JOURNAL OF BACTERIOLOGY, Aug. 1978, p. 713-716
0021-9193/78/0135-0713$02.00/0

Vol. 135, No. 2
Printed in U.S.A.

Presence of an Actin-Like Protein in Mycelium of *Neurospora crassa*

E. D. ALLEN[1]* AND A. S. SUSSMAN[2]

Veterans Administration Hospital[1] and Department of Biological Sciences, University of Michigan,[1,2] Ann Arbor, Michigan 48105

Received for publication 17 March 1978

Addition of ATP, $CaCl_2$, and KCl to supernatants prepared from mycelia of Snowflake (strain 507), a morphological mutant of *Neurospora crassa*, results in the formation of filaments 70 nm in diameter. The "decorated" appearance of these filaments after incubation with heavy meromyosin from rabbits suggests they are actin-like.

Actin-like proteins have been shown to be present in a large number of nonmuscle cells and tissues (4, 6, 8, 11), including platelets (3, 17), *Acanthamoeba castellanii* (14), *Amoeba proteus* (12, 13), and *Chaos carolinensis* (7). However, to our knowledge the presence of such proteins in fungi has been described only for nonfilamentous forms. These include the slime molds, *Dictyostelium discoideum* (5, 16) and *Physarum polycephalum* (1, 10).

The presence of actin-like proteins can be demonstrated by using heavy meromyosin (HMM), a subfragment prepared by the digestion of myosin. HMM binds to actin and actin-like proteins and, when visualized by electron microscopy, reveals "decorated filaments" which often show the HMM bound to the filaments in an "arrowhead" configuration.

This note describes the presence of actin-like proteins in extracts prepared from the mycelium of *Neurospora crassa*. We have previously reported that Snowflake (strain 507), a morphological mutant of *Neurospora*, possesses large numbers of cytoplasmic microfilaments approximately 70 nm in diameter. (2).

The preparation of extracts to visualize filaments similar in size to those observed in intact cells was done by using mycelia of Snowflake harvested after several days of growth in liquid culture (2% sucrose with 2% 40× Vogel salts) (15). After washing with distilled water, the mycelia were squeezed dry between paper towels and ground by hand with a mortar and pestle. After dilution with fresh culture medium (5 ml/g of mycelium), the suspended slurry was centrifuged two to three times (15,000 × *g*, 20 min.) to remove extraneous materials. Several centrifugations were required to remove a lipid layer which floated on the supernatant. Grinding of mycelia and centrifugation were carried out at 0 to 4°C.

ATP (1 mM), $CaCl_2$ (1 mM), and KCl (100 mM) were added to the supernatants which were then left for 1 h at room temperature (20°C) or in a water bath at 37°C, as suggested by Adelman (1). After standing, the supernatants were fixed for electron microscopy (1.5% of both paraformaldehyde and glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, for 1 h) and centrifuged (10,000 × *g*, 10 min) to obtain pellets. The pellets were further fixed and prepared for electron microscopy as previously described (2).

Myosin from rabbit hind-leg muscle (9) was used to prepare HMM by tryptic digestion according to the procedures of Pollard et al. (14). HMM (2 to 3 mg/ml) in 0.007 M sodium phosphate, pH 7.0, was added to supernatants to yield a final concentration of 0.5 to 1.0 mg/ml approximately 1 h after ATP, $CaCl_2$, and KCl were added. These supernatants were left standing at room temperature or 37°C for 1 to 2 h before being fixed and prepared for electron microscopy as above.

Figure 1 shows the appearance of filaments in supernatants after addition of ATP, $CaCl_2$, and KCl. The filaments are similar in size and appearance to those seen in intact cells. The results obtained following addition of HMM to such extracts are seen in Fig. 2. No filaments of the type seen in Fig. 1 are observed after addition of HMM; instead, there are decorated filaments closely resembling the actin-like proteins bound with HMM in the other systems mentioned above.

The appearance of negatively stained filaments present in supernatants after addition of ATP, $CaCl_2$, and KCl (Fig. 3) is similar to actin-like proteins observed in other systems. Figure 4 reveals the presence of negatively stained decorated filaments (some with arrowhead complexes) following the addition of HMM. Methods employed for negative staining were taken

