ORIENTED THICK AND THIN FILAMENTS IN *AMOEBA PROTEUS*

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Actin and myosin filaments as a foundation of contractile systems are well established from ameba to man (3). Wolpert et al. (19) isolated by differential centrifugation from *Amoeba proteus* a motile fraction composed of filaments which moved upon the addition of ATP. Actin filaments are found in amebas (1, 12, 13) which react with vertebrate heavy meromyosin (HMM), forming arrowhead complexes as vertebrate actin (3, 9), and are prominent within the ectoplasmic tube where some of them are attached to the plasmalemma (1, 12). Thick and thin filaments possessing the morphological characteristics of myosin and actin have been obtained from isolated ameba cytoplasm (18, 19). In addition, there are filaments exhibiting ATPase activity in amebas which react with actin (12, 16, 17). However, giant ameba (*Chaos-proteus*) shapes are difficult to preserve, and the excellent contributions referred to above are limited by visible distortions occurring in the amebas (rounding up, pseudopods disappearing, and cellular organelles swelling) upon fixation. Achievement of normal ameboid shape in recent glycerination work (15) led us to attempt other electron microscope fixation techniques, resulting in a surprising preservation of *A. proteus* with a unique orientation of thick and thin filaments in the ectoplasmic region.

MATERIALS AND METHODS

Amoeba proteus was grown in Chalkley's solution, and the food source was *Colpidium*. Amebas were starved for at least 48 h to reduce cytoplasmic inclusions. The fixation solution for electron microscopy consisted of: 0.001 M ethylene glycol bis(β-aminoethyl ether)N,N,N,'N'-tetraacetic acid (EGTA), 0.5% acrolein, 5% glutaraldehyde and cacodylic buffer, adjusted to a pH of 7.4. The objectives of fixation were: (*a*) achieving rapid penetration by adding large volumes of fixation solution to 10 amebas, with judicious stirring; and (*b*) preventing contraction of amebas with EGTA. Fixation time was 2 h at 25°C. Satisfactory fixation was based on retention of ameboid shape as judged by light microscope examination. Selected specimens were washed and stained with 1% osmium tetroxide for 1.5 h, dehydrated, and embedded in Epon (6). Thin sections were cut with a diamond knife on an LKB ultramicrotome, and the sections

were then stained with lead citrate and uranyl acetate and examined in a JEM 10.0 B electron microscope.

RESULTS

Fig. 1 illustrates the preponderance of rough endoplasmic reticular (RER). Oriented thick and thin filaments (*Fi*) are seen along the periphery of this cell, but no oriented thick and thin filaments are visible in the left cell extension. In Fig. 2, the thick and thin filaments near the periphery overlap and they follow the contours of the cell. RER is present immediately below the thick and thin filaments. Fig. 3 illustrates a striking overlapping of thick and thin filaments (left side), and shows a continuous layer of these filaments near the periphery of this section. The arrow indicates a possible attachment of filaments to the plasmalemma. Note that the oriented filaments do not appear to enter the prominent cell extension at *Y*.

DISCUSSION

Filaments as a possible basis for contraction in amebas have been reported by many investigators (1–5, 10, 12–14, 16, 17). With HMM labeling of thin filaments there was found a specific orientation of labeled filaments within the ectoplasmic tube positioned near and/or attached to the plasmalemma (1, 13). With HMM labeling in ameba, thin filaments are beautifully illustrated but thick filaments are rarely found within the ectoplasmic tube (1, 13). ATPase activity in contracting ameba has been reported (12, 16, 17, 19). In extracted cytoplasm of ameba exhibiting streaming, Taylor et al. and Wolpert et al. (18, 19) demonstrated

