The Mechanism of Cytoplasmic Streaming in Characean Algal Cells: Sliding of Endoplasmic Reticulum along Actin Filaments

Bechara Kachar* and Thomas S. Reese

Laboratory of Neuro-otolaryngology* and Laboratory of Neurobiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205

Abstract. Electron microscopy of directly frozen giant cells of characean algae shows a continuous, tridimensional network of anastomosing tubes and cisternae of rough endoplasmic reticulum which pervade the streaming region of their cytoplasm. Portions of this endoplasmic reticulum contact the parallel bundles of actin filaments at the interface with the stationary cortical cytoplasm. Mitochondria, glycosomes, and other small cytoplasmic organelles enmeshed in the endoplasmic reticulum network display Brownian motion while streaming. The binding and sliding of endoplasmic reticulum membranes along actin cables can also be directly visualized after the cytoplasm of these cells is dissociated in a buffer containing ATP. The shear forces produced at the interface with the dissociated actin cables move large aggregates of endoplasmic reticulum and other organelles. The combination of fast-freezing electron microscopy and video microscopy of living cells and dissociated cytoplasm demonstrates that the cytoplasmic streaming depends on endoplasmic reticulum membranes sliding along the stationary actin cables. Thus, the continuous network of endoplasmic reticulum provides a means of exerting motive forces on cytoplasm deep inside the cell distant from the cortical actin cables where the motive force is generated.

THE coordinated movement of intracellular elements, the cytoplasmic streaming, in giant characean algae cells was discovered by Corti in 1774 (for review see reference 14). The remarkable cytoplasmic streaming in these algae has long served as a model system to study mechanisms of intracellular transport and organelle movement. Several observations indicate that the motive force for streaming is a shear force produced at the interface between the stationary cortex and the moving endoplasm where parallel bundles of actin filaments (13–15, 17, 18) are attached to the stationary cortical cytoplasm. However, the streaming cytoplasm exhibits a nearly uniform velocity profile (14, 19) so the shear force at the interface with the cortical cytoplasm is apparently exerted throughout the endoplasm.

The effect on streaming of agents that inhibit actin–myosin systems (5, 7, 14, 18, 23, 24), along with the ability of myosin molecules (20, 21, 25) to reconstitute in vitro (1, 11, 13, 14, 23) movements reminiscent of cytoplasmic streaming, suggests that myosin (7, 14) or myosin-like translocator molecules participate in force production by interacting with the stationary bundles of actin filaments. However, the locations and distributions of these molecules, as well as the nature of their association with the cytoplasmic elements, remains unknown. Another unanswered question is how cytoplasm distant from the actin cables can display the same streaming velocity as the cytoplasm near the actin cables.

Several reports (2, 3, 5, 14, 16, 19) have dealt with the question whether the active movement of putative organelles, using the translocator molecules on their surfaces to move them along the actin cables, could exert enough viscous drag to produce streaming of the whole endoplasm. Hydrodynamic models of viscous coupling between a motile translocator and the endoplasm in characean algae cells indicate that only a fibrous or membraneous network system extending into the whole endoplasm could generate or extend the motive force from the actin cables to the endoplasm (19).

An early paper (5) based on thin section views of fixed cells, which showed the extensive sheets of endoplasmic reticulum membranes present in the streaming region of the endoplasm (17) suggested that the endoplasmic reticulum could participate in the generation of the integrated streaming of the endoplasm by two possible mechanisms. For example, if actomyosin cross-bridge attachments were made between the actin filaments and sites on the endoplasmic reticulum, then the reticulum would move along the filaments; the viscous cytoplasm could then be propelled by frictional forces transmitted to it by the large area of sliding membranes of endoplasmic reticulum (5). A second possibility that was considered is that suitable mechanical coupling between the filament bundles and the reticulum could generate traveling waves along the membranes of endoplasmic reticulum that would propel the endoplasm (5).

