Cytoskeleton-Secretory Vesicle Interactions During the Docking of Secretory Vesicles at the Cell Membrane in *Paramecium tetraurelia* Cells

H. PLATTNER, C. WESTPHAL, and R. TIGGEMANN
Faculty of Biology, University of Konstanz, Federal Republic of Germany

ABSTRACT Stationary-phase cells of *Paramecium tetraurelia* have most of their many secretory vesicles ("trichocysts") attached to the cell surface. Log-phase cells contain numerous unoccupied potential docking sites for trichocysts and many free trichocysts in the cytoplasm. To study the possible involvement of cytoskeletal elements, notably of microtubules, in the process of positioning of trichocysts at the cell surface, we took advantage of these stages. Cells were stained with tannic acid and subsequently analyzed by electron microscopy. Semithin sections allowed the determination of structural connections over a range of up to 10 μm. Microtubules emanating from ciliary basal bodies are seen in contact with free trichocysts, which appear to be transported, with their tip first, to the cell surface. (This can account for the saltatory movement reported by others). It is noteworthy that the "rails" represented by the microtubules do not directly determine the final attachment site of a trichocyst. Unoccupied attachment sites are characterized by a "plug" of electron-dense material just below the plasma membrane; the "plug" seems to act as a recognition or anchoring site; this material is squeezed out all around the trichocyst attachment zone, once a trichocyst is inserted(Westphal and Plattner, in press. [53]). Slightly below this "plug" we observed fasciae of microfilaments (identified by immunocytochemistry using peroxidase labeled F(ab) fragments against *P. tetraurelia* actin). Their arrangement is not altered when a trichocyst is docked. These fasciae seem to form a loophole for the insertion of a trichocyst. Trichocysts remain attached to the microtubules originating from the ciliary basal bodies—at least for some time—even after they are firmly installed in the preformed attachment sites. Evidently, the regular arrangement of exocytotic organelles is controlled on three levels: one operating over a long distance from the exocytosis site proper (microtubules), one over a short distance (microfilament bundles), and one directly on the exocytosis site ("plug").

Cytoskeletal elements are known to be involved in exocytosis (5, 20, 23, 29, 30, 31, 43). Microtubules serve as guiding structures for the transport of secretory vesicles to the cell membrane. This was concluded from the conspicuous arrangement of secretory vesicles along microtubules in some cells (23, 29, 30, 45, 54) and from microcinematographic analyses of vesicle movement in other cells (31), including paramecia (6, 7). The participation of a contractile actomyosin complex or of microfilaments (5, 16, 19, 39, 42) was also postulated. Finally, the interplay of cytoskeletal elements could allow for pulling the secretory vesicles to the cell membrane to establish the close contact required for subsequent membrane fusion (13, 39).

The generally accepted model for the interaction between cytoskeletal elements, secretory vesicles, and the cell membrane would have two important consequences. Firstly, the stable microtubular "rails" would determine the final destination of the vesicles and, thus, account for the topological specificity of exocytosis, which is an obvious phenomenon in many cells (c.f. 13, 23, 29, 30, 34). Secondly, microfilaments would have to be actively involved in exocytosis. Unfortunately, experiments with tubulin- and actin-directed drugs do not yield unequivocal results (11, 20, 27, 32, 40), so that it is difficult to manipulate these structures without unwanted side-effects. Therefore, it is still largely unknown how microtubules and microfilaments contribute precisely to the final steps of exocytosis (c.f. 34, 49).

The analysis presented here tries to approach these questions from a different viewpoint. The cells analyzed here, *Parame-*

THE JOURNAL OF CELL BIOLOGY · VOLUME 92 FEBRUARY 1982 368-377

cium tetraurelia, display a highly evolved and redundant exocytotic apparatus with an incomparable degree of regularity and with the unique situation that exocytosis is arrested just before the very last steps of this complex process (c.f. 2, 9, 21, 37). These special features of the present system greatly facilitate the analysis of questions such as whether microtubules and/or microfilaments could be (directly or indirectly) involved in the determination of the sites of exocytosis.

It was necessary to abandon "standard" electron microscopic techniques to achieve sufficient contrast and to allow for determining long-range structural connections. Therefore, we used tannic acid staining (51) and then prepared semithin sections for subsequent electron microscopic analysis.

MATERIALS AND METHODS

P. tetraurelia cells, strain *K 401* (derived from stock 51), were cultivated at 25°C in a decoct dry lettuce medium under monoxenic conditions (with *Enterobacter aerogenes* added as a food bacterium). Cells were harvested two (middle logarithmic growth phase of cultures) or 4 d (early stationary phase) after inoculation. Cells were concentrated by filtration through a 40-μm sieve and washed with 10 mM Tris-maleate buffer pH 7.0.

For electron microscopy, cells concentrated to ~10^5 cells/ml were supplemented first with 4 mM EDTA (30 s), then for up to 30 s with 0.3% (wt/vol) saponin for permeabilization (35), and then were fixed with an equal volume of 2.5% (vol/vol) glutaraldehyde (in 0.1 M cacodylate buffer pH 7.0, 22°C). The same buffer was added 1 min later for a 10-fold dilution of the fixative, in which the cells remained for another 14 min. After washing with the same buffer, the samples were postfixed with 1% (wt/vol) OsO_4. 4% (wt/vol) tannic acid was added to both concentrations of glutaraldehyde as a mordant dye for proteins (44, 51). Samples were stained overnight in 1% (wt/vol) aqueous uranyl acetate, routinely dehydrated in a series of acetone, and embedded in Spurr medium (48). We prepared ultrathin sections of grey interference color (~40 nm) and semithin sections of blue-green color (~250 nm), which were then routinely stained with alkaline lead citrate. All sections were analyzed in a ZEISS EM 10 (Oberkochen, Germany) electron microscope, operated in the conventional transmission mode at 100 kV acceleration voltage and with a 30-μm objective aperture.

For immunocytochemistry we added 4 mM EDTA (30 s) to concentrated cells before they were fixed for 1 min in a cacodylate (0.1 M, pH 7.0)-buffered 2.5% formaldehyde + 0.5% glutaraldehyde solution (0°C); after 1 min the fixative was diluted 1:10 (14 min, 22°C). Cells were rendered permeable with 10% saponin in cacodylate buffer for 5 min, extensively washed in the same buffer, and suspended twice in 10 mM sodium phosphate buffer pH 6.8. The immunolabeling was done as follows. Actin, isolated from *Paramecium* cultures, was used for the preparation of monospecific antibodies (50), from which we prepared F(ab) fragments by papain cleavage, which were subsequently tagged with horseradish peroxidase isoenzyme VII (F(ab)-HRPOX) from Sigma Chemical Co. (St. Louis, Mo.) by standard toluene-2,4-diisocyanate coupling (50). The purified F(ab)-HRPOX conjugate (0.5 mg/ml) was added to stabilized and permeabilized *Paramecium* cells (see above) at 22°C. Unspecific adsorption was efficiently prevented by adding a cocktail of proteins (BSA, ovalbumin, myoglobin, lactoglobulin, glycine, each 1 mg/ml) to the incubation medium. Cells were repeatedly washed overnight, incubated in a 3,3'-diaminobenzidine (3 mg/ml)-H_2O_2 (0.01%) mixture for 30 min at 22°C, postfixed with 1% OsO_4, and further processed for electron microscopy as indicated above. Preincubation with specific but unlabeled F(ab) fragments before F(ab)-HRPOX incubation gave negative results, just as the treatment with the HRPOX VII marker alone. As a further control we identified the isolated actin (in addition to using the usual biochemical standard techniques) by routine decoration with heavy meromyosin in conjunction with negative staining (1% uranyl acetate).

RESULTS

For the identification of microtubules and microfilaments we measured their diameters and also, visualized the subunit structure of microtubules (by tannic acid staining) and the antiactin F(ab) fragment (HRPOX tagged) binding capacity of microfilaments. This led us to the terminology and the summary of results contained in the scheme of Fig. 21.