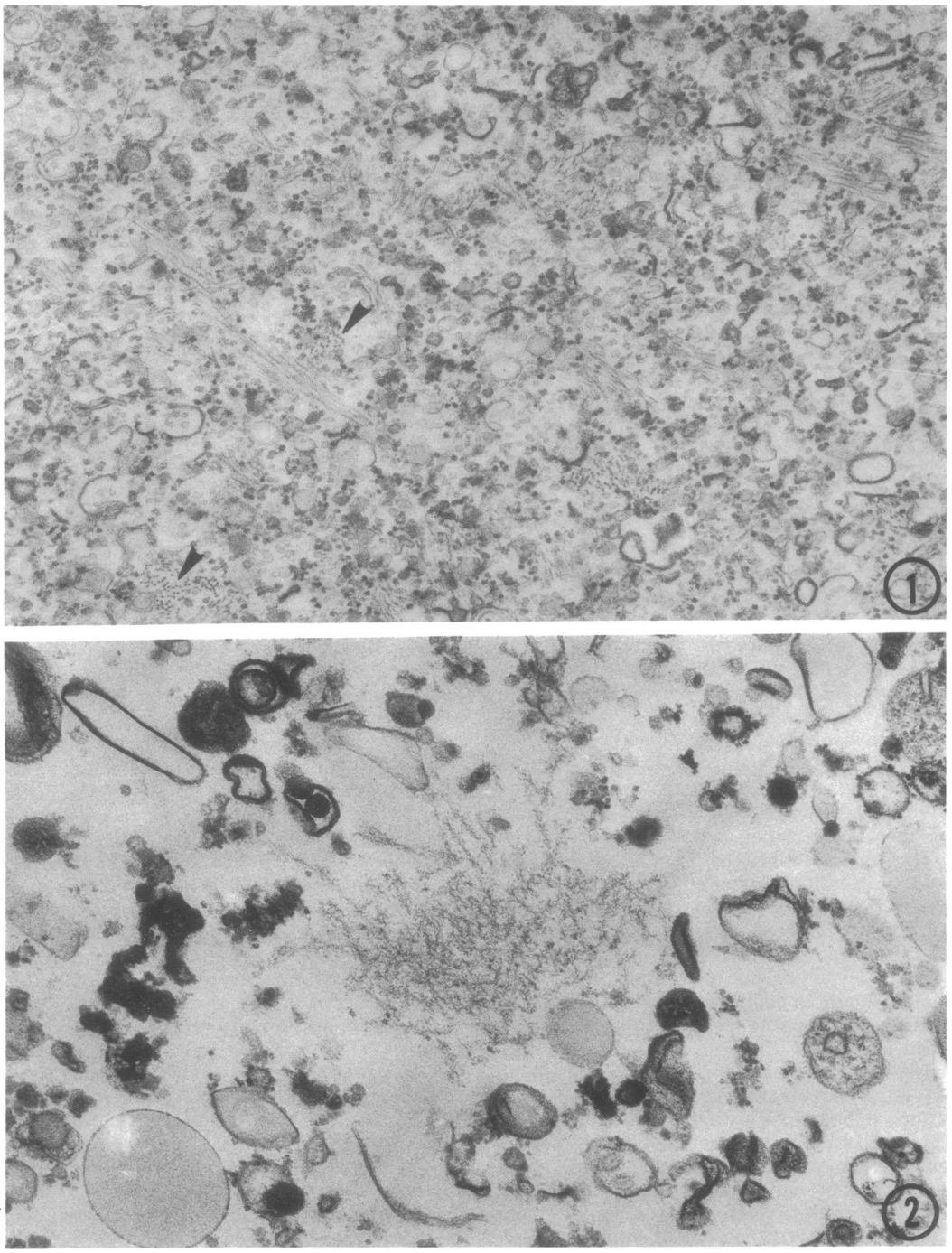
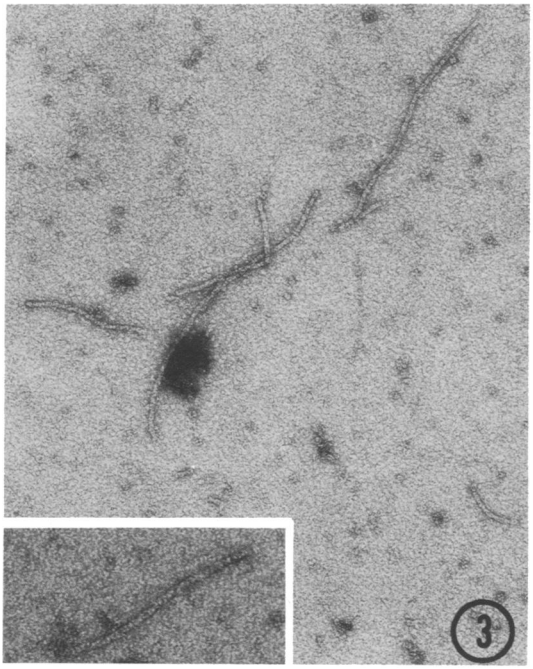


FIG. 1. *Appearance of filaments in pellet obtained from fixed supernatant after addition of ATP, $CaCl_2$, and KCl. Some filaments are seen in transverse view (arrows). ×26,400.*

FIG. 2. *Appearance of "decorated" filaments after incubation of supernatants with HMM prepared from rabbit. ×40,000.*



