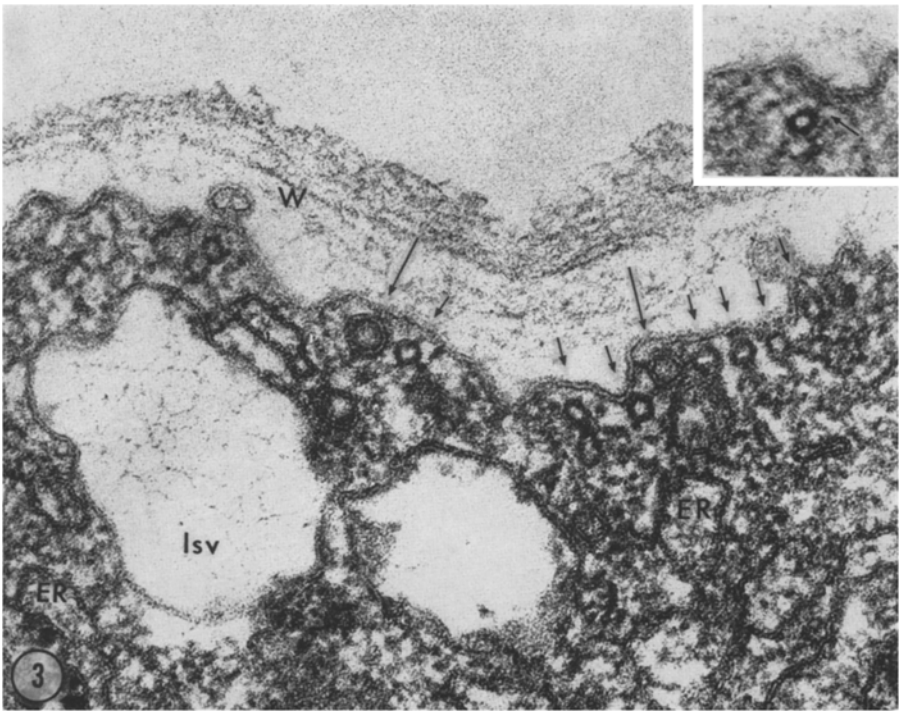
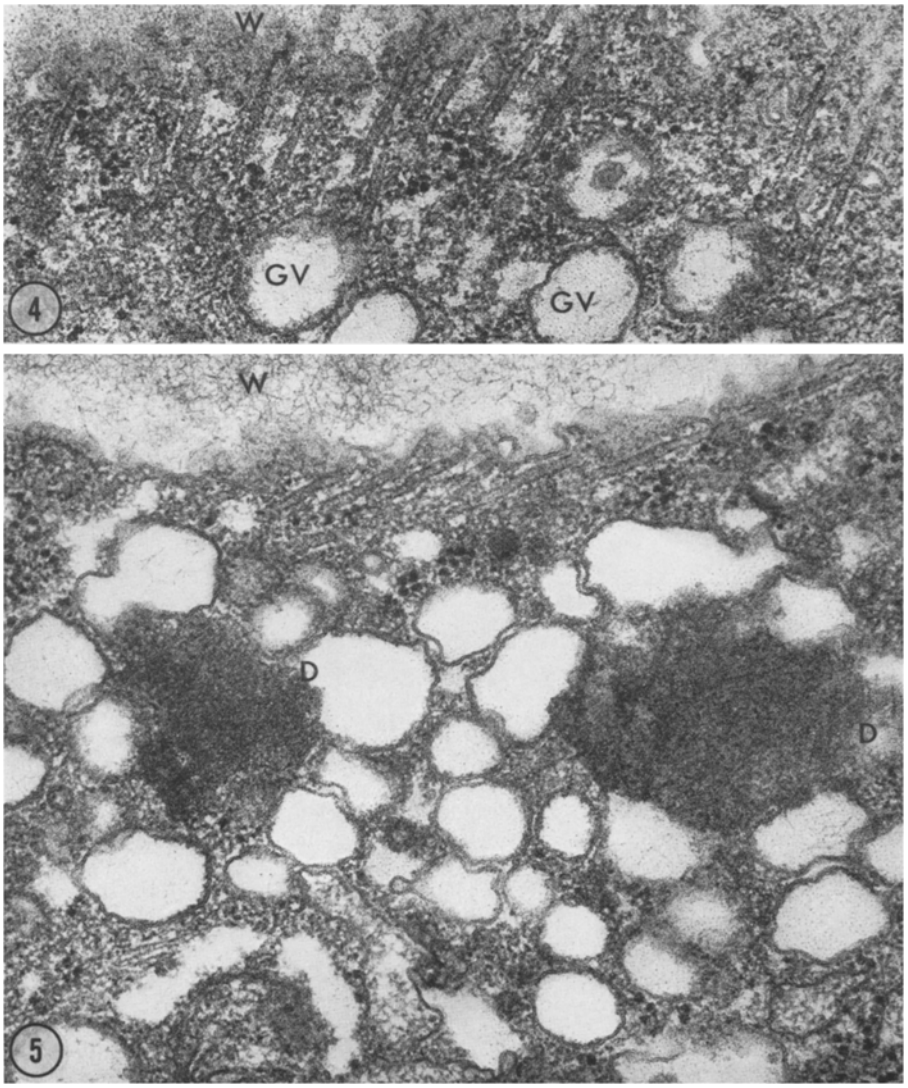


Fig. 3. Subapical part of the *Lilium longiflorum* pollen tube in cross-section. Note the rows of cortical microtubules (small arrows) which are laterally linked to each other as well as to the plasma membrane (e. g., at the first small arrow from the left; see also inset). Note that the membraneous vesicles can occur in register with such cortical microtubules (long arrow) and are also linked with cross-bridges to the microtubules as well as to the plasma membrane (e. g., the one indicated by the right long arrow). *ER* endoplasmic reticulum cisterna; *W* cell wall; *lsv* large smooth vesicle. Magnifications. ×138000

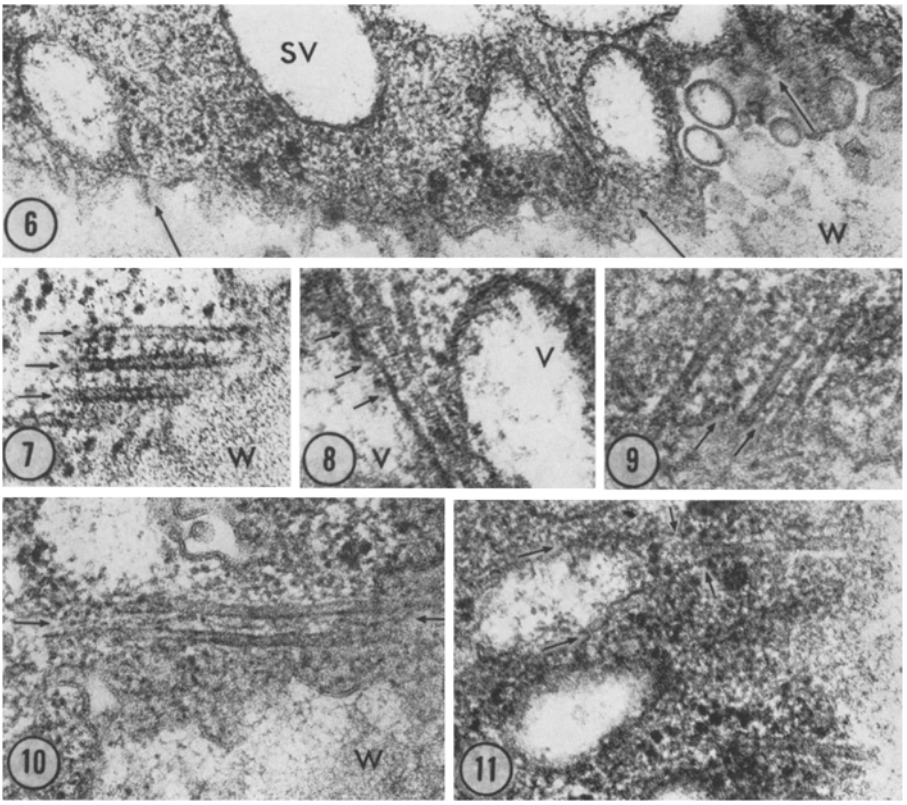
the exception of the very apical zone, i. e. up to approximately 2 microns from the tip. Topologically, these microtubules can be divided into:

a) Cortical Microtubules (Figs. 1—3). Many of these microtubules (outer diameter 160–240 Å) underlie the plasma membrane and are oriented parallel to the longitudinal axis of the pollen tube. Others are clearly not axially oriented (Fig. 1). Such cortical microtubules are regularly spaced in groups consisting of up to five or six elements with a minimal lateral separation of about 150 Å. The elements may be cross-linked to one another (e. g. Fig. 7). The microtubule-microtubule cross-bridges can be up to 280 Å long and are spaced laterally along the microtubules with a periodicity of about 225 Å. A helical subarchitecture of the micro-



