IS THERE A ROLE FOR ACTIN IN VIRUS BUDDING?

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ABSTRACT

Electrophoretic data from both sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) and acid-urea gels reveal a protein in purified murine mammary tumor virus (MuMTV) which co-migrates with purified chick skeletal muscle actin. ^{125}I-labeling of intact and disrupted virus preparations shows that the actin-like protein is not artifactually adsorbed to the outside of virions during isolation. Quantitative SDS-PAGE and examination of negatively stained preparations show that the actin cannot be accounted for by a contaminating population of virus-free vesicles. The ultrastructure of mammary epithelial cells and of Rous sarcoma virus-transformed chick embryo fibroblasts shows that virus extrusion is associated with filament-containing cellular processes. In particular, MuMTV is released from the ends of long microvilli which contain a bundle of 6–8-nm microfilaments and share other structural features with intestinal microvilli. We suggest that virus nucleoids require an interaction with host cell contractile proteins for their extrusion from the cell.

KEY WORDS mammary tumor virus · actin · microvilli · virus budding · virus assembly

It has been assumed without much experimental basis that the interaction of a virus nucleoid with the cell surface membrane produces a localized alteration, sufficient for the virus to be extruded and pinched off without the need for a contractile apparatus (2–4, 15). We report here the presence of an actin-like protein associated with purified preparations of murine mammary tumor virus (MuMTV), a B-type RNA-containing, surface-budding, enveloped virus produced by mammary tumor epithelium in vivo and in vitro. The ultrastructural and electrophoretic data presented in this report[1] show that the actin present in purified virus preparations is an internal protein of the virion, and not a contaminant of the isolation procedure, and suggest that it is derived from host microvillus microfilaments. Our results raise the possibility that interaction of the virus nucleoid with actin is required for the virus to be extruded from the cell. Biochemical evidence for the presence of actin in other enveloped viruses has been independently reported by Wang et al. (32).

[1] Preliminary reports of this work have appeared in abstract form: Damsky et al. (5, 6).

MATERIALS AND METHODS

An established line of mammary tumor cells in culture derived from a BALB/cfC3H mouse mammary tumor (13) was used for electron microscopy, and as a source of MuMTV. Cells were grown to a confluent monolayer in Hanks' balanced salt solution and Eagle's minimum essential medium (MEM) with 10% fetal calf serum, and then stimulated to produce MuMTV with 10^{-8} M dexamethasone (13, 20).

Isolation of Virus

MuMTV is isolated either from the milk of lactating female mice of the high tumor strain RIII or from the fluids of mammary tumor epithelial cells in culture (BALB/cfC3H) by sequential rate zonal and isopycnic centrifugation (7). Negatively stained preparations of purified virus particles were analyzed morphometrically for the presence of contamination by virus-free vesicles. 20 fields from each of two preparations were examined at a magnification of 50,000 with a Weibel multi-purpose test grid (33). At least 30 particles were present in each field.

Isolation of Virus-Free Membrane Vesicles

Isolation of vesicles lacking virus was performed as previously described, using milk from mice which do not produce MuMTV particles (C57BL). The polypeptide composition of these vesicles was compared with that of MuMTV by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Iodination of Intact and Disrupted MuMTV

Intact virus preparations were iodinated with ^{125}I by the lactoperoxidase method (10). The labeled virus particles were then separated from the unbound ^{125}I and lactoperoxidase by centrifugation through a cushion of 15% sucrose. To label disrupted virus, virus preparations were first disrupted physically by homogenization in a "no clearance" homogenizer (Kontes Co., Vineland, N. J., [16]), and then labeled with ^{125}I. Unreacted iodine was removed by dialysis. 150,000 cpm, each of disrupted and intact preparations, were analyzed by SDS-PAGE. The gels were stained, destained, pressed, and exposed to Kodak X-ray film RP/R-54 for 5 days. The autoradiographs were scanned with an EC densitometer (E-C Apparatus Corp., St. Petersburg, Fla.).