Fig. 1 documents how a microtubule, originating from a basal body, contacts the lower portion of a trichocyst, which

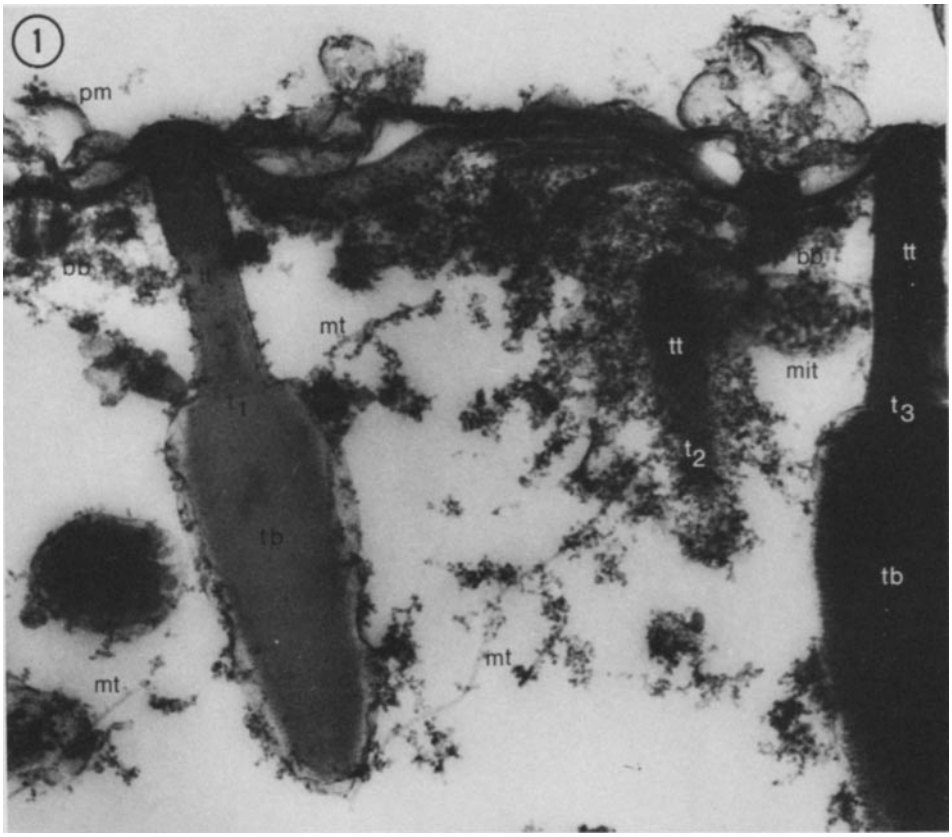


FIGURE 1 Semithin section of a stationary-phase cell. Two trichocysts (t_1, t_3; *tb*: trichocyst body; and *tt*: trichocyst tip) are seen in contact with the plasma membrane (*pm*). Among the four microtubules (*mt*) present, the longest one appears to be in contact with the lower body region of t_1, with the tip of another trichocyst (t_2) and a mitochondrion (*mit*); this microtubule ends close to a ciliary basal body (*bb*). × 25,000.

itself is attached to the cell surface. Fig. 2 provides similar evidence and shows, moreover, that one microtubule can contact more than one trichocyst and that one presumably attached trichocyst may be in contact with more than one microtubule. This is also shown for a free trichocyst (Fig. 3) that appears to be transferred, with its tip first, along several microtubules in parallel arrangement towards the cell surface. Although these long-range interactions are seen more clearly in semithin sections, ultrathin sections document more clearly that microtubules actually emanate from the ciliary basal bodies (Figs. 4 and 5) and then may contact a more or less remote site of a trichocyst (Fig. 6) or a mitochondrion (Fig. 5). We did not observe microtubules emanating from the trichocyst attachment site proper. Figs. 4 and 5 also show the association of some electron-dense material with the ciliary basal bodies (where we had shown before the presence of actin by immuno- and affinity-fluorescence labeling; see reference 50). Other microfilament bundles surround the upper region of the trichocyst tip (Figs. 7–10). The presence of actin in these microfilaments was identified by immunocytochemistry (Figs. 19, 20). These microfilaments do not end at the trichocyst membranes but appear to run as bundles or fasciae without interruption, almost perpendicular to one another, from one epiplasmic layer to the other (Figs. 9 and 10). We could not ascertain whether individual filaments span the whole width between two epiplasmic layers. This structural arrangement is present already before a trichocyst is inserted (Figs. 11–13) and remains unaltered afterwards. Therefore, the free space in the middle between the filamentous bundles (see asterisk in Fig. 12) could function like a loophole that would determine the site for the insertion of a trichocyst into the cell surface.

Around the upper tip region the trichocyst membrane is surrounded by a special structure (Figs. 7–9), the "collar", made up of a densely staining "collar matrix" and ~30 bright "collar tubules". Although the latter have a diameter comparable to that of microtubules, they lack persistently the 13 protofilament construction after tannic acid staining (Fig. 14); therefore, they can not be true microtubules. The "collar tubules" might, therefore, serve for local structural stabilization (rather than for intracellular transport), and Figs. 7–9 clearly show the intimate connection between "collar" and epiplasmic layer. In contrast, "genuine" microtubules in cilia (Fig. 15) and cytoplasm (Fig. 17) display the 13 protofilaments very clearly.

We tried to estimate the number of microtubules associated with trichocysts and basal bodies in log-phase cells. These estimates were made with ultrathin and semithin sections. On ultrathin sections, 12 trichocysts (7 attached and 5 free) out of 143 were closely associated with one or two microtubules; the percentage of basal bodies with attached microtubules was higher (11 out of 58). If one takes into account the different diameter of trichocysts and basal bodies in relation to the thickness of an ultrathin section (see Materials and Methods), one can roughly extrapolate that the number of contacts (microtubules with trichocysts or with basal bodies) per one or-

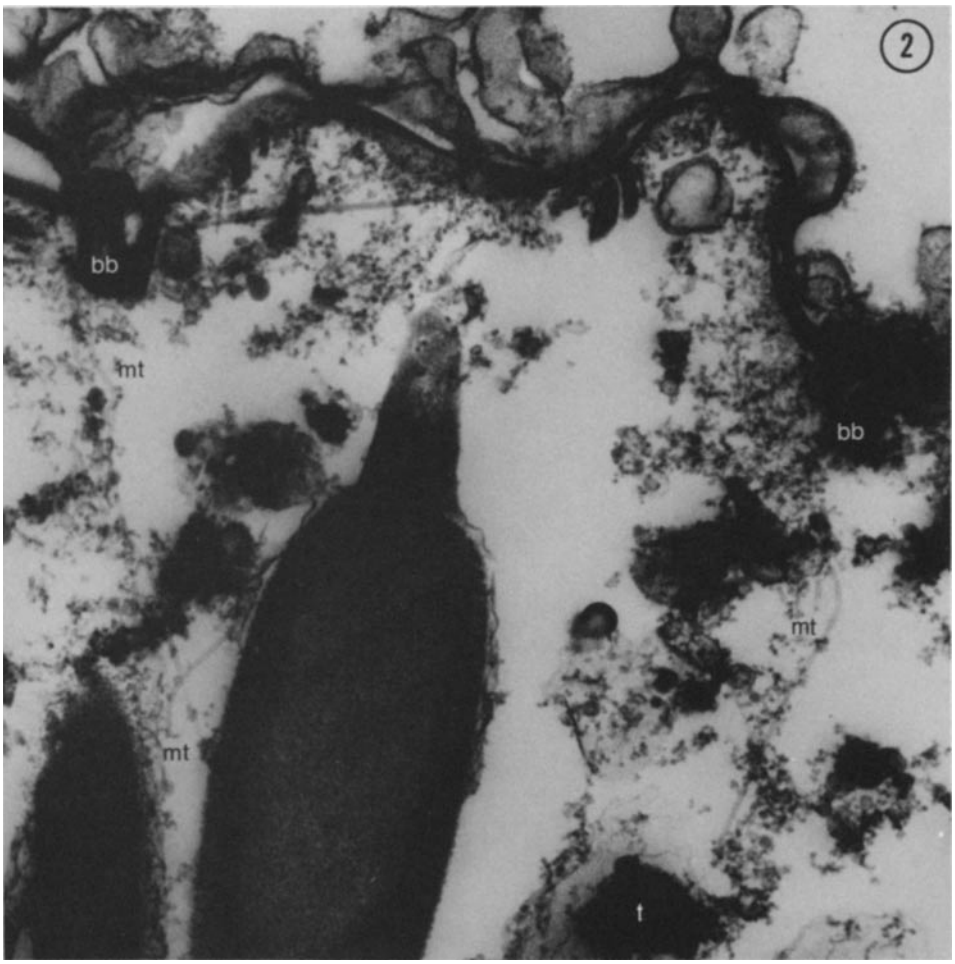


FIGURE 2 Semithin section of a stationary phase cell. From two ciliary basal bodies (*bb*), microtubules (*mt*) are seen to emanate and to come in contact with trichocysts (*t*), i.e. their body region. On the lower left, one microtubule is approaching two trichocysts. × 25,000.

