THE VISUALIZATION OF ACTIN FILAMENT POLARITY IN THIN SECTIONS

Evidence for the Uniform Polarity of Membrane-Associated Filaments

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ABSTRACT

We have developed an improved method for visualizing actin filament polarity in
thin sections. Myosin subfragment-1 (S-1)-decorated actin filaments display a
dramatically enhanced arrowhead configuration when fixed in a medium which
contains 0.2% tannic acid. With the exception of brush borders from intestinal
epithelial cells, the arrowhead periodicity of decorated filaments in a variety of
nonmuscle cells is similar to that in isolated myofibrils. The periodicity of
decorated filaments in brush borders is significantly smaller. Actin filaments
which attach to membranes display a clear, uniform polarity, with the S-1
arrowheads pointing away from the plasma membrane, while those which
comprise the stress fibers of myoblasts and CHO cells have antiparallel polarities.
These observations are consistent with a sliding filament mechanism of cell
motility.

KEY WORDS   actin   ·   membranes   ·   cell
motility   ·   myosin S-1   ·   filament polarity

The demonstration that cytoplasmic actin and
myosin are involved in the movement of cells and
of cell organelles (9, 17, 26) has led to the
formulation of a number of conceptually similar
models of cell motility (2, 13, 25, 30). These
theories postulate that the polarized interaction of
membrane-associated actin with cytoplasmic
myosin generates the force for motility, in a
manner analogous to the mechanism of skeletal
muscle contraction. If cell motility is based upon a
musclelike interaction of actin and myosin, the
actin filaments would be expected to attach to
membranes with the same polarity with which
they attach to Z lines in muscle (13, 22).

The polarity of actin filaments can be deter-
mined from the specific binding of heavy mero-
myosin (HMM) to form an arrowhead configura-
tion (12). The arrowheads point in the direction
in which the filament generates force by interact-
ing with myosin. Although the polarity of actin-
HMM complexes can be clearly visualized in
negatively stained preparations of isolated fila-
ments, negative staining techniques have only
limited applicability to the study of the distribution

and polarity of actin filaments *in situ*.

Ishikawa et al. (15) developed a method which
permits the determination of actin filament polar-
ity in thin sections of glycerinated cells. This
technique has been used successfully to determine
the polarity of the highly ordered bundles of actin
filaments which occur in the brush border of
intestinal epithelial cells (22) and in the acrosomal
process of Mytilus sperm (31). However, the
microfilaments which are associated with most
types of cell motility are not found in ordered
arrays, but occur individually or in loosely orga-
nized groups. Although in a number of cases these
filaments have been shown to "decorate" with
HMM or myosin subfragment-1 (S-1), their polar-
ity has not been clearly demonstrated in thin
sections (e.g., references 7, 15, 24, 27, and 28).

We have found that the inclusion of 0.2%
tannic acid in the primary glutaraldehyde fixative
results in a clear demonstration of the arrowhead
configuration of S-1-decorated actin filaments in
thin sections of a variety of cell types. In all of the
examples studied, actin filaments which attach to
the plasma membrane show a uniform polarity,
with the arrowheads pointing away from the mem-
brane, while bundles of actin filaments which
occur in the cytoplasm contain antiparallel fila-
ments.

MATERIALS AND METHODS

Preparation of Myosin S-1

Rabbit skeletal muscle myosin was prepared by the method of Kielley and Harrington (16) and was digested with soluble papain to produce myosin S-1 (19). S-1 was stored at a concentration of 25 mg/ml in 50% glycerol, 10 mM imidazole, pH 7.0, 1 mM K$^+$EDTA, and 1 mM dithiothreitol at $-20°$C.

S-1 Decoration of Actin Filaments

Samples of material from each cell type studied were incubated for 10–15 min at 24°C with myosin S-1 at a concentration of 1–2 mg/ml, washed twice with incubation medium, and processed for electron microscopy. Control samples were incubated in the same concentration of S-1 with either 10 mM pyrophosphate (PPi) or 4 mM ATP.

