

# Dynamic Shape Changes of Cytoplasmic Organelles Translocating along Microtubules

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**Abstract.** Transient shape changes of organelles translocating along microtubules are directly visualized in thinly spread cytoplasmic processes of the marine foraminifer, *Allogromia laticollaris*, by a combination of high-resolution video-enhanced microscopy and fast-freezing electron microscopy. The interacting side of the organelle flattens upon binding to a microtubule, as if to maximize contact with it. Organelles typically assume a teardrop shape while moving, as if

they were dragged through a viscous medium. Associated microtubules bend around attachments of the teardrop-shaped organelles, suggesting that they too are acted on by the forces deforming the organelles. An 18-nm gap between the organelles and the microtubules is periodically bridged by 10-nm-thick cross-bridge structures that may be responsible for the binding and motive forces deforming organelles and microtubules.

**D**IRECTED movement of cytoplasmic organelles and chromosomes occurs in most cells. Recently, it has become clear that there are at least two classes of motility machinery for moving cellular organelles. The first class, which is characteristically microtubule-based and supports bidirectional movement, can be directly visualized in cultured epithelial cells (11), in the squid giant axon (2, 22), in a protozoan (17), and on isolated flagellar microtubules (9). The second class of motility mechanism characteristically utilizes actin filaments, promotes unidirectional movement, and can be directly visualized in characean algae cells (14, 16). The isolation of the functional motile machinery from cytoplasm has provided new insights into the mechanisms of both classes of organelle movement (1, 2, 14, 18, 22, 23). It now seems clear that the common principle is that motive forces are generated at an interface between an organelle and a microtubule (2, 18, 22) or actin filament (1, 14, 16, 23), and that the direction of organelle movement, for a particular motor, depends on the polarity of the filament (14, 23, 28). Other cytomatrix components are not necessary and may even hinder organelle translocation, explaining in part the dependence of the velocity of movement on organelle size and shape (22). Detailed light-microscopy studies have also shown that large organelles may undergo shape changes related to the shear force produced during their translocation (10, 15, 19).

More recently, it has been demonstrated that purified proteins can generate movements similar to native movements along the surfaces of isolated, purified microtubules. These molecular motors translocate beads along microtubules, or the microtubules themselves, along the surface of a glass coverslip (28). However, it is not yet clear what role these