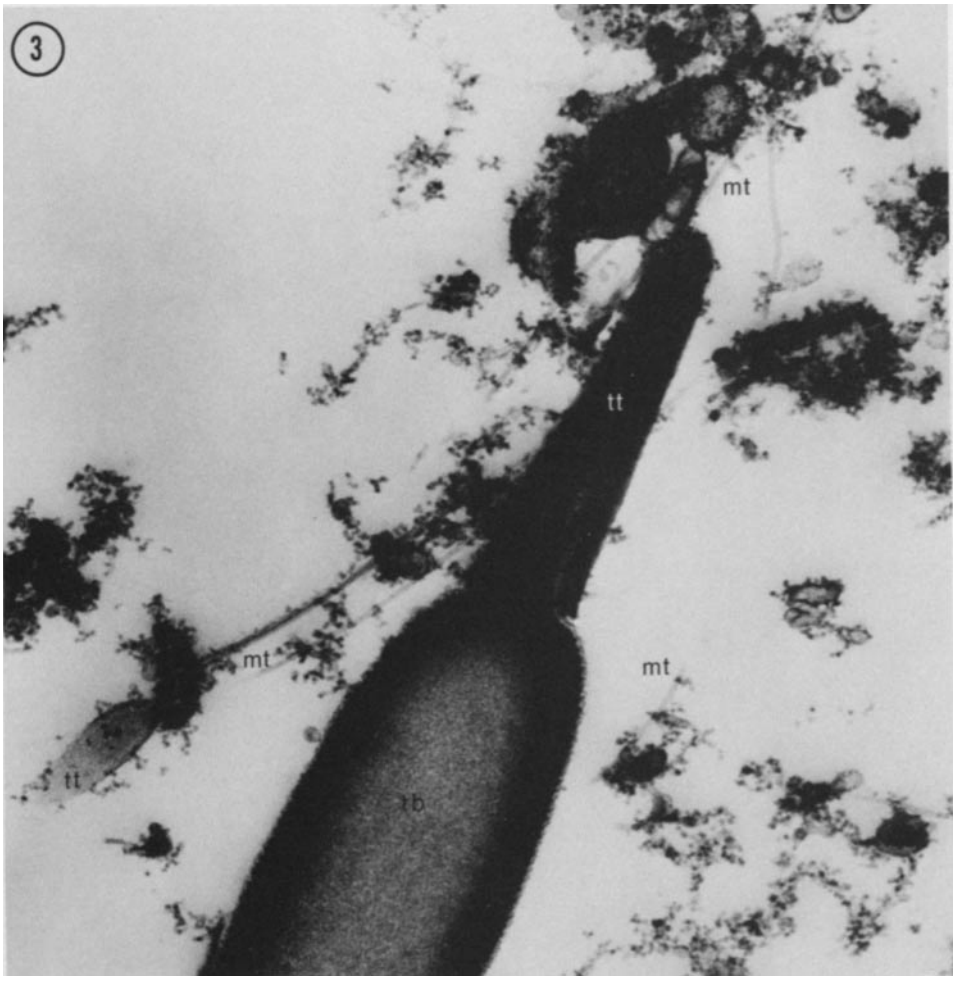
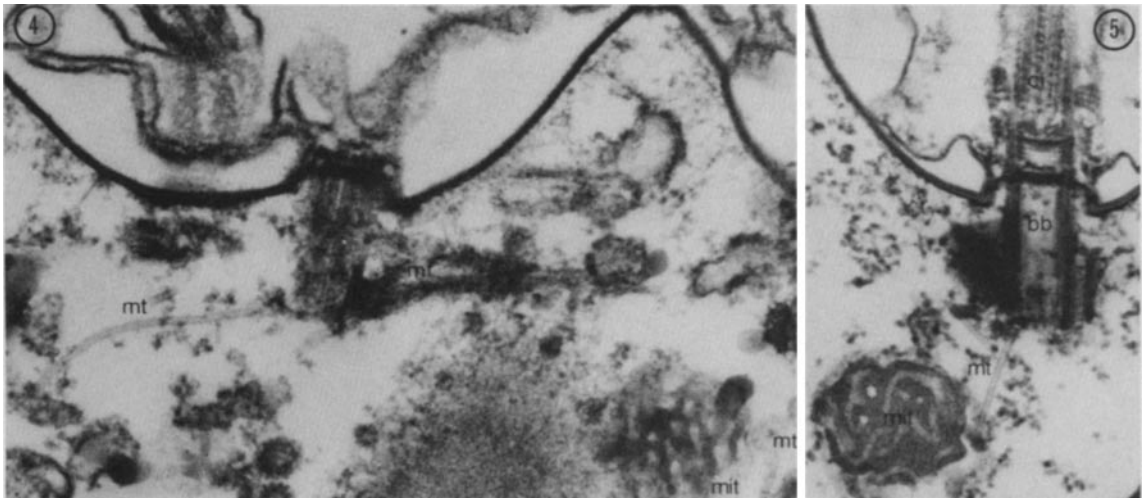


FIGURE 3 Semithin section of a log-phase cell. Two free trichocysts are cut, but of the lower left trichocyst only the tip (*tt*) is contained in this section. Several microtubules (*mt*) run parallel to the trichocysts to which they come very close, mainly in the tip and less evident in the body region (*tb*). The cell surface would be close to the upper end. × 25,000.



FIGURES 4 and 5 Ultrathin sections of stationary-phase cells. Fig. 4 shows a ciliary basal body (*bb*) with a microtubule (*mt*) and with microfilament-like structures (*mf*) that appear closely associated, at almost right angles, with the basal body. At the lower right there is a mitochondrion (*mit*) with closely approaching or touching microtubules. × 51,000. Fig. 5 shows a ciliary (*ci*) basal body (*bb*) with microtubules (*mt*) and an attached mitochondrion (*mit*). × 51,000.

ganelle would be identically ~1.5 for each organelle. When we repeated such estimates from counts on semithin sections we found again an average of about 1.5 or 2 microtubules associated with one trichocyst or one basal body, respectively (though such estimates from semithin sections appear to us less reliable).

The above numbers for microtubule contacts with attached trichocysts indicate at least a temporary persistence of such contacts after docking. (Extensive time-sequence studies would be required to ascertain how long this contact would be maintained). We also emphasize that for the above countings we

