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Polarity of actin filaments in Characean algae

(microfilaments/cytoplasmic streaming)

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ABSTRACT Heavy meromyosin from rabbit muscle combines with oriented *Nitella* and *Chara* actin *in vitro* to form arrowhead structures directed opposite to the cytoplasmic flow in the living plant cell. All filaments and all bundles of filaments in the apically directed stream are similarly oriented; polarity with respect to the axis of the thallus is reversed in the downward stream. The actin filaments are attached to the chloroplasts at the ectoplasm-endoplasm interface, where the motive force for streaming is known to be generated.

To our knowledge, the polarity of actin filaments has never before been revealed in any nonmuscle cell where a clearly defined cause and effect relationship exists between cytoplasmic movement and the presence of actin filaments. Internodal cells of the Characean algae are ideal for the study of this problem for the following reasons: first, actin filaments in these cells occur in bundles in which all members have the same polarity (1–3); second, bulk flow of cytoplasm can be easily determined and is unidirectional over half of the cell surface; and third, the cells are exceptionally large and the contents favorably disposed so that it is possible to excise cell segments containing large blocks of chloroplasts and associated actin filaments while retaining their orientation with respect to cytoplasmic movement. We have negatively stained oriented actin filaments decorated with heavy meromyosin (HMM) from rabbit muscle and have found that all discernible arrowheads in all filament bundles within a cytoplasmic stream are oriented with arrowheads directed opposite to the cytoplasmic flow.

MATERIALS AND METHODS

Nitella flexilis **and** ***Chara australis*** were grown in soil-water medium (2 cm of soil in 30 cm of solution height) and washed in running distilled water before use. We found that only healthy, vigorously streaming plants yielded good results in these experiments. A few shoots were placed in a disposable plastic petri dish (Falcon Plastics, Los Angeles, Calif.) containing either 0.1 M KCl or a standard solution consisting of 0.1 M KCl plus 0.05 M $MgCl_2$ and 0.01 M potassium phosphate buffer, pH 7. Under a binocular microscope, an actively streaming internode approximately 500–700 μm in diameter was vitally stained with a spot extending between clear zones at the boundary between ascending and descending cytoplasmic streams (see Fig. 1). The stained spot allowed orientation of the cell cortex with respect to streaming direction during subsequent operations (see Fig. 1 and legend). The stain was applied by means of a needle dipped into dry methylene blue powder (methylene blue chloride, National Aniline Division, Allied Chemical, New York). The internode was then cut immediately above the spot in the direction of streaming and about 1.5 mm below it (see Fig. 1). The cytoplasm was allowed to flow from the cut segment prior to its removal to a droplet of fresh salt solution. The segment was then cut longitudinally in such a manner that the stained area was contained in one side of the sectioned cylinder, with care being taken to avoid including material across the clear zones. The stained portion, consisting largely of cell wall, aligned chloroplasts, and actin filaments, was then transferred to a droplet of 0.1 M KCl on a Formvar- and carbon-coated directional grid (for example, Maxtaform H-4, obtainable from Ernest F. Fullam, Inc., Schenectady, N.Y.), previously made hydrophilic by means