Figs. 4 and 5. Sections somewhat grazing to the pollen tube cortex in a subapical zone. Note how closely the cortical microtubules can be associated with Golgi-derived vesicles (Fig. 4, *GV*) or with the dictyosomal periphery (Fig. 5). Both figures. $\times 66000$

tubules was discernable in some sections (Fig. 1, inset). In cross sections through the pollen tube the microtubules are seen to be structurally linked to the inner aspect of the plasma membrane (e. g., Fig. 3) by



Figs. 6–11. Structural details of the cortical microtubules of *Lilium* and *Clivia* pollen tubes. Cross-linking between adjacent parallel microtubules (arrows) is demonstrated in Fig. 7. Figs. 9 and 10 show the microtubular associations with strand-like elements of microfilamentous dimensions. Here the situation of a microfilament-like strand lying equidistantly in between the microtubules is especially frequent. The association of the cortical microtubular profiles with smooth Golgi apparatus vesicles (*sv*) is recognized in the *Clivia* pollen tubes shown in Fig. 6. A more detailed view in Fig. 8 suggests lateral connections of microtubules with the vesicle membranes through thin cross-bridges (at sites indicated by arrows). Fig. 11 shows another unexplained vesicle-microtubular profile relationship in which a contrast continuity of the vesicular membrane with the tubular wall is suggested. Fig. 6, ×55000; Fig. 7, ×76000; Fig. 8, ×100000; Fig. 9, ×92000; Figs. 10 and 11, ×75000

cross-bridges similar to those which have been described in other plant and animal cells (e. g., Dickson *et al.*, 1966; Cronshaw, 1967; Kiermayer, 1968; Robards, 1968; Olson and Kochert, 1970). A special type of small and smooth tubulo-vesicular profiles with a plasma membrane-like

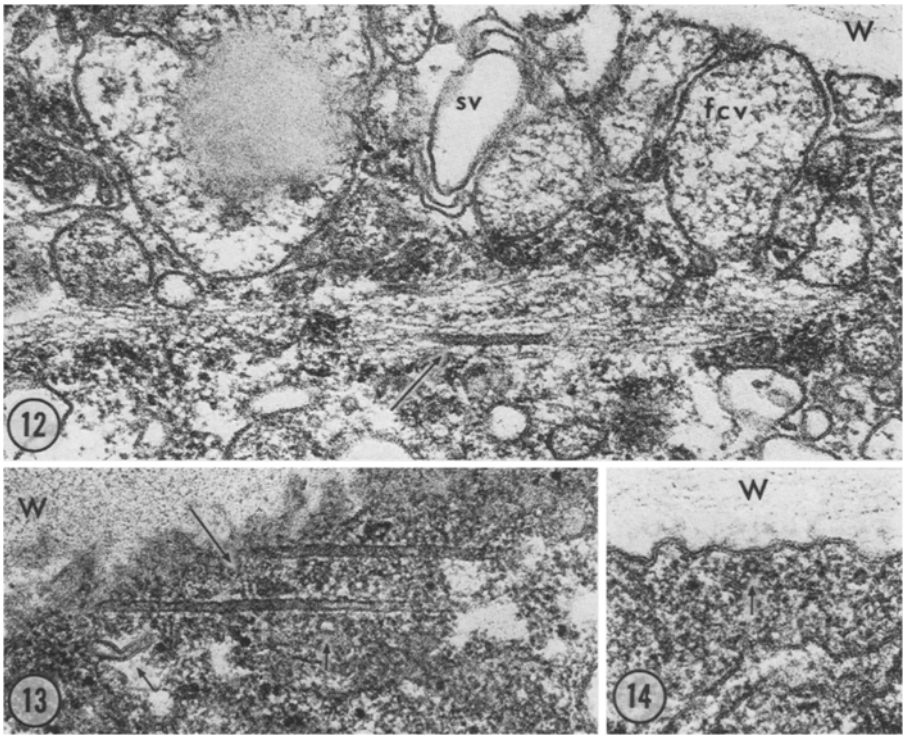
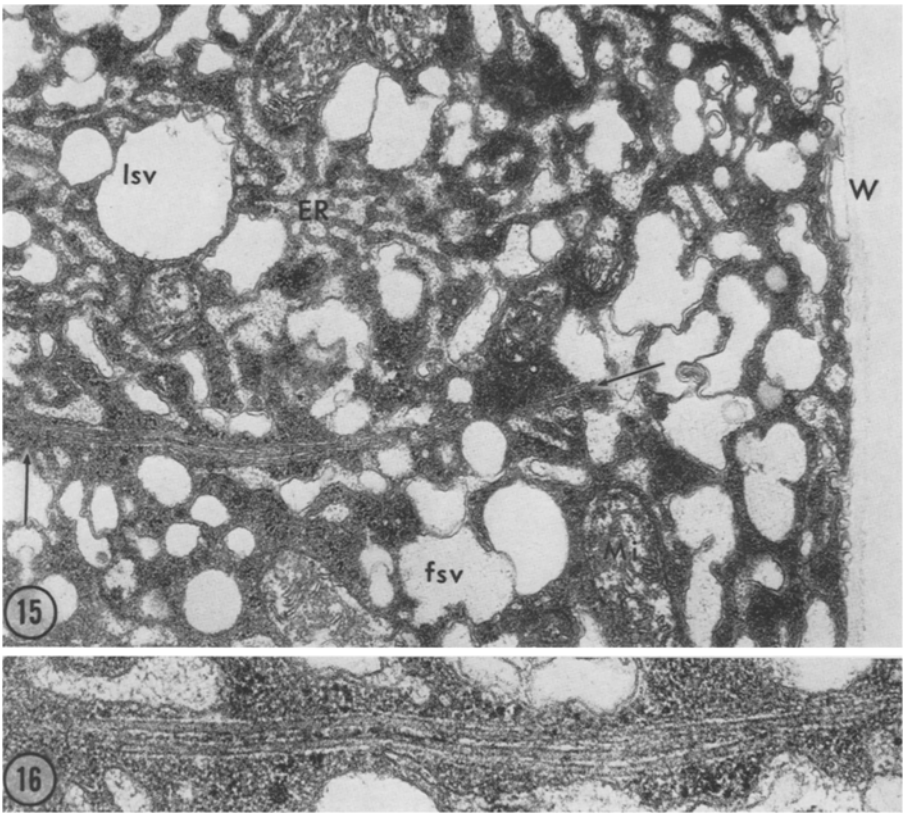


Fig. 12. Pollen tube of *Lilium* in which a microtubular piece (arrow) is embedded in (and may be continuous with) tangles of microfilaments. *sv*, smooth vesicle; *W* wall. Magnification, ×65000

Fig. 13. Cortical microtubules in the pollen tube are often laterally associated with the bridge-like dense filaments (long arrow) and a distinct class of vesicles which is smooth and occasionally can exhibit a more tubular appearance (e. g., indicated with the small arrows). Magnification, ×65000

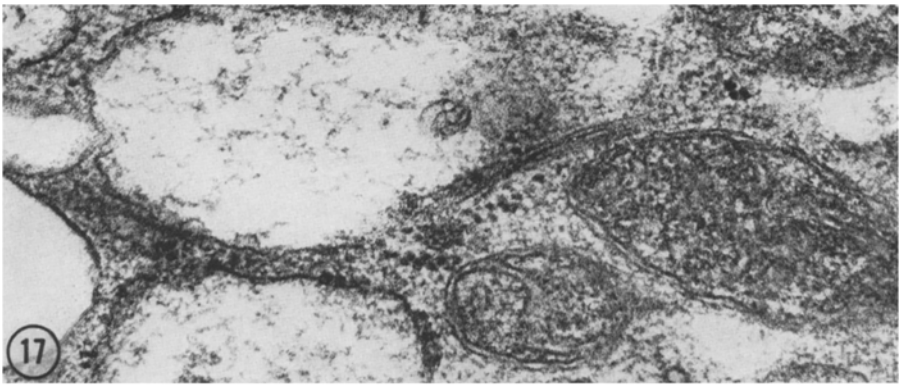
Fig. 14. "Minitubular" structures (arrow) which are clearly different from microtubules are sometimes recognized in the tube cortex. These "tubules" are insensitive to colchicine. Magnification, ×100000