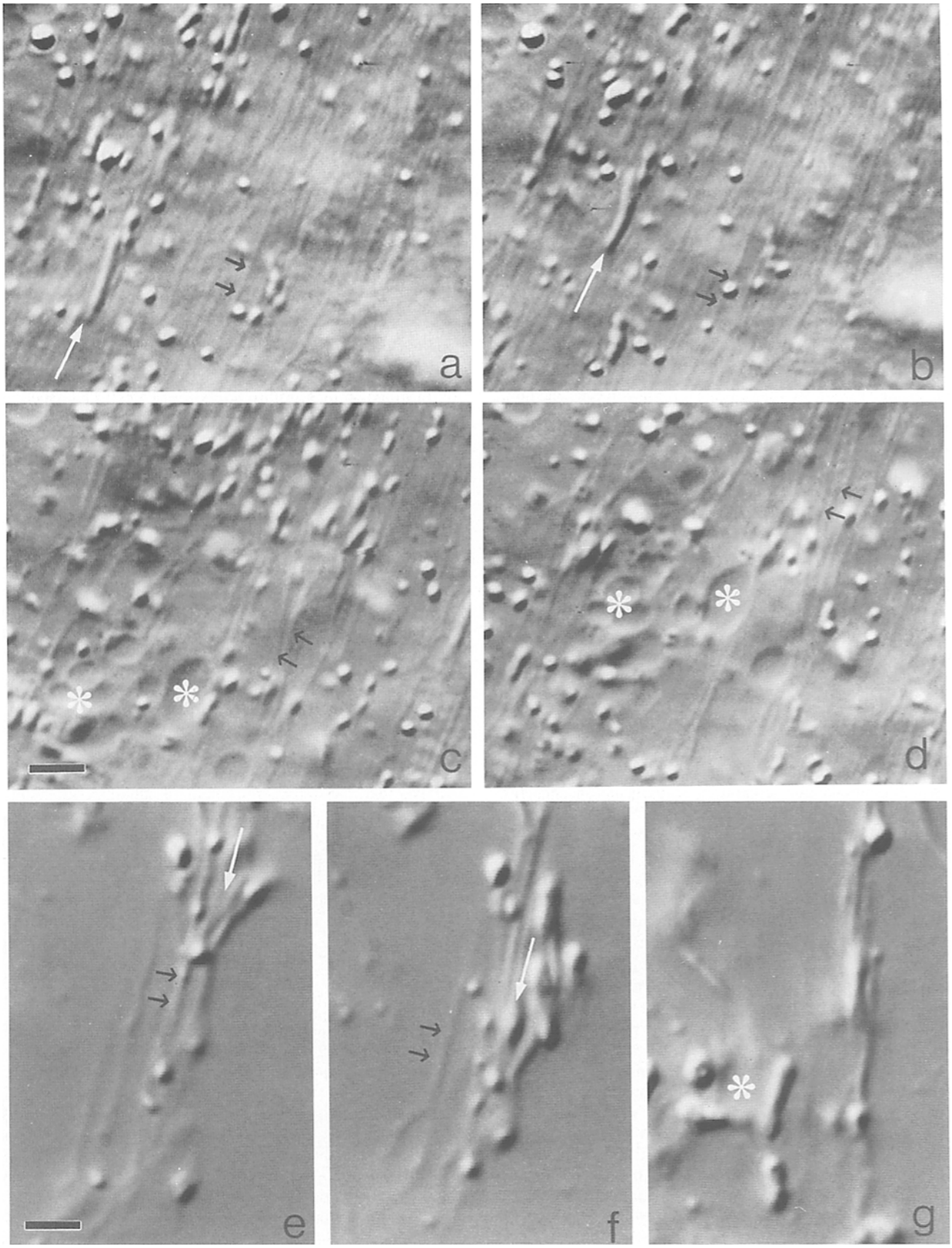

Figure 1. Sequential video micrographs showing cytoplasmic streaming in an intact (*a–d*) and extruded (*e–g*) internodal cell of the algae *Chara*. *a* and *b* represent sequential optical sections exactly at the interface of the stationary cortical actin cables and the moving endoplasm. Most organelles are glycosomes (spherical, birefringent structures) and mitochondria (*white arrow*) that are bound to and move along the

We now report structural findings from complementary video-enhanced light microscopy and fast-freezing electron microscopy of intact cells and extruded endoplasm which demonstrate that a network of endoplasmic reticulum membranes is indeed the element that couples the propelling forces produced along the actin cables to the whole streaming endoplasm. However, the sliding forces propelling cytoplasmic streaming appear to be generated by translocator molecules associated with the surface of the endoplasmic reticulum membranes which move them along the stationary cortical actin cables. Thus, the continuous network of endoplasmic reticulum system in these cells provides a means of mobilizing cytoplasm distant from the cortical actin cables.

Materials and Methods

Dissociation of Cytoplasm

Giant internodal cells of the algae *Chara* and *Nitella* (Carolina Biological Supply Co., Burlington, NC) were isolated from neighboring cells, rinsed, and incubated for 20 min in a solution containing 4 mM EGTA, 25 mM KCl, 4 mM $MgCl_2$, and 10 mM imidazole at pH 7.6. Individual internodal cells were blotted on tissue paper, transected at one end in order to drain the contents of the giant central vacuole, and then grasped with a pair of forceps whose tips were covered with Teflon tubing. By sliding the forceps forward, a cylinder of cytoplasm was gently extruded into a drop of isotonic buffer, containing 4 mM EGTA, 25 mM KCl, 4 mM Mg Cl, 5 mM Tris, and 200 mM sucrose at pH 7.6, on a thin microscope slide (11). Such a cylinder of cytoplasm, a protoplast, still has an intact plasma membrane that prevents dispersion of the cytoplasmic contents. At this point, the buffer was replaced with fresh isotonic buffer solution with 2 mM ATP, and fine needles were used to break the protoplast and disperse the cytoplasmic content into the buffer solution. Drops of the extruded cytoplasm at different degrees of mechanical dispersion were viewed by video-enhanced light microscopy or placed on gelatin and fast frozen for electron microscopy.

Video Microscopy

A Zeiss Axiomat microscope in the differential interference contrast mode and equipped with an internally corrected 100 × 1.3 NA planapochromatic objective was used. The aperture of a 1.4-NA condenser was fully illuminated with a 100 W mercury lamp aligned for critical illumination (12). The change from Kohler illumination to critical illumination is obtained by defocusing the image of the light source seen at the condenser aperture plane by adjusting the collection lens in the lamp housing (12). By doing so, the image of the light source now becomes focused in the specimen plane. Because the image of the light source at the condenser aperture plane is replaced by an evenly and fully illuminated field, the effective aperture is maximal and consequently the resolution is also maximal. The stronger illumination of the image plane in the center of the field is not a problem because only a small area in the center of the microscope field of view is projected onto the surface of the video tube of the camera at the very high magnifications used in video-enhanced microscopy. This illumination condition increases the signal-to-noise ratio of the video image, allowing very high magnifications to be used (the magnification of the image on the television monitor was 20,000×). A Newvicon video camera (Dage-MTI, Michigan City, IN) was used for recording and contrast enhancement (10).