SEA URCHIN EGG CORTEX: Fertilized eggs from the sea urchin *Strongylocentrotus purpuratus* were demembranated by passing them through 60-μm bolting cloth (TETKO Inc., Elmsford, N. Y.). The demembranated eggs were incubated as a 0.1% suspension in artificial seawater at 17–18°C and stirred constantly. 1 h after insemination, the eggs were washed once in 0.53 M Ca^{++}/Mg^{++}-free saline (19 parts NaCl to 1 part KCl, pH 7.8) to remove divalent cations. The eggs were then washed at 0°C in one change of isolation buffer and resuspended in fresh isolation buffer as a 10% suspension. Cortex isolation buffer is composed of 0.35 M glycine, 0.1 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (Hepes), pH 7.5, 5 mM ethylene glycol-bis (β-aminoethylether)*N*,*N'*-tetraacetic acid (EGTA), 5 mM MgCl$_2$, and 1 mM dithiothreitol; and contains 0.5 mM phenylmethylsulfonyl fluoride and 0.1 mg/ml soybean trypsin inhibitor to reduce proteolysis. The eggs were gently homogenized at 0°C with 1–3 passes of a loose-fitting teflon homogenizer. Cortices were sedimented at 200 *g* for 5 min, washed three times in cold isolation buffer, and decorated with myosin S-1 in isolation buffer. The method of cortex isolation and the morphology and protein composition of the isolated cortex will be dealt with in detail in a forthcoming paper (Begg, Morell, and Rebhun, manuscript in preparation).

ISOLATED MYOFIBRILS: Glycerinated myofibrils from rabbit psoas muscle were prepared by the method of Herman and Pollard (10). Isolated myofibrils were decorated with myosin S-1 in standard salt solution (12).

ISOLATED BRUSH BORDERS: Brush borders were isolated from intestinal epithelial cells of the jejunum of 10- to 15-day-old Sprague-Dawley rats by the method of Rodewald et al. (27), and incubated with myosin S-1 in phosphate-buffered (10 mM Na$^+$-phosphate, pH 7.2) saline.

CHO CELLS: CHO-K$_1$ cells were cultured in F12 medium containing 10% fetal calf serum at 37°C in an atmosphere of 6% CO$_2$. Cells were converted from an epithelioid to a fibroblast-like morphology by the addition of 1 × 10^{-3} M dibutyryl cyclic AMP (dBCAMP) (11) 12–18 h before use. Cells were rinsed three times with the modified standard salt solution (MSS) of Schloss et al. (28) and glycerinated at room temperature (28). Myosin S-1 decoration was carried out in MSS.

MYOBLASTS: Secondary cultures of myoblasts from 10-day-old quail embryo (*Coturnix coturnix*) breast muscle were prepared by the method of Emerson and Beckner (5). Cells were cultured by the method of Bowman and Emerson (1). 3- to 5-day-old cultures were washed three times with MSS, glycerinated as above, and decorated with S-1 in MSS.

Electron Microscopy

S-1 decorated samples were fixed in 1% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, Pa.), 0.1 M Na$^+$-phosphate buffer, pH 7.0, and 0.2% tannic acid (Type AR, code no. 1764, Mallinckrodt, Inc., St. Louis, Mo.) for 30 min at 24°C. The tannic acid was added to the fixative as a powder immediately before use. Fixed specimens were rinsed several times with 0.1 M phosphate buffer, pH 7.0, and postfixed in 1% OsO$_4$, 0.1 M Na$^+$-phosphate, pH 6.0, for 20 min on ice. After a distilled water rinse, samples were stained en bloc with 1% aqueous uranyl acetate for 30–45 min at 24°C, dehydrated in a graded series of ethanol, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and were examined with either a Hitachi HU-11E electron microscope operated at an accelerating voltage of 75 kV or a Philips 200 electron microscope operated at 60 kV. The microscopes were calibrated by photographing a grating replica with 54,800 lines per inch. Measurements of filament dimensions were made from negatives with a Nikon Model 6C profile projector (Erenreich Photo Optical Industries, Garden City, N. J.).

