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Actin in the Green Alga, *Nitella*

(heavy meromyosin/microfilaments/electron microscopy/cytoplasmic streaming/plants)

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ABSTRACT **Bundles of microfilaments very similar in appearance to actin are present in cytoplasmic suspensions obtained from *Nitella flexilis*. The microfilaments bind rabbit heavy meromyosin in arrowhead arrays similar to those produced on muscle actin. The arrowheads are removed with ATP. The results provide evidence that actin is present in green plants, probably in the form of microfilaments thought to be involved in cytoplasmic streaming.**

Actin has now been identified in many eukaryotic cells (1, 2), and actin-containing microfilaments have been implicated in a variety of motility phenomena, including cell locomotion (3), ameboid movement (4–6), and cytoplasmic streaming (7, 8).

A major gap in our knowledge of the distribution of actin, however, centers on the green plants. Although microfilaments are present in algae and higher plants and are strongly implicated in cytoplasmic streaming, they have never been shown to consist of actin. In addition, actin has never been purified from plants, although crude actomyosin preparations have been obtained (9, 10). We now report evidence that microfilaments in the green alga, *Nitella*, consist of actin. Actin-like microfilaments present in cytoplasmic suspensions on coated grids bind rabbit heavy meromyosin (HMM) in typical arrowhead arrays that can be removed by ATP. The results extend our knowledge of the distribution of actin in eukaryotes and add further evidence that actin-containing microfilaments are responsible for cytoplasmic streaming in plants.

MATERIALS AND METHODS

Preparation and Examination of Cytoplasmic Suspensions. Actively streaming samples of *Nitella flexilis* were obtained from cultures grown on soil media and rinsed carefully in several changes of distilled water. Segments of internode cells were excised at room temperature in a few drops of salt solution containing 0.1 M KCl, 5 mM $MgCl_2$, and 5 mM potassium phosphate buffer, pH 7.0. The segments were then gently crushed with the blunt end of a stirring rod until the solution turned slightly green. Drops of this cytoplasmic suspension were placed on Formvar–carbon-coated copper grids. After 1 min, the excess was drawn off with a wedge of filter paper. The sample was rinsed and negatively stained or a drop of HMM solution (0.6 mg of protein per ml) was applied for 1 min. After the excess HMM was drawn off, the grid surface was rinsed with several drops of salt solution before the sample was negatively stained for 1 min with 2% aqueous uranyl acetate, pH 4.2. The grids were immediately examined on a Hitachi HU-11E electron microscope at 50 kV. The microscope magnification was calibrated with a grating replica, 1134 lines per mm. Samples of cytoplasmic suspension were also examined on slides with differential interference contrast optics. Whole internode cells were also inspected in this manner.

Preparation of HMM. Myosin was prepared from New Zealand White rabbit back striated-muscle, as described by Tonomura *et al.* (11), except that piperazine-*N*,*N'*-bis (2-ethanesulfonic acid) (PIPES) was substituted for histidine as a buffering agent and all salt solutions contained 1 mM dithiothreitol. HMM was obtained by tryptic digestion and separated from light meromyosin and residual myosin by dialysis against low-ionic-strength buffer (12). The soluble HMM was further purified by ammonium sulfate fractionation. The material that precipitated between 40 and 60% saturation was collected by low-speed centrifugation, dissolved in a buffer consisting of 5 mM Tris·HCl, 0.5 mM EGTA [ethyleneglycol - bis(β - aminoethylether)*N*,*N'* - tetraacetic acid], 1 mM dithiothreitol (pH 8.0, 25°) and dialyzed against 1 liter of the same buffer (two changes). The HMM was mixed with an equal volume of glycerol and stored at −20°. Samples containing 10 μg of protein were applied to sodium dodecyl sulfate–polyacrylamide gels (13) and judged to be free of contamination with actin. Protein was determined by the biuret reaction (14). Before use, the HMM stock was diluted 10-fold with salt solution.

RESULTS

In negatively stained preparations of the cytoplasmic suspension, bundles of filamentous material can easily be discerned. Some of the bundles are associated with large dense structures that are probably chloroplasts. Chains of chloroplasts linked by fibers or filaments of varying diameter and which often fray out into finer elements were observed in the suspensions viewed with Nomarski optics. When viewed after negative staining, many of the bundles fray out into individual microfilaments approximately 50 Å in diameter (Fig. 1). Small groups and individual microfilaments are also commonly observed. The alternating beaded nature of the microfilaments, with an axial repeat of approximately 375 Å, is quite evident (Fig. 1).

After treatment with HMM, many of the microfilaments appear decorated with arrowheads, all of which point in the same direction (Figs. 2–4). Arrowheads are present on free microfilaments (Fig. 4) as well as on those frayed out from

Abbreviations: HMM, heavy meromyosin; salt solution, 0.1 M KCl, 5 mM $MgCl_2$, and 5 mM potassium phosphate buffer, pH 7.0.

