IDENTIFICATION AND ORGANIZATION OF THE COMPONENTS IN THE ISOLATED MICROVILLUS CYTOSKELETON

PAUL T. MATSUDAIRA and DAVID R. BURGESS. From the Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755

ABSTRACT

We have examined the effects of ATP and deoxycholate (DOC) on the cytoskeletal organization of Triton-demembranated microvilli (MV) isolated from chicken intestine brush borders. Isolated MV are composed of a core of tightly bundled microfilaments from which arms project laterally to the plasma membrane with a 33-nm periodicity. These lateral arms spiral around the core microfilaments as a helix with a 25° pitch. Demembranated MV consist of four polypeptides with mol wt of 110,000, 95,000, 68,000, and 42,000, present in molar ratios of 1.1:1.6:1.3:10.0. After addition of 50 μM ATP and 0.1 mM Mg^{++}, the cytoskeletons are organized as a tight bundle of microfilaments from which lateral arms are missing. In these ATP-treated cytoskeletons, the 110-kdalton polypeptide is reduced in amount and the 95,000, 68,000, and 42,000 polypeptides are present in a 1.3:1.2:10.0 ratio. In contrast, after incubation with 0.5% DOC, the core microfilaments are no longer tightly bundled yet the lateral arms remain attached with a distinct 33-nm periodicity. These DOC-treated cytoskeletons are depleted of the 95,000 and 68,000 polypeptides and are composed of the 110,000 and 42,000 polypeptides in a 2:10 molar ratio. These results suggest that the microfilaments are associated into a core bundle by the 95- and 68-kdalton polypeptides and from this core bundle project the lateral arms composed of the 110-kdalton polypeptide.

KEY WORDS microfilaments · microvillus · cytoskeleton · actin-binding proteins · actin-membrane interactions

Microvilli (MV) are fingerlike plasma membrane specializations found on the free surfaces of most cells. The microvillus cytoskeleton is composed, in part, of a core bundle of actin microfilaments (14) which inserts end-on into an electron-dense cap at the microvillus tip. The remainder of the microvillus cytoskeleton, revealed by thin-section and freeze-fracture electron microscopy, includes cross-links which appear to link the microfilaments into a bundle and periodic arms (cross-filaments or cross-bridges) which connect the bundle laterally to the microvillus membrane (21, 18). We use the term lateral arms when referring to membrane-microfilament interactions to avoid confusion when using the term cross-links in describing the microfilament-microfilament interactions. The absorptive surface of some epithelial cells, such as those of the intestine, are covered with hexagonally packed MV organized into a structure termed the brush border (BB) (7). BB MV, because of their uniformity and abundance, are excellent models for the study of actin-actin and actin-membrane interactions.

Recent biochemical analysis has provided information about the proteins involved in the organization of the microvillus cytoskeleton. The core of actin filaments are all unidirectionally polarized with respect to the tip when decorated with myosin subfragment, S1 (18, 1). Alpha-actinin, another muscle protein, has been localized only in the terminal web region of the BB (10, 13) rather than along the sides and the tips of the MV as suggested

earlier (19, 23). Bretscher and Weber (4, 6) have reported that isolated BB MV contain only two proteins, actin and a 95-kdalton protein which they named villin. Villin and α-actinin have different electrophoretic mobilities and are immunologically distinct (5, 6). Antibodies to the 95-kdalton protein appear to bind to the periodic arms linking the bundle of filaments laterally to the membrane (6). In addition, it has been proposed that this same 95-kdalton protein serves the added role of cross-linking the filaments together into a bundle (18, 2, 3, 6).

We have been studying the organization of isolated MV, using an approach fundamentally different than that taken by previous workers. We have developed methods that selectively remove either the components which cross-link the actin filaments into a bundle or the elements which connect the bundle laterally to the membrane. These methods have allowed us to distinguish between proteins participating in actin-actin association and those responsible for actin-membrane association within MV. In this paper, we have reexamined the composition and organization of the microvillus cytoskeleton and have identified the major proteins of both the actin bundle and the laterally projecting arms.