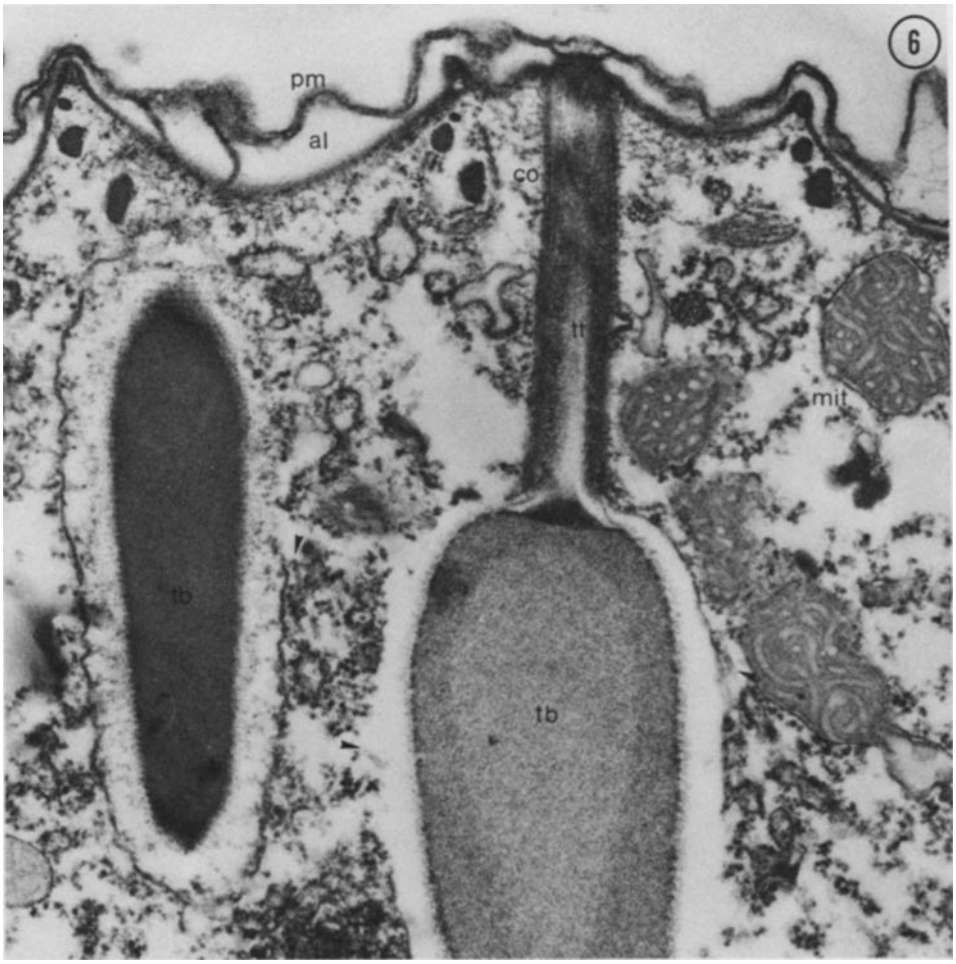
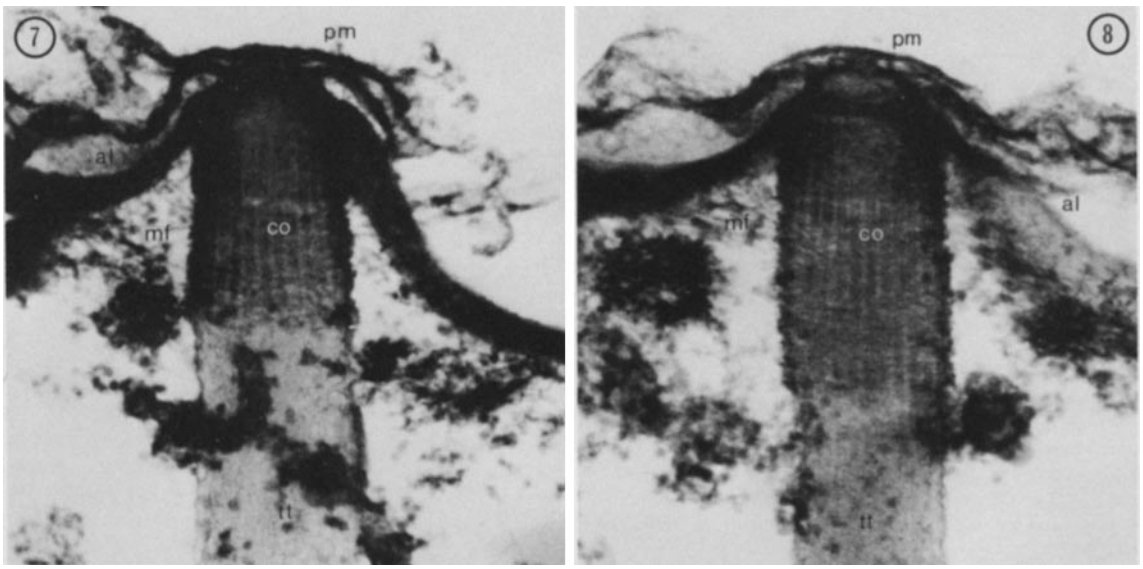


FIGURE 6 Ultrathin section of a log-phase cell. Below the plasma membrane (*pm*) there are alveolar cavities (*al*) present, except for the regions where a trichocyst is attached directly to the *pm*. Microtubules are absent from the attachment site of a trichocyst, whereas microtubules (*mt*) can be occasionally seen to come in contact with the trichocyst body (*tb*) remote from the attachment site. *co*: collar; *tt*: trichocyst tip; and *mit*: mitochondrion. × 39,000.



FIGURES 7 and 8 Semithin sections of stationary-phase cells. The trichocyst tip (*tt*) in Fig. 8 shows a kind of a cap (just below the plasma membrane, *pm*) which is from the rims of alveolar cavities (*al*) that encircle here the uppermost trichocyst tip. The collar structure (*co*) around the upper trichocyst tip region displays vertical striations represented by "collar tubules". This tip region is surrounded by a web of microfilament-like structures (*mf*), more distinctly visible in Fig. 7, whereas microtubules are unequivocally absent. × 70,000.

