Direct Visualization of Organelle Movement Along Actin Filaments Dissociated from Characean Algae

Abstract. *A system has been developed in which organelle transport can be studied without the influence of an organized cellular cytoplasm. Binding and continuous unidirectional movement of organelles along isolated cellular transport cables were directly visualized by video light microscopy after the dissociation of the cytoplasm of characean algae cells in a Ca^{2+}-free buffer containing adenosine triphosphate. Individual organelles had more than one attachment site and moved at mean rates of 11.2 or 62.1 micrometers per second along multiple parallel pathways on each cable. Electron microscopy of these cables after direct freezing demonstrated that they consist of compact bundles of actin filaments. Under these conditions, characteristics of organelle movement should reflect directly the underlying molecular processes of binding and force generation.*

Intracellular transport is a general term for phenomena that participate in cell division, metabolism, secretion, and motility. Directional movement of membrane-limited organelles is a common type of intracellular transport and has been observed in many different cell types (*1*). A direct assessment of the dynamics of the motile mechanism in intact cells is hindered by the presence of the many organelles and filamentous structures that coexist with the motile elements within the cytoplasm. Changes in the rate of organelle movement during experimental manipulations may be attributed not only to alterations of the motive force but also to changes in the viscosity of the cytoplasm. Another problem is the presence of the plasma membrane which insulates the cell from external influences and biochemical and pharmacological manipulations. Giant characean algae cells have been used as a model system to study organelle movement (*2*). Many characteristics of cytoplasmic streaming in intact characean cells (*2, 3*), as well as rotational and translational movement of chloroplasts or chloroplast chains in extruded droplets of cytoplasm (*2, 4*), have been studied. I now describe a system made of discrete actin cables, isolated free of extraneous material from a characean alga, that continues to transport membrane-bound organelles for long periods of time.

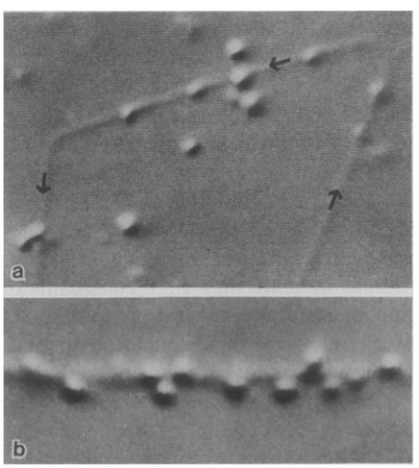

Fig. 1. (a) Dissociated *Chara* cytoplasm showing several organelles either free or associated to an isolated transport cable. In this ATP-containing preparation, all the organelles attached to the cable moved continuously (as in a conveyor belt) in the direction indicated by the arrows. Organelles that were not attached to the cables displayed free Brownian motion and constantly appeared and disappeared from the plane of focus, which corresponded to a 200-nm optical section in the specimen. Magnification, ×4500. (b) Immobile organelles attached to a segment of a transport cable from a preparation without added ATP. Magnification, ×7500.

Giant internodal cells of the alga *Chara* (Carolina Biological Supply Co.) were isolated from neighboring cells and rinsed and incubated for 20 minutes in a buffer containing 4 m*M* EGTA, 25 m*M* KCl, 4 m*M* $MgCl_2$, and 5 m*M* imidazole at *p*H 7.0. An internodal cell was blotted on tissue paper and transected at one end in order to drain the contents of the giant central vacuole. The cell was then lifted at one end with a pair of forceps and, with the aid of another forceps whose tips were covered with Teflon tubing, a cylinder of cytoplasm was gently extruded into a drop of isotonic buffer (4 m*M* EGTA, 25 m*M* KCl, 4 m*M* $MgCl_2$, 5 m*M* tris, and 200 m*M* sucrose at *p*H 7.6) on a thin microscope slide. The cylinder of cytoplasm without the cell wall is a protoplast, with an intact plasma membrane that prevents the dispersion of the cytoplasmic contents (*2*). Fine needles were used to break this membrane and gently disorganize the cytoplasm. The buffer was then replaced with fresh isotonic buffer solution containing 2 m*M* adenosine triphosphate (ATP) by slow, continuous, addition and removal of solution from the edges of the drop. Finally, the cytoplasm was further disorganized until its components were dispersed into the small drop of buffer containing ATP. The drop was covered with a cover glass and sealed with Vaseline. Samples were then viewed by video-enhanced differential interference contrast microscopy on a Zeiss Axiomat inverted light microscope (*5*).