MATERIALS AND METHODS

All experiments were performed on ice except where noted. Freshly prepared phenylmethylsulfonyl fluoride from a 0.1-M stock in isopropanol was added, at a final concentration of 0.1 mM, immediately to each resuspended pellet.

Isolation of BBs

Chicken intestine BBs were isolated using methods modified from Mooseker et al. (20). The intestine from the pylorus to the caecum was excised, slit down the side, rinsed in ice-cold saline, and cut into 4- to 5-cm-long pieces. Intestine pieces were stirred in phosphate-buffered saline (made 0.1 M in sucrose and 20 mM in EDTA) for 30 min at room temperature. The segments of intestine were then discarded and the remaining cell suspension (primarily consisting of sheets of epithelial cells) was centrifuged for 10 min at 750 *g* in a Sorvall RC-5B centrifuge (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.). The pellet was resuspended in 4 mM EDTA, 1 mM EGTA, 10 mM imidazole, pH 7.3, and homogenized by four 15-s bursts at full speed in a Sorvall Omnimixer. The homogenate was centrifuged for 10 min at 750 *g* to pellet the BBs. The pellet was washed again in the EDTA-EGTA-imidazole solution and once in solution I (75 mM KCl, 1 mM EGTA, 0.1 mM MgCl, 10 mM imidazole, pH 7.3). The pellet (consisting of BB, nuclei, and cellular debris) was layered on a 45/55/60% sucrose step gradient made up in solution I and centrifuged in a Sorvall SS-90 vertical rotor at 22,700 *g* for 45 min, setting the rate controller at 70. The BBs banded at the 45/55% interface and were collected by centrifugation at 12,000 *g* for 10 min and washed free of sucrose by centrifuging at 750 *g* for 10 min. From ~1.0 g of cells, 40 mg of pure BB was routinely isolated in 3.5–4 h.

Isolation and Demembranation of MV

MV were isolated from BB using the protocol outlined by Bretscher and Weber (4). MV were then demembranated by resuspending the pellet in 1% Triton X-100 in solution I for 15 min at room temperature. Approximately 7.5 mg of demembranated MV were isolated from 40 mg of pure BB. The demembranated MV were collected by centrifugation for 10 min at 12,000 *g* in an SS-34 rotor, resuspended in the above Triton-solution I, divided into aliquots, and repelleted. MV were stored on ice and used within 2 h after demembranation.

Experimental Treatments

Demembranated MV were gently resuspended in the experimental buffer solutions and incubated for 5 min on ice. Samples (10 μl) were withdrawn for negative-stain EM and the balance was sedimented by centrifugation at 10,000 rpm for 5 min in a Sorvall SS-34 or 0.5 min in a Beckman microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The pelleted material was resuspended in solution I, and samples were removed for negative-stain EM or solubilized in sample buffer for SDS gel electrophoresis. The experimental buffer solutions were made in solution I to which one of the nucleotides ATP, ADP, AMP, GTP, ITP, or CMP (Sigma Chemical Co., St. Louis, Mo.) was added at 1-mM concentration. In addition, the responses to differing ATP (Na_2 salt, vanadate free, Sigma Chemical Co.) levels (0.05, 0.1, 0.15, 1.0, and 5.0 mM) and differing Mg^{++} levels (0.1, 1.0, 5.0 mM) were tested. The effects of the detergent, deoxycholate (DOC) (free acid, Sigma Chemical Co.) 0.5% (wt/vol), in solution I were tested. DOC, recrystallized from ethanol, was made fresh as a 10% stock solution, adjusted to pH 8.0, and diluted just before use.

Electron Microscopy

Demembranated MV were fixed as pellets using the fixative described by Begg et al. (1) and processed for embedding in Epon-Araldite. Thin sections were cut with diamond knives on a Sorvall MT-1 ultramicrotome.