Fast-freezing Electron Microscopy

Intact or extruded cytoplasm from internodal cells of *Chara* or *Nitella*, as well as drops of cytoplasmic material extruded and mechanically dissociated into the buffer solution, were placed on the surface of a gelatin support, directly frozen by contact with the surface of a copper block cooled by liquid helium (9), and then processed for freeze fracture, freeze etching, or freeze substitution. For freeze fracture, the specimens were fractured and immediately replicated at −130°C. For freeze etching, the specimens were fractured at −100°C and allowed to etch until a thin layer of water was removed and then replicated. Freeze-substitution was done according to methods described elsewhere (6). Due to the insulating effect of the thick cell wall in the intact cells, the rate of success for the freezing was very low for the larger internodal cells. However, the thinner walls of internodal cells or cells at the growing tips of algal cells permitted better freezing. For the freeze substitution of intact cells, the cell wall had to be scraped off under liquid nitrogen to allow substitution and fixation chemicals to penetrate. Electron micrographs were taken with a JEOL 200CX electron microscope.

Results

Video-enhanced Microscopy

The use of the critical illumination procedure and the selection of fields lacking chloroplasts permitted optical section views of the cytoplasm with sufficient clarity to see many pertinent cytoplasmic structures in the intact cells. At the boundary between the streaming endoplasm and the cortical ectoplasm, organelles displayed continuous movements along the parallel array of stationary actin cables (Fig. 1, *a* and *b*). In this boundary region, organelles that came off the cable moved off with the adjacent streaming endoplasm either immediately or after remaining there briefly in Brownian motion. Deeper in the streaming cytoplasm, elongated tubular and vesicular structures also streamed continuously and these were accompanied by spherical particles making Brownian motion while streaming (Fig. 1, *c* and *d*). The elongated tubular and vesicular elements appeared to be connected to form a membranous network distributed throughout the whole streaming endoplasm; these were distinct from the actin cables which are stiff, immobile, and appear in regular arrays in only one plane of optical section. Larger organelles, such as the nuclei of this multinucleated algal cell, also moved along embedded in the membrane network at their typical streaming rate of 50–60 μm/s. Stretching and recoil movements of the membrane network occurred upon collision with organelles that momentarily lagged behind the streaming.

Aggregates of tubular and vesicular elements (Fig. 1, *d–f*) continued to move along isolated actin cables even after cells were cut open and the cytoplasm extruded. This in vitro movement could only be maintained if the buffer in which the cytoplasm is dissociated contained ATP (11). Organelles maintained a predominantly elongated shape while moving (Fig. 1, *d* and *e*) and formed retracted aggregates when they stopped their movement after they were released from the actin cables (Fig. 1 *f*). The leading process of isolated membrane elements moved along stationary actin cables that attached to the surface of the microscope slide, even in extruded and well-dissociated cytoplasm (Fig. 2, *a–c*). Elon-

parallel array of stationary actin cables (*black arrows*). *c* and *d* are sequential views at an optical section deep in the streaming endoplasm. Tumbling glycosomes, mitochondria, and other organelles move together within a ubiquitous network of elongated tubular (*arrows*) and vesicular (*asterisks*) structures at 50–60 μm/s. Sequential video micrographs of cytoplasm after gentle dispersion in a buffer containing ATP (*e–g*) show the network of elongated membranes (*black arrows*) and entrapped spherical organelles rapidly moving in the direction of the long white arrow along an actin cable (not clearly visible due to the overlap of structures). Asterisk in *g* indicates an aggregate of the network structure and the enmeshed organelles that stopped as it was released from the actin cable on the right, along which other organelles were moving. Bars: (*a–d*) 2 μm; (*e–g*) 1.7 μm.