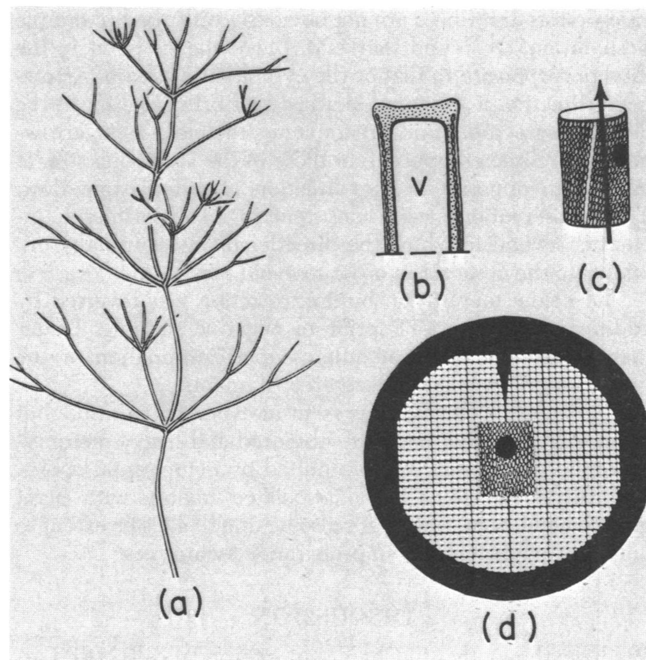

FIG. 1. (a) Shoot tip, *Nitella flexilis*. (b) Diagram of top of an internodal cell showing large central vacuole (v), peripheral cytoplasm, and cortical chloroplasts. (c) Segment of an internodal cell. Black spot to the right represents methylene blue used to mark the streaming direction in the living cell. The cell is cut immediately above the spot in the direction of streaming and about one to two mm below in the opposite direction. A second cut (arrow) is made to remove the cortical area containing the upward streaming path. (d) The excised cell segment is placed cell wall upward in a droplet of 0.1 M KCl on a directional grid, with the methylene blue spot used for orientation with respect to the streaming path. In the figure, this path is directed toward the top of the grid.

Abbreviation: HMM, heavy meromyosin.

of a glow discharge. With the cell wall upward, the sample was oriented on the grid in such a manner that the methylene blue spot representing the upward streaming direction faced the top of the grid, and the sample was pressed gently against the Formvar-carbon surface. The cell wall was easily pulled away, leaving rows of chloroplasts connected by oriented actin filament bundles, which we will refer to as *fibrils*, adhering to the grid surface (Fig. 2). The grids were washed several times with 0.1 M KCl, followed by a drop of HMM (0.2–0.5 mg/ml) in buffer, washed again, and negatively stained in 1% uranyl acetate. Some grids were rinsed in 2 mM ATP prior to negative staining.

Grids were examined with a Hitachi HU-11E electron microscope operated at 75 kV and were photographed at several magnifications from approximately 1,200× to 33,000 or 45,000× to determine the direction of HMM arrowheads relative to cytoplasmic streaming.

To insure the reliability of our results, several preparations were made and examined by one of us with no prior knowledge of fibril orientation.

RESULTS

Rows of densely stained chloroplasts connected by actin fibrils are visible in negatively stained preparations viewed at low magnifications (Fig. 2). The preparative technique in which methylene blue is used as a marker of streaming direction in the transfer of the sample to a directional grid (Fig. 1) allows one to easily ascertain the original direction of cytoplasmic streaming relative to fibril orientation. At high magnifications, in areas in which the configuration of chloroplasts and fibrils are not obviously disturbed in sample preparation, we found that HMM arrowheads point in the direction *opposite* to that of the cytoplasmic stream. Arrowhead direction is the same in adjacent fibrils (Fig. 3). In the descending cytoplasmic stream across the clear zone, arrowheads are directed opposite to those in the ascending stream and again opposite to the direction of cytoplasmic flow. These observations were consistent in all experiments, including several in which the directionality was initially unknown to the observer in order to avoid bias.

The heavy meromysin binding reaction was reversed by addition of 2 mM ATP prior to negative staining. Under these conditions, electron microscopic examination of the preparations revealed undecorated filaments.

Aligned actin fibrils are present in every preparation, but often individual filaments are obscured and heavy meromyosin binding appears to be inhibited by cytoplasmic debris. This difficulty seems to be associated mainly with plant growth conditions, but might also be due to a lesser extent to unidentified differences in preparative techniques.