Experimentally treated MV were pipetted onto Formvar- and carbon-coated grids, rinsed with 10 drops of solution I, four drops of 0.02% cytochrome *c* in 1% amyl alcohol, and stained for 5 s in 1% aqueous uranyl formate. Grids were examined in a JEOL 100CX electron microscope operating at 60 kV with a 40-μm objective aperture.

SDS Polyacrylamide Gel Electrophoresis

Proteins were analyzed by SDS polyacrylamide gel electrophoresis using the microslab method described by Matsudaira and Burgess (17). Gels were poured with a 5–20% gradient of acrylamide using the buffer formulations of Laemmli (15). The amounts of protein loaded on the gels were adjusted to insure linear binding of stain with Coomassie Blue (Fig. 8 in reference 17). The stained gels were scanned with a Joyce-Loebl microdensitometer (Joyce, Loebl and Co., Ltd., Gateshead-on-Tyne, England), and the area under the peaks was totaled as described earlier (17). The areas under the peaks representing the amount of protein were compared to known amounts of standard proteins and converted to molar ratios.

RESULTS

Triton-Demembranated MV

The microvillus cytoskeleton in both intact MV and isolated demembranated MV is organized as a core bundle of microfilaments from which arms project laterally to the membrane (Fig. 1 *a–c*). The core bundle contains 15–30 microfilaments and varies in diameter from 24 to 30 nm. The 12- to 16-nm-long lateral arms are spaced approximately every 33 nm along the side of the bundle. In grazing thin sections of demembranated MV, the lateral arms are seen as transverse stripes which spiral around the bundle in a helical array with a pitch of ~25° (Fig. 1 *a* and *b*). While the handedness of the helix generally remains the same along the length of the bundle (Fig. 1 *b*), we have seen a few cases where the handedness reverses within