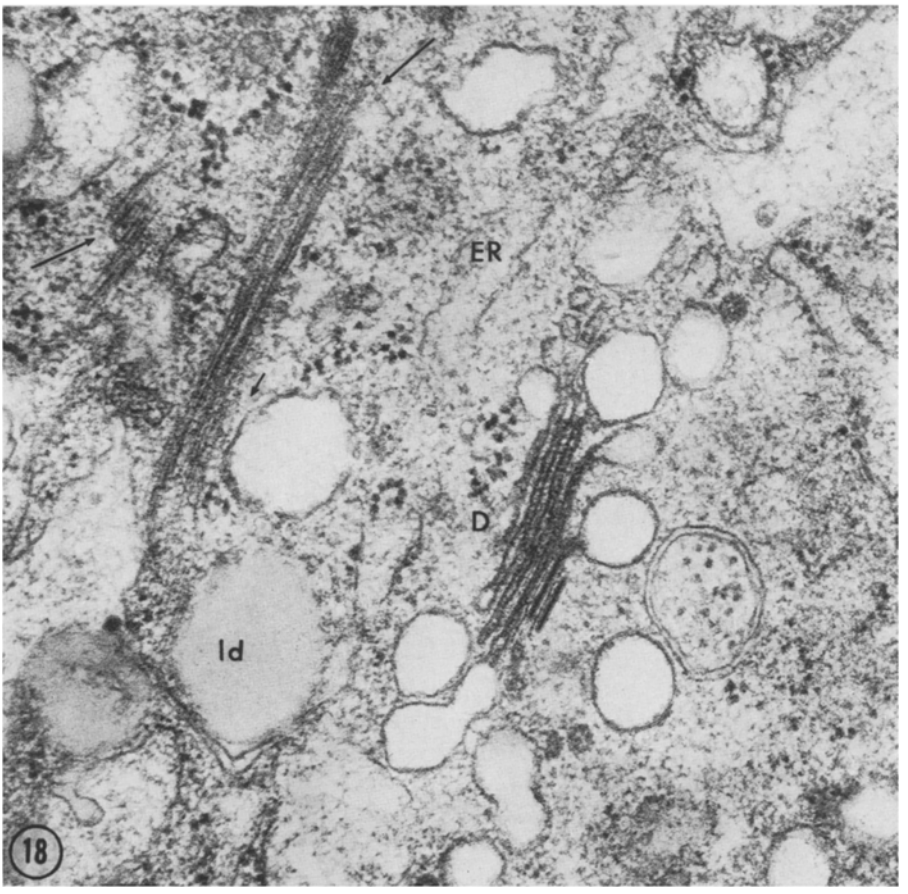
ultrastructural appearance (ca. 100 Å membrane showing a clear dark-light-dark pattern, c. f. Grove *et al.*, 1968) is frequently associated with the cortical microtubular bundles in positions commonly occupied by microtubules and can be in register with these (Figs. 3 and 13). Most interestingly, cross-bridge connections are also found to link the membranes of these cortical tubules and vesicles with those of the plasma membrane and with the adjacent microtubules (Fig. 3). This points to the relationship of microtubular and membraneous structures (for discussion



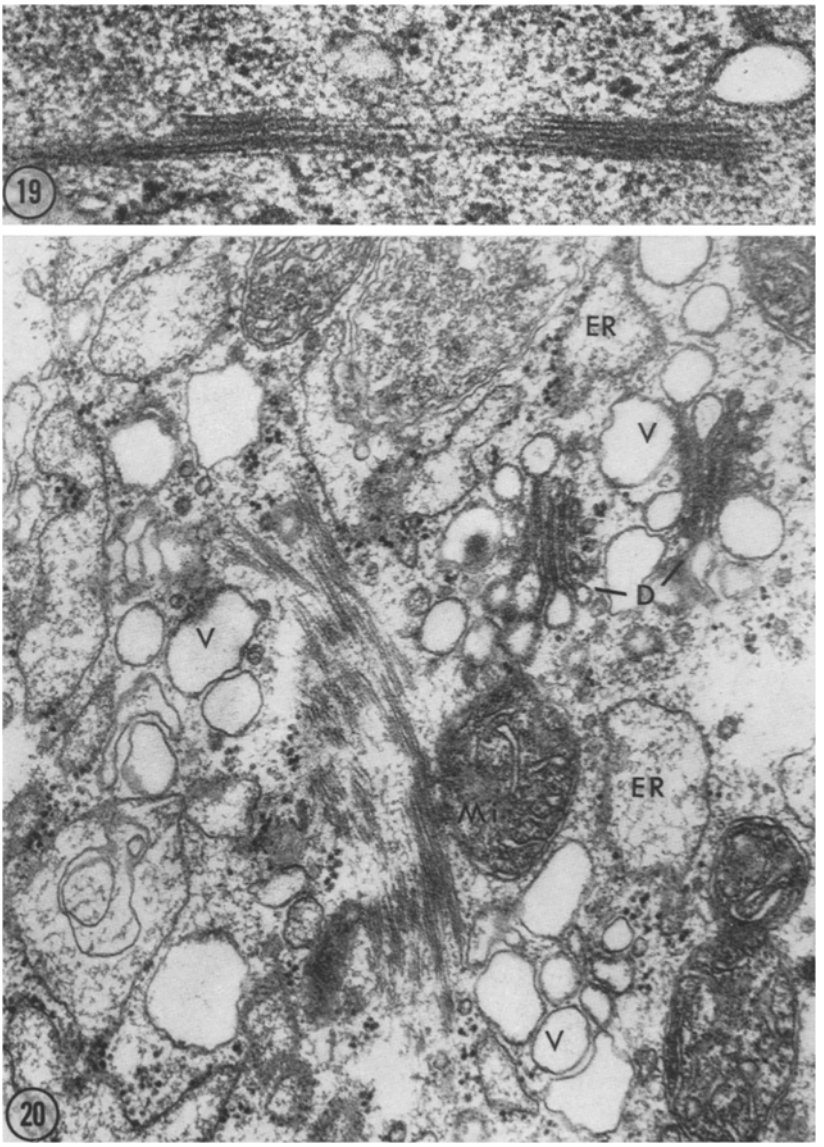
Figs. 15 and 16. Bundles of non-cortical microtubules also occur in the subapical zone of the pollen tubes of the two species studied as shown in Fig. 15 (e. g., between the arrows) and at higher magnification in Fig. 16. *W* wall; *lsv* large smooth vesicles; *fcv* fibrillar material containing vesicles. Fig. 15, ×25000; Fig. 16, ×62000

see Mazia and Ruby, 1968; Franke, 1971a, b; Stadler and Franke, 1972) and provides another example of membrane-to-membrane cross-bridges (Franke *et al.*, 1971c). Individual microtubules could be traced for as long as 0.8 micron (Fig. 2). In association with the microtubules, and frequently in the space between them, ca. 60–70 Å broad microfilament-like strands were observed (e. g., Figs. 1, 9, 10). Lateral electron-opaque cross-bridges were also present in these associations (Fig. 9). The cortical microtubules which usually abut the plasma membrane at a very low angle, came very close to Golgi-apparatus derived vesicles (Fig. 4) as well as to the dictyosomal periphery itself (Fig. 5). Occasionally, the wall of a cortical microtubule appears to be continuous with other cyto-