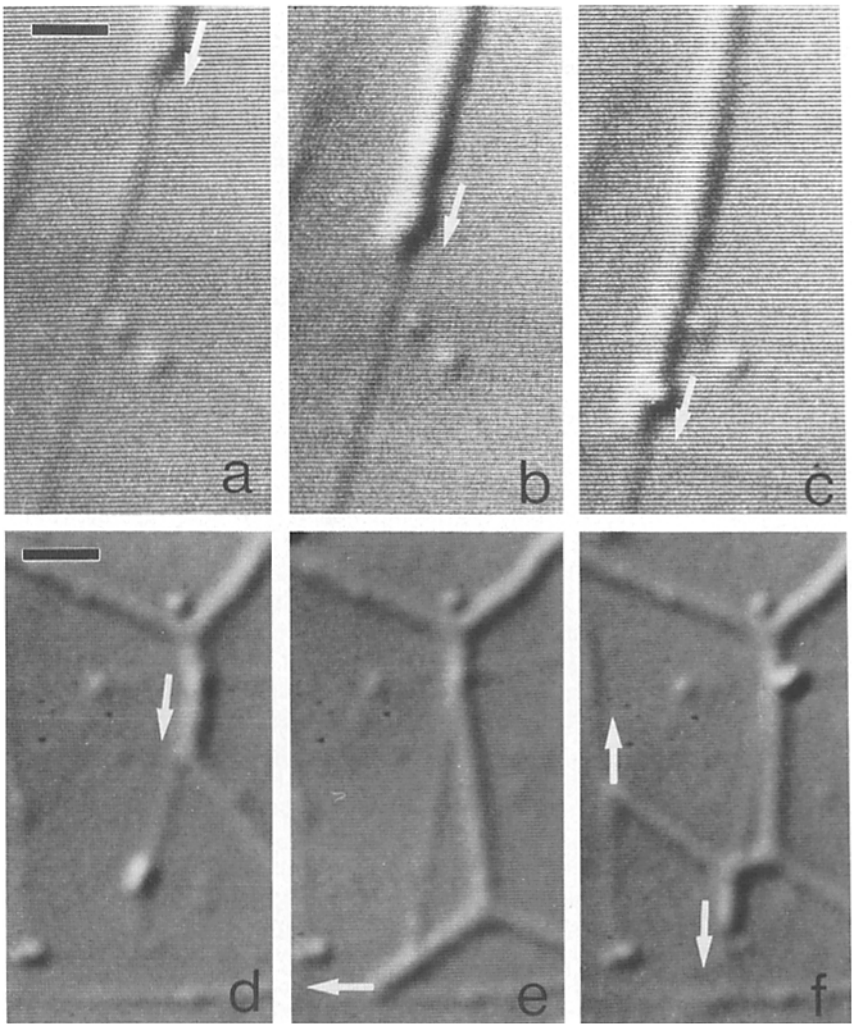

Figure 2. Sequential video micrographs (*a–c*) showing the leading process of an endoplasmic network moving along an actin cable that adhered to the microscope slide surface in an extruded and well-dispersed piece of cytoplasm. Sequential video micrographs (*d–e*) show movements of multiple branches of dissociated endoplasmic network along actin cables. In the field of view are three actin cables attached to the surface of the glass slide. They are seen as faint straight lines. The arrows indicate their position and their polarity, which is determined by the direction of movement of the endoplasmic membrane network and other organelles. Force generation along the actin cables pulls the membranes, deforms the network, and forms new branches. The forces producing the translocations pull out the membranes until the tension overcomes the binding to the actin cable, allowing the membrane to recoil. This release always occurs before the membrane network breaks. Binding, branching, stretching, and release occur in repetitive cycles. Bars: (*a–c*) 1.3 μm; (*d–f*) 1.6 μm.

gated processes of a network of membranes also interacted with actin cables that had attached to the glass surface (Fig. 2, *d–f*). Forces generated during movements of the tubular processes along different actin cables stretched each process in different directions thereby stretching the membranes and sometimes forming new branches. The tubular membrane processes typically stretched until they broke away from the cable and recoiled (Fig. 2, *d–f*). Thus, the forces generated during movement are strong enough extensively to reshape the membranes but not strong enough to rupture them.

Electron Microscopy

A continuous, cisternal network of membranes was a predominant feature of the gently extruded cytoplasm seen in freeze-etch replicas of material prepared by direct freezing (Fig. 3 *A*). Freeze fracture (Fig. 3 *B*) through this membrane showed spiral arrays of intramembrane particles in patterns similar to those in the spiral arrays of ribosomes seen in tangential thin sections of these same membranes after freeze substitution (Fig. 3 *C*). These polyribosome sites on the surfaces of the tubular membranes are identical to those previously observed in endoplasmic reticulum of another green alga (8), and they identify them as components of the rough endoplasmic reticulum. Segments of the membrane network associated with the bundles of actin filaments could be seen in freeze-etch images of the gently extruded cytoplasm (Fig. 4). Unfortunately, the granular material that forms the background of the freeze-etched cytoplasm did not permit detailed definition of the relationships between the endoplasmic reticulum membranes and the actin cables (Fig. 4 and 5 *c*); these relationships will be treated separately (Kachar, B., and R. Urrutia, manuscript in preparation). Freeze-etch images of intact cells also showed the well-characterized parallel bundles of actin filaments adjacent to the membranes of the chloroplasts lining the cortex of the cytoplasm (Fig. 5 *A*). The array of elongated tubular and vesicular cisternae of endoplasmic reticulum interconnected into a tridimensional continuous network of endoplasmic reticulum (Fig. 5 *B*) was also apparent in cells frozen while the cytoplasm was actively streaming. These networks have their elongated segments oriented along the actin bundles and therefore parallel with the direction of streaming. The elongated endoplasmic reticulum processes that are directly interacting with the stationary actin cables can be visualized by freeze etching (Fig. 5 *C*, *arrows*) or freeze substitution (Fig. 5 *D*, *arrows*) after the direct freezing of active intact cells.

The images of cytoplasm frozen while streaming was in progress are directly comparable to the video images of streaming. The direct freezing permitted the arrest of the