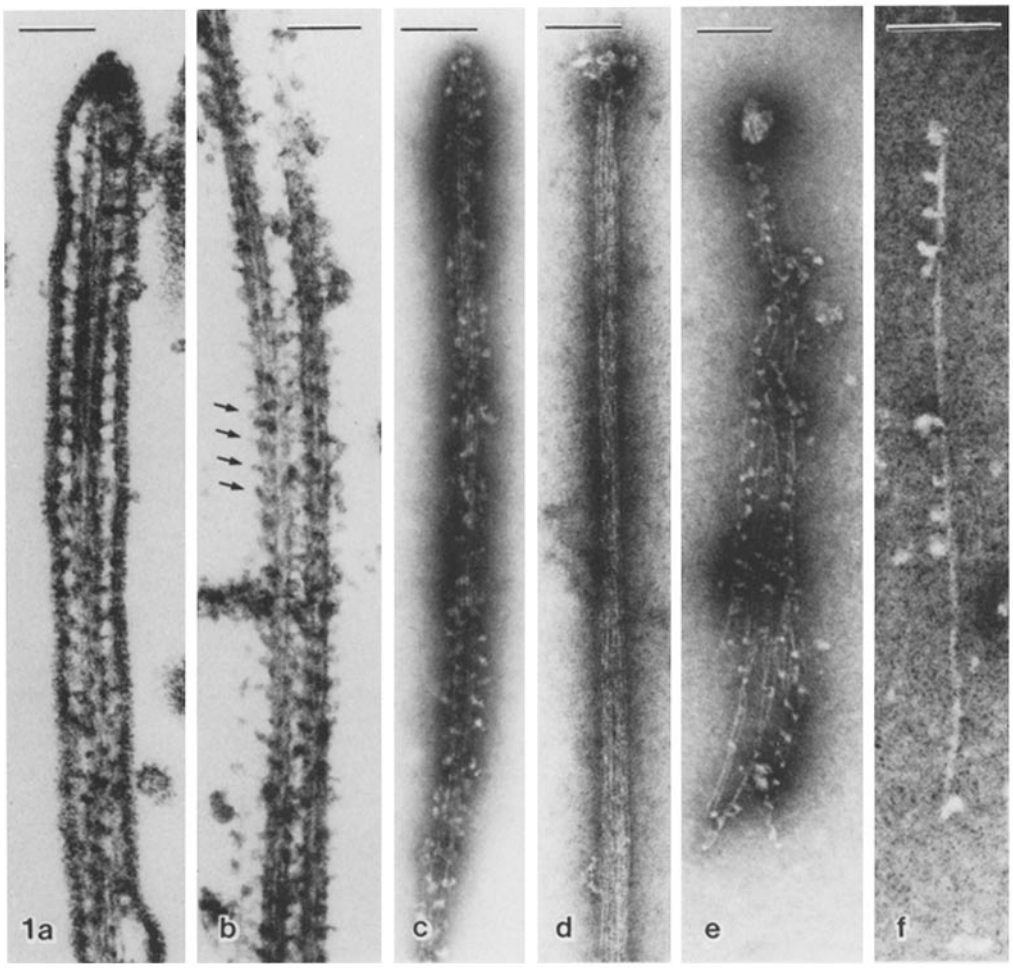

FIGURE 1 (*a*) Thin section of an isolated microvillus before demembranation, showing the periodic lateral arms. Bar, 100 nm. × 100,000. (*b*) Thin section through demembranated MV, revealing cytoskeleton still retaining the lateral arms organized in a helical array (arrows). Bar, 100 nm. × 100,000. (*c*) Isolated demembranated cytoskeleton as seen in negative stain with lateral arms demonstrating 33-nm periodicity. Bar, 100 nm. × 100,000. (*d*) Isolated cytoskeleton after ATP treatment, showing smooth bundle of microfilaments lacking lateral arms. Triton-resistant cap is present at one end of the bundle. Bar, 100 nm. × 100,000. (*e*) Microvillar cytoskeleton treated with DOC, showing unbundled microfilaments still gathered at one end by the Triton-resistant cap. Some microfilaments possess lateral arms and others are bare. Bar, 100 nm. × 100,000. (*f*) Individual microfilament still possessing many periodic lateral arms after DOC treatment. Bar, 100 nm. × 150,000.

the bundle.

After Triton treatment the demembranated MV aggregate together, apparently by associations of their lateral arms (Fig. 1 *b*). This aggregation occurs even in the presence of 1% Triton X-100. Unlike those in previous reports (4), our preparations of demembranated MV remain stable and intact for up to 3 h as monitored by negative-stain EM (data not shown).

Demembranated microvillus cytoskeletons consist of four major polypeptides, with mol wt of 110,000, 95,000, 68,000, and 42,000 (actin) (Fig. 2, slot *d*) in molar ratios of 1.1:1.6:1.3:10 (Table I). In addition to these four polypeptides are five polypeptides with mol wt of 12,500, 14,500, 15,200, 15,700, and 16,500. As expected, the proteins which compose the microvillar cytoskeletons are also among the major components of isolated demembranated BBs and isolated intact MV (Fig. 2, slots *a* and *c*).

Nucleotide-treated MV

Incubation of demembranated microvillus cytoskeletons in solution I containing 0.1 mM Mg^{++} and 50 μM ATP causes the loss of lateral arms from the core microfilaments (Fig. 1 *d*). The microfilaments within the cores remain tightly bundled and range in diameter from 24 to 30 nm. Because the microfilaments are tightly bundled we presume that actin-actin cross-links are present; however, a regular transverse periodicity suggestive of these cross-links within the bundle is not apparent. The ATP-treated MV, in comparison to the untreated controls, are no longer aggregated. Supernates of the pelleted cytoskeletons are enriched in large globular aggregates which are also seen in negative stain as persistent contaminants of the pellets.

The ATP-treated cytoskeletons are composed primarily of the 95-, 68-, and 42-kdalton proteins, and four of the low molecular weight proteins (Fig. 2, slot *e*). Both the 110- and 16.5-kdalton proteins are greatly reduced in amount while the 95-, 68-, and 42-kdalton proteins remain in a 1.3:1.2:10 molar ratio (Table I). The amount of the 110,000 polypeptide lost after ATP treatment varies between 30 and >80% among experiments and is correlated with the presence of the globular aggregates contaminating the pelleted bundles. It must be stressed that in all cases the microfilament bundles are completely devoid of lateral arms. In parallel experiments, ATP-treated demembranated BBs also lack the lateral arms and the 110- and 16.5-kdalton polypeptides (Fig. 2, slot *b*). The supernates from ATP-treated cytoskeletons contain the 95,000 and 42,000 polypeptides and are enriched in the 110,000 polypeptide.