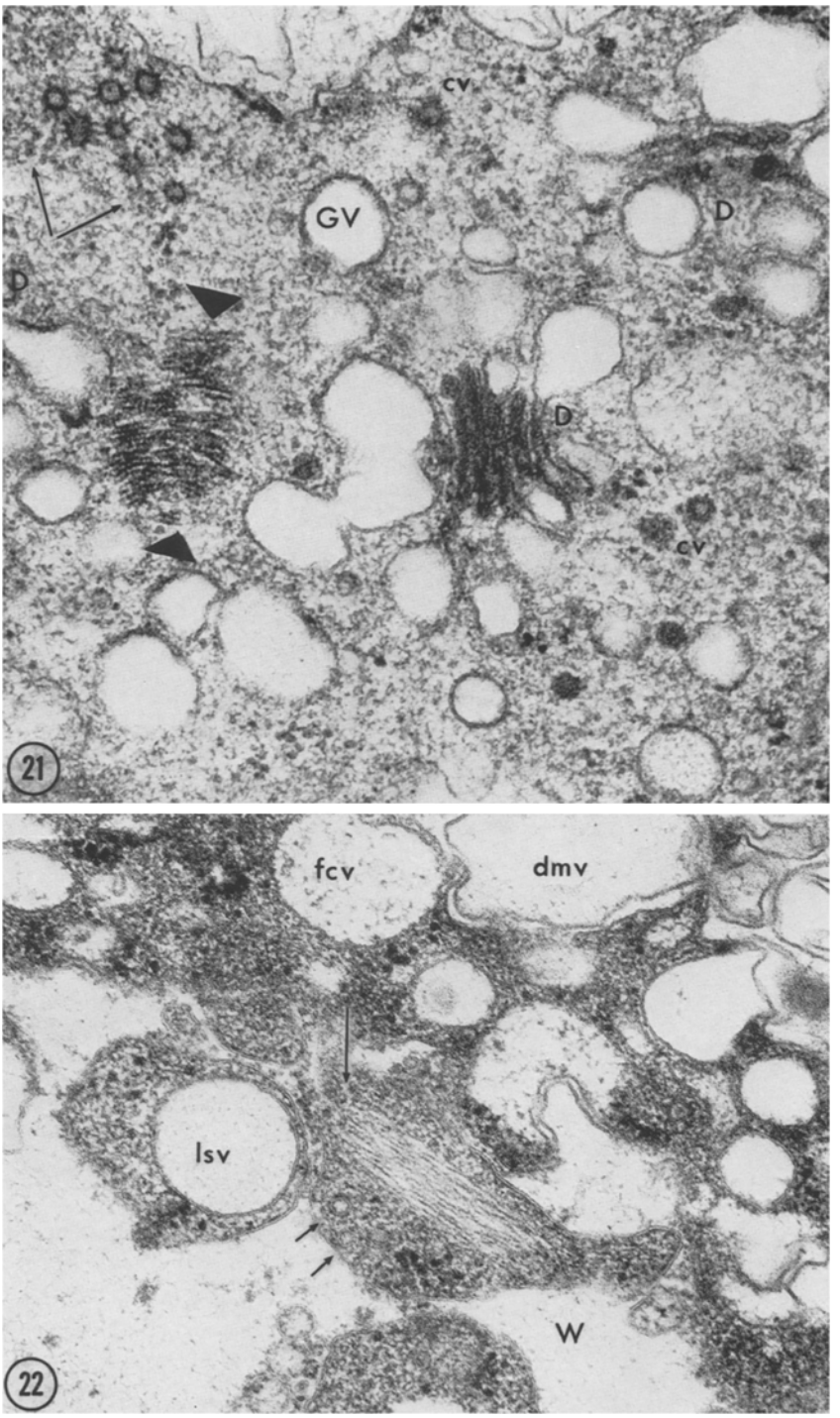




Figs. 17 and 18. In the *Lilium* pollen grains (Fig. 18) and tubes (Fig. 17) bundles of thread-like elements are frequent which are predominantly made up of microfilaments but sometimes reveal additionally the presence of microtubule-like structures. Such bundles are very often found in the vicinity of dictyosomes (*D*) as demonstrated in Fig. 18, and in close association with the membranes of the vesicles (Fig. 17). *ld* lipid droplet. Fig. 17, ×72000; Fig. 18, ×60000



Figs. 19 and 20. Bundles of microfilaments similar to those shown in the previous figure are abundant in the cytoplasm of the *Clivia* pollen tube, again in the same preferential association with vesicles and dictyosomes. Fig. 20 gives a survey, and details of the bundle organization are recognized in Fig. 19, including a relatively regular lateral distance of the filaments within the bundle. Fig. 19, $\times 80000$; Fig. 20, $\times 40000$



Figs. 21 and 22

plasmic membranes (Fig. 11), probably parts of the endoplasmic reticulum (ER, for possible explanations of continuity of tubule-membrane profiles see the discussion in Franke, 1971c). Not infrequently, the cortical microtubules revealed lateral cross-bridges also with the large Golgi-apparatus derived vesicles (Figs. 6 and 8), especially in more apical zones.

b) Non-Cortical Microtubules. Bundles of microtubules as well as single tubules were also common deep in the cytoplasm. Figs. 15 and 16 show such a bundle of microtubules in a region subjacent to the vesicle aggregates at the tip. Associations of microtubules with membranes and filaments such as those described for the cortical microtubules were also found in the pollen tube interior.

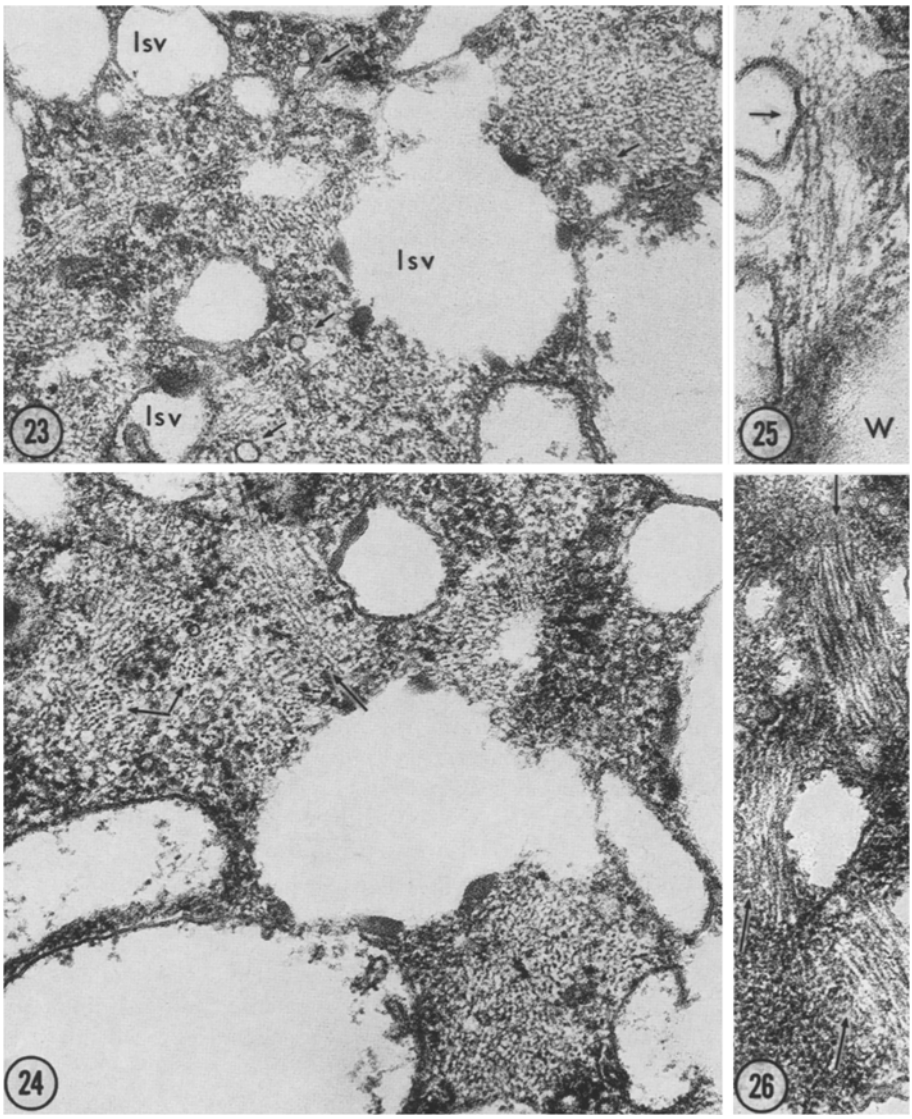
Infrequently, some "minitubular" structures were encountered (outer diameter ca. 150 Å, Fig. 14). These can occur in the cortex (Fig. 14) as well as with Golgi membrane structures (Fig. 5), but are clearly different from the microtubules. It is conceivable that these minitubular structures are related to the ca. 100 Å tubular "filaments" which have been described by Goldman and Follett (1969) to occur interassociated with microtubules and microfilaments in cell processes of baby hamster kidney fibroblasts.

B. Microfilaments

Fibrillar structures resembling typical microfilaments and having diameters as small as 50 Å are abundant in the pollen tube and grain. (Their occurence in the pollen grain was mentioned in the work of Hoefert, 1969a, b, on the pollen of *Beta vulgaris*.) They are especially conspicuous where they occur in bundles (Figs. 17–20). Such bundles can be closely associated with Golgi-apparatus-derived vesicles and ER-elements (Figs. 17—19) as well as with mitochondria (Fig. 20). Microtubular structures can be integrated in such filament bundles as well (Fig. 12, 17—19). Sometimes the filaments are parallel and exhibit lateral thin fishbone-pattern-like thread interconnections (Fig. 20). As with the microtubule-vesicle relationship, the association of the filaments with the vesicle membranes appears to be also mediated by cross-links (Fig. 18). A different type of filamentous aggregates is occasionally

Fig. 21. Filamentous aggregate (triangle) in the dictyosomal vicinity in the *Clivia* pollen tube with a relatively ordered spacing of filamentous components suggesting lateral cross-bridging. The pair of arrows in the upper left points to a group of "spiny vesicles". *D* dictyosome; *GV* Golgi apparatus derived vesicles; *cv* coated vesicles. Magnification, ×65000