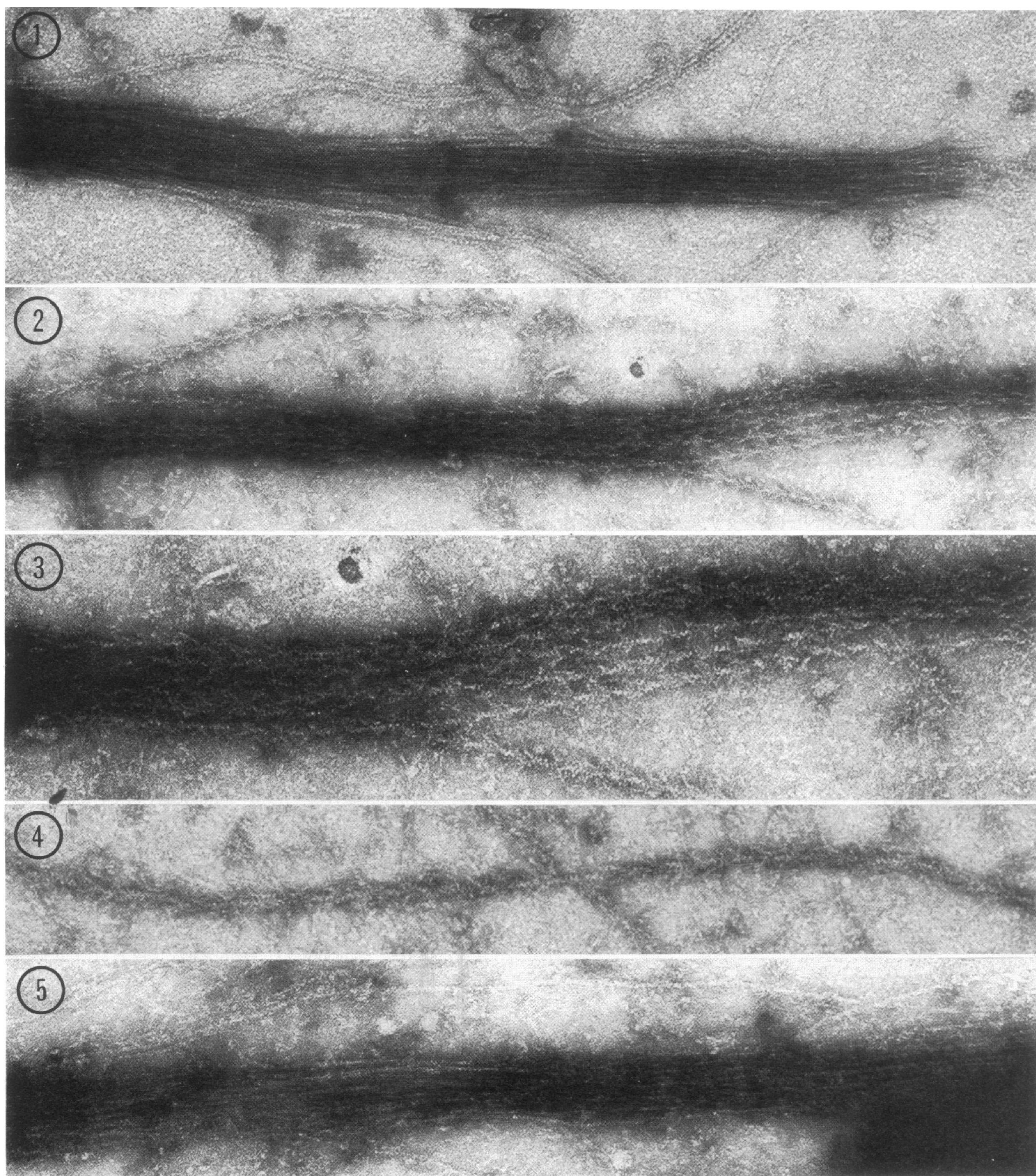

Fig. 1. A bundle of 50-Å beaded microfilaments on a grid not treated with HMM. ×170,000.

Fig. 2. A similar bundle of microfilaments decorated with HMM arrowheads. All of the arrowheads in the bundle point in the same direction. ×92,000.

Fig. 3. An enlargement of part of Fig. 2. ×170,000.

Fig. 4. Individual decorated microfilament after HMM treatment. ×170,000.

Fig. 5. A bundle of beaded microfilaments treated with 1 mM ATP after treatment with HMM. The arrowheads are no longer evident. ×162,000.

bundles (Figs 2 and 3). Many of the decorated individual microfilaments seem linked to or associated with the large dense structures (probably chloroplasts). Our observations indicate that the arrowheads on almost all the microfilaments in a bundle point in the same direction (Figs. 2 and 3), indicating that the polarity of all microfilaments in a bundle may be the same.