RESULTS

Appearance of S-1 Decorated Actin Filaments after Tannic Acid Fixation

Decorated actin filaments which are fixed without tannic acid (Fig. 1*a*) have the typical morphology described in many previous studies (e.g., references 7, 15, 24, 27, and 28). They appear thicker than undecorated filaments, with a fuzzy or serrated border, but do not show a consistent pattern of arrowheads. The filaments often appear disrupted, as if poorly fixed. In dramatic contrast to these results, S-1-decorated filaments which have been fixed in the presence of tannic acid show a clear, uniform arrowhead configuration (Fig. 1*b*). The polarity of longitudinally sectioned filaments can be determined unambiguously, except in areas of very close packing. No filament

decoration was observed when S-1 incubation was performed in the presence of 10 mM PPi or 4 mM ATP.

Fixation in the presence of 0.2% tannic acid does not detectably alter the dimensions of the various classes of cytoplasmic microfilaments. Un-decorated actin filaments have a diameter of 60–70 Å, while decorated filaments range in diameter between 180 and 230 Å (Fig. 3 b). The decorated filaments are easily distinguished from intermediate filaments (tonofilaments) which measure 95–115 Å in diameter (Fig. 2) and from filaments
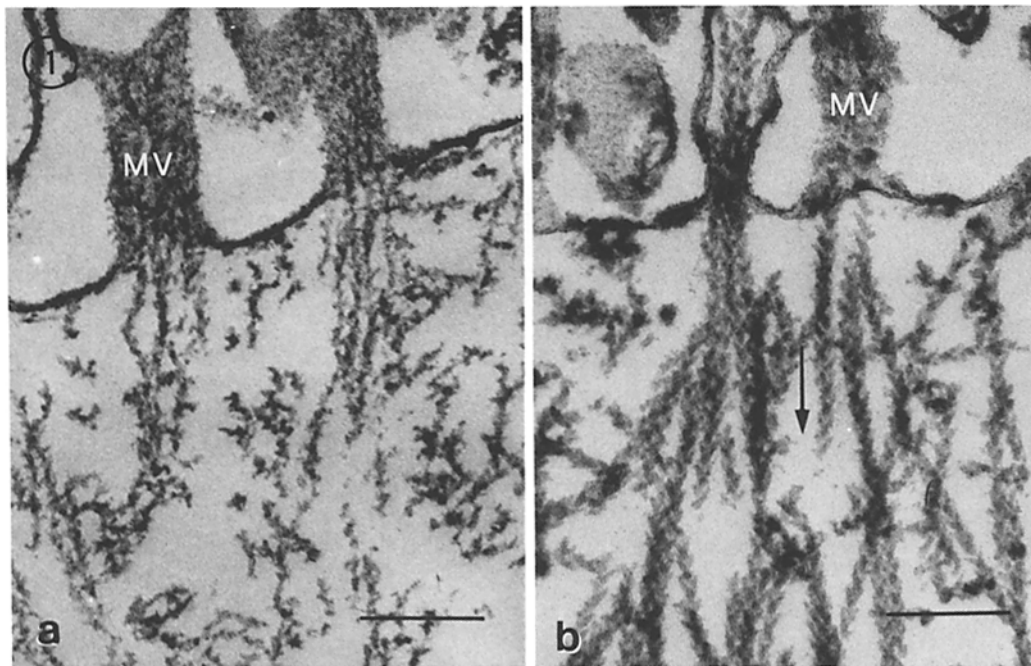
FIGURE 1   Isolated cortices from fertilized sea urchin eggs decorated with S-1. (a) Fixed without tannic acid. (b) 0.2% tannic acid included in the fixative. Arrow indicates polarity of actin filaments. MV, microvillus. The actin S-1 complex is well-preserved in Fig. 1 b and shows a clear arrowhead structure. Bars, 0.2 µm. × 80,000.
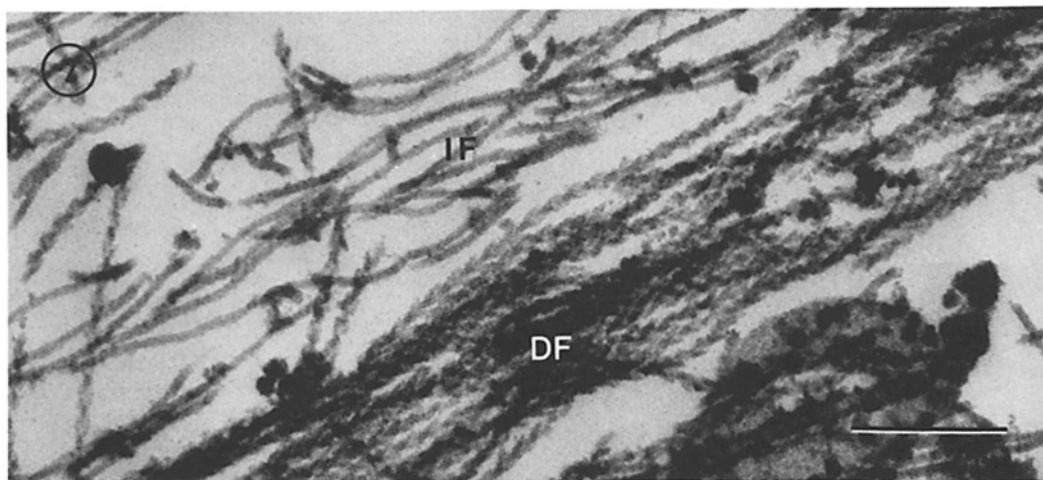