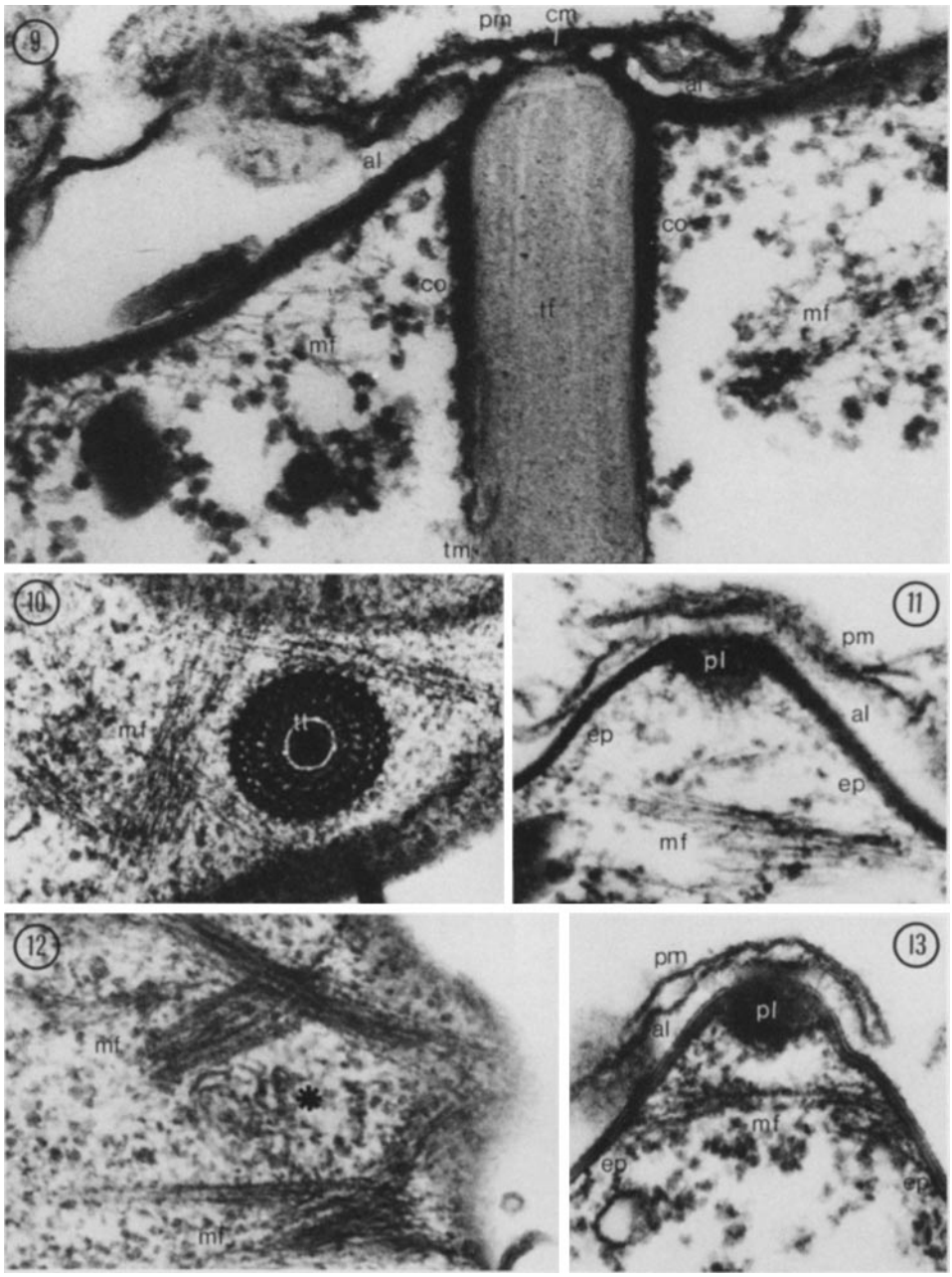


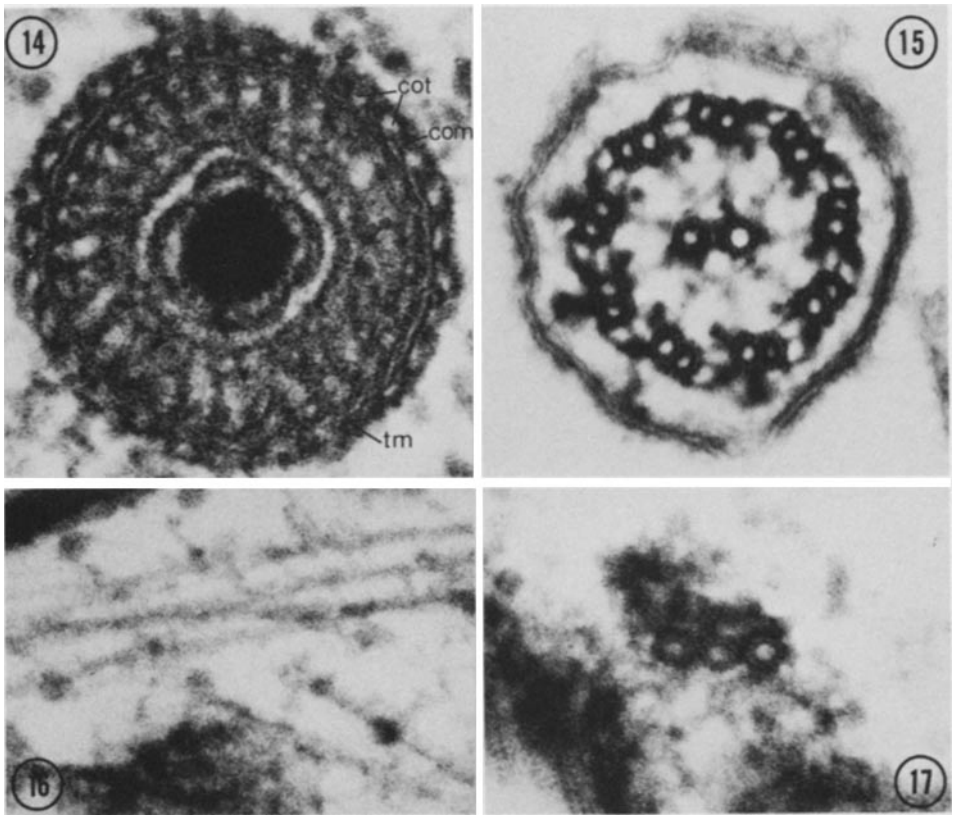
FIGURE 9 Ultrathin section of a stationary-phase cell. *pm:* plasma membrane; *tm*: trichocyst membrane; and *al*: alveolar cavities. The inner side of alveoli is enforced by a densely stained epiplasmic layer (*ep*) that appears continuous with the "collar" (*co*) around the uppermost trichocyst tip (*tt*). *cm* designates connecting material, i.e. a small mass of electron-dense material between plasmamembrane and trichocyst membrane. Microtubules are absent, whereas microfilament-like structures (*mf*), which apparently radiate from the epiplasmic layer, surround the trichocyst tip, mostly without coming into visible contact with the trichocyst. × 86,000.

FIGURE 10 Same situation as in Fig. 9 but in cross-section. Clearly, the microfilament-like structures (*mf*) do not directly abut onto the trichocyst tip (*tt*), but they surround it in the form of fasciae arranged perpendicular to one another. × 68,000.

FIGURES 11–13 Ultrathin sections of log-phase cells with free trichocyst attachment sites in longitudinal (Figs. 11 and 13) and perpendicular (Fig. 12) direction. *pm:* plasmamembrane, *al*: alveolar cavities, *ep*: epiplasmic layer. *pl:* plug of electron-dense material contained in all free potential trichocyst attachment sites. *mf* labels fasciae of microfilaments (see Figs. 19 and 20) emanating from the epiplasmic layer in a region slightly below the attachment site of the trichocyst. The fasciae span the whole width; whether this holds also for the individual filaments that make up the fasciae can not be seen. Note the presence of bundles of microfilament structures before a trichocyst is put in position (asterisk in Fig. 12) and the absence of any rearrangement or direct contact after docking of a trichocyst (Fig. 10). × 68,000.

have used, selectively, cells from experiments with as little material extraction (by the brief saponin treatment, mandatory for intracellular tannic acid fixation and staining) as possible; the samples used for counting were of the kind presented in Fig. 6. This assures us that microtubules would not simply get in contact with a trichocyst due to an artifact caused by material extraction; moreover, all subcellular constituents seem to have maintained their intracellular position in these cells.

Microfilaments were identified as follows. Their diameter after tannic acid staining was 6–8 nm, i.e. compatible with



FIGURES 14–17 Ultrathin sections presented for ultrastructural identification of microtubules and microfilament-like structures. Fig. 14 is a cross-sectioned trichocyst tip, the membrane of which (*tm*) is surrounded by a "collar" composed of a "collar matrix" (*com*) and by regularly interspersed "collar tubules" (*cot*). These are devoid of the 13 protofilament subunits which are otherwise recognized in ciliary (Fig. 15) and cytoplasmic (Fig. 17) "genuine" microtubules. Fig. 16 shows microfilament-like structures (near a trichocyst tip) with a thickness of ~6–8 nm. × 175,000.

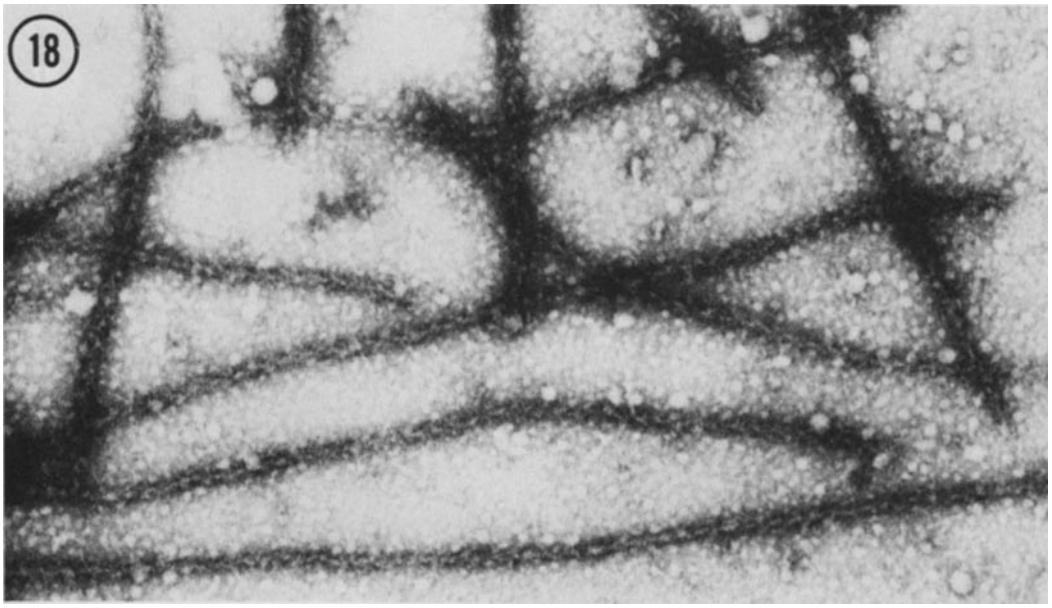
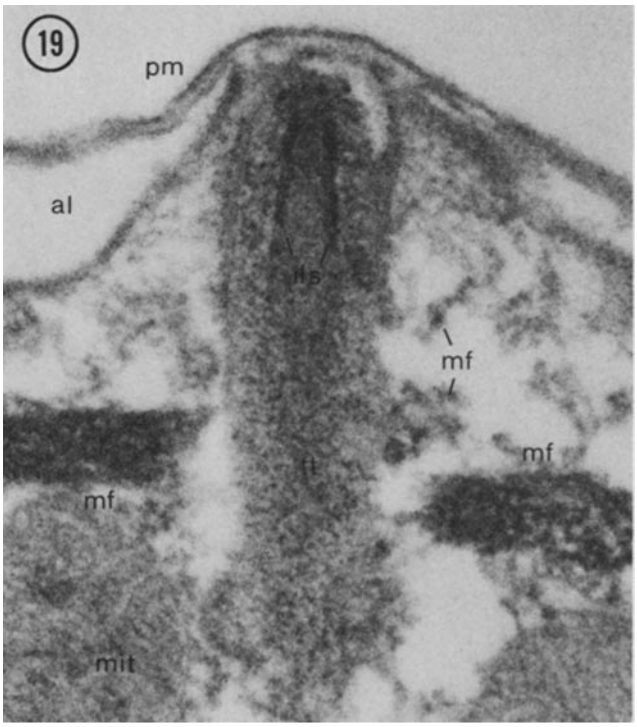