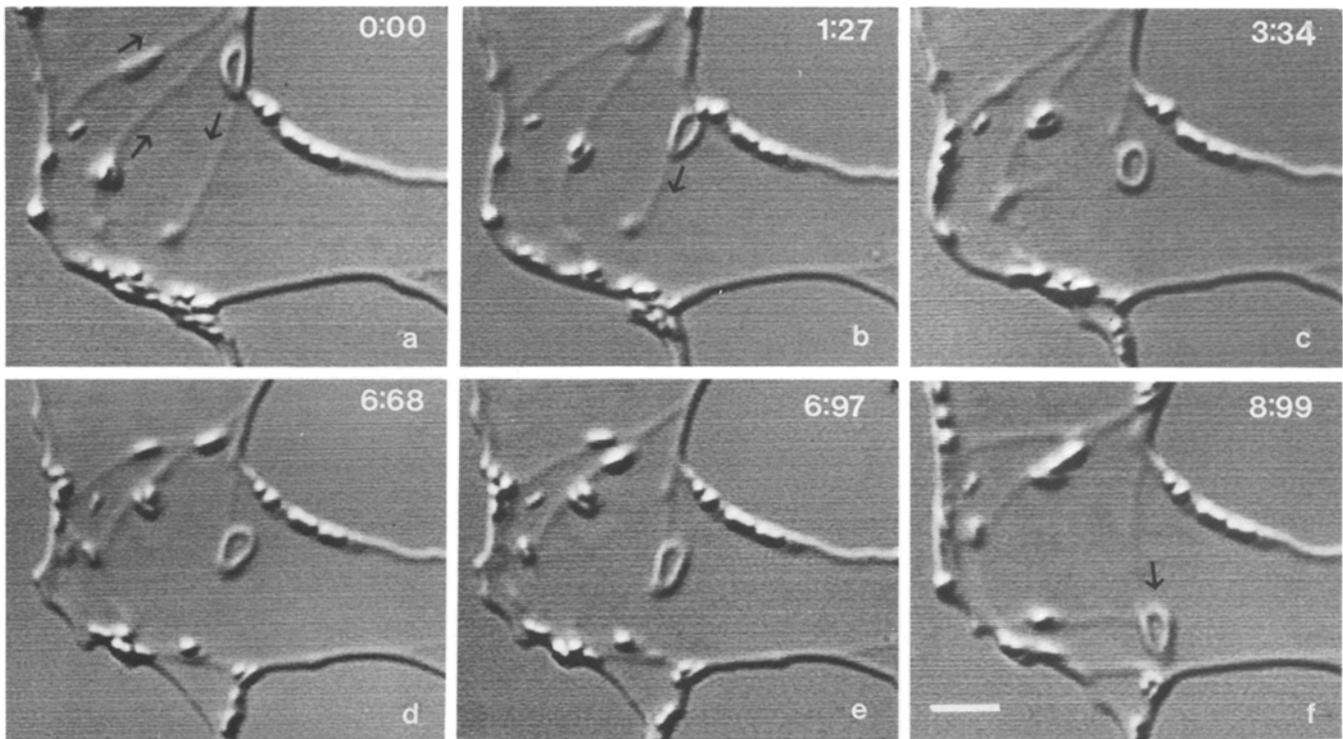
molecular motors have and how they are organized at the interfaces between microtubules and endogenous organelles in intact cells.

*Allogromia laticollaris* is a marine foraminifer that displays extraordinary directed movements of cytoplasmic organelles in all parts of its reticulopodial network (3, 25, 26). These movements appear to be associated with microtubules (25). The high frequency of movement and the large size of the organelles in *Allogromia* provide an ideal system for studying the structural basis of organelle movement in intact cytoplasm. We have used video-enhanced microscopy (13) with an improved illumination procedure coupled with electron-microscopic cryotechniques (4, 5, 12) to investigate the structural basis of organelle translocation. The rapidity of freezing and freedom of the cryotechniques from artifacts of direct chemical fixation is presumed to provide a more realistic picture of organelle-substrate interactions. Images of fast-frozen cells show characteristic deformations of organelles and microtubules that correlate with the transient deformations of organelles that occur during movements along microtubules seen directly with video microscopy. On the basis of these organelle deformations, we can infer properties of their interactions with microtubules which are the basis of organelle translocation.

## Materials and Methods

### Cell Culture

*Allogromia laticollaris* were grown in flasks containing Alga-gro seawater (Carolina Biological Supply Co., Burlington, NC) where they were fed a diet of cocultured algae, diatoms, and bacteria. Individual *Allogromia* were



**Figure 1.** Sequential video micrographs showing movements of organelles along transport tracks in a thin, lamellipodial extension of cytoplasm of *Allogromia*. Time elapsed in seconds between each video frame is indicated in top right corner. (a) Organelles initially were moving along three distinct tracks in the direction indicated by the arrows. The organelle moving from top to bottom in the center of this frame is large and has a translucent content so that its membrane contour is clearly visualized. The side of the organelle next to the transport track is flattened, as if to maximize contact. (b) This same organelle moves down and switches to the other side of the transport track, but remains flattened against it. In addition, the contact angle with the transport track differs at its front and rear ends, so the organelle now has a teardrop shape that is maintained while it is moving. (c) The transport track is no longer visible and the immobile organelle returns to its spherical shape. (d-f) The organelle progressively deforms as contact develops with the transport track and directional movement begins again. Bar, 1.3  $\mu\text{m}$ .

removed from the culture flasks, rinsed, and placed on a drop of calcium-free artificial seawater on the surface of polylysine-coated coverslips to stimulate the formation of thin lamellipodia (25, 26).