FIGURE 2   Glycerinated myoblast decorated with S-1. DF, decorated actin filaments. IF, 100-Å intermediate filaments. The 100-Å filaments do not bind S-1. Bar, 0.2 µm. × 101,000.
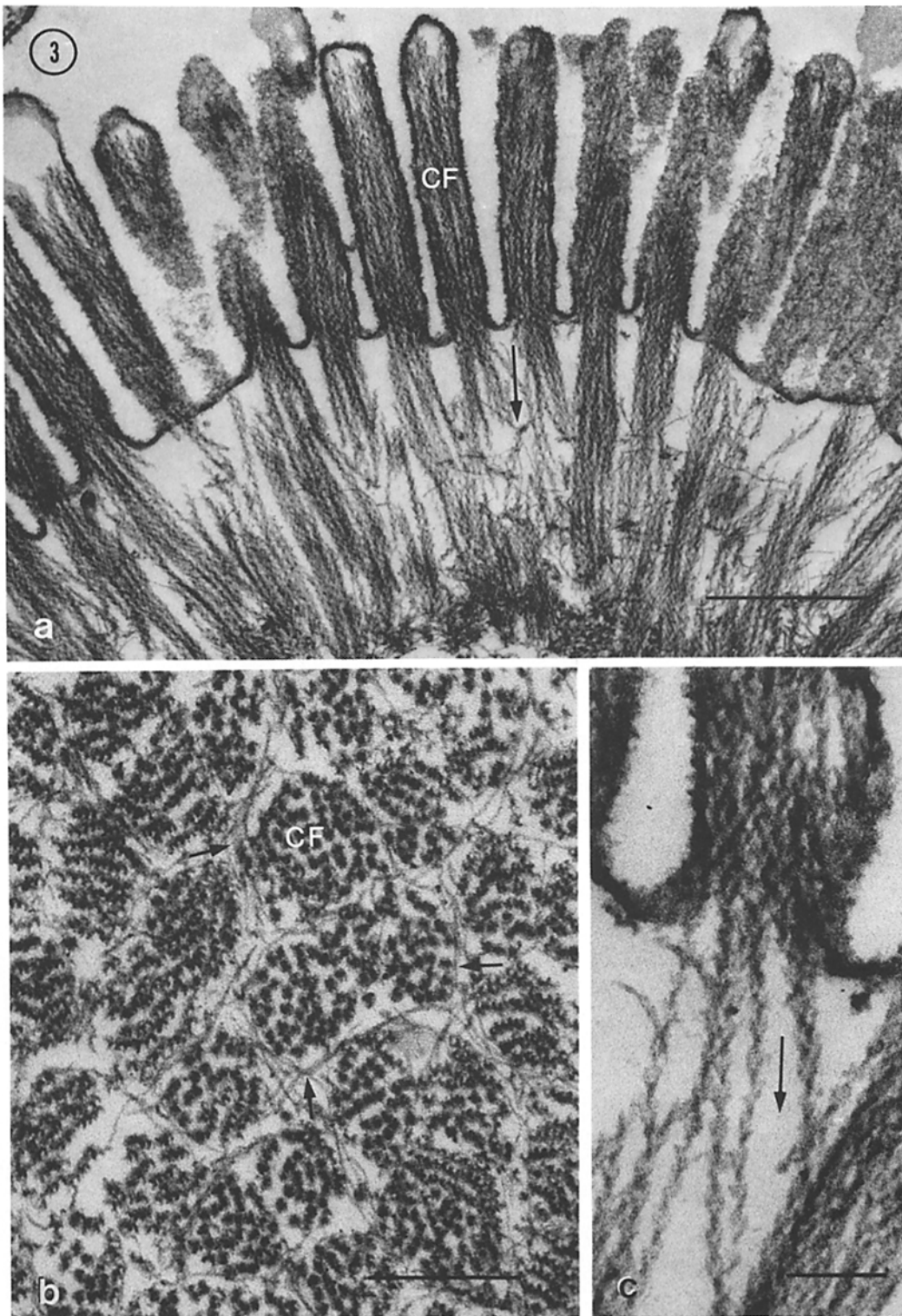
FIGURE 3 Isolated brush borders decorated with S-1 and fixed in the presence of 0.2% tannic acid. (*a*) Low magnification view showing longitudinal profiles of decorated microvillar core filament bundles (*CF*). The filaments are well-decorated even where they are densely packed in the microvilli. Note the thin, undecorated filaments which run perpendicular to the core filaments. Arrows indicate polarity of the actin filaments. Bar, 0.5 μm. × 49,500. (*b*) Cross section of core filaments in apex of cell body. Actin filaments appear as dense circles with fuzzy borders. Filament bundles are surrounded by a network of thin filaments, 35–50 Å in diameter (arrows). Bar, 0.2 μm. × 115,000. (*c*) High magnification of longitudinally sectioned core filaments, showing basally directed polarity. Arrow indicates filament polarity. Bar, 0.1 μm. × 144,000.

