

## FILAMENTS OF *AMOEBA PROTEUS*

### II. Binding of Heavy Meromyosin by Thin Filaments in Motile Cytoplasmic Extracts

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*Amoeba proteus* has two types of cytoplasmic filaments that are thought to be important in cell motility, cytoplasmic streaming, and changes in cytoplasmic consistency: thin, 50–70 Å wide filaments, and short, thick, 160 Å wide rods (Pollard and Ito, 1970). The thin filaments resemble the actin filaments of muscle (Hanson and Lowy, 1963), *Acanthamoeba* (Weihing and Korn, 1969; Pollard et al., 1970), and *Physarum* (Hatano and Oosawa, 1966).

Cell-free extracts of *Amoeba proteus* show characteristic patterns of streaming when they are warmed to room temperature in the presence of 3 mM adenosine triphosphate (ATP) (Thompson and Wolpert, 1963; Pollard and Ito, 1970). Fibrils become visible by phase-contrast microscopy after about 10 min, and continue to increase in size and number for about 1 hr. These fibrils are birefringent when viewed in a polarizing microscope. Extracts treated with 0.5 mM [ethylenebis-(oxyethylenitrilo)]tetraacetic acid (EGTA) instead of ATP also form fibrils at room temperature but do not stream. The fibrils have been identified by electron microscopy as bundles of thin filaments

(Morgan et al., 1967) that are apparently identical with the thin filaments of the intact amebas (Pollard and Ito, 1970).

We have now demonstrated that these thin filaments form specific ATP-dissociable complexes with muscle heavy meromyosin (HMM) that are indistinguishable from the complexes of HMM with the F actins from muscle (Huxley, 1963), *Acanthamoeba* (Pollard et al., 1970), and *Physarum* (Nachmias et al., 1970). This indirect method provides tentative, but reasonable, identification of the *Amoeba proteus* thin filaments as F actin.

#### METHODS

The motile cytoplasmic extract from *Amoeba proteus*, extract 1, was prepared by the method of Pollard and Ito (1970). Details of the electron microscopic methods and the preparation of muscle actin, *Acanthamoeba* actin, and rabbit muscle heavy meromyosin have been described previously (Pollard et al., 1970).

#### RESULTS

Motile extract 1 was allowed to stand for 1 hr at room temperature in the presence of either 3 mM

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FIGURES 1–6 All illustrations are electron micrographs of preparations negatively stained with 1% uranyl acetate.

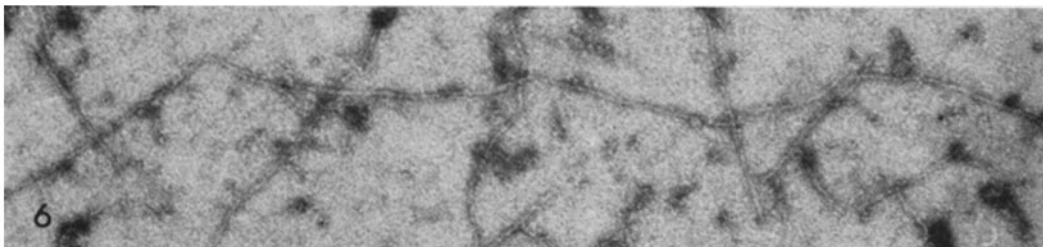
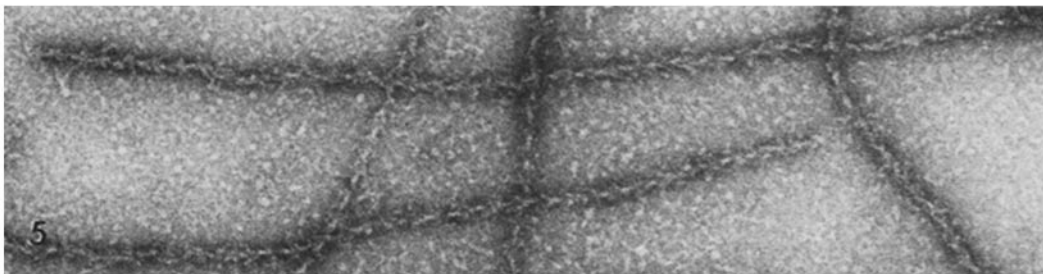
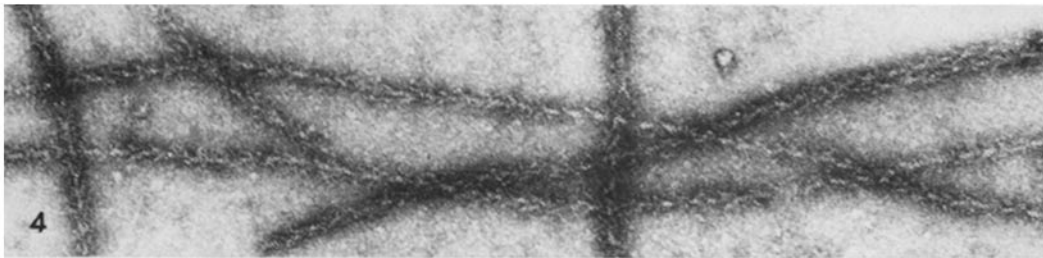
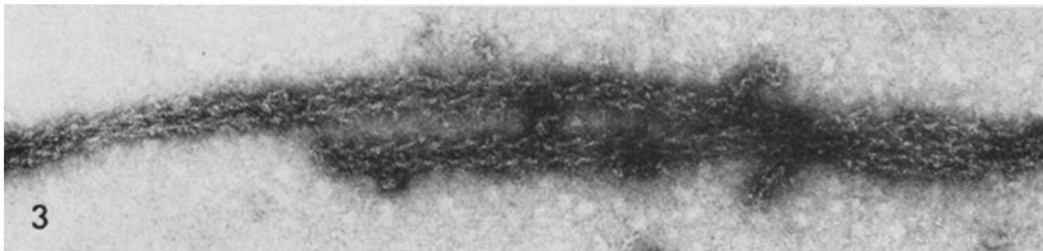
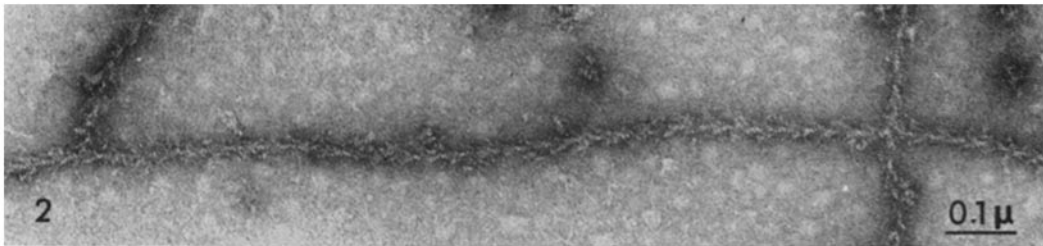
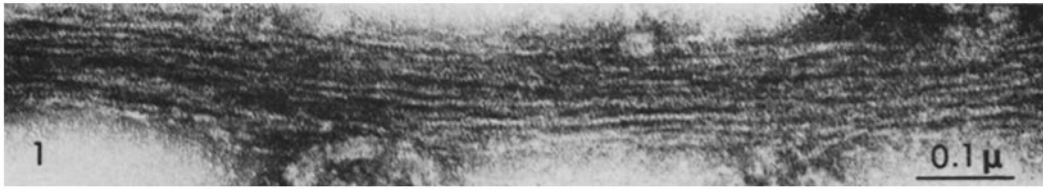
FIGURE 1 A bundle of thin, 70 Å filaments found in extract 1 of *Amoeba proteus*.  $\times 130,000$ .