### Critical Illumination Video Microscopy

The aperture of a 1.4 NA condenser in a Zeiss Axiomat microscope (Carl Zeiss, Inc., Thornwood, NY) in the differential interference contrast (DIC) mode, equipped with an internally corrected  $\times 100$  1.3 NA planapochromatic objective, was fully illuminated with a 100-W mercury lamp aligned for critical illumination (8). The change from Kohler illumination to critical illumination is obtained by means of defocusing the image of the light source at the condenser aperture plane by adjustments in the collector lens inside the lamp housing. The image of the light source now becomes focused in the specimen plane. When the image of the light source at the condenser aperture plane is replaced by an evenly and fully illuminated field, the effective aperture and the resulting resolution become maximal (8). The image plane becomes more strongly illuminated in the center of the field which is not a problem in that only a small area from the center of the microscope field of view is projected onto the surface of the video tube of camera at the very high magnification used in video-enhanced microscopy. This illumination condition provides the intense illumination that is needed to increase the signal-to-noise ratio of the video image at very high magnifications (the magnification of the image on the TV monitor was 20,000). The video camera used for the contrast enhancement (13) was a Newvicon Dage-68 (Dage-MTI, Inc., Michigan City, IN). Further manipulations of the gray scale were produced with the aid of a Image I digital image processor (IVS, Concord, MA).

### Fast Freezing for Electron Microscopy

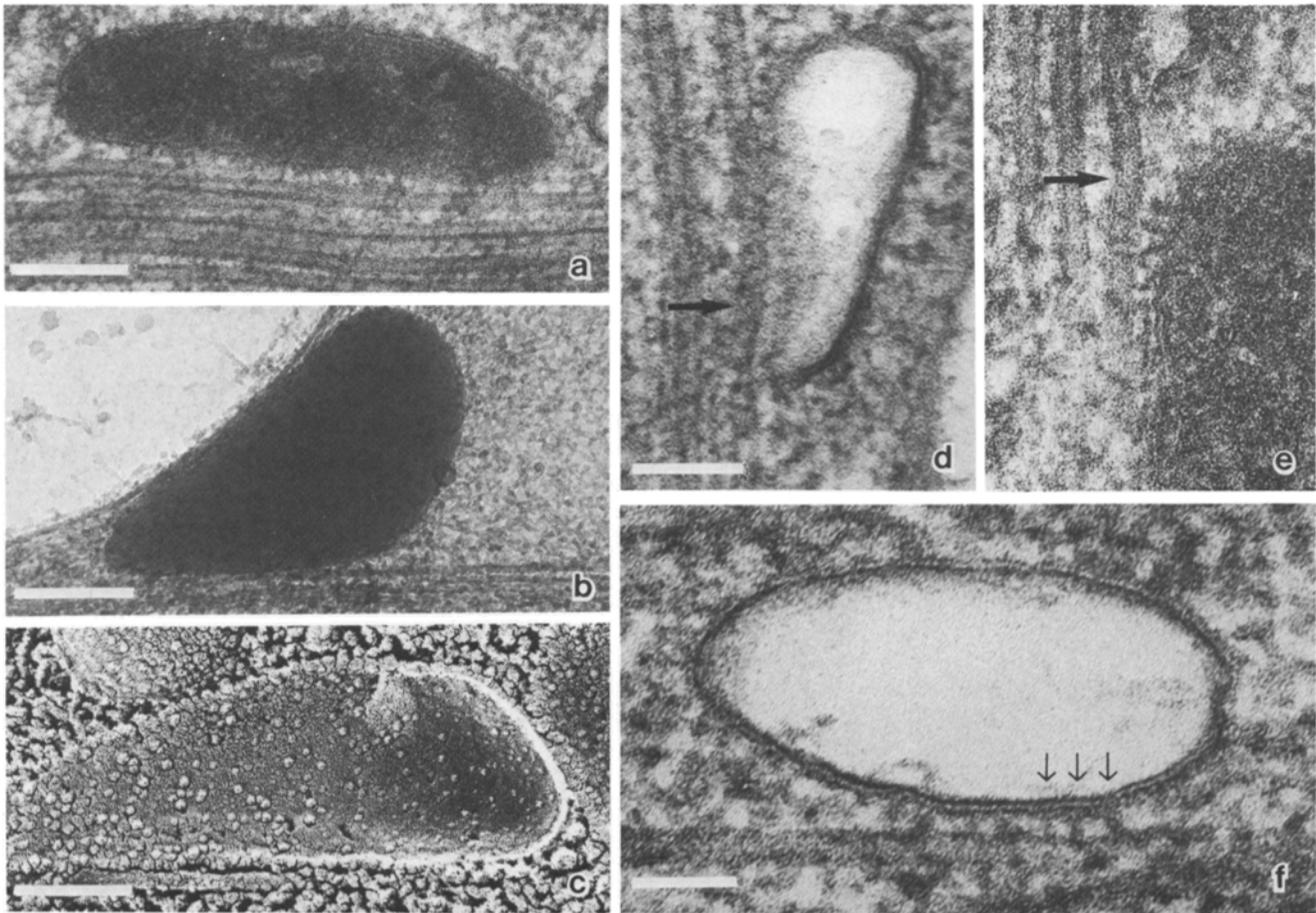
Fast freezing in combination with freeze-fracture (12) or freeze-substitution