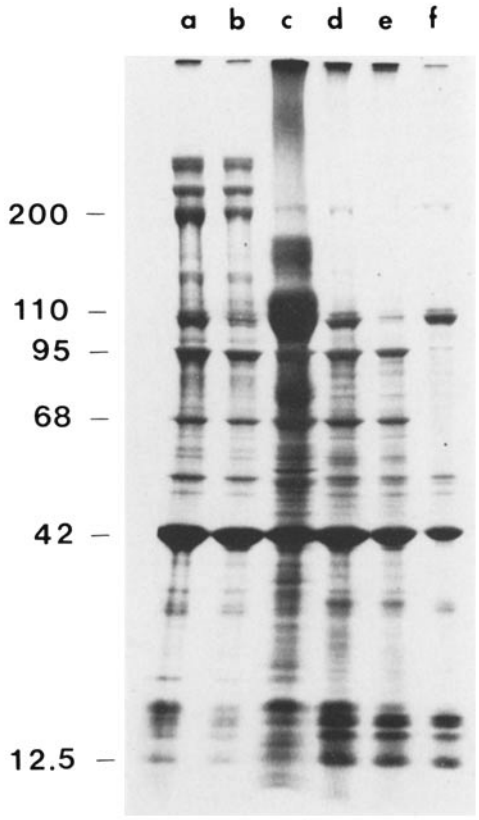

FIGURE 2 Electrophoresis is carried out on SDS microslab gel poured with a 5–20% gradient of acrylamide. Slot *a*: Isolated demembranated whole BBs. Slot *b*: Isolated demembranated BBs after ATP treatment, showing loss of 110,000 polypeptide. Slot *c*: Isolated microvilli. Slot *d*: Isolated demembranated microvilli. Slot *e*: Pellet of ATP-treated microvillar cytoskeletons, showing reduction in 110- and 16.5-kdalton polypeptides. Slot *f*: Pellet of DOC-treated microvillar cytoskeletons revealing marked loss of 95-, 68-, and 16.5-kdalton polypeptides.

The stability of microfilament bundles after removal of lateral arms is dependent on Mg^{++} concentration. In 0.1 mM Mg^{++}, the ATP-treated MV remain tightly bundled with very few loose microfilaments seen in negative-stain EM. The amount of actin pelleted after ATP treatment is almost equal to the amount pelleted in untreated controls. When cytoskeletons are incubated in ATP solutions containing $\geq$1 mM Mg^{++}, the resulting bun-

TABLE I

Protein Composition of Treated Microvillar Cytoskeletons

	mol Protein/mol actin		
Treatment	110,000	95,000	68,000
Control	0.11	0.16	0.13
+ATP	0.02	0.13	0.12
+DOC	0.20	0.05	0.00

The relative molar amounts of the proteins composing the microvillus cytoskeleton before and after treatments. The samples shown in slots *d*, *e*, and *f* of Fig. 2 were scanned, the area under the peaks was calculated and converted to molar amounts relative to actin.

dles are unstable. In negative stain, many free filaments in addition to smooth bundles are observed. Also, the amount of actin pelleted after addition of ATP is much less than in either the untreated controls or the low Mg^{++} preparations.

