EVIDENCE FOR FIRM LINKAGES BETWEEN MICROTUBULES
AND MEMBRANE-BOUNDED VESICLES

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Microtubules are known to be located in many cells and organisms in regions and orientations paralleling the axes of a variety of intracellular vectorial movements of membrane-bounded particles. The relationship of these microtubules to the particles varies and thus the roles proposed for the microtubules have also varied. In some cases, such as in killifish melanophores (5, 12, 28, 29), melanosomes move within a channel more or less framed by microtubules. Here the microtubules may simply form a channel through which particle movement is directed, although the possibility that the microtubules provide the motive force for aggregation of melanosomes has also been suggested. In other cases, such as in the axons of neurons, the microtubules at times are obviously joined to the synaptic vesicles by 6–10-nm long bridges (24). In these situations a more intimate role of the microtubules in particle movement is suspected.

Additional evidence for the role of microtubules in the movement of membrane-bounded particles comes from the use of microtubule-active agents. These studies, using colchicine, vinblastine, and vincristine, show that the cessation of vesicular movement is at times correlated with the disappearance of microtubules (15, 16, 18, 27, 29, 30). However, other reports have also shown that these alkaloids may cause the cessation of movement without having any apparent effect on microtubules (8, 9, 17).

Many of the above examples of vesicular movement occurring in the presence of microtubules are
found in long narrow cell processes where the lateral movement of particles would naturally be restricted by the covering plasma membrane. This situation is true of neuron axons (e.g., 24, 25), of the heliozoan axopodia (22, 26), and of the suctorian tentacles (4, 23) and, to a lesser extent, it is true in the processes of melanophores (5, 12, 19, 28, 29) and chromatophores (21). It could be argued that the proximal association between the microtubules and particles in these cells is more a result of the small diameter of the cell process than of an actual physical linkage between the two. Thus in these examples, with the possible exception of some axons where bridges are seen, the microtubules can be viewed as structural components only which do not contribute in any way to the motive force which initiates and sustains particle movement.

Other examples not subject to this criticism have been reported where microtubules do lie within open, unbounded cytoplasm and where membrane-limited organelles and vesicles move in patterns paralleling these arrays of microtubules (11, 16, 18, 27). However, no links have been seen between the microtubules and the particles in these cases, so the role of the microtubules here remains in doubt.

Three questions regarding the relationship of microtubules to particles will be considered here. First, do membrane-bounded particles bind to microtubules in open cytoplasm; second, are bridges the only method by which microtubules are attached to intracellular particles; and third, how strong are the observed microtubule-membrane associations? To the author's knowledge the strength of such associations has not yet been tested. On the other hand, it has been shown that nonmembrane-bounded adenovirus particles are attached to microtubules firmly enough to withstand the tubulin crystallization effects of vinblastine and the subsequent isolation of these crystals (7).

Observations confirming a strong attachment between microtubules and membrane-bounded particles within the open cytoplasm of a large cell will be reported in this paper. However, this attachment may not be by way of bridges. These observations permit a reevaluation of, and can be used as further support for, the suggestion that microtubules are intimately involved in moving vesicles and cell organelles in the cytoplasm.

MATERIALS AND METHODS

Paramecium caudatum cells grown in hay infusion were used. Observations were made on cells fixed at different times over the past several years. Two principal fixations were used with some minor variations. First, cells were fixed in a 2% solution of 0.034 or 0.05 M collidine-buffered glutaraldehyde before postfixation in 1% osmium tetroxide. Cells in this group had either no pretreatment or a 1-h pretreatment in 1 mM KCl + 1 mM CaCl₂ solution (1). Others were starved for 2 h and then refed (3). In the second group, cells were fixed in 2% glutaraldehyde buffered with 0.05 M cacodylate and postfixed in 1% OsO₄ (1). Finally, the most recent fixation combines the above procedures. This fixation consisted of a 3-4-min pretreatment in 0.05 M cacodylate-buffered 1%...
glutaraldehyde before a longer 15-min fixation in 0.05 M collidine-buffered 1% glutaraldehyde which was then followed by the usual OsO₄ postfixation and in block staining with a 0.5% solution of uranyl acetate.