(3, 4) was used to prepare tissue for thin sectioning or for electron microscopy of whole mounts. Before freezing, individual *Allogromia* were placed on the surface of either formvar-coated electron-microscopic grids or gelatin in calcium-free artificial seawater for 1–3 h. During this time *Allogromia* grew an extensive network of processes that displayed frequent movements of organelles. Cultures on grids were frozen by rapid injection of the grid into a stirred mixture of propane/ethane (3:1) cooled by liquid nitrogen (4, 5). Specimens were then freeze-substituted and either critical-point-dried to prepare them for whole-mount observation (4, 5) or embedded in plastic to prepare them for thin sectioning. Cultures on gelatin, fast-frozen by slamming them against a copper block cooled by liquid helium (12), were either freeze-fractured and etched, or embedded in plastic for thin sectioning by conventional techniques. Freeze-etching replicas, thin sections, and whole mount preparations were examined in a JEOL 200-CX electron microscope (JEOL USA, Peabody, MA).

## Results

### Video Microscopy

Video microscopy of thinly spread cytoplasmic processes of *Allogromia* shows organelles being transported at rates up to 5  $\mu\text{m/s}$  (3, 25, 26) along linear tracks (Fig. 1). The range of diameters of organelles in transport is quite broad. Many organelles appear as faint structures without defined boundaries and with an apparent diameter of 0.1–0.2  $\mu\text{m}$ . These structures are very likely to have an actual size of 50–100 nm (2, 11), which is below the theoretical limit of resolution of



**Figure 2.** Electron microscopy of organelles moving along microtubules after direct freezing. (a) Thin section of a mitochondrion, (b) whole mount of an electron-dense granule, and (c) freeze-fracture replica of a large vesicular organelle all show that the side of the organelle in contact with the microtubule is flattened. The degree of deformation varies for different organelles. (b) The electron-dense granules and (c, d) the translucent vesicular organelles show the most deformation and make the largest contacts with the microtubules. Organelles often show an asymmetry in relation to their long axis, displaying a teardrop shape which corresponds to images of organelle deformation observed in the video images of living cells. (d, e) Sharp deflections of the microtubule occur at each end of an organelle contact and can be seen in different orientation in thin sections. (f) Filamentous structures, 10 nm in diameter and 18 nm long, bridge the gap between microtubules and organelles at periodic spacings of 23 nm (arrows). Bars, 140 nm (a); 260 nm (b); 160 nm (c); 110 nm (d, e); 80 nm (f).

the light optical system, yet they are detected with this technique (11). The trajectory of these organelles can be visualized but details of their shapes and associations with the tracks cannot be observed. However, a large number of organelles that are moving along the tracks have diameters of 0.5–1.0  $\mu\text{m}$ , permitting subtle shape changes occurring during translocation to be resolved by the video microscope set up for critical illumination microscopy.