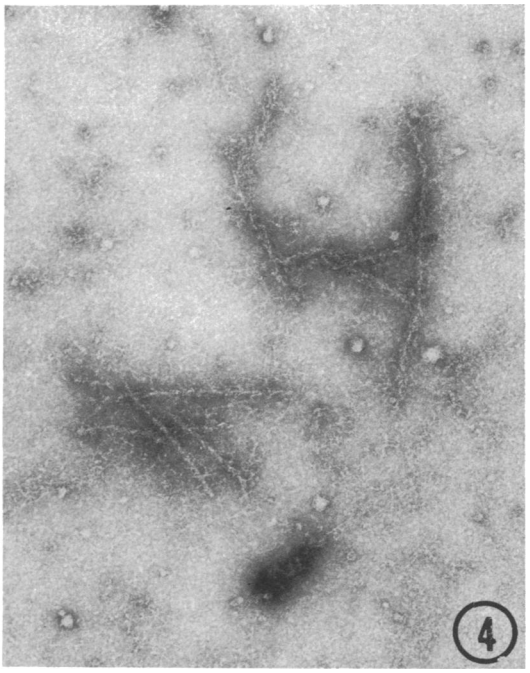


FIG. 3. *Negatively stained filaments present in supernatant after addition of ATP, $CaCl_2$, and KCl. ×65,000. Inset shows the "beaded" appearance seen in some filaments. ×105,000.*

FIG. 4. *Negatively stained "decorated" filaments following incubation with HMM. ×49,000.*

from Wooley (16).

Although ATP alone precipitates the filaments as well as ATP, KCl, and $CaCl_2$, binding with HMM is accomplished better when the latter substances are used as a precipitant. On the other hand, $CaCl_2$ and KCl precipitate the filaments much less effectively than ATP alone or in combination with these salts.

The resemblance of filaments from *Neurospora* to actin-like proteins from other organisms, as well as the appearance of decorated filaments following HMM binding, suggests the presence of an actin-like protein. Filaments similar in appearance to those from Snowflake were prepared from the wild-type strain, 74a, by the same procedures described here. We are currently attempting to purify and compare the actin-like proteins from Snowflake with those found in wild-type strains of *Neurospora*.

We thank Patricia Permoad for excellent technical assistance.

This study was supported by the Medical Research Service of the Veterans Administration Hospital.

LITERATURE CITED

1. **Adelman, M. R.** 1977. *Physarum* actin. Observations on its presence, stability, and assembly in plasmodial extracts and development of an improved purification procedure. Biochemistry **16**:4862–4871.
2. **Allen, E. D., R. Lowry, and A. S. Sussman.** 1974. Accumulation of microfilaments in a colonial mutant of *Neurospora crassa*. J. Ultrastruct. Res. **48**:455–464.
3. **Bettex-Galland, M., E. Probst, and O. Behnke.** 1972. Complex formation with heavy meromyosin of the isolated actin-like component of thrombosthenin, the contractile protein from blood platelets. J. Mol. Biol. **68**:533–535.
4. **Bray, D.** 1973. Cytoplasmic actin: a comparative study, p. 567–571. *In* The mechanism of muscle contraction. Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, N.Y.
5. **Clarke, M., G. Schatten, D. Mazia, and J. A. Spudich.** 1975. Visualization of actin fibers associated with the cell membrane in amoeba of *Dictyostelium discoideum*. Proc. Natl. Acad. Sci. U.S.A. **72**:1758–1762.
6. **Clarke, M., and J. A. Spudich.** 1977. Nonmuscle contractile proteins: the role of actin and myosin in cell motility and shape determination. Annu. Rev. Biochem. **46**:797–822.
7. **Comly, L. T.** 1973. Microfilaments in *Chaos carolinensis:* membrane association, distribution and HMM binding in the glycerinated cell. J. Cell Biol. **58**:230–237.
8. **Ishikawa, H., R. Bischoff, and H. Holtzer.** 1969. Formation of arrowhead complexes with heavy meromyosin in a variety of cell types. J. Cell Biol. **43**:312–328.
9. **Kielley, W. W., and L. B. Bradley.** 1956. The relationship between sulfhydryl groups and the activation of myosin adenosinetriphosphatase. J. Biol. Chem. **218**: 653–659.
10. **Nachmias, V. T., D. Kessler, and H. E. Huxley.** 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**:83–90.
11. **Pollard, T. D.** 1976. Cytoskeletal functions of cytoplasmic contractile proteins. J. Supramol. Struct. **5**:317–334.

12. **Pollard, T. D., and E. D. Korn.** 1971. Filaments of *Amoeba proteus*. II. Binding of heavy meromyosin by thin filaments in motile cytoplasmic extracts. J. Cell Biol. **48**:216–219.
13. **Pollard, T. D., and E. D. Korn.** 1973. Electron microscopic identification of actin associated with isolated *Amoeba* plasma membranes. J. Biol. Chem. **248**: 448–450.
14. **Pollard, T. D., E. Shelton, R. R. Weihing, and E. D. Korn.** 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**:91–97.
15. **Vogel, H.** 1964. Distribution of lysine pathways among fungi: evolutionary implications. Am. Nat. **98**:435–446.
16. **Woolley, D. E.** 1972. An actin-like protein from Amoebae of *Dictyostelium discoideum*. Arch. Biochem. Biophys. **150**:519–530.
17. **Zucker-Franklin, D., and G. Grusky.** 1972. The actin and myosin filaments of human and bovine blood platelets. J. Clin. Invest. **51**:419–430.