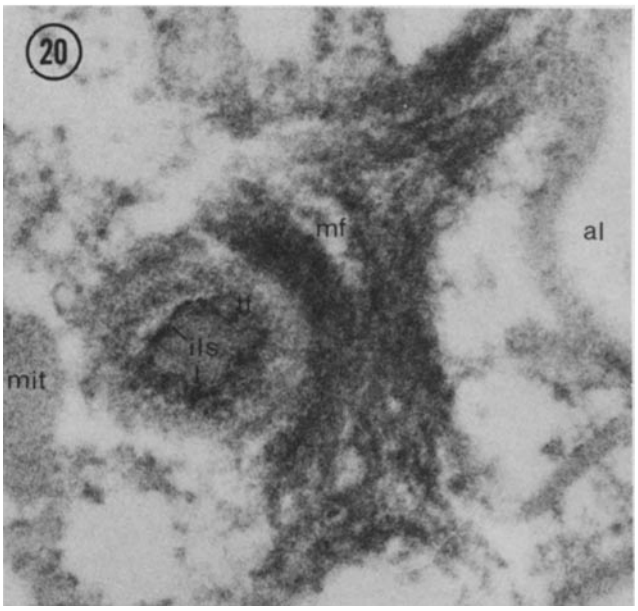
FIGURE 18 Actin isolated from *Paramecium cells*, decorated with heavy meromyosin and negatively stained. × 50,000.

actin filaments (Fig. 16). Moreover, actin was isolated from *P. tetraurelia* cells and identified in vitro by heavy meromyosin decoration (Fig. 18). This actin was also used for antibody production and for the subsequent identification of microfilaments in situ by immunocytochemistry (Figs. 19 and 20).

All data presented (Figs. 1–20) show the following essential points. Microtubules and microfilaments do not end directly on the presumptive exocytosis site, but they come in contact with the secretory organelle laterally, i.e. at sites that are more (microtubules) or less (microfilaments) remote from the zone of plasmamembrane-trichocyst interaction. Before a trichocyst is attached, one can recognize a "plug" of electron-dense material at the potential docking site below the cell membrane (Figs. 11, 13). The nature of the "plug", which must be a

recognition signal for attachment or an attachment structure, and its transformation during trichocyst attachment (see summarizing Fig. 21) was analyzed in a separate study (52, 53).





FIGURES 19 and 20 Immunocytochemical identification of actin in the microfilament fasciae (*mf*) which surround a trichocyst tip (*tt*) without being attached to it. In Fig. 19, a few scattered small microfilament bundles are also labeled. These, together with the fasciae, will probably correspond to the "striated fibers" and the "infraciliary lattice" (1). (A possible cross-connection between these two microfilament systems may be suggested by the semithick section presented in Fig. 7, but this was not analyzed in detail). The HRPOX-labeled F(ab) fragments used for these assays were obtained from IgG directed against *P. tetraurelia* actin. The two situations presented here correspond to Figs. 9 and 10, where tannic acid staining was used. Except for the "inner lamellar sheath" (*ils*) structures, which are known for their endogenous contrast, there are no other structures with any remarkable contrast. This holds especially for the plasmamembrane (*pm*)-trichocyst tip (*tt*) contact zone. *al*: alveoli, *mit*: mitochondria. × 80,000.

DISCUSSION

As in other systems (29, 31) microcinematographic investigations of *Paramecium* cells show that secretory organelles are transferred from the cytoplasm to the cell surface by saltatory movement (6, 7). Aufderheide reported that a small number of trichocysts are always moving in the cytoplasm with the cyclosis stream. He observed that when free trichocyst attachment sites became available by exocytosis the individual free trichocysts seemed to receive an unknown signal and suddenly moved along their longitudinal axis towards the cell surface (6, 7). Our findings suggest this signal to be an encounter with a free microtubule. If there is a free trichocyst docking site available, the corresponding microtubules would hang like a fishing-line from their anchoring point, the ciliary basal bodies, into the cytoplasm. Once a free trichocyst "bites", it would be immediately "pulled out", tip first, from the cyclosis stream. Because the microtubules remain at least for some time attached to the trichocysts, even after the latter are docked (see Results), there would be no "signal" going from an occupied site to any free trichocyst in the cytoplasm. This would occur again only at a certain time after exocytosis, as the ghost membrane vesiculates (24) in the course of membrane recycling (3). Alternatively, microtubules could be depolymerized after trichocyst docking and be formed anew when required; however, this possibility was not analyzed in detail in the present study. (The emphasis here is on the docking mechanism rather than on what might happen over long time periods afterwards. Nevertheless, this aspect would certainly deserve further analysis.)

The fact that some cytoplasmic microtubules originate from ciliary basal bodies has been documented (4). However, we should like to emphasize that our observation that these microtubules participate in the transfer of secretory organelles to the cell surface is new. In fact, older (46) and newer (9) reviews have repeatedly postulated a self-organizing capacity for the regular structural units of the ciliate cell cortex but have not given any details of how this can be realized. On the other hand, it was shown that basal bodies isolated from *Tetrahymena* cells serve as organizing centers for mitotic aster formation after microinjection into *Xenopus* oocytes (25). The close association between mitochondria and ciliary basal bodies has also been seen in *Tetrahymena* (8). All this evidence illustrates the potential of ciliate basal bodies as structure-organizing centers in general; according to our data, this seems to hold also for microtubule-guided trichocyst positioning.

The nature of the interaction between a trichocyst and a microtubule remains unclear. As in other systems (38), this could involve actin filaments (see also introduction) which could generate the force required for intracellular transport (cf. 28). However, this aspect is not yet settled in the literature. Our observations on the arrangement of microfilaments indicate only their involvement as guiding structures, but this would not preclude that actin (outside the fasciae) could also participate in active movements. (We did not analyze this aspect.)

Cross-sections stained with tannic acid can serve to identify microtubules by their 13 protofilaments (51), which we found only with ciliary (Fig. 15) and cytoplasmic microtubules (Fig. 17), but not with the tubular structures contained in the "collar" around attached trichocysts (Fig. 14). Therefore, these "collar" structures, which were previously classified as microtubules (10), should no longer be considered as such. For this reason and because they cover only the uppermost portion of the trichocyst membrane, to which they are tightly apposed, these "collar" structures can not be expected to contribute to the movement of trichocysts. In fact, Cohen et al. (18) showed that

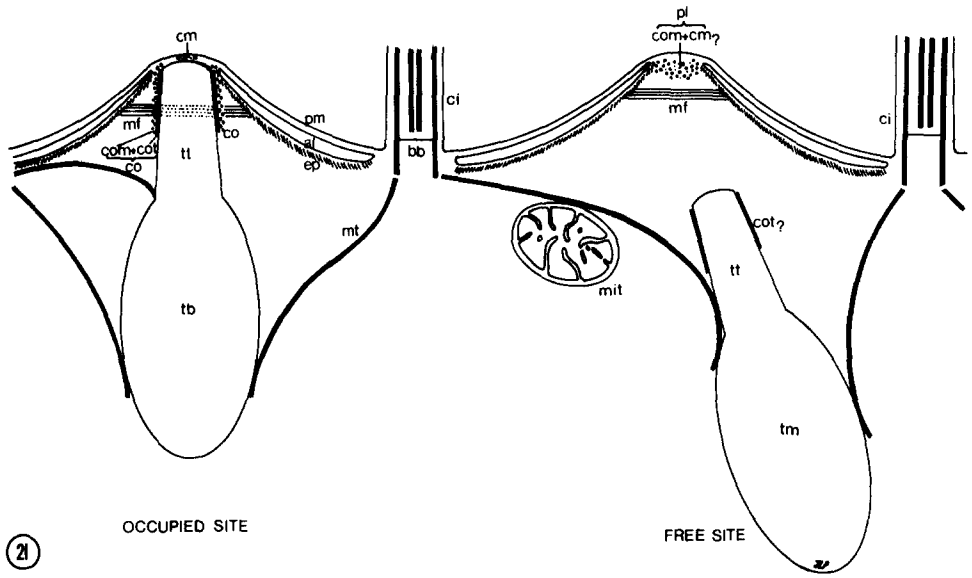


FIGURE 21 Summarizing scheme. *al*: alveolar cavities; *bb*: basal bodies; *ci*: cilia; *cm*: connecting material (electron-dense bridge material between trichocyst tip and plasmamembrane); *co*: collar (cytoplasmic dense layer around trichocyst tip, composed of "collar matrix", *com*, and "collar tubules", *cot*, which are not genuine microtubules); *ep*: epiplasmic layer; *mf*: microfilament-like structures; *mit*: mitochondria; *mt*: microtubules; *tb*: trichocyst body; *tt*: trichocyst tip.—On the right side, the scheme shows the transport of a free trichocyst, tip first, along microtubules (emanating from neighboring ciliary basal bodies) to a free trichocyst docking site. We also indicate the possibility that the "collar tubules" could be attached to a trichocyst before it becomes attached to the surface, but this is not precisely known. To be docked, a trichocyst tip has to pass through the loophole of the fasciae, made by the microfilaments just below the attachment site proper. The docking itself involves the cytoplasmic components called the "plug" (*pl*), which is probably transformed into "connecting material" and "collar matrix" (see above and reference 52). Microtubules remain—at least for some time—associated with the trichocysts also after their insertion (left side). The scheme also indicates the attachment of a mitochondrion to a microtubule close to a ciliary basal body. It becomes evident, from the scheme, that the regular arrangement of exocytotic organelles along the cell surface is controlled at three levels (microtubules, microfilament-like structures, and docking site proper).

these structures surround also the tips of free trichocysts of strain *tam 8*, which can not dock at the cell membrane.