TABLE I

Arrowhead Periodicity

Cell type	Mean	Standard deviation	No. measurements
Epithelial cell brush border	339	±30	22
Myoblast	358	±21	17
Sea urchin egg cortex	360	±24	17
CHO-K$_1$	366	±23	19
Rabbit skeletal muscle	362	±23	17

with a diameter of 35–50 Å (Fig. 3a and b).

Table I gives the mean arrowhead periodicity of decorated actin filaments in the cell types that we have examined. Our observed value for rabbit skeletal muscle actin in thin sections is 362 Å. This value is very close to that obtained by Huxley (12) for negatively stained preparations of HMM-decorated actin from rabbit skeletal muscle (366 ± 15 Å). With the exception of actin from the brush border, a protected t test of the mean arrowhead periodicities shows no significant difference (P > 0.30). However, the periodicity of the brush border filaments differs significantly from the filament periodicity of the other four cell types (P < 0.025).

Actin Filament Polarity

In each of the cell types that we have studied, actin filaments that appear to attach to the plasma membrane exhibit a clear, uniform polarity, with their arrowheads pointing away from the membrane. In both the microvilli of the brush border (Fig. 3) and those associated with the sea urchin egg cortex (Fig. 1), all the filaments point in a basal direction, away from the microvillar tips. There is no evidence in either case for a second set of antiparallel filaments within the microvilli. However, a network of 35- to 50-Å-diameter filaments which do not bind S-1 are found in the brush border (Fig. 3a and b). These filaments surround each of the core bundles of actin filaments where they emerge from the microvilli into the apex of the cell.