DISCUSSION

We have shown that in Characean internodal cells HMM arrowhead complexes point in the direction opposite to that of the cytoplasmic stream. Thus the cytoplasm moves in the same direction relative to polarized actin filaments as myosin moves in the muscle sarcomere; i.e., counter to the tips of arrowheads on the actin filaments. These observations lead to the prediction that in other nonmuscle systems actin will also be polarized so that HMM arrowheads are opposite to the direction of cytoplasmic motion.

The presence of actin in bundles polarized opposite to the direction of streaming immediately raises the question of how these filaments are involved in producing motion. Cytoplasmic movement could presumably occur through a sliding filament mechanism in which actin of one polarity interacts through myosin with actin of opposite polarity free in the cytoplasm or attached to organelles or to endoplasmic reticulum. However, thin sections of *Chara* cells (4–6) suggest that actin is found in discrete bundles localized at the ectoplasm-endoplasm interface. Moreover, actin filaments appear to be vanishingly scarce in freshly removed, negatively stained endoplasm (Y. M. Kersey, unpublished results), and when they are found it is difficult to rule out ectoplasmic contaminants. Of course it might be that the mobile actin filaments are labile, or that they are difficult to visualize by conventional preparative techniques. Alternatively, movement could conceivably occur without actin filaments of opposite polarity by means of myosin molecules which move along the fixed actin filament bundles as monomers, dimers, or in aggregates, or in combinations of the above, either associated with organelles or endoplasmic reticulum, or free in the cytoplasm.

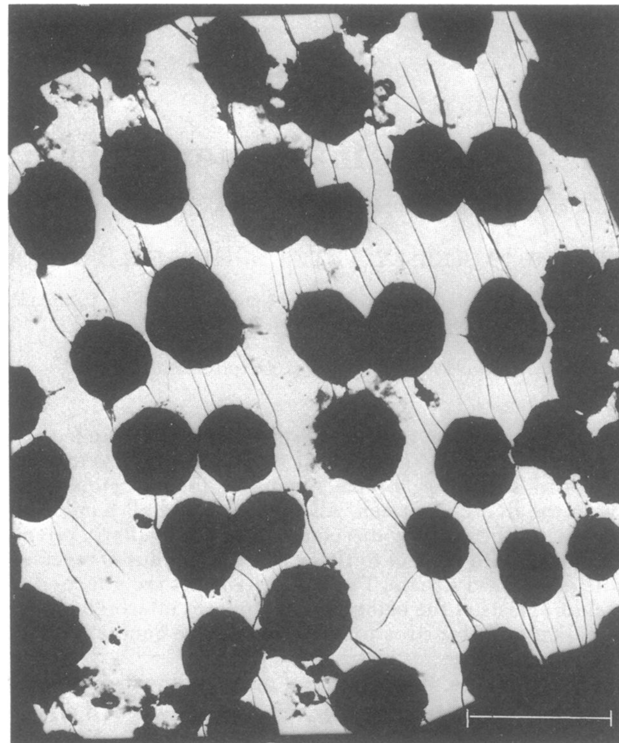

FIG. 2. Low power electron micrograph of negatively stained chloroplasts and associated actin fibrils prepared as described in Fig. 1. Bar = 10 μm.

Although the mechanism of motility is unknown, preliminary biochemical evidence suggests that a myosin-like protein is present in green plants (Y. M. Kersey, T. D. Pollard, and S. Ito, unpublished results), and therefore it seems reasonable that it participates in generating the force for cytoplasmic streaming.