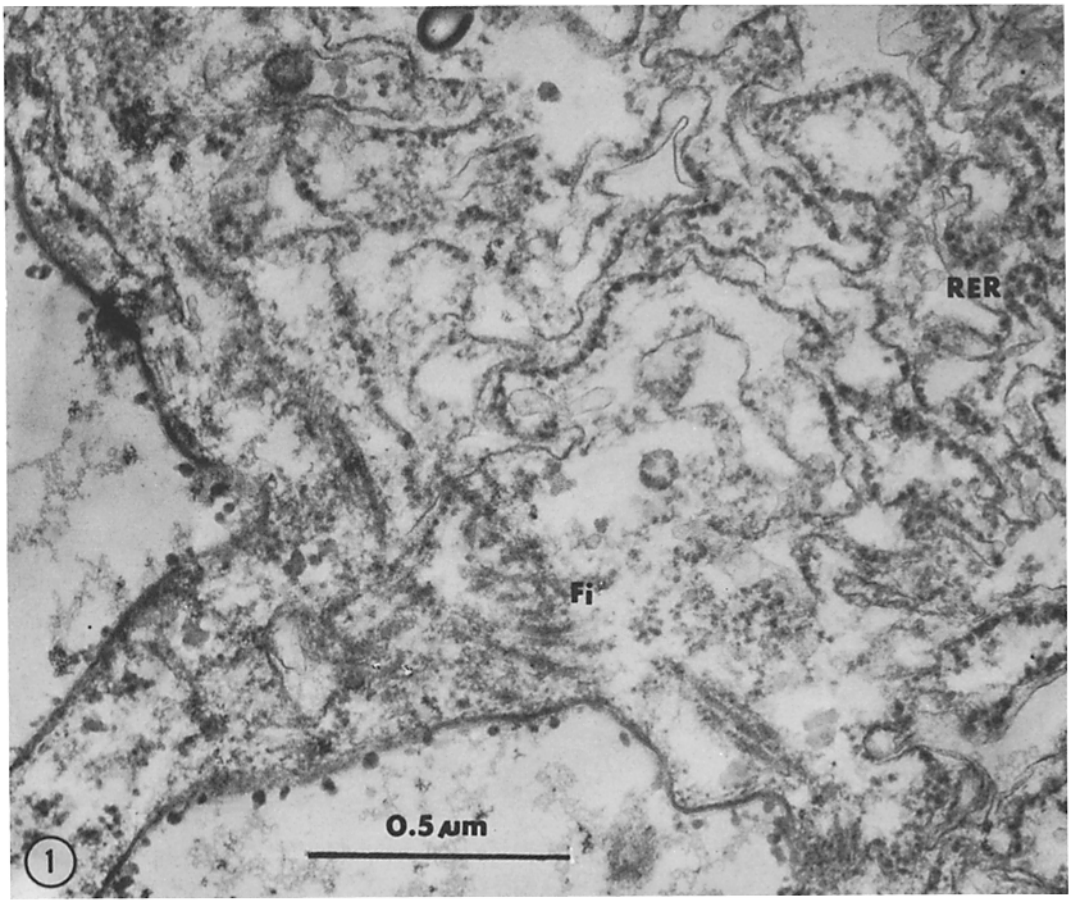


FIGURE 1 The filaments (*Fi*) along the periphery of the ameba. Note the lack of filaments extending into the cell extension at lower left. There is abundant RER in this section from the front end of a pseudopod. ×78,000.