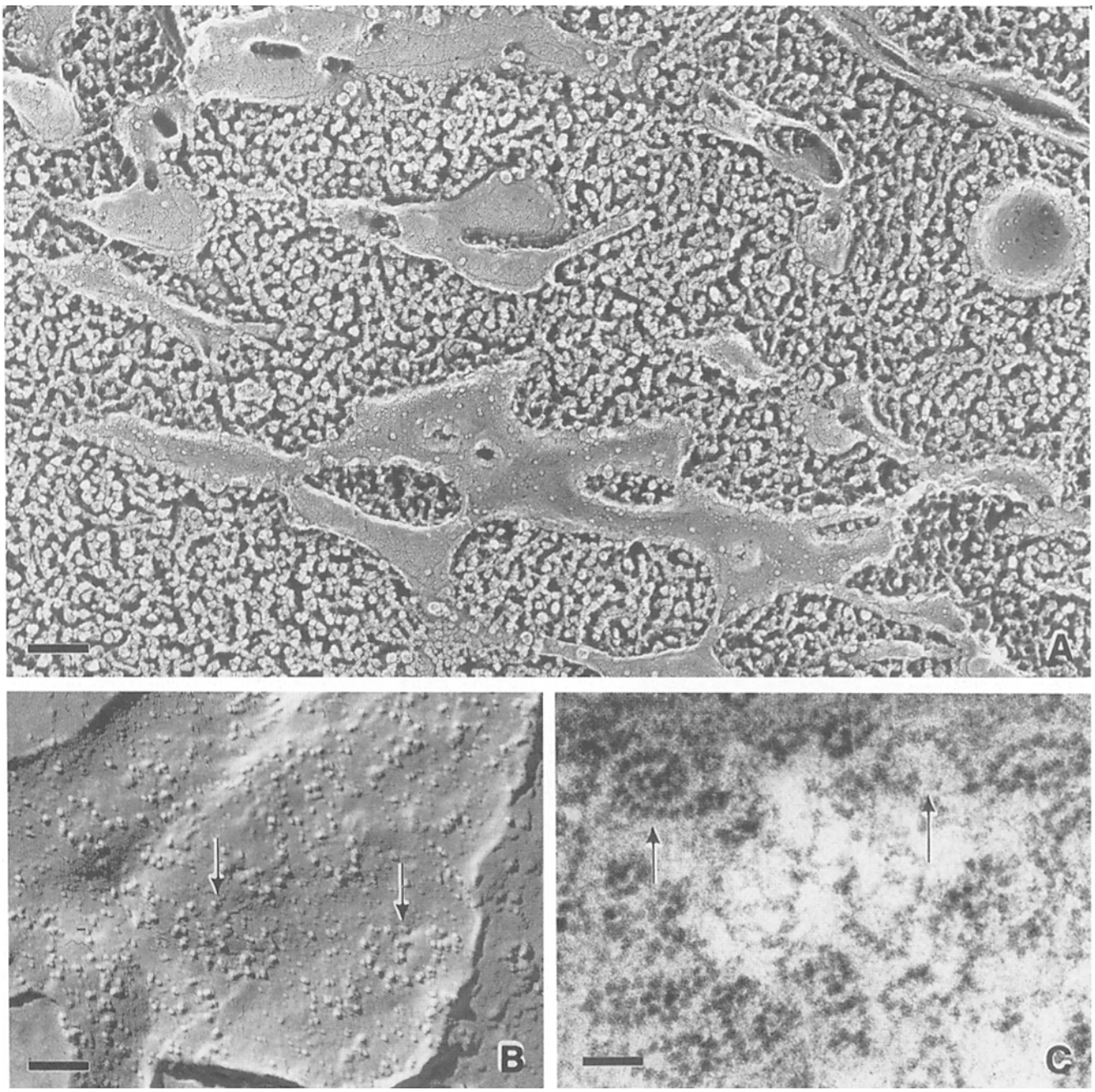

Figure 3. (*A*) Freeze-etch replica of directly frozen droplet of gently dissociated cytoplasm shows the ubiquitous network of anastomosing tubular and vesicular structures against the granular background consisting of salts and soluble proteins. (*B*) Freeze fracture of a portion of the endoplasmic network of membranes showing spiral arrays of intramembrane particles. (*C*) Analogous spirals of ribosomes are seen in tangential view in thin sections through these membranes, which indicates that these cisternae belong to the endoplasmic network. Bars: (*A*) 0.18 μm; (*B*) 0.12 μm; (*C*) 0.12 μm.

dynamic stretching of the endoplasmic reticulum network occurring when the shear forces produced at the region of contact with the actin cables are transmitted to the whole network.

Discussion

We provide here direct evidence that a continuous network of membranes of the rough endoplasmic reticulum is present in the cytoplasm of characean algae cells and that this network provides the means of integrating and mobilizing the streaming cytoplasm. Fig. 6 diagrams the relationships of this endoplasmic network to the stationary actin cables during streaming. The relationships depicted in this diagram lead us to conclude that streaming of the cytoplasm depends on shearing forces generated at the interface between the stationary cortical actin cables and the surface of the endoplasmic reticulum.