The release of lateral arms from the cytoskeleton is specific for ATP and GTP, among the nucleotides tested. Incubation with ATP in excess of 50 μM and $Mg^{++} \geq 0.1$ mM results in the release of all lateral arms from microvillar cytoskeletons while the absence of Mg^{++} (1 mM EDTA) prevents their release. The effects of other divalent cations were not tested. In the case where microvillar cytoskeletons are treated with 1 mM GTP (1 mM Mg^{++}), some lateral arms are released resulting in bundles with bare patches. However, the loss of the lateral arms does not occur to the same extent as in ATP-treated MV under the same conditions, and totally smooth bundles are not observed. In the presence of 1 mM Mg^{++}, lateral arms are not released when cytoskeletons are treated with 1 mM each of either ADP, AMP, ITP, or CMP, nor is there loss of the 110-kdalton protein as detected by gel electrophoresis.

DOC-treated MV

DOC-treated microvillus cytoskeletons are composed of a loose bundle of filaments to which lateral arms remain attached. In negatively stained preparations these MV are not organized into tight bundles but rather appear as single microfilaments gathered together at one end by a dense plaque (Fig. 1*e*). Within the loosely associated filaments are found some bare microfilaments and some microfilaments possessing periodic globular projections. These globular projections have the same dimensions as the lateral arms decorating the untreated controls. More importantly, the globular projections have a 33-nm periodicity along the length of a single filament (Fig. 1*f*).

The DOC-treated cytoskeletons are composed of the 110,000 and 42,000 proteins and four of the low molecular weight proteins (Fig. 2, slot *f*). The 95,000, 68,000, and 16,500 proteins are greatly reduced in amount. The 110-kdalton protein is present in a 2:10 molar ratio with actin (Table I) which represents a twofold enrichment of the 110,000 protein over the untreated controls.

DISCUSSION

The shape of the microvillus is maintained through interactions which hold the actin filaments in a bundle and others which attach the bundle to the surrounding membrane. Our results suggest that the proteins involved in microfilament-microfilament associations differ from those involved in microfilament-membrane associations since treatments which disrupt one type do not affect the other. This differs from the proposals of others (18, 3, 6) where a single protein species was postulated to play these dual roles.

The protein composition of the microvillus cytoskeleton is more complex than has been previously reported and reflects the distinct structural roles needed for maintenance of the microvillus shape. Our preparations of MV are composed of the 110-, 95-, 68-, 42-, and the 16.5- through 12.5-kdalton proteins. These proteins are also major components of intact isolated BBs and probably do not represent proteolytic artifacts generated during microvillus isolation. In a recent study of isolated microvillar vesicles, Geiger et al. (13) report that detergent-resistant microvillar cores contain the 105-, 95-, and 42-kdalton proteins, a finding consistent with our results. In contrast, Bretscher and Weber (4) find only the 95- and 42-kdalton proteins and report that the isolated demembranated MV remain intact only in the presence of polyethylene glycol. In our preparations it was unnecessary to stabilize the demembranated MV with polyethylene glycol. The reason for the differences between our results is as yet unknown.

Our results strongly suggest that the lateral arm linking the core of actin filaments to the membrane is composed of the 110-kdalton protein. First, lateral arms are lost from cytoskeletons treated with ATP; this morphological finding is correlated with a sharp decrease in the amount of the 110-kdalton protein in the cytoskeletons. The molar ratios of the other major proteins (95,000 and

68,000) in relation to actin do not change markedly, suggesting that they are not a major part of the lateral arms. Secondly, DOC-treated cytoskeletons retain both lateral arms and the 110-kdalton protein, yet become unbundled and lack the 95- and 68-kdalton proteins. These experiments argue against the earlier proposals (18, 3) that the 95-kdalton protein is the lateral arm.

Direct evidence that the lateral arms are periodic along the length of an individual filament (Fig. 1*f*) confirms the assumption of Bretscher and Weber in calculating the number of lateral arms per actin expected for a microvillus. Based on a hexagonally packed bundle of 19–34 microfilaments where lateral arms are attached only to the peripheral microfilaments with a 33-nm periodicity, there should be 3.5 to 4.5 lateral arms per 100 actin monomers. Yet the apparent molar ratio determined by densitometry of stained gels shows that there are eleven 110-kdalton proteins per 100 actins. Thus, each lateral arm may be composed of at least a dimer of the 110-kdalton protein. Experiments (to be reported elsewhere) reassociating the lateral arms to the microfilament bundle should provide direct evidence of the composition of the lateral arms.