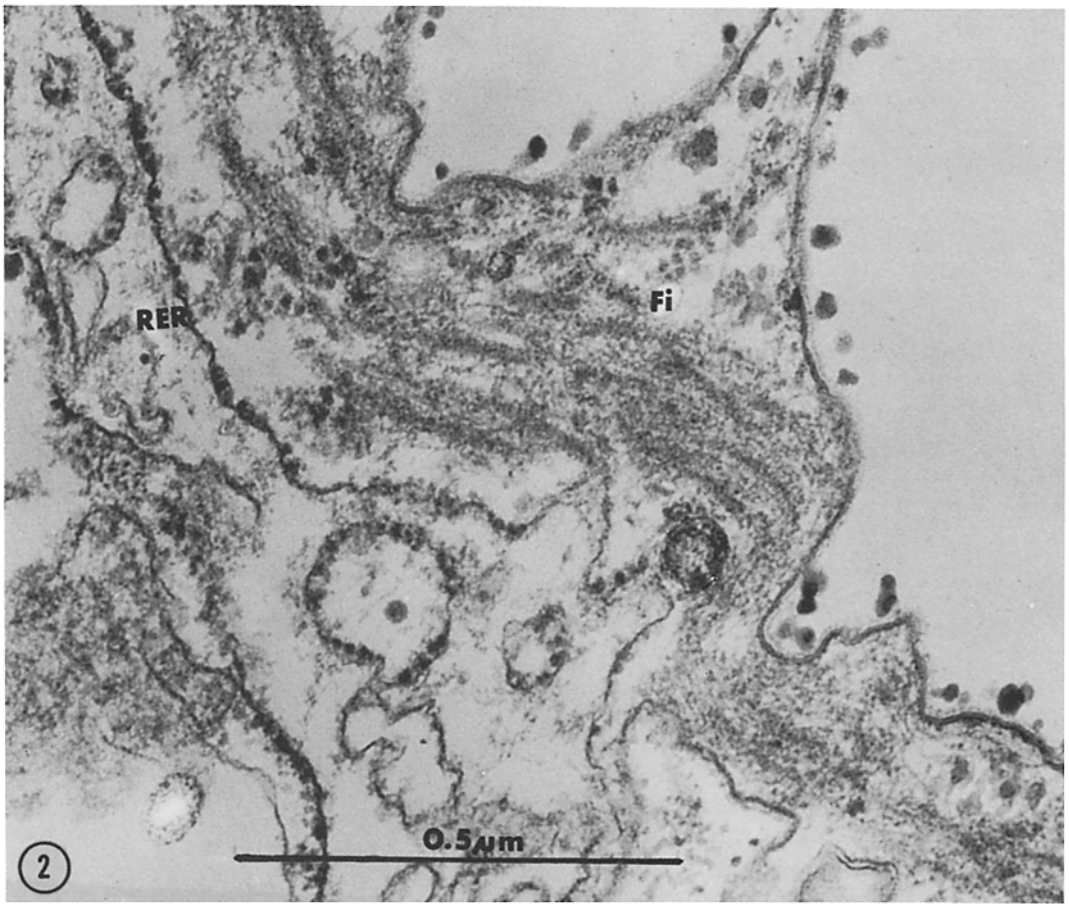
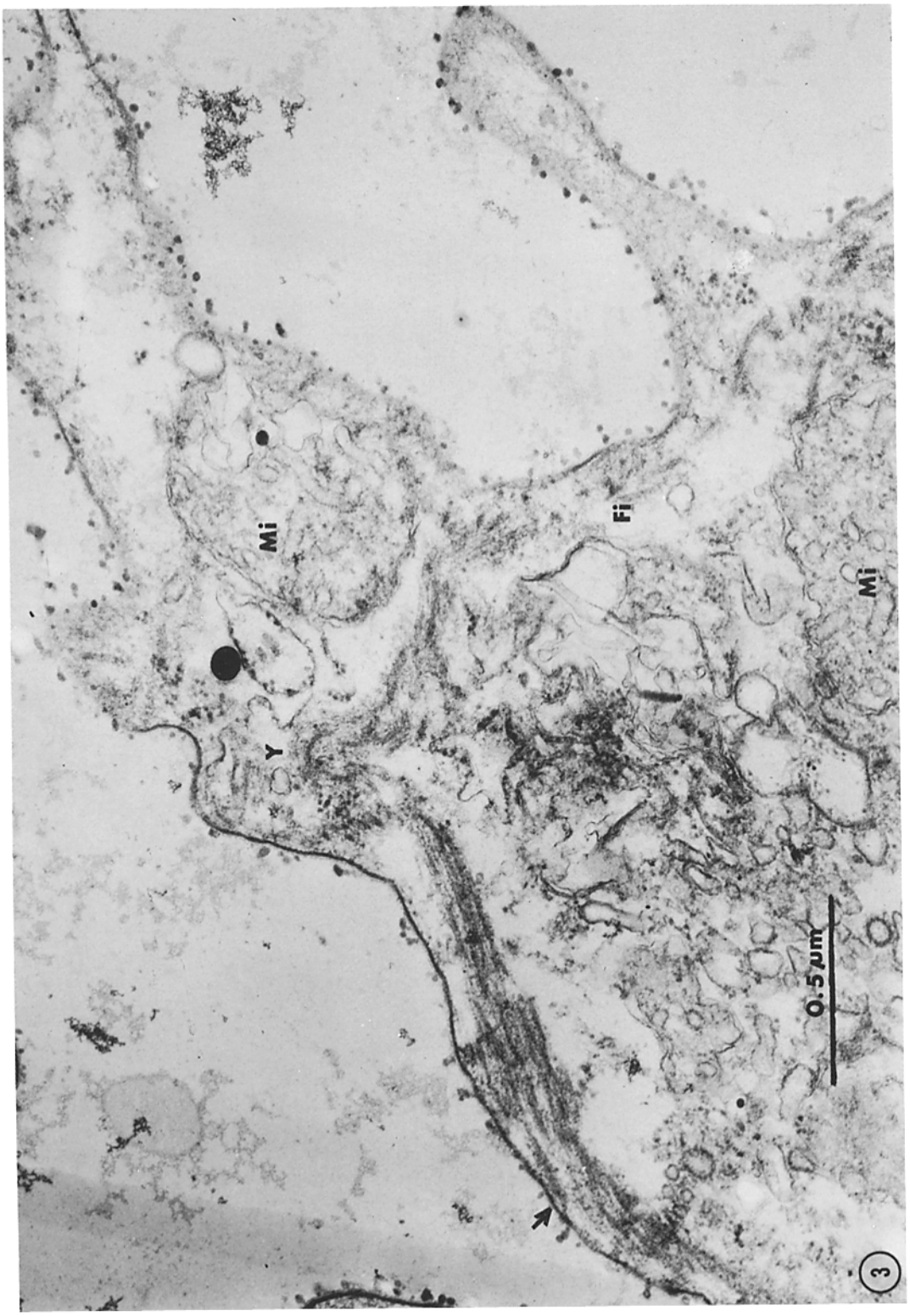