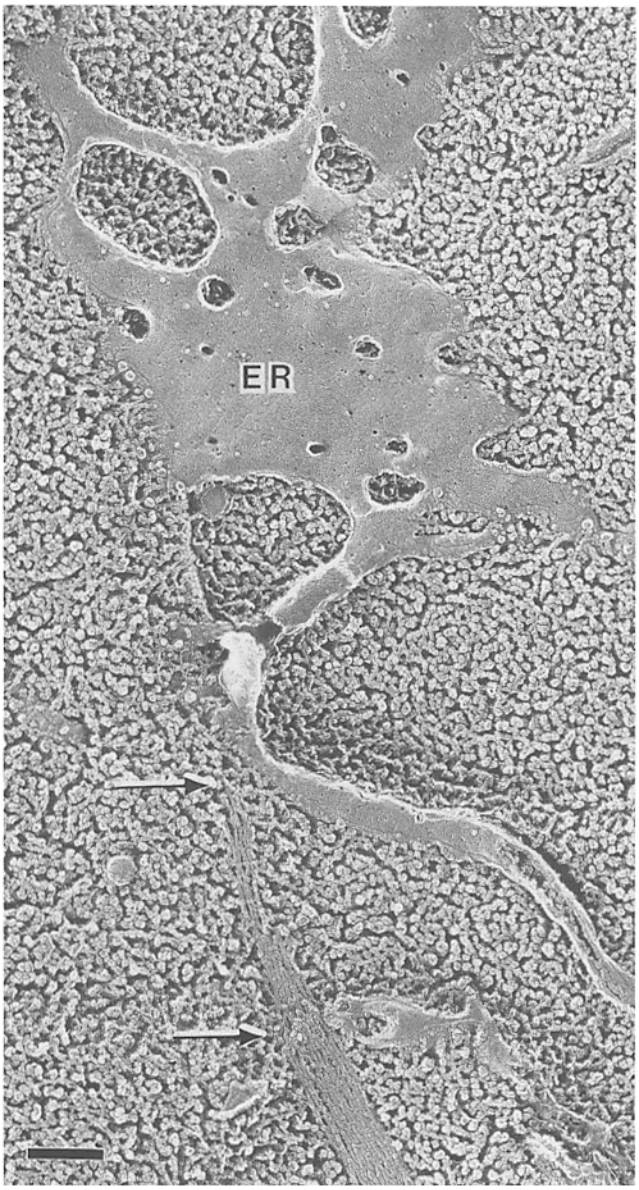

Figure 4. Freeze-etch replica showing lamellar and tubular portions of extruded endoplasmic reticulum network interacting with actin filament cables (*arrows*). Bar, 0.2 μm.

In the intact active cell, the network of endoplasmic reticulum is stretched and most of its anatomosing elements become elongated in the direction of the streaming. As observed in the extruded cytoplasm, the shear force produced along actin cables is capable of moving large aggregates of endoplasmic reticulum membranes and other organelles. While the shear force produced during movement is capable of extensively deforming the endoplasmic reticulum, it is not capable of rupturing these membranes. Separation of the interacting portion of the endoplasmic reticulum membrane always occurs before rupture, sometimes after a brief period of apparent isometric tension. The continuous endoplasmic reticulum network in the intact cells thereby provides the effective means of mobilizing cytoplasm distant from the cortical actin cables where the moving force is generated.

While an early report (5) has suggested that the endoplasmic reticulum could be implicated in the mechanism of streaming, the question of how the streaming cytoplasm moves as a coherent mass remained open because the endoplasmic reticulum had not been directly observed in living cells. However, images previously interpreted as filaments branching off of the stationary actin cables into the moving endoplasm (2, 3), which were proposed to propel the cytoplasm by their undulating action, could actually have been images of elongated branches of the endoplasmic reticulum network.

Conventional fixatives do not provide a rapid and undistorted arrest of dynamic structures moving at rates of 60 μm/s, as they do in the characean cells (11, 14), and definitive electron microscopic data based on rapid nonchemical preservation of functioning cells have also been lacking. An early report using direct freezing by immersion in liquid freon (4) describes a three-dimensional network of 7–8-nm filaments characterized as actin filaments that interacts with 4–5-nm filaments presumed to be involved with the process of streaming. In our specimens, which are frozen by the copper block method, the actin filaments appear as parallel bundles at the interface between the streaming cytoplasm and the layer of chloroplast in the stationary cortical cytoplasm (Fig. 5 *a*). The organization of the actin filaments in parallel bundles has been observed using several other microscopy techniques (11, 14, 17, 18, 23).

Putative "balloon-shaped" organelles found associated with the actin filaments under ATP-deficient conditions have also been suggested as candidates for the production of the streaming effect (16). Indeed, vesicular and tubular structures (0.2–5 μm long) translocate at rates of 60 μm/s along actin cables when cytoplasm is extensively dissociated in presence of ATP (11). We did find similar structures in electron microscopy of the disrupted cytoplasm but no balloon-shaped structures were recognized in the intact, cryofixed cytoplasm except as components integrated into processes of the continuous tubular network of endoplasmic reticulum.

The plethora of hypothetical mechanisms to explain the uniform velocity profile of streaming endoplasm of characean cells (2–5, 14, 16, 19, 24) led to a rigorous consideration of the hydrodynamic characteristics of the cortical cytoplasm (19). The conclusion was that only a continuous fibrous or membraneous network extending into the endoplasm could transfer motive force from the actin cables to the endoplasm (19). This requirement, based on hydrodynamic characteristics, could be met by the endoplasmic reticulum.

Our electron microscopic images do not permit a clear definition of the nature of the interaction between the endoplasmic reticulum membranes and the actin filament cables in the intact cells. We cannot confirm the possibility that a filamentous form of myosin (24) is present on the surfaces of the endoplasmic reticulum membranes. Indeed, in light of recent work depending on the application of myosin-containing organelles (1) and myosin-coated beads (20, 21) to characean actin cables, the presence of filamentous myosin would appear to be unnecessary.