Most dispersed cytoplasmic elements could be identified directly. Chloroplasts appeared to be isolated or associated end to end in the form of chains by three to six barely visible, parallel, subcortical cables (*6*). Many of these cables were partially or totally detached from the associated chloroplasts. Small spherical organelles were suspended in the medium and exhibited Brownian motion. When ATP was present, some organelles moved unidirectionally along the chloroplast chains or along the barely visible, isolated cables. By means of the higher magnification and the improved contrast of the video image, isolated cables, with diameters below the limit of resolution of the optical image (*7*), were visualized. Isolated cables were thus observed in extensive areas in which they and the small organelles were the only components present (Fig. 1a). Typically, the cables appeared as stiff segments with smooth surfaces, apparent diameters of 0.2 to 0.3 μm (*7*), and lengths ranging from 1 to >100 μm. They appeared either unconnected or attached to the glass surface, with sharp bends along their lengths (Fig. 1a). Every cable which had these morphological characteristics supported movement of organelles. In the absence of ATP, organelles did not display directional movement and a high proportion of them appeared to be attached to the cables (Fig. 1b).

The dynamic interaction between organelles and transport cables was studied by analyzing slow motion or frame-by-frame playbacks of recorded video images from several regions of at least 60

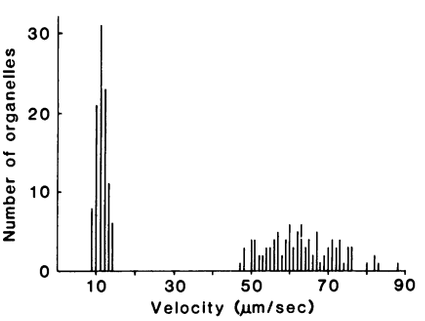

Fig. 2. Histogram of the velocities for organelles moving along the cables from several preparations containing 2 m*M* ATP. A total of 100 velocity measurements were made for each of the two classes of organelles; the data were plotted at intervals of 1 μm/sec. The average velocity for the slower moving organelles was 11.2 ± 0.1 μm/sec and for the faster moving organelles was 62.3 ± 1.1 μm/sec (*8*).

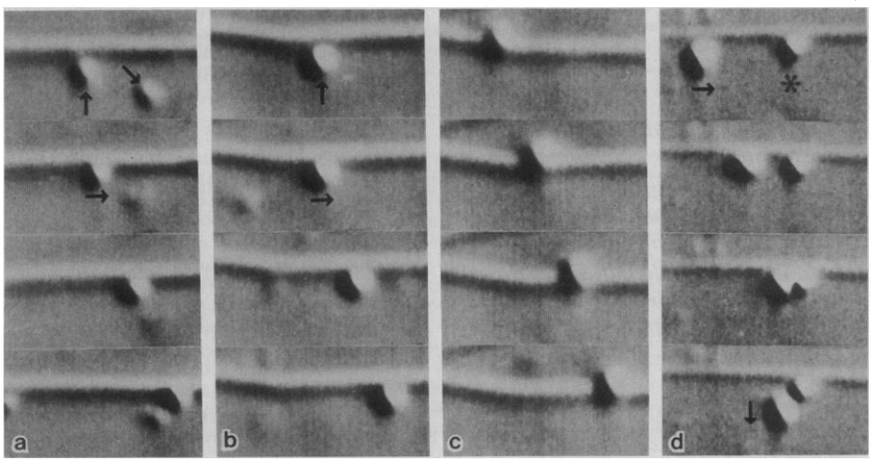

Fig. 3. Sequential micrographs in each panel (a to d) show binding and directional movement of spherical organelles along discrete transport cables. The time interval between two sequential pictures in each panel was 60 msec, equal to the duration of two frames. In (a), one of the two organelles (in Brownian motion) in the field moved toward the cable to which it bound and initiated directed movement, while the other organelle disappeared from the field, going out of focus. (b) Shows sequential pictures of the binding and movement of an organelle along another transport cable. The rate of displacement was the same as that in (a). (c) Shows the movement of another organelle, along the same cable as in (b), but along a different track at the upper portion of the cable. (d) Shows a moving organelle (arrow) that encountered another organelle (asterisk) which was permanently attached to the cable (and probably to the glass surface). Immediately after the encounter, the moving organelle left the cable (arrow in bottom picture) and began Brownian motion. Magnification, ×11,000.

preparations. The organelles moving along the cables could be divided into two categories. Organelles of the first category had a spherical shape and an apparent diameter ranging from 0.2 μm to 0.6 μm (*7*), and moved along the cables at a mean rate of 11.2 ± 0.1 μm/sec (*8*) (Fig. 2). This category represented more than 99 percent of the moving organelles. The characteristic birefringence of their content, spherical shape, dimensions, and frequency suggest that they correspond to glycosomes (*9*). Organelles of the second category moved along the cables at rates of 62.3 ± 1.1 μm/sec (*8*) (Fig. 2). These relatively rare organelles had a low contrast image and varied greatly in size and shape. In most cases, they appeared as faint tubular structures (0.2 to 5 μm long) that could change shape during movement (*10*).