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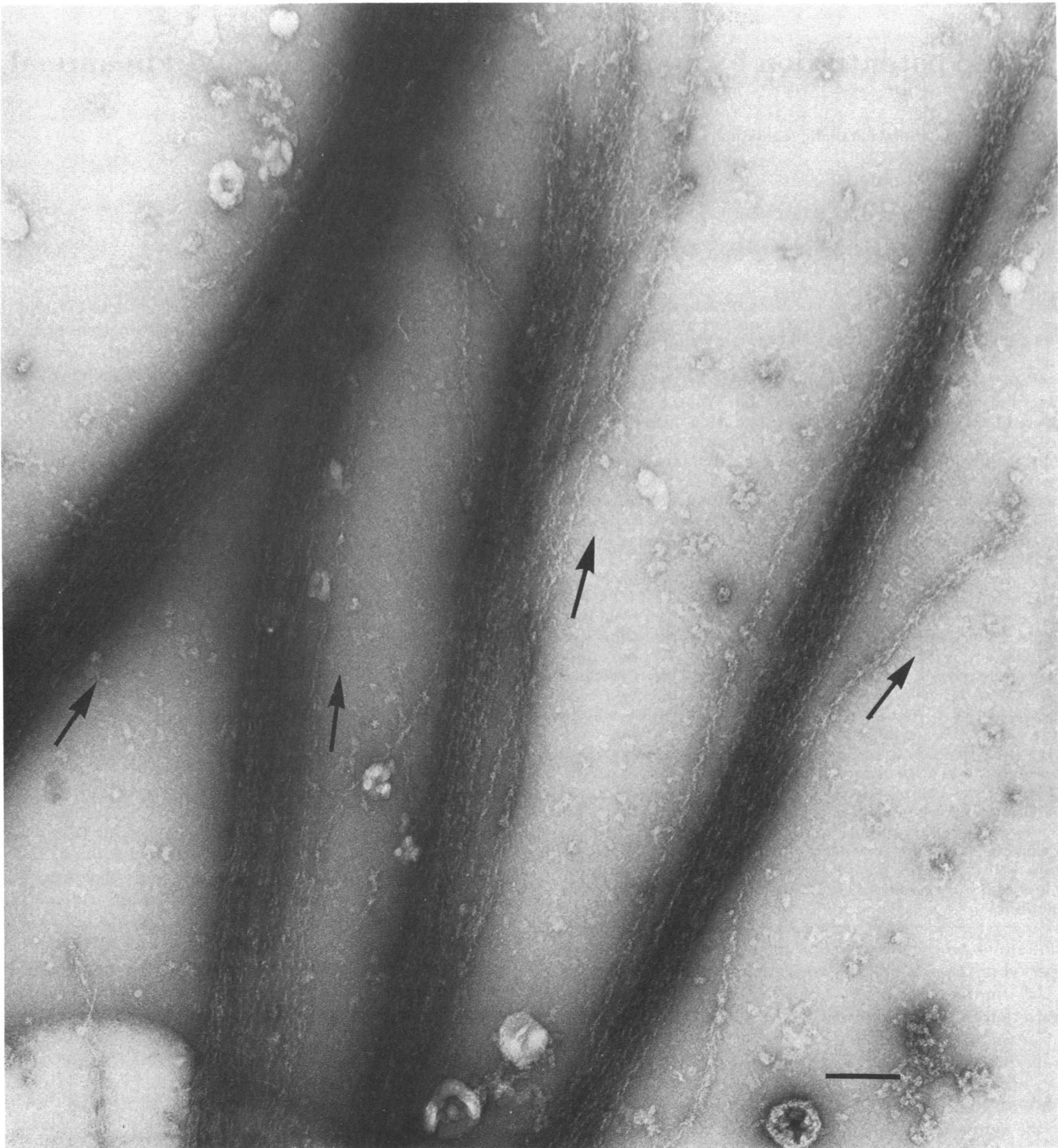

FIG. 3. High magnification of adjacent actin fibrils decorated with heavy meromyosin from rabbit muscle. Printed arrows represent orientation of HMM arrowheads. Note that arrowheads in adjacent fibrils are similarly oriented, facing the top of the page. The streaming path would then be directed toward the bottom. Bar = 0.1 μm.

P.K.H. A preliminary account of this research was presented at the November, 1974 meeting of the American Society for Cell Biology in San Diego, Calif.

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