Fig. 22. Bundle of microfilaments in the tip zone of the lily pollen tube (long arrow). Note the occurence of very small smooth vesicles in this zone (small arrows). *fcv* fibrillar material containing vesicle; *lsv* large smooth vesicle; *dmv* double membrane surrounded vesicle; *w* cell wall material. Magnification, ×70000



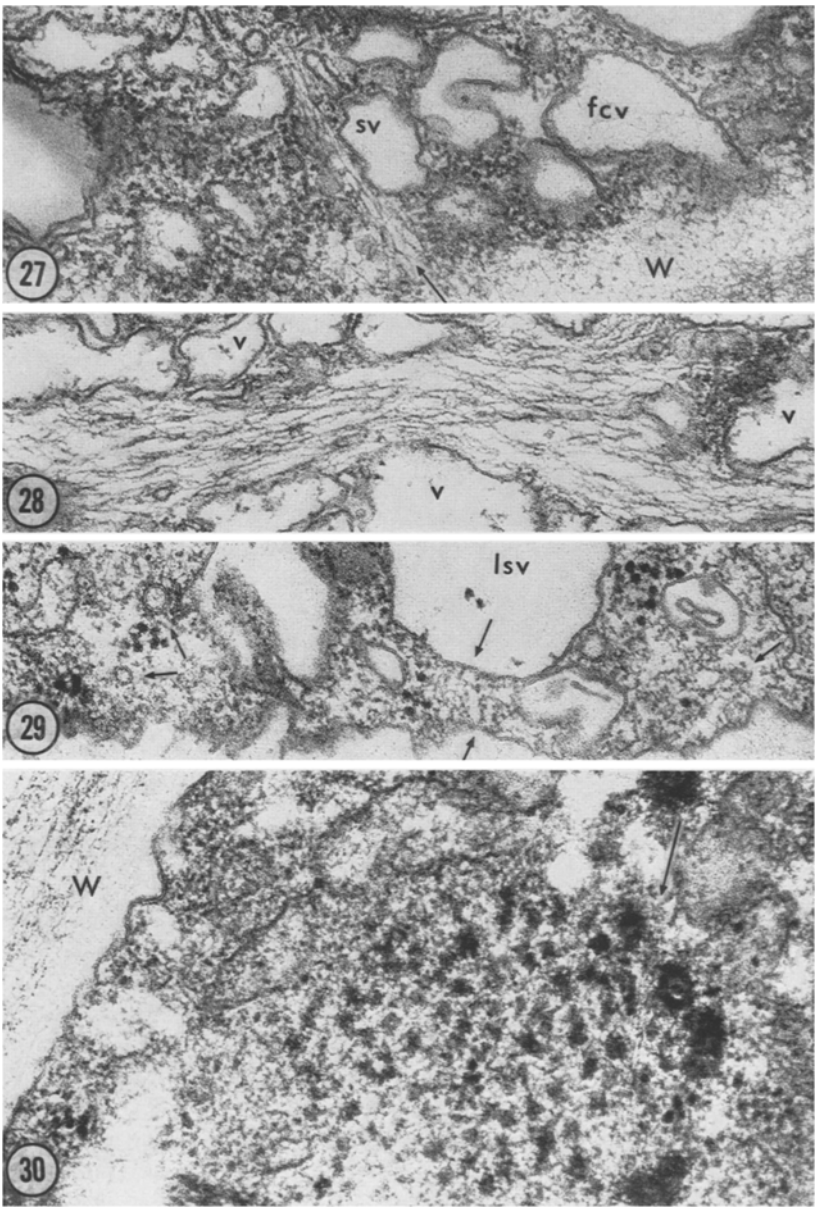
Figs. 23—26. Appearance of more or less ordered arrangements of microfilaments as seen after various times of glycerol extraction. The filamentous bundles are now more easily recognized in cross (e. g., at the pair of arrows in Fig. 24) as well as in longitudinal section (long arrow). Note also the frequent associations with membrane surfaces (Figs. 25 and 26) and the occurrence of the distinct "small and smooth" vesicles in the microfilament containing areas. For further details see text. Figs. 23 and 24, ×65000; Fig. 25, ×86000; Fig. 26, ×65000

observed in the *Clivia* pollen tubes and is presented in Fig. 21. It is also found in association with dictyosome vesicles and is characterized by a higher degree of regularity in lateral spacings and interfilamentous cross-bridges. While microtubules never have been observed in the tip regions, filamentous aggregates can occur in this area where the rate of vesicle extrusion seems to be especially high as suggested in Fig. 22 for *Lilium*. The filaments described are not exclusively oriented in parallel to the tube axis although sometimes such a direction appears to be predominant. Microfilaments are also present in the peripheral regions of the pollen tube and thus may function in cytoplasmic streaming. Microfilaments of the pollen tube cytoplasm are most apparent after glycerol treatment (Figs. 23—28). In such preparations they occur also in bundles (e. g., Figs. 24 and 26). Their proximal association with vesicular membranes is still recognizable after glycerol treatment of the pollen tubes as is their extension to cortical zones (e. g., Figs. 27 and 28). It is not clear whether the 500–1 000 Å long strand-like connection spanning from vesicle membranes to the adjacent plasma membrane (e. g., Fig. 29) are related to such microfilaments. Such connecting strands are numerous in the pollen tube cortex.

Structures hitherto unidentified in the pollen grain and tube cytoplasm are the aggregates consisting of thin fibrils and dense clumps (Fig. 30). These aggregates exhibit a close resemblance to the fibrillar arrays described in mammalian oviducts which have been regarded as "aggregate pools" of microtubular precursor protein (Anderson and Brenner, 1971; Dirksen, 1971).

C. Endoplasmic Reticulum Elements

A structural relationship regularly observed with both *Clivia* and *Lilium* pollen tubes is the association of cortical ER-cisternae with the plasma membrane similar to that described for a variety of animal cells (Kumegawa *et al.*, 1968; Pitelka, 1969; Hufnagel, 1969; Hanaoka and Friedman, 1970; Allen, 1971; Campbell and Campbell, 1971) and also from plant tissue culture (Franke and Herth, unpublished observations) and onion stem (Morré and VanDerWoude, 1972). The orientation of such "sub-surface cisternae" or tubules is always longitudinal (e. g., Fig. 31). The membrane faces of plasma membrane and ER may be as close as 90 Å and appear linked to each other by cross-bridge like elements (e. g., Figs. 34 and 35). This mode of connection closely resembles the plasma membrane linkage of the cortical small tubules and/or vesicles as already described above (see e. g., also Figs. 3 and 35). A tight mechanical linkage of the two membranes is also suggested from the observation that the ER-cisternae follows even the slightest curvatures of



Figs. 27—29. Further details of the microfilament-vesicle membrane relationship in the lily pollen tube subapical zone as revealed with (Fig. 27, 28) and without glycerol treatment. At many instances microfilamentous bundles can be recognized

the plasma membrane (Figs. 33 and 35). The ER regions linked to the plasma membrane display a polarization in that the cisternal surface adjacent to the plasma membrane is smooth while the inner cisternal surface may display attached ribosomes (e. g., Fig. 35).

D. Effects of Drug Treatments

a) Cycloheximide in concentrations higher than 10^{-5} moles/l prevents germination. Growth rates of tubes of germinated grains were nearly normal after the addition of 10^{-4} M cycloheximide but subsequent retardation of growth did occur (80% inhibition of growth after $3^1/_2$ h). No change in the structure of microtubules, microfilaments, and cortical endoplasmic reticulum was observed.