Organelles in Brownian motion in the fluid phase became attached to the cables after random collisions and immediately moved at a smooth constant rate along the cable surface (Fig. 3a). Occasionally organelles would pause before starting to move or even return to the fluid phase without displaying any directed movement. The rate of the directional organelle movement was the same from preparation to preparation (Fig. 2) and remained constant within a preparation for up to 2 hours, after which time there was a gradual decay in rate (*11*).

Several observations indicated that cables have multiple structural tracks for organelle movement. The video-enhanced images permitted direct visualization of organelles moving along different parallel pathways on the surface of the same cable (Fig. 3, b and c). Subtle lateral changes of pathway along a cable were often observed. Organelles moving at different speeds on the same cable frequently passed each other without interference. Sometimes organelles stopped at a barrier attached to the filament (Fig. 3d) while other moving organelles could pass undisturbed along another side of the same filament.

The transport cable would move in opposite direction to the movement of the organelles if it was not anchored to a fixed substrate, with the velocity of the organelles relative to the cable remaining constant. Organelles often moved from one cable to another nearby or overlapping cable. In some cases, organelles pulled an attached cable while moving along another one, or even showed movement associated with two cables simultaneously. Thus, each organelle may have more than one site for attachment and force generation.

Moving organelles often changed cables or tracks in the same cable without any pause or hesitation detectable with the temporal resolution of video scanning (the interval between frames was 30 msec). When an organelle was forced to stop its directional movement it immediately became detached from the cable and returned to Brownian motion. This typically occurred under two circumstances: (i) when it collided with an organelle that was in Brownian motion in the medium or (ii) when it encountered an obstacle attached to the cable (Fig. 3d). In the latter case, it detached either immediately or after a brief pause. These findings indicate that the mechanisms of binding and force generation are interrelated and work in a concerted manner during movement. However, in the absence of ATP, organelle binding to the cable was independent of the movement. This situation may be analogous to the state of rigor between myosin and actin in muscle in the absence of ATP (*12*).

Organelle movement was undisturbed when rhodamine-phalloidine (0.2 μ*M*), a fluorescent toxin that binds specifically to actin (*13*), was present in the medium. The fluorescent compound specifically labeled the transport cables (Fig. 4a). Electron microscopy of freeze-etched samples of directly frozen (*14*), extruded cytoplasm of *Chara* cells showed organelles closely associated with the surfaces of long cables made of compact bundles

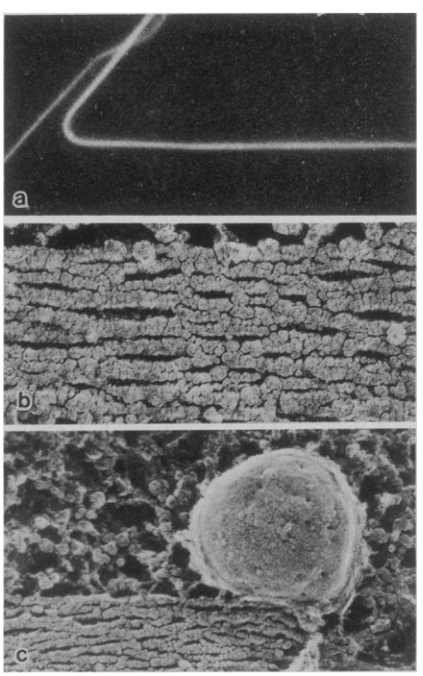

Fig. 4. (a) Fluorescence micrograph of isolated transport cables labeled with rhodamine-phalloidine. Magnification, ×6,100. (b) Electron micrograph of a freeze-etched replica of fast-frozen transport cable. The closely associated, parallel, 7- to 8-nm filaments have a 5- to 6-nm periodic substructure along the length typical of actin filaments (*15*). Magnification, ×229,000. (c) Spherical organelle at the surface of an actin cable. Magnification, ×130,000.

of 6- to 8-nm thick filaments (Fig. 4, b and c). Individual filaments had substructural periodicity similar to that of actin filaments (*15*). The estimated number of filaments per cable varied from 10 to more than 80 (*16*).