FIGURES 2 and 3 Rough, 250 Å wide filaments composed of polarized arrowhead-shaped complexes spaced every 380 Å along the filaments from a sample of extract 1 of *Amoeba proteus* treated with rabbit muscle HMM (0.01 mg/ml).  $\times 90,000$ .

FIGURE 4 The hybrid rabbit HMM—*Acanthamoeba* F actin complex of polarized arrowhead-shaped complexes. Note the similarity of this complex to the complex of HMM with *Amoeba proteus* thin filaments.  $\times 90,000$ .

FIGURE 5 The homologous complex of rabbit HMM—rabbit F actin which is indistinguishable from the preparations shown in Figs. 2–4.  $\times 90,000$ .

FIGURE 6 Same preparation as Figs. 2 and 3 except treated with 5 mM ATP, 0.5 mM  $MgCl_2$  before negative staining. Only bare, 70 Å filaments are visible in this preparation. No rough arrowhead-bearing filaments are observed.  $\times 90,000$ .



ATP or 0.5 mM EGTA, during which time numerous fibrils and tactoids formed. The fibrils and clusters of particles in the extract were pelleted by centrifugation at 1000 *g* for 10 min and then resuspended in 1.5 volumes of 0.05 M KCl–0.005 M Tris-maleate, pH 7.0. This step removed soluble proteins and other cytoplasmic constituents which interfere with the negative staining of the pellets with 1% uranyl acetate. These suspensions contained clusters of thin filaments in parallel arrays (Fig. 1), ranging in size from small bundles of five or six filaments to large bundles containing many filaments 2  $\mu$  wide and several microns long. Individual thin filaments were seen most easily after dispersing the tightly packed bundles of filaments by gentle homogenization of the samples. Individual thin filaments were 70 A (SD 11 A) wide and up to 4  $\mu$  long.

To determine whether the thin filaments formed specific complexes with rabbit muscle HMM, the 1000 *g* pellet was resuspended in 0.2 volumes of KCl–Tris-maleate and incubated with HMM (0.10 mg/ml) for 3 hr at 22°C. Samples of this reaction mixture were then diluted 1:10 in KCl–Tris, gently homogenized, and negatively stained with 1% uranyl acetate. Treatment with HMM converted the thin filaments to rough filaments about 250 A wide, which consisted of linear arrays of polarized, arrowhead-shaped complexes spaced at intervals of 380 A along the thin filaments (Figs. 2 and 3). All arrowheads on individual filaments pointed in the same direction. Some filaments were not completely labeled with arrowheads, leaving short regions of bare, 70 A filaments between the arrowhead complexes. A small number of 70 A filaments in samples treated with HMM had no arrowheads, a situation which was also observed in samples of purified muscle and *Acanthamoeba* F actin treated with HMM. The arrowhead complexes along the thin filaments of *Amoeba proteus* were indistinguishable from the complexes of HMM with *Acanthamoeba* F actin (Pollard et al., 1970, and Fig. 4) and muscle F actin (Huxley, 1963, and Fig. 5).