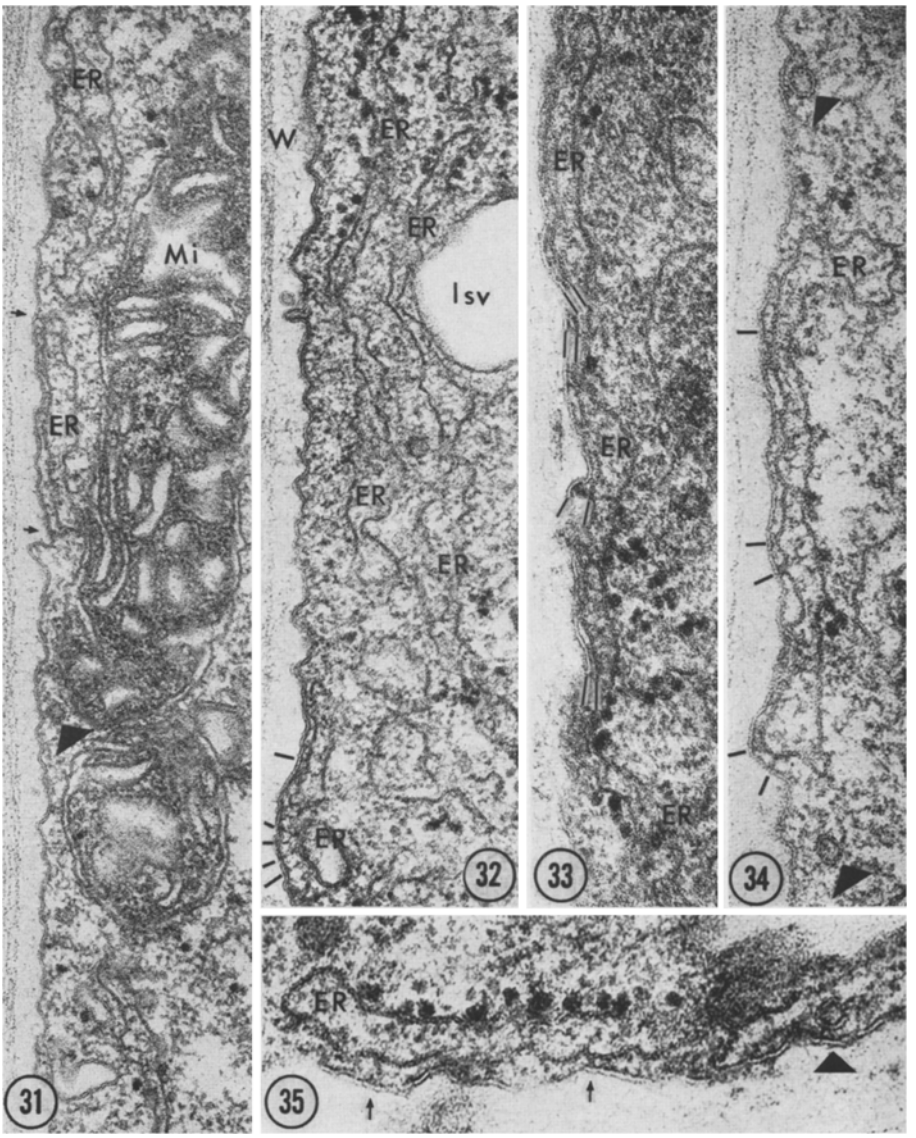
b) Colchicine in concentrations from 10^{-8} moles/l to 2×10^{-4} moles/l does not affect pollen germination and pollen tube growth up to a total length of ca. 5 mm. These are concentrations in which no microtubules have been observed.

c) Vinblastine sulfate in concentrations as low as 5 μg/ml inhibited both germination and pollen tube growth. Vinblastine treated grains and tubes displayed a coarse, unspecific aggregation of cytoplasmic structures, particularly of rough ER. Regular arrays of filaments as they have been demonstrated in vinblastine treated animal cells (e. g., Malawista *et al.*, 1969; Marantz and Shelanski, 1970; Krishan and Hsu, 1969; Krishan, 1971) were not observed.

d) Cytochalasin B in concentrations down to 0.1 μg/ml completely prevented pollen germination. A concentration of 0.03 μg/ml allowed germination of 5% of the pollen grains within two hours, compared with 60% germination in the control assay as well as in the DMSO-control in which a 1% final concentration of DMSO did not prevent normal growth and cytoplasmic streaming. In growing pollen tubes, cytochalasin B in concentrations down to 0.1 μg/ml stopped cytoplasmic streaming completely within 5 min. The cytoplasmic streaming was reestablished within less than 30 min by diluting the culture medium 1:30 with fresh culture medium. The crude effects of high concentrations of cytochalasin (9–30 μg/ml) such as concentration of the cytoplasm and its retraction from the cell wall apparently were not reversible. A concentration of 0.07 μg/ml slows down cytoplasmic streaming of the pollen tube and

to abut the cell periphery and the plasma membrane (Fig. 27) and sometimes thin strands are observed to span the cytoplasmic interspace between vesicles and the plasma membrane (e. g., in Fig. 29 at the arrows). All Figs., ×65000

Fig. 30. Thin filamentous strands are also recognized within the aggregates of electron opaque clumps which are frequent in the pollen tubes of both species. Magnification, ×65000



Figs. 31—35. Close associations of specialized cisternae of endoplasmic reticulum (*ER*) in the pollen tube of *Lilium longiflorum*. "Subsurface cisternae" are parallel with large parts of the inner face of the plasma membrane (Figs. 31, 33, 34). As has been demonstrated for the association of the plasma membrane with microtubules and cortical vesicles and tubules (see, e. g., the triangles of Figs. 31, 34, 35) these subsurface ER-cisternae are also linked to the plasma membrane by electron dense membrane-to-membrane cross-bridges (e. g., the arrowheads and bars of Figs. 31,

stopped growth within 15 min. Pollen tubes treated in this way showed an enlarged "clear cap", no tip growth, but still some very slow cytoplasmic streaming in which saltatory movement seemed to predominate. After several hours, the tubes burst. Electron microscopic examination of cytochalasin B treated pollen tubes displayed no marked changes in cytoplasmic ultrastructure: Microtubules were recognized, though they appeared somewhat less frequent. Changes in microfilamentous structures could not be observed with significance.

Discussion

The present study documents that microtubules, microfilaments, and membranes of the ER-system are found in the pollen tube. All three candidates for an involvement in tipward translocation of the secretory vesicles are frequently oriented parallel to the long axis of the tube. Moreover, all three are interassociated by a system of cross-linking elements.

From the colchicine-insensitivity of pollen tube growth, secretory flow and cytoplasmic streaming we conclude that in germinating pollen of *Lilium* and *Clivia* microtubules are not necessary for the vectorial translocation of secretory vesicles. Such an insensitivity of pollen germination and tube tip growth to colchicine and other spindle poisons has also been shown by the classical karyological experiments of Swanson, 1940, and Conger, 1953 (quoted in Darlington and LaCour, 1963). Additionally, protoplasmic streaming in *Chara* has also been demonstrated to be insensitive to colchicine (Pickett-Heaps, 1967). In these studies, sections of 8 days colchicine-treated tissue revealed the presence of cytoplasmic filaments, but very few microtubules.

The experiments of Nagai and Rebhun (1966) in *Nitella* first suggested that microfilaments are essential for promoting cytoplasmic streaming. Cytochalasin-sensitivity of cytoplasmic streaming in *Nitella* and *Avena* led Wessels *et al.* (1971) to suggest also that microfilaments are the causal agents of streaming in plant cells. Our results with cytochalasin when taken together with the current cytological concepts of cytochalasin action (e. g., Krishan, 1971; Wessels *et al.*, 1971) point to microfilaments as potential guide elements but do not prove them responsible for directional migration of secretory vesicles in pollen tubes. This is in

32, 34, 35). The upright bars of Fig. 33 demonstrate the close and precise parallelity of this membrane-membrane-arrangement of ER and the plasma membrane. Fig. 35 gives an example of the polarity of the subsurface ER cisternae with the inner (cytoplasmic) face being set by ribosomes and the outer face being smooth and bearing only the bridging elements (arrowheads in Fig. 31). Magnifications: Fig. 31, ×63000; Fig. 32, ×72000; Fig. 33, ×96000; Fig. 34, ×90000; Fig. 35, ×126000

agreement with information on the directional flow of vesicles toward the growth zone of neuronal cells (Yamada *et al.*, 1971) and with the result of cytochalasin treatment of various other forms of tip growth including Brassicacean root hairs and rhizoids of the coenocytic green alga *Caulerpa prolifera* (Herth *et al.*, 1972). Yet, there are examples where cytoplasmic streaming is sensitive to both cytochalasin and colchicine, e. g., *Caulerpa* rhizoids (Herth, unpublished observation). The inhibition of tube growth as well as of germination in the presence of vinblastine (which also results in a total disappearance of microtubules) is difficult to interpret since many other structures seemed to be affected by this vinca alkaloid. Particularly evident was the production of large clumped aggregates from the rough ER (for the non-specificity of vinblastine action compare also Wilson *et al.*, 1970).

On the other hand we cannot definitely exclude that oriented membranes such as the cortical ER-elements may be involved in such processes as well (compare also Rebhun, 1963, and Larson, 1965). However, the observation that they are not significantly altered in the presence of cytochalasin suggests that they do not play the major role as locomotory organelles of vesicle transport. We ascribe special importance to the observation that all three classes of potential guide structures appear intimately associated with each other as well as with secretory vesicles by lateral cross-links. A close interaction of the guide elements with the vesicle membrane has also been reported in other cell situations where directional vesicle flow occurs, in particular in neuronal axons (Smith, 1970, for further references see Yamada *et al.*, 1971) and during cell plate formation (Hepler, *et al.*, 1970). It is possible that, just as with the microtubule-microtubule and the microtubule-plasma membrane interaction (ref. e. g., Grimstone and Cleveland, 1965; Cronshaw, 1967; Kiermayer, 1968; Allen, 1968; Warner, 1970; Olson and Kochert, 1970), something like a sliding filament mechanism, mediated by cross-links, plays a role in the translocations of secretory vesicles (McIntosh *et al.*, 1969; Smith, 1970; Yamada *et al.*,, 1971). This process is conceivable for a vesicle-ER membrane-to-membrane interaction as well as for the association of the vesicles with microtubular elements. From this, the further possibility is raised then, that cytochalasin B inhibition of streaming and secretion results from a direct interaction with cross-links. We were yet unable, however, to confirm this with electron-microscopic observations of cross-bridge breakdown in the presence of cytochalasin.