FIGURE 2 Thick and thin filaments (*Fi*) overlapping near the periphery of the cell and following the contours of the surface. The RER is particularly noticeable in this photograph. ×125,000. Section taken from front end of a pseudopod.

thick and thin filaments. Comly (1) found thick and thin filaments in glycerinated amebas but the filaments had no specific orientation because the ameboid shape is lost after most fixation techniques (1, 5, 10, 11, 13). Pollard and Ito (12) found thick- and thin-filament clusters in the ectoplasmic tube in *A. proteus* but they did not have the orientation or appear as a relatively continuous layer of filaments as in this report.

Recently, glycerinated contractile models of *A. proteus* and *C. chaos* have been achieved with preservation of ameboid shape (15). Upon addition of ATP-Ca-Mg to these models, the amebas contracted to as much as 50% of their original volume. Preliminary electron microscope examination of these glycerinated models demonstrated that thick and thin filaments were present in the ectoplasmic tube of *A. proteus* similar to those found in glycerinated *C. chaos* (5, 16).

The present investigation demonstrates, for the first time in *A. proteus* with conventional electron microscope fixation, that thick and thin filaments can be seen in a unique orientation around the periphery of the cell directly underneath the plasmalemma. Further, these filaments in *A. proteus* are so oriented that they appear to resemble (Fig. 3) in their orientation the thick and thin filaments found in muscle cells. They form a continuous region of contractile material which is usually positioned directly under the plasmalemma within the ectoplasmic gel, and some of the thin filaments appear to be attached to the plasmalemma (1, 13). These filaments are disposed predominantly parallel to the plasmalemma. These oriented arrays of


Mi
Fi
Mi
Y
0.5 µm
3

filaments do not appear to extend into small pseudopods or cell extensions (Figs. 1–3). Further, we did not find them within the frontal zone of an advancing pseudopod in *A. proteus*. The orientation and distribution of the thick and thin filaments in *A. proteus* are similar to those found for the small ameba *Thecamoeba sphaeronucleolus* by Haberey (2).

Contraction by the association of thick (myosin-like) and thin (actin-like) filaments was proposed in the sliding filament model (SFM) for ameboid motion (14). Other workers have suggested the interdigitation of filaments for ameboid motion (3, 13). A major assumption for the SFM is that actin-like and myosin-like filaments reach their contractile states within the ectoplasmic tube of an ameba, which is inseparably based on the ectoplasmic tube contraction theory advanced by Mast (7, 8). Muscle-like contraction, one assumes, requires a highly organized interaction between thick and thin filaments. Therefore, we suggest that the ectoplasmic tube may be a matrix permitting precise orientations of these two filaments relative to one another to achieve contraction.

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FIGURE 3 Note the obvious overlapping of thick and thin filaments on the left side of this photograph, in contrast to the more sparse numbers of filaments on the right side of the photograph. The arrow at the left indicates a spot where the filaments come into close proximity with (and possibly attach to) the plasmalemma. *Y* indicates a place where oriented thick and thin filaments turn and do not extend into the small pseudopod. Mitochondria (*Mi*) are prominent. Section taken from the uroid region. ×62,500.

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