Active intracellular translocation of the endoplasmic reticulum is not unique to characean cells; it has been recently observed in cultured cells. In the algal cell endoplasmic reticulum moves along actin filament bundles, while in cultured cells endoplasmic reticulum has been reported to be

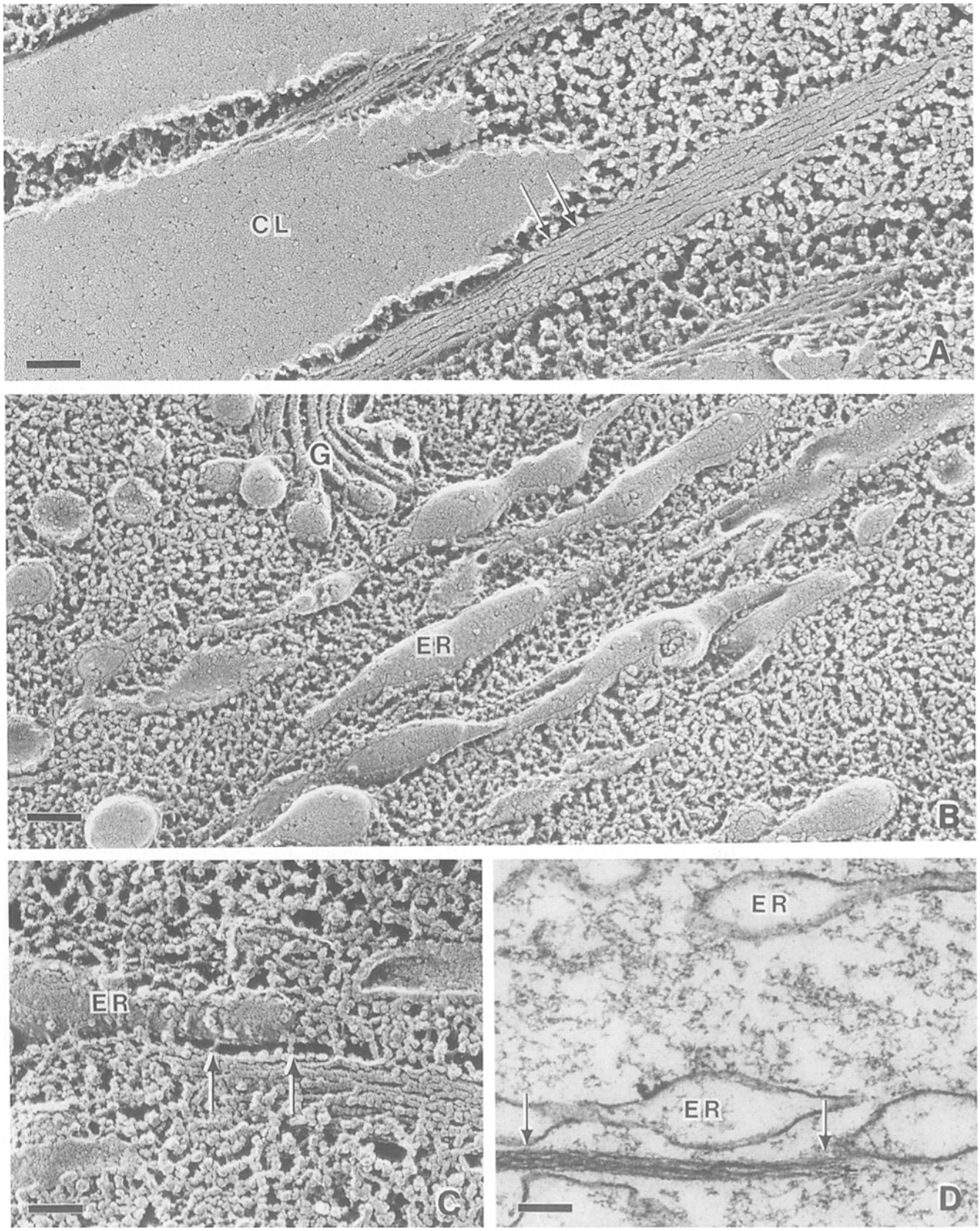

Figure 5. Freeze-etch (*A–C*) and thin section (*D*) views of fast frozen intact cytoplasm of *Nitella*. (*A*) The fracture plane crosses the interface of the parallel actin filament cables (*arrows*) with the membrane of the chloroplasts (*CL*). (*B*) The fracture plane passes through the streaming cytoplasm beyond the actin cables. The endoplasmic reticulum (*ER*) forms a network of anastomosing (*arrows*) tubular and vesicular cisternae sheared in the direction of the streaming. *G*, Golgi apparatus. (*C*) The fracture plane crosses the interface (*arrows*) between the actin filament bundles and the interacting portions of the endoplasmic reticulum membranes (*ER*). In *D* is a thin section of freeze-substituted, intact *Nitella* at the interface between the actin cables (*arrows*) and the streaming endoplasmic reticulum network (*ER*). Bars: (*A*) 0.12 μm; (*B* and *D*) 0.17 μm; (*C*) 0.10 μm.

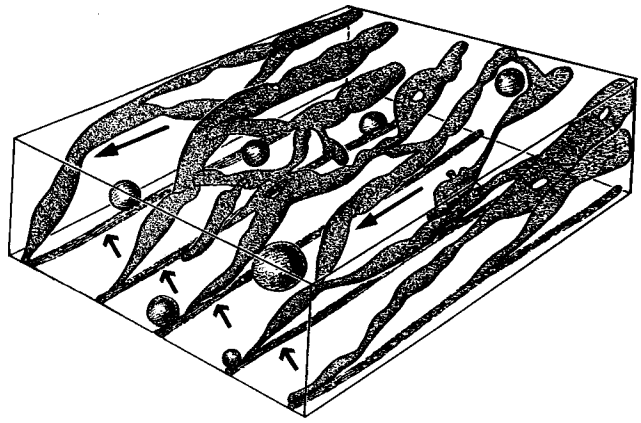

Figure 6. Summary diagram showing the relationships of the stationary actin filament cables (*small arrows*) and the cytoplasm composed of the continuous endoplasmic reticulum network and the enmeshed organelles streaming in the direction of the long arrows.

associated (22) and to move along microtubule tracks. Thus, interactions between the endoplasmic reticulum and various intracellular motility systems can be involved in shaping the endoplasmic reticulum and in characean cells, at least, in bulk transport of cytoplasm.