In most preparations serial sections were picked up on Formvar-supported single-hole grids, stained with uranyl acetate followed by lead citrate, and observed in either a Philips 300 or a Hitachi HU-11A electron microscope.

OBSERVATIONS

After cacodylate-buffered fixations the cytoplasm appears relatively electron opaque (Fig. 1). Glycogen which is preserved by this technique tends to mask the cytoplasmic microtubules. However, ribbons of cytopharyngeal microtubules (see reference 2) can be detected in the cytoplasm when disk-shaped vesicles (d in Fig. 1) are aligned in single file along the tubules (cr in Fig. 1). After this fixation, the microtubules are either straight or curved in wide arcs. This seems to be their normal or undisturbed appearance.

Disk-shaped vesicles lie flat against the broad face of the ribbons. As many as 12–15 vesicles were observed to lie edge to edge along a single ribbon (Fig. 2), but the maximum number that can occur is unknown since the ribbons rarely remain in the plane of a single section for more than a few micrometers.

These vesicles do not touch the microtubules but remain at a distance of 30–40 nm from the tubule. The space between the vesicle and microtubule does not appear to be empty but is definitely less electron opaque than either the membrane or tubule wall (Fig. 5). A finely filamentous material occupies this space, whereas bridges such as those seen by Smith (24) are not present. There seems to be an asymmetry to the two sides of the ribbon even in areas not closely associated with vesicles (Figs. 1, 2, 5). The 40-nm coating of filamentous material lines the ribbons on the side next to the vesicles, even where vesicles do not occur. However, on the opposite side of the same ribbon, cytoplasmic ribosomes and glycogen granules closely approach the microtubules. Vesicles generally appear to lie only on the side of the ribbon with the 40-nm filamentous coat except at the ribbon's cytostomal end where vesicles lie close to both sides of the ribbons (see reference 2).

Collidine-buffered fixatives seem to disturb the cytoplasm more than the cacodylate-buffered fixatives (Figs. 3, 4). With the collidine-buffered fixatives, the glycogen is not retained, the endoplasmic reticulum swells and vesiculates, and the trichocysts discharge internally. The cytoplasm is, therefore, less electron opaque so that microtubules are easier to see. The profiles of these microtubules, however, often exhibit unnatural bends and folds (Figs. 3, 4). In regions where vesicles are associated with these cytopharyngeal ribbons, the ribbons sometimes appear compressed into a zigzag outline. Significantly, the vesicles continue to maintain their close, 40 nm, association with the microtubules and to follow the same zigzag path. If the microtubules bend at intervals close to the same length as the diameter of the vesicles, the vesicles themselves may remain unbent (Fig. 3). However, sometimes the bends occur at intervals less than the diameter of the vesicles and, in these cases, the vesicles may bend as the microtubules bend (Fig. 4). This suggests that the vesicles must be attached to the microtubules at many points to maintain such a constant distance from the tubules.

The space between the microtubules and vesicles after this fixation is also filled with a finely filamentous material. This material is of the same density as the background cytoplasm (Fig. 3) or is slightly more electron opaque (Fig. 4). The asymmetrical location of this filamentous material on one side of the microtubules is not evident after the collidine-buffered fixation. This fixation does not seem to preserve this material as well as the cacodylate-buffered fixative.

A specific search was made for bridges such as those that have been described between microtubules and membrane-bounded organelles and vesicles (10, 13, 24). Occasionally an 8-nm wide link of low contrast (arrows, Figs. 6, 7) could be seen crossing this 30–40-nm space but such “bridges” were only seen infrequently. At other times a thin filamentous line was found halfway between the microtubules and the vesicles (Fig. 7). This line may be an artifact due to a compression of the surfaces of two electron-transparent coatings, one next to the microtubules and one coating the vesicle, when they lie next to each other.

DISCUSSION

The observations presented here give strong support to the concept that membrane-limited particles can be firmly bound to microtubules. The very fact that the flattened vesicles accumulate at a constant distance from the microtubules and that these vesicles also become oriented in special ways to the microtubular band suggests that the two are
FIGURE 6 An enlarged portion of Fig. 3. The space between the microtubule and vesicles is little different from the background cytoplasm. An occasional bridge, in this case 8 x 30 nm, can be detected (arrow). x 100,000.