In glycerinated myoblasts and CHO cells, many actin filaments are closely associated with the cytoplasmic face of the plasma membrane. An example of a glycerinated CHO cell which has been incubated with myosin S-1 is shown in Fig. 4a. The arrowheads on those actin filaments which appear to originate on the plasma membrane all point away from the membrane (Fig. 4c).

CHO cells that are treated with 1 × 10^{-3} M

dBCAMP undergo a change in shape from their normal epithelioid morphology to an elongated, fibroblast-like shape (11). This drug-induced cell elongation is accompanied by the development of stress fibers, composed of bundles of actin filaments, which are oriented parallel to the long axis of the cell (Fig. 4b). Although it is difficult to determine the polarity of the tightly packed filaments which comprise these bundles, individual filaments with opposite polarities can be seen in regions where the bundles splay slightly (Fig. 4d and e).

DISCUSSION

The fixation technique we have described improves dramatically the preservation and visualization of the arrowhead structure of S-1-decorated actin filaments. Indeed, all classes of cytoplasmic filaments show enhanced preservation when fixed in this concentration of tannic acid, which supports the previous suggestion that tannic acid itself may have fixative properties (21) in addition to acting as a mordanting agent (29). Tannic acid may also improve fixation by binding to the filaments, thereby protecting them from the degradative action of OsO$_4$ (20) during postfixation.

In all of the cell types we have examined, actin filaments attach to membranes with a uniform polarity, with the S-1 arrowheads pointing away from the membrane. However, antiparallel actin filaments occur within the stress fibers of CHO cells and myoblasts. Stress fibers have been shown to contain several types of muscle proteins in addition to actin, including myosin, tropomyosin, and α-actinin (6, 18, 32). This leads to the speculation that they may serve a contractile as well as a cytoskeletal role in the cell (6, 8, 32), a suggestion supported by the recent demonstration that stress fibers in glycerinated rat mammary adenocarcinoma cells undergo a dramatic contraction in response to the addition of ATP (14). Our observations of filament polarity in stress fibers is consistent with the idea that this contraction results from a sliding filament mechanism similar to that of skeletal muscle.