Finally, we draw attention to ER of the tip zone of pollen tubes. This is at least partially rough surfaced and appears generally to be filled with some fibrillarly textured electron-opaque material (e. .g, Figs. 4 and 5), presumably of proteinaceous nature. Although vesicle flow from dictyosomes is a well established mechanism

for transport to the extracellular space we see nothing contradictory to the view that secretion of extracellular material can, additionally, take place directly from ER. Moreover, proteinaceous components have to be postulated for Golgi and ER cisternae of the pollen tube tip from our recent analysis of newly synthesized pollen wall material which contains up to ca. 30% of dry weight as protein, partially in stable linkage to the polysaccharide moieties.

Note added in proof. Since this manuscript was finished a series of relevant publications have appeared. These include in particular the report by M. V. Parthasarathy and K. Mühlethaler [Cytoplasmic microfilaments in plant cells. J. Ultrastruct. Res. **38**, 46–62 (1972)] on the widespread occurence of 50–60 Å filaments in elongating plant cells and various articles on cytochalasin B effects on microfilamentous systems (F. J. Manasek, B. Burnside and J. Stroman: The sensitivity of developing cardiac myofibrils to cytochalasin B. Proc. Nat. Acad. Sci. (Wash.) **69**, 308–312 (1972). N. B. Thoa, G. F. Wooten, J. Axelrod, and I. J. Kopin: Inhibition of release of dopamine-β-hydroxylase and norepinephrine from sympathetic nerves by colchicine, vinblastine, or cytochalasin B. Proc. Nat. Acad. Sci. (Wash.) **69**, 520–522, (1972). R. Wagner, M. Rosenberg, and R. Estensen: Endocytosis in Chang liver cells. Quantitation by sucrose-^{3}H uptake and inhibition by cytochalasin B. J. Cell Biol. **50**, 804–817 (1971). However, the interpretation of the findings that cytochalasin B inhibits both streaming and tip growth in pollen tubes is further complicated in the view of reports of the non-effect of cytochalasin B on microfilament-type structures [A. Forer, J. Emersen and O. Behnke: Cytochalasin B: Does it affect actin-like filaments? Science **175**, 774–776 (1972). See, however, also: J. A. Spudich and S. Lin: Cytochalasin B, its interaction with actin and actomyosin from muscle. Proc. Nat. Acad. Sci. (Wash.) **69**, 442–446 (1972)] and the *in vivo* inhibition of mucopolysaccharide synthesis by cytochalasin B (J. W. Sanger and H. Holtzer: Cytochalasin B: Effects on cell morphology, cell adhesion, and mucopolysaccharide synthesis. Proc. Nat. Acad. Sci. (Wash.) **69**, 253–257, 1972). Preliminary results show a membrane-associated glucan synthetase of pollen tubes to be inhibited by cytochalasin *in vitro* over approximately the same range of cytochalasin concentrations as the *in vivo* response when assayed at low substrate concentrations. These findings plus the observation that microfilaments were not altered ultrastructurally by cytochalasin B in this study, emphasize the need for caution in specifically associating all processes inhibited by cytochalasin B only with microfilaments.

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References

Allen, R. D.: A reinvestigation of cross sections of cilia. J. Cell Biol. **37**, 825–831 (1968).

Allen, R. D.: Fine structure of membraneous and microfibrillar systems in the cortex of *Paramecium caudatum*. J. Cell Biol. **49**, 1–20 (1971).

Anderson, G. W., Brenner, R. M.: The formation of basal bodies (centrioles) in rhesus monkey oviduct. J. Cell Biol. **50**, 10–34 (1971).

Bikle, D., Tilney, L. G., Porter, K. R.: Microtubules and pigment migration in the melanophores of *Fundulus heteroclitus*. Protoplasma (Wien) **61**, 322–345 (1966).

Brown, R. M., Franke, W. W., Kleinig, H., Falk, H., Sitte, P.: Scale formation in chrysophycean algae. I. Cellulosic and noncellulosic components made by the Golgi apparatus. J. Cell Biol. **45**, 246–271 (1970).

Campbell, R. D., Campbell, J. H.: Origin and continuity of desmosomes. In: Origin and continuity of cell organelles. Results and problems in cell differentiation, ed. by Reinert, J., Ursprung, H., vol. **2**, p. 261–298. Berlin-Heidelberg-New York: Springer 1971.

Crang, R. E., Miles, G. B.: An electron microscope study of germinating *Lychnis alba* pollen. Amer. J. Bot. **56**, 398–405 (1969).

Cronshaw, J.: Tracheid differentiation in tobacco pith cultures. Planta (Berl.) **72**, 78–90 (1967).

Dahlström, A.: In: Cellular dynamics of the neuron, ed. Barondes, S. H., p. 153–174. New York and London: Academic Press 1969.

Darlington, C. D., LaCour, L. F.: Methoden der Chromosomenuntersuchung, S. 76. Stuttgart: Kosmos-Verlag 1963.

Dashek, W. V., Rosen, W. G.: Electron microscopical localisation of chemical components in the growth zone of lily pollen tubes. Protoplasma (Wien) **61**, 192–204 (1966).

Dickson, M. R., Mercer, E. H.: Fine structure of the pedal gland of *Philodina roseola* (Rotifera). J. Microscopie **5**, 81–90 (1966).

Dirksen, E. R.: Centriole morphogenesis in developing ciliated epithelium of the mouse oviduct. J. Cell Biol. **51**, 286–302 (1971).

Franke, W. W.: Cytoplasmic microtubules linked to endoplasmic reticulum with cross-bridges. Exp. Cell Res. **66**, 486–489 (1971a).

Franke, W. W.: Membrane-microtubule-microfilament relationship in the ciliate pellicle. Cytobiologie **4**, 307–316 (1971b).

Franke, W. W.: Relationship of nuclear membranes with filaments and microtubules. Protoplasma (Wien) **73**, 263–292 (1971c).

Franke, W. W., Kartenbeck, J., Zentgraf, H., Scheer, U., Falk, H.: Membrane-to-membrane cross-bridges. A means to orientation and interaction of membrane faces. J. Cell Biol. **51**, 881–888 (1971).

Freed, J. J., Lebowitz, M. M.: The association of a class of saltatory movements with microtubules in cultured cells. J. Cell Biol. **45**, 334–354 (1970).

Goldman, R. D., Follett, E. A. C.: The structure of the major cell processes of isolated BHK 21 fibroblasts. Exptl. Cell Res. **57**, 263–276 (1969).

Grimstone, A. V., Cleveland, L. R.: The fine structure and function of the contractile axostyles of certain flagellates. J. Cell Biol. **24**, 387–400 (1965).

Grove, S. N., Bracker, C. E., Morré, D. J.: An ultrastructural basis for hyphal tip growth in *Pythium ultimum*. Amer. J. Bot. **57**, 245–266 (1970).

Gullvåg, B. M.: The fine structure of pollen grains and spores: A selective review from the last twenty years of research. Phytomorphol. **16**, 211–227 (1966).

Hanaoka, H., Friedman, B.: Paired cisternae in human tumor cells. J. Ultrastruct. Res. **32**, 323–333 (1970).

Hepler, P. K., Jackson, W. T.: Microtubules and early stages of cell-plate formation in the endosperm of *Haemanthus katherinae* Baker. J. Cell Biol. **38**, 437–446 (1968).

Hepler, P. K., McIntosh, J., Cleland, S.: Intermicrotubule bridges in mitotic spindle apparatus. J. Cell Biol. **45**, 438–444 (1970).

Herth, W., Franke, W. W., VanDerWoude, W. J.: Cytochalasin stops tip growth in plants. Naturwissenschaften **59**, 38a (1972).

Hoefert, L. L.: Ultrastructure of *Beta* pollen. Amer. J. Bot. **56**, 363–368 (1969).

Hoefert, L. L.: Fine structure of sperm cells in pollen grains of *Beta*. Protoplasma (Wien) **68**, 237–240 (1969).

Hoffmann-Berling, H.: Adenosintriphosphat als Betriebsstoff von Zellbewegungen. Biochim. biophys. Acta (Amst.) **14**, 182–194 (1954).