The loss of lateral arms is specific for ATP and GTP in the presence of Mg^{++} among the nucleotides tested. This effect is also a characteristic of the myosin head fragment, S1, in its association with actin filaments. While it is possible that the lateral arms are a proteolytic contaminant of myosin from the terminal web region, we consider it unlikely for several reasons. MV are isolated free of the terminal web before demembranation and show the periodic lateral arms. In fact, periodic microvillar lateral arms are noted in freeze-fracture studies of intact intestinal epithelial cells (21, 18). Others report that myosin antibodies which stain the terminal web do not react with the microvillus (20, 5).

Our results also suggest that the actin microfilaments are associated into a bundle by the 95- and 68-kdalton proteins. Not only are the smooth microfilament bundles after ATP treatment composed of the 95- and 68-kdalton proteins, but DOC-treated cytoskeletons, which are unbundled, are devoid of these two proteins. Thus, while it is clear that the 95- and 68-kdalton proteins are components of the bundled actin filaments, we do not yet know which protein actually cross-links the filaments together along their lengths. The dense labeling of demembranated microvillar cytoskeletons with ferritin-conjugated antibodies to a 95,000 polypeptide was originally attributed to binding to the lateral arms (6), but it is also consistent with the 95,000 protein's being a component of the microvillar bundle.

Actin filament bundles from other systems resembling microvillar bundles have been well characterized in terms of the proteins involved in cross-linking actin together. In both in vitro prepared actin needles from sea urchin egg extracts and microspikes from sea urchin coelomocytes, a 58-kdalton protein, termed fascin, is the cross-linking protein (12, 8, 22). This protein may play a similar role in the MV from fertilized sea urchin eggs (9, 24). In these systems a clear transverse 11-nm periodicity is present in the bundles and represents the cross-linking between adjacent filaments of every four or five actin monomers by a fascin (11, 24). In intestinal MV the 95,000 and 68,000 proteins are present in lesser amounts in relation to actin as compared to the fascin/actin ratio, which may explain the lack of a clear transverse periodicity within ATP-treated microvillar cytoskeletons.

The end-on insertion of microfilaments into the dense tip of the microvillus is similar in appearance to the insertion of microfilaments into adhesion plaques in tissue culture cells. While α-actinin has been localized at these adhesion plaques (16), its presence in the microvillus tips is questioned (4, 13). In contrast to this example, the lateral arms connect the membrane along the length of the filaments and, as pointed out by Mooseker and Tilney (18), this represents a different class of actin-membrane interactions. This lateral connection to the membrane is the type expected during formation of filopodia in the sea urchin coelomocyte and human platelet and possibly in other types of cell shape changes such as cytokinesis. The identification of the 110,000 protein as a possible component of these lateral filament-membrane associations should provide another probe for studying the role of the cytoskeleton in the development and maintenance of cell shape.

We would like to thank Dr. Roger Sloboda for stimulating discussions and helpful comments on the manuscript. We would also like to give special thanks to Dr. Mark Mooseker for his leadership in this area of research and for many helpful discussions.

This study was supported in part by National Institutes of Health grant AM20021 to D. R. Burgess.

Received for publication 13 August 1979, and in revised form 25 September 1979.