Our results, summarized in Fig. 21, indicate that the next step would be to get the trichocyst tip to the proper site. In this regard the following facts appear relevant: (*a*) trichocysts are transported with the tip first, (*b*) they are positioned with the help of several microtubules from different basal bodies, and (*c*) about four bundles of microfilaments (see Figs. 10–13) are arranged perpendicular or at an angle to one another just below the attachment sites. The free space between them suggests a loophole for directing a trichocyst tip to its final docking site.

It was shown in Figs. 19 and 20 that the attachment sites of trichocysts do not contain actin. Therefore, the uppermost trichocyst attachment structures called "plug" or "connecting material" (see Fig. 21) are actin-free. The fasciae of microfilaments represented in Figs. 7–13 correspond to the "striated fibers" described by Allen (1) for *Paramecium caudatum* cells. We show here for the first time that they contain actin. Their association with the epiplasmic layer is in agreement with electrophoretic data obtained with *Tetrahymena* cells (55). So far we have not found any indications that microfilaments would be arrayed in a mode that would allow them to pull a trichocyst into its final position, because the bundles mentioned before are not attached to the trichocyst membrane.

From experiments with cytochalasin B-treated insulin-secreting cells, Orci (36) also assumed that microfilaments might be involved in exocytosis regulation in a more passive way, i.e., by controlling the accessibility of the cell surface for secretory organelles. This would be another possible way to explain the inhibitory effect of cytochalasin B treatment on the docking of trichocysts (12). However, experiments with "anti-cytoskeletal" drugs are generally difficult to interpret in a straightforward manner, not only because of additional side-effects (c.f. 11, 20), possibly related to some nonspecific binding to a variety of biomembranes (33, 41), but also because the binding properties might be quite different in protozoan cells (c.f. 9). The literature is contradictory concerning the effect of such drugs on secretory activity (49). Recent findings suggest that such drugs inhibit intracellular transport rather than the final exocytosis events (see references 14, 15, 22, 26, and 47). This would be in line with our conclusion that cytoskeletal elements are responsible more (or only?) for organelle positioning than for the active extrusion process. The frequently repeated postulate of the involvement of an actomyosin "mechanocomplex" (13, 39) in the actual membrane fusion process during exocytosis appears speculative, at least in the trichocyst system. The fact that microtubules remain attached to trichocysts even after docking—at least for some time—was interpreted above in the sense of a signal theory and would not necessarily, therefore, imply their direct involvement in exocytosis; the same would hold if they were depolymerized after docking at a later time (which we did not analyze).

A third point of attachment regulation involves the "plug" of electron-dense material at the potential attachment sites of trichocysts. This material will be characterized separately by cytochemical methods (52, 53). When a trichocyst is inserted, the "plug" material appears to be squeezed out and transformed partly into the "collar matrix" around the trichocyst tip and perhaps partly also into the small mass of "connecting

material" between trichocyst tip and plasma membrane (see Fig. 21).

We believe that the findings presented here are a useful ultrastructural basis for further investigations on the regulation of intracellular transport and the topological arrangement of presumptive exocytosis sites. In future work the collection of 24 known mutations of *P. tetraurelia* (17) should be investigated. All these mutations have defects in exocytosis regulation, and some of these may reside in cytoskeletal functions.

So far, our data provide a clear-cut ultrastructural support for the involvement of different "signals" in the positioning of trichocysts: a long-range signal (microtubules from ciliary basal bodies), a short-range signal (loopholes of microfilament fasciae), and possibly a target signal ("plug") in close apposition to the cell membrane.

We thank Mrs. D. Bliestle for photographic assistance, Mrs. R. Hildebrand for skillful technical assistance, and Dr. H. Matt for his help in standardizing the culture conditions. Part of the present work is from a thesis (C. Westphal) in partial fulfillment of the requirements for a PhD.

This study was supported by grants from SFB 138.

Received for publication 20 February 1981, and in revised form 28 July 1981.

Note Added in Proof: The labeled antibodies and Fab fragments directed against *P. tetraurelia* actin—as used here—have recently been characterized in detail by R. Tiggemann, H. Plattner, I. Rasched, P. Baeuerle, and E. Wachter (1981) in *J. Histochem. Cytochem.* 29: 1387–1396. Microfilaments thus labeled could perhaps account for the pivoting movements during trichocyst insertion (see reference 7).