Hufnagel, L. A.: Cortical ultrastructure of *Paramecium aurelia*: studies on isolated pellicles. J. Cell Biol. **40**, 779–801 (1969).

Kamiya, N.: Motilität des Plasmas. In: Die Zelle, Struktur und Funktion, ed. Metzner, H., S. 373–404. Stuttgart: Wissenschaftliche Verlagsgesellschaft 1971.

Kiermayer, O.: The distribution of microtubules in differentiating cells of *Micrasterias denticulata*. Planta (Berl.) **83**, 223–236 (1968).

Komnick, H., Stockem, W., Wohlfahrt-Bottermann, K. E.: Weitreichende fibrilläre Protoplasmadifferenzierungen und ihre Bedeutung für die Protoplasmaströmung. Z. Zellforsch. **109**, 420–430 (1970).

Kreutzberg, G. W.: Neuronal dynamics and axonal flow. IV. Blockage of intraaxonal enzyme transport by colchicine. Proc. nat. Acad. Sci. (Wash.) **62**, 722–728 (1969).

Krishan, A.: Fine structure of cytochalasine induced multinucleated cells. J. Ultrastruct. Res. **36**, 191–204 (1971).

Krishan, A., Hsu, D.: Observations on the association of helical polyribosomes and filaments with vincristine-induced cristals in Earle's L-cell fibroblasts. J. Cell Biol. **43**, 553–563 (1969).

Kumegawa, M., Cattoni, M., Rose, G. G.: Electron microscopy of oral cells in vitro. II. Subsurface and intracytoplasmic confronting cisternae in strain KB cells. J. Cell Biol. **36**, 443–452 (1968).

Larson, D. A.: Fine-structural changes in the cytoplasm of germinating pollen. Amer. J. Bot. **52**, 139–154 (1965).

Lentz, T. L.: The fine structure of differentiating interstitial cells in *Hydra*. Z. Zellforsch. **67**, 547–560 (1965).

Lentz, T. L.: Rhabdite formation in *Planaria*: The role of microtubules. J. Ultrastruct. Res. **17**, 114–126 (1967).

Luykx, P.: Cellular mechanisms of chromosome distribution. Int. Rev. Cytol. **2**, 1–171 (1970).

Maitra, S. C., De, D. N.: Role of microtubules in secondary thickening of differentiating xylem element. J. Ultrastruct. Res. **34**, 15–22 (1971).

Malaisse-Lagae, F., Greider, M. H., Malaisse, W. J., Lacy, P. E.: The stimulus-secretion coupling of glucose-induced insulin release. IV. The effect of vincristine and deuterium oxide on the microtubular system of the pancreatic beta cell. J. Cell Biol. **49**, 530–535 (1971).

Malawista, S. E., Sato, H., Creasy, W. A., Bensch, K. G.: Vinblastine produces uniaxial, birefringent crystals in starfish oocytes. Fed. Proc. **28**, 875 (1969).

Marantz, R., Shelanski, M. L.: Structure of microtubular crystals induced by vinblastine in vitro. J. Cell Biol. **44**, 234–238 (1970).

Mazia, D., Ruby, A.: Dissolution of erythrocyte membranes in water and comparison of the membrane protein with other structural proteins. Proc. nat. Acad. Sci. (Wash.) **61**, 1005–1012 (1968).

McIntosh, J. R., Hepler, P. K., Van Wie, D. G.: Model for mitosis. Nature (Lond.) **224**, 659–663 (1969).

Mollenhauer, H. H., Morré, D. J.: Golgi apparatus and plant secretion. Ann. Rev. Plant Physiol. **17**, 27–46 (1966).

Morré, D. J., VanDerWoude, W. J.: Origin and growth of cell surface components. Develop. Biol., in press.

Mühlethaler, K.: Ultrastructure and formation of plant cell walls. Ann. Rev. Plant Physiol. **18**, 1–24 (1967).

Nagai, R., Rebhun, L. I.: Cytoplasmic microfilaments in streaming *Nitella* cells. J. Ultrastruct. Res. **14**, 571–589 (1966).

Newcomb, E. H.: Plant microtubules. Ann. Rev. Plant Physiol. **20**, 253–288 (1969).

Northcote, D. H.: Organization of structure, synthesis and transport within the plant during cell division and growth. Symp. Soc. exp. Biol. **25**, 51–69 (1971).

Northcote, D. H., Pickett-Heaps, J. D.: A function of the Golgi apparatus in polysaccharide synthesis and transport in the root-cap cells of wheat. Biochem. J. **98**, 159–167 (1966).

O'Brien, T. P., Thimann, K. V.: Intracellular fibers in oat coleoptile cells and their possible significance in cytoplasmic streaming. Proc. nat. Acad. Sci. (Wash.) **56**, 888–894 (1966).

Olson, L. W., Kochert, G.: Ultrastructure of *Volvox carteri*. I. The kinetosome. Arch. Mikrobiol. **74**, 31–40 (1970).

Pickett-Heaps, J. D.: Ultrastructure and differentiation in *Chara* sp. I. Vegetative cells. Aust. J. biol. Sci. **20**, 539–551 (1967).

Pickett-Heaps, J. D.: Xylem wall deposition. Radioautographic investigations using lignin precursors. Protoplasma (Wien) **65**, 181–205 (1968).

Pitelka, D. R.: Fibrillar systems in protozoa. In: Research in protozoology, Tze Tuan Chen, ed., vol. 3, p. 279–388. Oxford: Pergamon Press 1969.

Pollard, T. D., Korn, E. D.: Filaments of *Amoeba proteus*. II. Binding of heavy meromyosin by thin filaments in motile cytoplasmic extracts. J. Cell Biol. **48**, 216–219 (1971).

Rebhun, L. I.: Saltatory particle movements and their relation to the mitotic apparatus. In: The cell in mitosis, ed. by Levine p. L., 67–106. New York-London: Acad. Press 1963.

Robards, A. W.: On the ultrastructure of differentiating secondary xylem in willow. Protoplasma (Wien) **65**, 449–464 (1971).

Rosen, W. G.: Ultrastructure and physiology of pollen. Ann. Rev. Plant Physiol. **19**, 435–462 (1968).

Sabnis, D. D., Jacobs, W. P.: Cytoplasmic streaming and microtubules in the coenocytic marine alga *Caulerpa prolifera*. J. Cell Sci. **2**, 465–472 (1967).

Schmitt, F. O.: Fibrous proteins neuronal organelles. Proc. nat. Acad. Sci. **60**, 1092–1101 (1968).

Simard-Duquesne, N., Coullard, P.: Ameboid movement. I. Reactivation of glycerinated models of ameba proteus with adenosin triphosphate. Exp. Cell Res. **28**, 92–98 (1962).

Smith, D. S.: Bridges between vesicles and axoplasmic microtubules. J. Cell Biol. **47**, 195a (1970).

Smith, D. S., Järlfors, U., Beranek, R.: The organization of synaptic axoplasm in the lamprey (*Petromyzon marinus*) central nervous system. J. Cell Biol. **46**, 199–219 (1970).

Stadler, J., Franke, W. W.: Colchicine binding protein in chromatin and membranes. Nature (Lond.), in press.

Tilney, L. G.: Origin and continuity of microtubules. In: Origin and continuity of cell organelles, ed. by Reinert, J., Ursprung, H., p. 222–260. Berlin-Heidelberg-New York: Springer 1971.

VanDerWoude, W. J., Morré, D. J., Bracker, C. E.: Isolation and characterization of secretory vesicles in germinated pollen of *Lilium longiflorum*. J. Cell Sci. **8**, 331–351 (1971).

Warner, F. D.: New observations on flagellar fine structure. The relationship between matrix structure and the microtubule component of the axoneme. J. Cell Biol. **47**, 159–182 (1970).

Weihing, R. R., Korn, E. D.: *Acanthamoeba* actin. Isolation and properties. Biochemistry **10**, 590–600 (1971).

Wessels, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T., Yamada, K. M.: Microfilaments in cellular and developmental processes. Contractile microfilament machinery of many cell types is reversibly inhibited by cytochalasin B. Science **171**, 135–140 (1971).

Wilson, L., Bryan, J., Ruby, A., Mazia, D.: Precipitation of proteins by vinblastine and calcium ions. Proc. nat. Acad. Sci. (Wash.) **66**, 807–814 (1970).

Wooley, D. E.: An actin-like protein from amoeba of *Dictyostelium discoideum*. Fed. Proc. **29**, 667 A (1970).

Yamada, K. M., Spooner, B. S., Wessels, N. K.: Ultrastructure and function of growth cones and axons of cultured nerve cells. J. Cell Biol. **49**, 614–635 (1971).

W. W. Franke
Institut für Biologie II
D-7800 Freiburg i. Br.
Schänzlestr. 9–15
Germany