REFERENCES

1. BEGG, D., R. RODEWALD, and L. REBHUN. 1978. The visualization of actin filament polarity in thin sections. Evidence for the uniform polarity of membrane-associated filaments. *J. Cell Biol.* **79**:846–852.
2. BOOTH, A., and A. KENNY. 1976. A morphometric and biochemical investigation of the vesiculation of kidney microvilli. *J. Cell Sci.* **21**: 449–463.
3. BOOTH, A., and A. KENNY. 1976. Proteins of the kidney microvillus membrane. Identification of subunits after sodium dodecyl sulphate/ polyacrylamide-gel electrophoresis. *Biochem. J.* **159**:295–407.
4. BRETSCHER, A., and K. WEBER. 1978. Purification of microvilli and an analysis of the protein components of the microfilament core bundle. *Exp. Cell Res.* **116**:397–407.
5. BRETSCHER, A., and K. WEBER. 1978. Localization of actin and microfilament-associated proteins in the microvilli and terminal web of the intestinal brush border by immunofluorescence microscopy. *J. Cell Biol.* **79**:839–845.
6. BRETSCHER, A., and K. WEBER. 1979. Villin: The major microfilament-associated protein of the intestinal microvillus. *Proc. Natl. Acad. Sci. U. S. A.* **76**:2321–2325.
7. BRUNSER, O., and J. LUFT. 1970. Fine structure of the apex of absorptive cells from rat small intestine. *J. Ultrastruct. Res.* **31**:291–311.
8. BRYAN, J., and R. KANE. 1978. Separation and interaction of the major components of sea urchin actin gel. *J. Mol. Biol.* **125**:207–224.
9. BURGESS, D., and T. SCHROEDER. 1977. Polarized bundles of actin filaments within microvilli of fertilized sea urchin eggs. *J. Cell Biol.* **74**: 1032–1037.
10. CRAIG, S., and J. PARDO. 1979. Alpha-actinin localization in the junctional complex of intestinal epithelial cells. *J. Cell Biol.* **80**:203–210.
11. DEROSIER, D., E. MANDELKOW, A. SILLIMAN, L. TILNEY, and R. KANE. 1977. Structure of actin-containing filaments from two types of nonmuscle cells. *J. Mol. Biol.* **113**:679–695.
12. EDDS, K. T. 1977. Dynamic aspects of filopodial formation by reorganization of microfilaments. *J. Cell Biol.* **73**:479–491.
13. GEIGER, B., K. TOKUYASU, and S. SINGER. 1979. Immunocytochemical localization of α-actinin in intestinal epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* **76**:2833–2837.
14. ISHIKAWA, H., R. BISCHOFF, and H. HOLTZER. 1969. The formation of arrowhead complexes with heavy meromyosin in a variety of cell types. *J. Cell Biol.* **43**:312–328.
15. LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (Lond.).* **227**:680–685.
16. LAZARIDES, E., and K. BURRIDGE. 1975. α-Actinin: Immunofluorescent localization of a muscle structural protein in nonmuscle cells. *Cell.* **6**: 289–298.
17. MATSUDAIRA, P., and D. BURGESS. 1978. SDS microslab linear gradient polyacrylamide gel electrophoresis. *Anal. Biochem.* **87**:386–396.
18. MOOSEKER, M., and L. TILNEY. 1975. Organization of an actin filament-membrane complex. Filament polarity and membrane attachment in the microvilli of intestinal epithelial cells. *J. Cell Biol.* **67**:725–743.
19. MOOSEKER, M. 1976. Brush border motility. Microvillar contraction in Triton-treated brush borders isolated from intestinal epithelium. *J. Cell Biol.* **71**:417–433.
20. MOOSEKER, M., T. POLLARD, and K. FUJIWARA. 1978. Characterization and localization of myosin in the brush border of intestinal epithelial cells. *J. Cell Biol.* **79**:444–453.
21. MUKHERJEE, T., and L. A. STAEHELIN. 1971. The fine structural organization of the brush border of intestinal epithelial cells. *J. Cell Sci.* **8**: 573–599.
22. OTTO, J., R. KANE, and J. BRYAN. 1979. Formation of filopodia in coelomocytes: localization of Fascin, a 58,000 dalton actin cross-linking protein. *Cell.* **17**:285–293.
23. SCHOLLMEYER, J., D. GOLL, L. TILNEY, M. MOOSEKER, R. ROBSON, and M. STROMER. 1974. Localization of alpha-actinin in nonmuscle material. *J. Cell Biol.* **63**(2, Pt. 2):304*a*. (Abstr.).
24. SPUDICH, J., and L. AMOS. 1979. Structure of actin filament bundles from microvilli of sea urchin eggs. *J. Mol. Biol.* **129**:319–331.