The diameter of the decorated filaments (around 260 Å), as well as the polarity, appearance, and repeat (350–360 Å) of the arrowheads, resemble those reported in other systems (4, 5, 8). The beaded nature of the underlying microfilaments shows through the decorations (Figs. 2–4), as it does with other actin filaments treated with HMM (8, 15). Treatment of grids with 1 mM ATP in salt solution immediately after treatment with HMM for 1 min dissociates the arrowheads, leaving nearly bare, breaded microfilaments (Fig. 5).

Another class of filamentous material seen in both control and HMM-treated grids consists of bundles of filaments that appear coated and contain adhering globules or vesicles. These do not appear to bind HMM, and their relation to decorated filaments is unclear.

DISCUSSION

Based on structural characteristics and HMM binding, we conclude that actin is present in cytoplasmic suspensions obtained from *Nitella*. Much of the work on the relation of microfilaments to cytoplasmic streaming in green plants has been done with this alga. Classic studies have shown that the motive force for cytoplasmic streaming is generated at the interface between the moving endoplasm and the stationary ectoplasm (16–18). Light and electron microscopic studies have shown that bundles of 50- to 70-Å microfilaments are present at this site (19, 20). The same bundles become motile when freed by cytoplasmic extrusion or centrifugation (16, 18, 20, 21). Similar bundles of microfilaments occur in higher plants (22, 23). Our results show that actin is present in *Nitella, in vivo,* probably in the form of these microfilaments. Light microscopic examination of our extracts shows the presence of chloroplasts aligned along fibers of filaments. Decorated microfilaments associated with presumptive chloroplasts were also seen on grids. Other studies have reported chloroplast–microfilament associations in *Nitella* (16, 17, 19). Although many of the microfilament bundles in our study have a coated or vesiculate appearance, many also clearly consist of microfilaments similar to F-actin. In addition to bundles, many of the microfilaments occur in small groups or as individuals, all of which reversibly bind HMM in typical arrowhead arrays. The decorated microfilaments are similar to those found in *Physarum* (8) and muscle actin preparations (15). Because the binding of HMM followed by its reversal with ATP are considered specific for actin, these results provide compelling evidence that the microfilaments described here are also actin.

The microfilaments observed on grids not treated with HMM and in those treated with ATP after treatment with HMM have approximately the same diameter as the F-actin identified in other cells (8, 15) as well as the microfilaments seen in *Nitella in vivo* (19). Moreover, the alternating beaded structure strongly resembles the twisted double helix of actin (15). Both the repeat measured on the bare microfilaments and the arrowhead repeat are similar (350–375 Å) and conform to reported values for the half-pitch of actin (around 355 Å).

Efforts were made to insure that the actin reported here is not a contaminant. Protozoa or other eukaryotic organisms were not found on or near whole *Nitella* internodes when examined with the light microscope after several washings in distilled water. Actin-like or arrowhead-bearing filaments were not observed on grids treated with HMM alone, although some filamentous material unlike actin and possibly consisting of aggregated chains of light meromyosin was found. Gels run with the HMM preparation indicated the presence of some light meromyosin, but no actin band was evident. Thus actin was not a contaminant of the HMM nor was it found on clean, negatively stained grids not treated with cytoplasmic suspension or HMM.

We cannot as yet ascertain the nature of the coated, vesiculate filaments. However, Nachmias *et al.* (8) have reported that incompletely extracted *Physarum* actomyosin contains associated organelles or globular material. Thus, the vesiculate filaments in *Nitella* may be a fraction of actin coated with cytoplasmic contaminants.

In addition to the structural characteristics used here, actin should also be identified by biochemical criteria, including molecular weight, amino-acid analysis, and activation of myosin ATPase (24–26). Preliminary evidence indicates the presence of a band with the same mobility as actin on gels loaded with material from an extract of an acetone powder prepared from *Nitella*.

The weight of evidence from *Nitella* indicates that the actin reported here corresponds to the microfilaments seen *in vivo*. Actin has never been identified in plants except for allied organisms such as *Physarum* (8) and *Dictyostelium* (27). That actin can be identified in green plants is not surprising in light of its ubiquity in other eukaryotes. It seems likely that further understanding of the production and control of cytoplasmic streaming in plants will depend on elucidation of the nature and distribution of myosin-like proteins in these cells.

Note Added in Proof. After submitting this paper we also found HMM binding in the characteristic arrowhead pattern to bundles of microfilaments *in situ* in glycerinated segments of *Nitella* internode cells. The microfilament bundles are associated with chloroplasts; within a bundle all the arrowheads show the same polarity and appear to lie in register, suggesting a paracrystalline packing.

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