Since actin filaments were the only detectable linear components of the cables, it is likely that single actin filaments are the structural units of the multiple tracks at the surface of the cables. The requirement of ATP to support organelle movement along the actin filaments suggests that an actin-activated adenosinetriphosphatase was directly involved in this process. Since movement did not require Ca^{2+}, it may have been independent of regulatory proteins associated with the actin filament such as troponin and tropomyosin (*17*). Perhaps only actin and an actin-activated adenosinetriphosphatase present at the surface of the organelle are involved in movement in this system. Evidence from several physiological and pharmacological experiments supports the contention that myosin or a myosin-like adenosinetriphosphatase is responsible for the generation of the motive force (*2, 18*). Further electron microscopy is needed to ascertain whether small cross-bridging structures, similar to the myosin arms in muscle (*19*), are involved in the interactions between organelles and actin filaments.

The observation that a single transport substrate could support movement of different organelles at different rates indicates that only the part of the motile machinery that is associated with an organelle may modulate its rate of movement (*20*). Although this may be the case in other systems where a variety of patterns of organelle movement has been observed (*1, 21*), microtubules appear to be the basic substrate for organelle motility in axons and cultured cells (*21*).

The preparation described here permits extensive analysis of cytoplasmic organelle movement in the absence of an organized cytoplasm and without the insulating effect of a plasma membrane. It permits direct visualization and measurement of organelle displacements along the transport substrate. This preparation is easy to obtain, highly reproducible, and amenable to biochemical, pharmacological, and mechanical manipulations.

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7. Structures with dimensions equal to or smaller than the resolution limit of the light microscope (0.1 to 0.2 μ) appear inflated by diffraction. It is possible to visualize structures as small as 50 nm with the video contrast enhancement (*5*); however, it is not possible to correlate the apparent diameter seen on the video images with the real diameter of the structures.
8. Velocity was determined directly from the television screen by measuring the time required for an organelle to travel 5- to 25-μm distances. The data were derived from 100 values and given as the mean ± standard error of the mean.
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20. An important question concerns the manner in which two distinct velocities are produced along the same actin substrate if we assume that the basic mechanism is the same in both organelle types. It is possible that there are two adenosinetriphosphatases with different activities or that there is a configurational difference in the arrangement of an adenosinetriphosphatase at the surface of each organelle type.
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22. I thank Dr. T. S. Reese for continuous support, Dr. P. Bridgman for assistance with the "fast-freezing," and J. Fex, R. Wagner, and M. Brightman for valuable discussions and revision of the manuscript. I dedicate this report to my parents, Claire and Mikhael Kachar.

26 October 1984; accepted 21 December 1984

Hormonal Control of the Anatomical Specificity of Motoneuron-to-Muscle Innervation in Rats

Abstract. *Motoneurons of the spinal nucleus of the bulbocavernosus innervate bulbocavernosus muscles in male rats. Adult female rats normally lack both the spinal nucleus and its target muscles. Prenatal treatment of females with testosterone propionate resulted in adults having, like males, both the spinal nucleus and its target muscles. However, prenatal treatment with dihydrotestosterone propionate preserves the muscles but not the motoneurons. This paradoxical condition might result from (i) bulbocavernosus muscles without innervation; (ii) muscles innervated by morphologically unrecognizable motoneurons; (iii) muscles innervated by a very few spinal nucleus cells, each innervating many bulbocavernosus fibers; or (iv) muscles innervated by motoneurons outside their normal anatomical locus in the spinal nucleus. The results of retrograde marker injections into the bulbocavernosus muscles of females treated with androgen refute the first three possibilities and confirm the last: the different androgen treatments result in anatomically distinct spinal motor nuclei innervating bulbocavernosus muscles.*

The anatomical specificity of neural connections is undoubtedly crucial to behavioral function, and the achievement of this specificity poses one of the fundamental questions for developmental neurobiology. Most studies addressing this question monitor the neural connectivity in surgically manipulated individuals and then make inferences about the mechanisms guiding the formation of connections in normal subjects. Another approach is to study neural systems that differ anatomically between the two sexes. These sexually dimorphic neural systems can follow either of two developmental programs: that which results in the masculine anatomical configuration, or that which produces the feminine con-