The results we report here confirm and extend the obsrvations of Mooseker and Tilney (22) that all microvillar actin filaments have the same polarity. In their study, the plasma membrane was removed with detergent before S-1 decoration. As these authors point out, detergent extraction introduces the possibility that a second set of oppositely polarized actin filaments might be preferentially lost. The plasma membrane remains intact
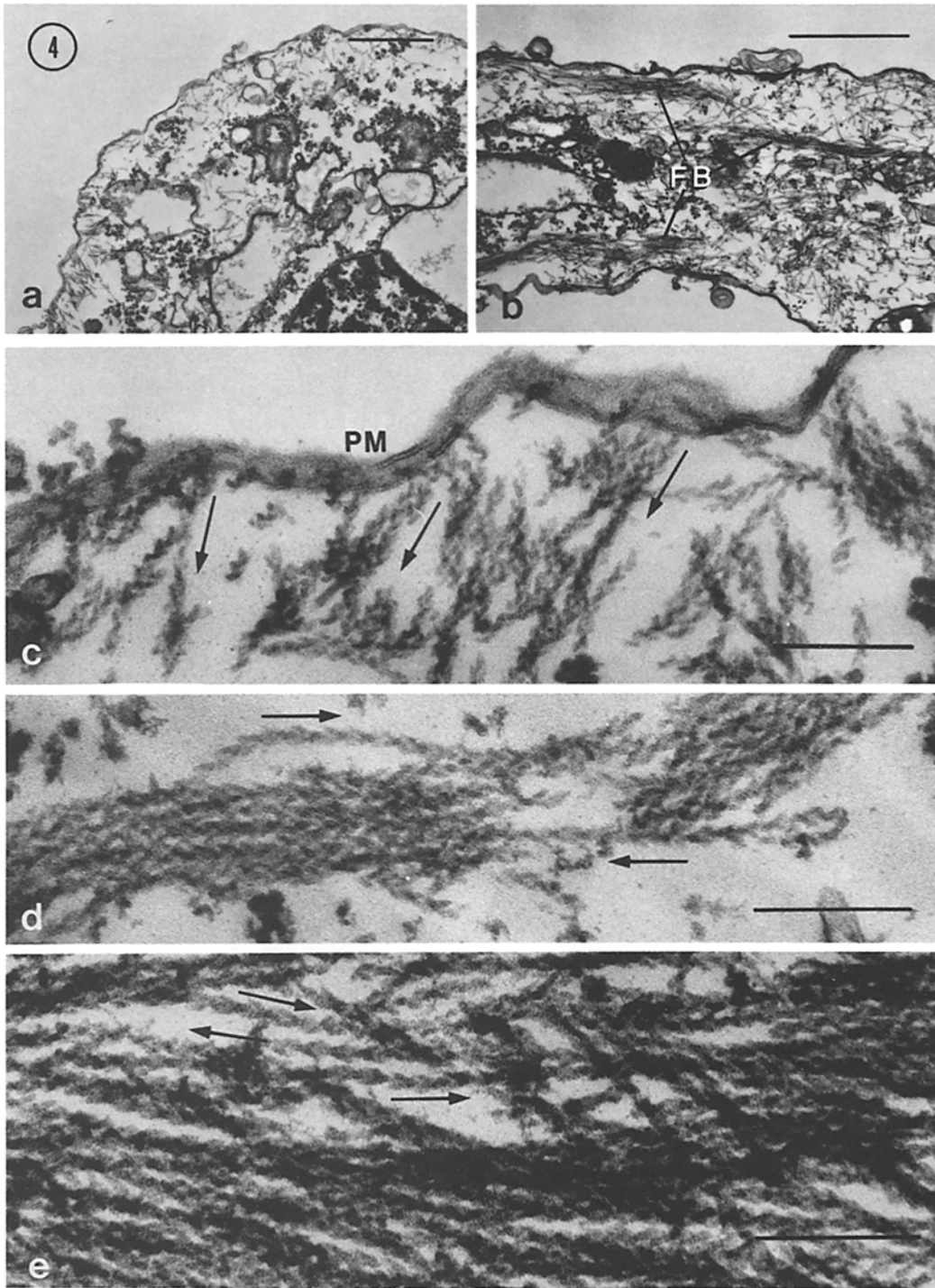
FIGURE 4 Glycerinated CHO-K$_1$ cells decorated with S-1. Arrows show polarity of actin filaments. (a) Normal cell showing epithelioid morphology. Many actin filaments project inward from the plasma membrane. Bar, 1.0 μm. × 12,000. (b) Cell treated for 12 h with 1 × 10^{-3} M dBCAMP. Cell has elongated and contains bundles of actin filaments parallel to its long axis. Bar, 1.0 μm. × 17,500. (c) Enlargement of cell shown in Fig. 4a. Filaments which appear to originate on the plasma membrane point away from the membrane. Bar, 0.2 μm. × 99,500. (d and e) Bundles of actin filaments from dBCAMP-treated cells. Bundles contain filaments with opposite polarities. Bar, 0.2 μm. (d) × 115,000. (e) × 117,000.

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in our preparations, thus supporting the conclusion that microvillar actin filaments have a single, uniform polarity. We do, however, find a second type of filament associated with the base of the bundle of microvillar core filaments. Each bundle is surrounded by a network of 35- to 50-Å filaments which do not bind S-1. The arrangement of these thin filaments suggests that they may be involved in holding the bundles of core filaments together. However, they may also actively function in brush border motility (22, 27).

Actin filaments in the mirovilli of isolated cortices from sea urchin eggs also show a uniform, basally directed polarity. Burgess and Schroeder (3) have reported identical results for a negatively stained preparation of HMM-decorated microvillar filaments from the sea urchin egg cortex. Similar studies using HMM decoration and negative staining have demonstrated that bundles of actin filaments in sea urchin coelomocytes (4) and human platelets (23) are also polarized away from the membrane. The observation that actin filaments exhibit a single polarity at sites of membrane attachment but antiparallel polarities at proposed sites of interaction is most easily interpreted in terms of a sliding filament mechanism of force generation.

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