When HMM-treated samples of the extract were treated with Mg-ATP before negative staining, no arrowhead-covered filaments were observed, but bare, 70 A filaments were seen in abundance (Fig. 6).

Characteristic ATP-dissociable arrowheads were also formed when HMM was incubated with thin filaments that had developed in extracts warmed in

the presence of EGTA instead of ATP. Arrowhead complexes formed more slowly with ATP extracts, probably because the reaction was limited by the rate of hydrolysis of residual ATP.

Thick filaments similar to those previously described in *Chaos* by Nachmias (1968) were observed in these preparations. Their appearance did not appear to be altered by HMM treatment, but this was difficult to determine with certainty because the thick filaments were usually obscured by debris.

In the course of this study we did not observe any of the very thin, 20 A filaments which Morgan et al. (1967) described in high-speed supernatant fractions of *Amoeba proteus*.

## DISCUSSION

The available evidence suggests that the reaction of filaments with HMM to form polarized arrowhead complexes which are dissociated by ATP is specific for F actin. All of the F actins which have been examined form these complexes with rabbit muscle HMM. These include rabbit muscle actin, thin filaments of insect indirect flight muscle, and *Mytilus* adductor muscle (a smooth muscle) (Huxley, 1963), *Acanthamoeba* actin (Pollard et al., 1970), *Physarum* actin (Nachmias et al., 1970), and *Dictyostelium* actin (Woolley, 1970). Unfortunately, only a few observations have been made to eliminate the possibility that some nonactin filaments might react with muscle HMM to form typical ATP-dissociable complexes. Ishikawa et al. (1969) noted that HMM did not bind to 100 A filaments in cultured muscle cells or to microtubules in glycerinated cells, and we have not observed binding of HMM to thick fibrillar or other components in extracts of *Amoeba proteus* or in glycerinated *Acanthamoeba*. Therefore, the demonstration that the thin filaments in extracts of *Amoeba proteus* form ATP-dissociable complexes with muscle HMM that are indistinguishable from the complexes of HMM with muscle and *Acanthamoeba* F actin provides strong evidence, but not final proof, that the thin filaments are also F actin.

This tentative identification of the thin filaments as a contractile protein, and their ability to interact specifically with another contractile protein from an animal far separated phylogenetically from that from which these thin filaments came, provide further support for the idea that these thin filaments are involved in the motility of *Amoeba proteus* and its cell-free extracts.

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#### REFERENCES

- HANSON, J., and LOWY, J. 1963. The structure of F-actin and of actin filaments isolated from muscle. *J. Mol. Biol.* 6:46.
- HATANO, S., and OOSAWA, F. 1966. Isolation and characterization of plasmodium actin. *Biochim. Biophys. Acta.* 127:488.
- HUXLEY, H. E. 1963. Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle. *J. Mol. Biol.* 7:281.
- ISHIKAWA, H., BISCHOFF, R., and HOLTZER, H. 1969. Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. *J. Cell Biol.* 43:312.
- MORGAN, J., FYFE, D., and WOLPERT, L. 1967. Isolation of microfilaments from *Amoeba proteus*. *Exp. Cell Res.* 48:194.
- NACHMIAS, V. T. 1968. Further electron microscope studies on the fibrillar organization of the ground cytoplasm of *Chaos chaos*. *J. Cell Biol.* 38:40.
- NACHMIAS, V. T., KESSLER, D., and HUXLEY, H. E. 1970. Electron microscope observations on actomyosin and actin preparations from *Physarum polycephalum*, and on their interaction with heavy meromyosin subfragment I from muscle myosin. *J. Mol. Biol.* 50:83.
- POLLARD, T. D., and ITO, S. 1970. Cytoplasmic filaments of *Amoeba proteus*. I. The role of filaments in consistency changes and movement. *J. Cell Biol.* 46:267.
- POLLARD, T. D., SHELTON, E., WEIHING, R. R., and KORN, E. D. 1970. Ultrastructural characterization of F-actin isolated from *Acanthamoeba castellanii* and identification of cytoplasmic filaments as F-actin by reaction with rabbit heavy meromyosin. *J. Mol. Biol.* 50:91.
- THOMPSON, C. M., and WOLPERT, L. 1963. Isolation of motile cytoplasm from *Amoeba proteus*. *Exp. Cell Res.* 32:156.
- WEIHING, R., and KORN, E. D. 1969. Ameba actin: the presence of 3-methylhistidine. *Biochem. Biophys. Res. Comm.* 35:906.
- WOOLLEY, D. E. 1970. An actin-like protein from amoebae of *Dictyostelium discoideum*. *Fed. Proc.* 29:667 A. (Abstr.)