One type of vesicular organelle, up to 1–2  $\mu\text{m}$  in diameter with a translucent content, is ideal for detection of subtle shape changes associated with its binding and translocation along the tracks. The tracks themselves also show translational movements and sometimes elongate, or shorten, or even disappear (Fig. 1). This dynamic assembly and disassembly of tracks provides an opportunity to observe the shape changes that occur during binding, translocation, and release of the organelles from these tracks. In a typical cycle of shape changes during translocation of an organelle, its interacting side is flattened against the track that it is moving along. When the track disassembles, the organelle is subsequently immediately released, becomes immobile, and as-

sumes a symmetrical shape (Fig. 1 c). Upon reassociation with a track, the organelle progressively deforms, as if to maximize contact with the microtubule. In addition, moving organelles often assume a teardrop shape (Fig. 1, b and d–f), which can be interpreted to result from the shear produced by their movement against the resistance of the viscous cytoplasm. Organelles often switched from one track to a nearby or overlapping track, and even moved along two tracks simultaneously (26), further suggesting that they form force-generating contacts with the microtubule-associated tracks at multiple locations and orientations on their surfaces.

### Electron Microscopy

Results from electron microscopy of thin sections, whole mounts after freeze-substitution, and freeze-etch replicas of directly frozen specimens (Fig. 2, a–c) are in agreement that the linear tracks associated with organelle translocation are individual microtubules or bundles of microtubules, and that no other long filaments are associated with the organelles.

The three most frequent types of large organelles are elongated mitochondria, electron-opaque granules, and large membrane vesicles with an electron-lucent core (Fig. 2).

Examination of thin sections (Fig. 2, *a* and *f*) and freeze-etch replicas (Fig. 2 *c*) showed the close associations of organelles with microtubules, either singly or in bundles. Typically the organelles are separated from the microtubules by a gap bridged by thin, periodically spaced filaments. The dimensions of these features measured from thin-section electron micrographs were as follows: the gap between organelle and microtubule,  $18.4 \pm 2.8$  nm ( $n = 50$ ); the diameter of the filaments that cross-bridge the gap between organelles and microtubules,  $10.1 \pm 1.5$  nm ( $n = 46$ ); the distance between successive cross-bridges,  $22.8 \pm 3.5$  nm ( $n = 66$ ). The diameter of the microtubules in the same set of micrographs was  $25.7 \pm 1.4$  nm ( $n = 41$ ).

The organelles associated with microtubules have an asymmetrical shape because the side of the organelle next to the microtubule is flatter. This deformation of the organelles is more apparent in the whole mount (Fig. 2 *b*) and freeze-etch (Fig. 2 *c*) preparations than in thin sections, where the plane of section is only occasionally optimal for exposing the flattened side of the organelle. There is often an additional asymmetry along the long axis of the organelle; typically, one end of the organelle displays a more pronounced curvature than the other, giving it a teardrop shape. The direction of movement of these organelles at the moment of freezing can be deduced in most instances by comparison with the images of organelle deformation observed in the live video images, where the more sharply curved end of the organelle is the leading end and the trailing end has the larger diameter. Occasionally, an organelle traveling along a microtubule bundle encounters other structures, such as the plasma membrane (Fig. 2 *b*) or other organelles, and appears to be even further deformed.