FIGURE 7 Microtubular bands (cr) close to the cytopharynx lie in close proximity to each other. Bridges (arrows) between the tubules and vesicles (d) can sometimes be imagined. A faint fibrillar-like line lying between one set of obliquely sectioned microtubules and a vesicle (indicated by brackets) may only be the interface between electron-transparent material coating the vesicle and that coating the tubules. x 100,000.

strongly attracted to each other. Furthermore, the presence of some type of bond is confirmed by the results obtained with the collidine-buffered fixation. When the bands are compressed into zigzag patterns during this fixation the vesicles are still 30–40 nm from the bands and, more important, they are found in an identical zigzag pattern. This means that during compression many of the vesicles were moved along the axis which paralleled the axis of compression and, in addition, were tilted with respect to this axis. The most logical explanation that would explain the identical patterns assumed by both the microtubules and the rows of vesicles is that the two are firmly bound to each other, firmly enough, in fact, to withstand the considerable forces of compression which buckled the ribbons. Other explanations, including the possibility that the vesicles and microtubules are confined together by the surrounding cytoplasm, are not probable since these ribbons are not found within narrow plasma membrane-limited cell processes but are found freely situated in the unbound fluid cytoplasm of a very large cell, about 180 x 50 μm in length and diameter. Also, a microtubular ribbon being flat and having about the same width as the vesicles could not form a channel to enclose these vesicles.

What is the nature of the attachment? This question cannot be answered at the present time. Links similar to bridges (10, 13, 24) were rarely seen so that it seems unlikely that bridges alone can account for the attachment. The possibility remains that the procedures used in this study neither preserve the bridges nor stain them sufficiently to make them visible in electron micrographs. However, bridges were seen in other areas of other Paramecium that were fixed in the same manner as those studied here. Bridges are present between microtubules of the postoral bundles and also between microtubules and the membrane of food vacuoles which are located near the cytoproct (3) as well as linking the cytopharyngeal microtubules to the cytopharyngeal membrane (2). Thus, if bridges are in fact present between the disk-shaped vesicles and cytopharyngeal ribbons, they should be detectable after the use of procedures identical with those that preserved bridges at other sites in Paramecium cells.

Although bridges were rarely seen in the interspace between the vesicles and microtubules this space was always filled with a low-contrast finely filamentous-to-amorphous material which may be similar to the filamentous material previously shown to coat microtubules in some axons (6, 14). This material seems to line the microtubular ribbons only on the side of the ribbon to which vesicles are attached and is present along this side of the microtubules whether vesicles are attached or not. It is this material which appears to attach the vesicles to the microtubules. This possibility that particles bind to microtubules in the absence of bridges may help in the interpretation of other observations (e.g. 11, 27) where microtubules parallel certain intracellular movements but where
bridges were not found. This present study clearly indicates that an apparent lack of bridges does not always indicate the absence of binding.

What would be the function of vesicle-to-microtubule binding? One strong possibility is that vesicle binding to microtubules is somehow involved in particle movement. Evidence for the movement of these disk-shaped vesicles in association with microtubular bands as a part of a process of recycling food vacuole membranes is presented in another paper (2). For such a movement to occur along flattened ribbons in open cytoplasm, a relatively firm binding must occur either permanently or temporarily between these two components. Then, whether the movement of these vesicles is subsequently due to a displacement of the microtubules as the microtubules grow from one end or to a shearing of the vesicles along stationary microtubules either by the repetitious formation and breaking of bonds that pass directly between vesicles and tubules (24) or, indirectly, through an intermediate microfilament (20), has yet to be determined.

SUMMARY

Direct evidence is presented in support of the widely held idea that membrane-bounded vesicles can bind firmly to microtubules. This is shown in P. caudatum which contains ribbons of straight microtubules located in open cytoplasm and uniquely associated with the disk-shaped vesicles. These vesicles frequently lie flat against the face of the ribbons at a constant distance of 30-40 nm. Under certain conditions the ribbons are compressed into zigzag patterns, but the vesicles continue to maintain their 30-40-nm spacing with the tubules and consequently they, too, assume the zigzag pattern. The author's interpretation of this phenomena is that the vesicles and the microtubules are strongly bound together. This interaction appears to be via a filamentous material rather than bridges.

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