We thank Dr. J. Fex for critical reading of the manuscript and Nina Allen for introducing us to the *Nitela* preparation in her microscopy course at the Marine Biological Laboratory.

Received for publication 21 September 1987, and in revised form 18 December 1987.

References

1. Adams, R., and T. D. Pollard. 1986. Propulsion of organelles isolated from Acanthamoeba along actin filaments by myosin-I. *Nature (Lond.).* 322: 754–756.
2. Allen, N. S. 1974. Endoplasmic filaments generate the motive force for rotational streaming in *Nitella*. *J. Cell Biol.* 63:270–287.
3. Allen, N. S. 1976. Undulating filaments in *Nitella* endoplasm and motive forces generation. *Cell Motil.* 3:613–621.
4. Allen, N. S. 1980. Cytoplasmic streaming and transport in the characean alga *Nitella*. *Can. J. Bot.* 58:786–796.
5. Bradley, M. O. 1973. Microfilaments and cytoplasmic streaming: inhibition of streaming with cytochalasin. *J. Cell Sci.* 12:327–343.
6. Bridgman, P., and T. S. Reese. 1984. The structure of cytoplasm in directly frozen cultured cells. *I.* Filamentous meshworks and the cytoplasmic ground substance. *J. Cell Biol.* 100:1655–1668.
7. Chen, J. C. W., and N. Kamiya. 1975. Localization of myosin in the internodal cell of *Nitella* a suggested by differential treatment with N-ethylmaleimide. *Cell Struct. Funct.* 1:1–9.
8. Gliddings, T. H., and L. A. Staehelin. 1980. Ribosome binding sites visualized on freeze-fractured membranes of the rough endoplasmic reticulum. *J. Cell Biol.* 85:147–152.
9. Heuser, J. E., T. S. Reese, M. J. Dennis, Y. Jan, and L. Evans. 1979. Synaptic vesicle exocytosis captured by quick freezing and correlated with quantal transmitter release. *J. Cell Biol.* 81:275–300.
10. Inoue, S. 1981. Video image processing greatly enhances contrast, quality, and speed in polarization-based microscopy. *J. Cell Biol.* 89:3346–3356.
11. Kachar, B. 1985. Direct visualization of organelle movements along actin filaments dissociated from Characean algae. *Science (Wash. DC).* 227: 1355–1357.
12. Kachar, B., P. C. Bridgman, and T. S. Reese. 1987. Dynamic shape changes of cytoplasmic organelles translocating along microtubules. *J. Cell Biol.* 105:1267–1271.
13. Kamitsubo, E. 1979. Motile protoplasmic fibrils in cells of Characeae. *Protoplasm.* 74:53–70.
14. Kamiya, N. 1981. Physical and chemical basis of cytoplasmic streaming. *Annu. Rev. Plant Physiol.* 32:205–236.
15. Kersey, Y. M., P. K. Hepler, B. A. Palevitz, N. K. Wessels. 1976. Polarity of actin filaments in Characean algae. *Proc. Natl. Acad. Sci. USA.* 73: 165–167.
16. Nagai, R., and T. Hayama. 1979. Ultrastructure of the endoplasmic factor responsible for cytoplasmic streaming in *Chara* internodal cells. *J. Cell Sci.* 36:121–136.
17. Nagai, R., and L. I. Rebhun. 1966. Cytoplasmic microfilaments in streaming *Nitella* cells. *J. Ultrastruct. Res.* 14:571–578.
18. Nothnagel, E. A., L. S. Barak, J. W. Sanger, and W. W. Webb. 1981. Fluorescence studies on modes of cytochalasin B and phallotoxin action on cytoplasmic streaming in *Chara*. *J. Cell Biol.* 88:364–372.
19. Nothnagel, E. A., and W. W. Webb. 1982. Hydrodynamic models of viscous coupling between motile myosin and endoplasm in characean algae. *J. Cell Biol.* 94:444–454.
20. Sheetz, M. P., R. Chasan, and J. A. Spudich. 1984. ATP-dependent movement of myosin in vitro: characterization of a quantitative assay. *J. Cell Biol.* 99:1867–1872.
21. Shimmen, T., and M. Yano. 1984. Active sliding movement of latex beads coated with skeletal muscle myosin on *Chara* actin bundles. *Protoplasma.* 121:132–137.
22. Terasaki, M., L. B. Chen, and K. Fugiwara. 1986. Microtubules and endoplasmic reticulum are highly interdependent structures. *J. Cell Biol.* 103:1557–1568.
23. Williamson, R. E. 1975. Cytoplasmic streaming in *Chara*: a cell model activated by ATP and inhibited by cytochalasin B. *J. Cell Sci.* 17:655–668.
24. Williamson, R. E. 1979. Filaments associated with the endoplasmic reticulum in the streaming cytoplasm of *Chara corallina*. *Eur. J. Cell Biol.* 20:177–183.
25. Yano, M. 1978. Observation of steady streamings in a solution of Mg-ATP and acto-heavy meromyosin from rabbit skeletal muscle. *J. Biochem.* 83:1203–1204.