## Discussion

The organelle shapes arrested by direct freezing parallel the dynamic configurations of moving organelles observed with video microscopy (compare Fig. 1, *d* and *e* to Fig. 2, *b-d*). Electron microscopy shows that these organelles are moving along microtubules. Although associations of cytoplasmic organelles with microtubules have been reported in a number of electron microscopic studies of conventionally fixed cells (2, 4-6, 11, 17, 18, 20, 22, 24) including *Allogromia* (3, 25), specific changes in shape have not previously been related either to these associations or to directed movements. The correlation of images from video microscopy of living cells with images from directly frozen specimens now shows dynamic changes in the shape of the organelles while they are moving along microtubules. In addition to flattening of the side of the organelle that interacts with the microtubule, they typically assume a teardrop shape, which is interpreted to result from the shear produced by their movement against the resistance of the viscous cytoplasm. This specific teardrop shape deformation of translocating organelles has been previously observed in fish eosinophils (10).

The interaction of an organelle and a microtubule also results in deformation of the microtubule. A sharp deflection of the microtubule occurs along the interface with the organelle (Fig. 2, *d* and *e*). This phenomenon appears to be

present also in squid axoplasm (see Figs. 5 and 7 in reference 20). Although microtubules are relatively stiff structures that provide support for the movement of organelles they can bend elastically, as seen here in intact cytoplasm as well as in dissociated cytoplasm preparations (2, 22, 28). The mutual bending of the microtubule and flattening of the associated organelle suggest that tight mechanical coupling extends the length of the interface between the organelle and microtubule and that viscous resistances are encountered by the organelle moving through the cytoplasm.

The filamentous structures that bridge the gap between organelles and microtubules in *Allogromia* have dimensions comparable to those of similar structures between organelles and microtubules in squid axoplasm (20). Although we cannot be sure that organelle binding and movement require multiple cross-bridges, these appear to be ubiquitous at large organelles. Multiple microtubule-binding sites on the surfaces of organelles have also been inferred from *in vivo* (15, 19) and *in vitro* (2, 22) observations of organelles being transported simultaneously along the surface of two or more microtubules.

Two ATPases that appear to be involved in many kinds of animal cell motility are myosin which, in conjunction with actin, is involved in many types of nonmuscle motility, and dynein which acts in conjunction with ciliary and flagellar microtubules. Dynein-like molecules may also play a role in other types of cell motility, including organelle movement (7). More recently, a third type of translocator, kinesin, has been characterized which promotes movement of beads and organelles along microtubules purified from squid brain (27), though the role of kinesin in organelle translocation in cells needs further clarification. The evidence presented here, showing deformation of both organelles and microtubules during translocation, suggests that the translocating organelles are tightly bound to microtubules during their translocation. The cross-bridges at these interfaces provide an adequate explanation of the binding and are likely to represent components of whatever translocator molecules are responsible for the microtubule-based organelle movements in *Allogromia*. The similarity of the cross-bridge attachment seen in organisms as diverse as *Allogromia* and squid (20) further supports the contention that microtubule-based vesicle transport is analogous to other cell motile systems such as muscle and axonemes which are based on a cross-bridge-mediated system (20).

Strong binding to the microtubules would explain why organelles moving along isolated microtubules show no Brownian movement (2, 22). The discontinuous or saltatory (21) nature of the movements typical of intact cytoplasm may result from interactions of moving organelles with the meshwork of cytoskeletal components (4, 5, 15), and might involve elastic deformations of microtubules and organelles while they squeeze by barriers in the cytoplasmic meshwork. Further shape changes including formation of branches may occur in some large, elongated organelles at points of intersections of microtubules (15, 19). Analysis of the deformational stress measured in particles with known mechanical properties could provide estimates of the adhesive forces exerted by the molecular mechanism for binding and force generation.

The extensive interface of interaction between an organelle and microtubule, the presence of cross-bridge elements, and

the tight binding of the organelle to the microtubule, as implied by the deformation of organelles and microtubules, together provide a framework within which to consider the molecular mechanism of how translocator proteins move organelles.

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