REFERENCES

1. Allen, R. D. 1971. Fine structure of membranous and microfibrillar systems in the cortex of *Paramecium caudatum*. *J. Cell Biol.* 49:1–20.
2. Allen, R. D. 1978. Membranes of ciliates: utlrastructure, biochemistry and fusion. *In:* Membrane Fusion. G. Poste, and G. L. Nicolson, editors. North Holland Publishing Co., Amsterdam. 657–763.
3. Allen, R. D., and A. K. Fok. 1980. Membrane recycling and endocytosis in *Paramecium* confirmed by horseradish peroxidase pulse-chase studies. *J. Cell Sci.* 45:131–145.
4. Allen, R. D., and R. W. Wolf. 1974. The cytoproct of *Paramecium caudatum*: structure and function, microtubules, and fate of food vacuole membranes. *J. Cell Sci.* 14:611–631.
5. Allison, A. C., and P. Davies. 1974. Mechanisms of endocytosis. *In:* Transport at the Cellular Level. M. Sleigh, and D. H. Jennings, editors. Cambridge University Press, Cambridge. 419–446.
6. Aufderheide, K. J. 1977. Saltatory motility of uninserted trichocysts and mitochondria in *Paramecium tetraurelia*. *Science* (*Wash. D.C.*). 198:299–300.
7. Aufderheide, K. J. 1978. Motility events of trichocyst insertion in *Paramecium tetraurelia*. *J. Protozool.* 25:362–365.
8. Aufderheide, K. J. 1980. Mitochondrial associations with specific microtubular components of the cortex of *Tetrahymena thermophila*. II. Response of the mitochondrial pattern to changes in the microtubule pattern. *J. Cell Sci.* 42:247–260.
9. Aufderheide, K. J., J. Frankel, and N. E. Williams. 1980. Formation and positioning of surface-related structures in protozoa. *Microbiol. Rev.* 44:252–302.
10. Bannister, L. H. 1972. The structure of trichocysts in *Paramecium caudatum*. *J. Cell Sci.* 11:899–929.
11. Bauduin, H., C. Stock, D. Vincent, and J. F. Grenier. 1975. Microfilamentous system and secretion of enzyme in the exocrine pancreas. Effect of cytochalasin B. *J. Cell Biol.* 66: 165–181.
12. Beisson, J., and M. Rossignol. 1975. Movements and positioning of organelles in *Paramecium aurelia*. *In:* Nucleocytoplasmic Relationship During Cell Morphogenesis in Some Unicellular Organisms. S. Puiseux-Dao, editor. Elsevier, Amsterdam. 291–294.
13. Berl, S., S. Puszkin, and W. J. Niklas. 1973. Actomyosin-like protein in brain. *Science* (*Wash. D.C.*). 179:441–446.
14. Boyd, A. E., W. Bolton, and B. R. Brinkley. 1979. Microtubules are necessary for sustained insulin secretion. *J. Cell Biol.* 83(Z.Pt.2):333*a* (Abstr.).
15. Boyd, A. E., W. E. Bolton, J. Kelly, M. Snabes, J. Bryan, and B. R. Brinkley. 1980. Contractile proteins and insulin secretion. *Eur. J.Cell Biol.* 22:345.
16. Burridge, K., and J. H. Phillips. 1975. Association of actin and myosin with secretory granule membranes. *Nature* (*Lond.*). 254:256–258.
17. Cohen, J., A. Adoutte, and J. Beisson. 1980. Genetic dissection of the control of nuclear movements and positioning in *Paramecium*. *Eur. J. Cell Biol.* 22:358.
18. Cohen, J., J. Beisson, and J. B. Tucker. 1980. Abnormal microtubule deployment during defective macronuclear division in a *Paramecium* mutant. *J. Cell Sci.* 44:153–167.
19. Drenckhahn, D., U. Gröschel-Stewart, and K. Unsicker. 1977. Immunofluorescence—microscopic demonstration of myosin and actin in salivary glands and exocrine pancreas of the rat. *Cell Tissue Res.* 183:273–279.
20. Dustin, P. 1978. Microtubules. Springer-Verlag, Berlin.
21. Ehret, C. F., and E. W. McArdle. 1974. The structure of *Paramecium* as viewed from its constituent levels of organization. *In:* Paramecium. A Current Survey. Elsevier Scientific Publishing Co., Amsterdam. 263–338.
22. Friedman, J. E., P. I. Lelkes, K. Rosenheck, and A. Oplatka. 1980. Possible involvement of actin filaments in the generation of transmembrane potential and in stimulus-secretion coupling in chromaffin cells. *Eur. J. Cell Biol.* 22:345.
23. Gray, E. G. 1975. Presynaptic microtubules and their association with synaptic vesicles. *Proc. R. Soc. Lond. B Biol. Sci.* 190:369–372.
24. Hausmann, K., and R. D. Allen. 1976. Membrane behavior of exocytic vesicles. II. Fate of the trichocyst membranes in *Paramecium* after induced trichocyst discharge. *J. Cell Biol.* 69:313–326.
25. Heidemann, S. R., and M. W. Kirschner. 1975. Aster formation in eggs of *Xenopus laevis*. Induction by isolated basal bodies. *J. Cell Biol.* 67:105–117.
26. Kelly, J., A. E. Boyd III, J. Bryan, W. E. Bolton, and B. R. Brinkley. 1980. Actin and insulin secretion. *J. Cell Biol.* 87(Z,Pt.2):306*a* (Abstr.).
27. Kern, H. F., W. Bieger, A. Völckl, G. Rohr, and G. Adler. 1979. Regulation of intracellular transport of exportable proteins in the rat exocrine pancreas. *In:* Secretory Mechanisms. C. R. Hopkins, and C. J. Duncan, editors. Cambridge University Press, Cambridge. 79–99.
28. Korn, E. D. 1978. Biochemistry of actomyosin-dependent cell motility (A review). *Proc. Natl. Acad. Sci. U. S. A.* 75:588–599.
29. Lacy, P. E. 1975. Endocrine secretory mechanisms. A review. *Am. J. Pathol.* 79:170–187.
30. Lacy, P. E., E. H. Finke, and R. C. Codilla. 1975. Cinamatographic studies on β granule movement in monolayer culture of islet cells. *Lab. Invest.* 33:570–576.
31. Lacy, P. E., S. L. Howell, D. A. Young, and C. J. Fink. 1968. New hypothesis of insulin secretion. *Nature* (*Lond.*). 219:1177–1179.
32. Lagunoff, D., and E. Y. Chi. 1976. Effect of colchicine on rat mast cells. *J. Cell Biol.* 71: 182–195.
33. Mayhew, E., G. Poste, M. Cowden, N. Tolson, and D. Maslow. 1974. Cellular binding of [^{3}H]cytochalasin B. *J. Cell. Physiol.* 84:373–382.
34. Meldolesi, J., N. Borgese, P. DeCamilli, and B. Ceccarelli. 1978. Cytoplasmic membranes and the secretory process. *In:* Cell Surface Reviews, Vol. V. G. Poste, and G. Nicolson, editors. North Holland Publishing Co., Amsterdam. 509–627.
35. Ohtsuki, I., R. M. Manzi, G. E. Palade, and J. D. Jamieson. 1978. Entry of macromolecular tracers into cells fixed with low concentrations of aldehydes. *Biol. Cell* 31:119–126.
36. Orci, L., K. H. Gabbay, and W. J. Malaisse. 1972. Pancreatic beta-cell web: its possible role in insulin secretion. *Science* (*Wash. D.C.*). 175:1128–1130.
37. Plattner, H., F. Miller, and L. Bachmann. 1973. Membrane specializations in the form of regular membrane-to-membrane attachment sites in *Paramecium*. A correlated freeze-etching and ultrathin-sectioning analysis. *J. Cell Sci.* 13:687–719.
38. Pollard, T. D. 1980. Association of contractile proteins with microtubules and their possible participation in microtubule-dependent movements. *Eur. J. Cell Biol.* 22:289.
39. Poste, G., and A. C. Allison. 1973. Membrane fusion. *Biochim. Biophys. Acta.* 300:421–465.
40. Prentki, M., C. Chaponnier, B. Jeanrenaud, and G. Gabbiani. 1979. Actin microfilaments, cell shape, and secretory processes in isolated rat hepatocytes. Effect of phalloidin and cytochalasin D. *J. Cell Biol.* 81:592–607.
41. Riordan, J. R., and N. Alon. 1977. Binding of [^{3}H]cytochalasin B and [^{3}H]colchicine to isolated liver plasma membranes. *Biochim. Biophys. Acta.* 464:547–561.
42. Röhlich, F. 1975. Membrane-associated actin filaments in the cortical cytoplasm of the rat mast cell. *Exp. Cell Res.* 93:293–298.
43. Sherline, P., Y. C. Lee, and L. S. Jacobs. 1977. Binding of microtubules to pituitary secretory granules and secretory granule membranes. *J. Cell Biol.* 72:380–389.
44. Simionescu, N., and Simionescu, M. 1976. Galloylglucoses of low molecular weight as mordant in electron microscopy. I. Procedure and evidence for mordanting effect. *J. Cell Biol.* 70:608–621.
45. Smith, D. S., U. Järlefors, and R. Beránek. 1970. The organization of synaptic axoplasm in the lamprey (*Petromyzon marinus*) central nervous system. *J. Cell Biol.* 46:199–219.
46. Sonneborn, T. M. 1970. Gene action in development. *Proc. R. Soc. Lond. B Biol. Sci.* 176: 347–366.
47. Specian, R. D., and M. R. Neutra. 1980. Mucous granule transport and secretion: effects of colchicine and cytochalasin B. *J. Cell Biol.* 87(Z,Pt.2):300*a* (Abstr.).
48. Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26:31–43.
49. Stoessel, T. P., M. S. Bretscher, B. Ceccarelli, S. Dales, A. Helenius, J. E. Heuser, A. L. Hubbard, J. Kartenbecle, R. Kinne, D. Papahadjopoulos, B. Pearse, H. Plattner, T. D. Pollard, W. Reuter, B. H. Satir, M. Schliwa, Y. J. Schneider, S. C. Silverstein, and K. Weber. 1978. Membrane dynamics. *In:* Transport of Macromolecules in Celullar Systems. S. C. Silverstein, editor. Dahlem Konferenzen, Berlin. *Life Sci. Res. Rep.* 11:503–516.
50. Tiggemann, R., and H. Plattner. 1981. Localization of actin in the cortex of *Paramecium tetraurelia* cells by immuno- and affinity-fluorescence microscopy. *Eur. J. Cell Biol.* 24: 184–190.
51. Tilney, L. G., J. Bryan, D. J. Bush, K. Fujiwara, M. S. Mooseker, D. B. Muphy, and D.H. Snyder. 1973. Microtubules: evidence for 13 protofilaments. *J. Cell Biol.* 59:267–275.
52. Westphal, C., and H. Plattner. Ultrastructural analysis of the cell membrane-secretory organelle interaction zone in *Paramecium tetraurelia* cells. I. In situ characterization by electron "staining" and enzymatic digestion. *Biol. Cell.* In press.
53. Westphal, C., and H. Plattner. Ultrastructural analysis of the cell membrane-secretory organelle interaction zone in *Paramecium tetraurelia* cells. II. Changes involved in the attachment of secretory organelles. *Biol. Cell.* In press.
54. Westrum, L. E., and E. G. Gray. 1976. Microtubules and membrane specializations. *Brain Res.* 105:547–550.
55. Williams, N. E., P. E. Vaudaux, and L. Skriver. 1979. Cytoskeletal proteins of the cell surface in *Tetrahymena*. I. Identification and localization of major proteins. *Exp. Cell Res.* 123:311–320.