SDS and Acid-Urea-Polyacrylamide Gel Electrophoresis

SDS-PAGE of MuMTV and other preparations was run on linear or gradient slab gels, using the discontinuous tris-glycine buffer system of Laemmli (12). Gels were stained with 0.25% Coomassie Blue in 25% isopropyl alcohol and 10% acetic acid, and destained in 10% acetic acid. To compare the behavior of the viral 42,000-dalton protein with that of purified chick skeletal muscle actin on a gel system that separates primarily on the basis of net charge, the 42,000-dalton bands were cut out of fixed and stained preparative slab gels of MuMTV and chick muscle actin. The proteins were electrophoretically eluted from the gels into a solution containing 1% SDS and 0.06 M Tris-HCl (pH 7.8). The SDS was removed by electrophoresis in the presence of 8 M urea and 1% Triton X-100 (Rohm & Haas Co., Philadelphia) to prevent the precipitation of the protein.[2] The samples were made acidic with acetic acid, and run on a 7.5% polyacrylamide, continuous acid-urea gel system at pH 2.5 (1). The proteins are positively charged at this pH and migrate toward the negative electrode. The gels were stained and destained as described for SDS-PAGE.

Isolation of Actin and Myosin Subfragment 1

Chick skeletal muscle was purified from an acetone powder by the method of Spudich and Watt (27). Myosin subfragment 1 was isolated from purified chicken skeletal muscle myosin (the gift of Dr. E. Elgart, see acknowledgements) by the method of Margossian and Lowey (17). The material precipitating between 42 and 60% ammonium sulfate was pelleted and stored as such at −80°C until use. Before use, ammonium sulfate was removed by dialysis against a solution containing 60 mM KCl, 10 mM phosphate buffer, 1 mM dithiothreitol (DTT), 1 mM ethylene glycol bis (β-aminoethyl ether) *N,N,N,N'* tetraacetate (EGTA), and 5 mM Mg^{++}, pH 7.3 (solution A; [18]).

Electron Microscopy

Small pieces of mammary tumor from the RIII strain of mouse and confluent cultures of BALB/cfC3H mammary tumor cells were fixed for 1 h in 1% glutaraldehyde (Polysciences, Inc., Warrington, Pa.) at room temperature in 0.1 M phosphate buffer, pH 7.2, followed by exposure to 1% osmium tetroxide in 0.1 M phosphate for 15 min at pH 6.0, and to 1% aqueous uranyl acetate for 2 h as suggested by Mooseker and Tilney (18) for improved preservation of actin filaments. Cells were removed from the plastic dish in propylene oxide, and treated as a pellet for embedding in Epon. Sections were cut with a diamond knife on an MT2 microtome (Ivan Sorval, Inc., Wilmington, Del.) and examined in a JEOL JEM 100B electron microscope. For decoration of tumor cells by myosin subfragment 1, cultures were washed *in situ* with solution A and treated with 0.25% Nonidet P-40 (Shell Chemical Co., New York) in solution A for 10 min. After washing with solution A, myosin subfragment 1 in solution A at 1 mg/ml was added to the cultures for 1 h, after which cultures were washed twice with solution A, fixed with 1% glutaraldehyde, and processed for electron microscopy as previously described.

Negative Staining

Negative staining of virus and virus-free vesicle preparations was performed using 1% neutral sodium phos-

[2] Details of the elution and electrodialysis procedures will be presented in a separate manuscript (29). For a discussion of the removal of SDS from proteins, see Tuszynski and Warren (30).

photungstate (21). The critical-point drying technique was performed as described in reference 21.

RESULTS

Identification of Actin in MuMTV

Fig. 1 *a* shows SDS-PAGE patterns of MuMTV isolated from milk, skimmed milk from virus-free mice, and molecular weight standards. A band in the virus and a prominent band in the virus-free skimmed milk protein patterns line up with the purified chick muscle actin in the standard well.

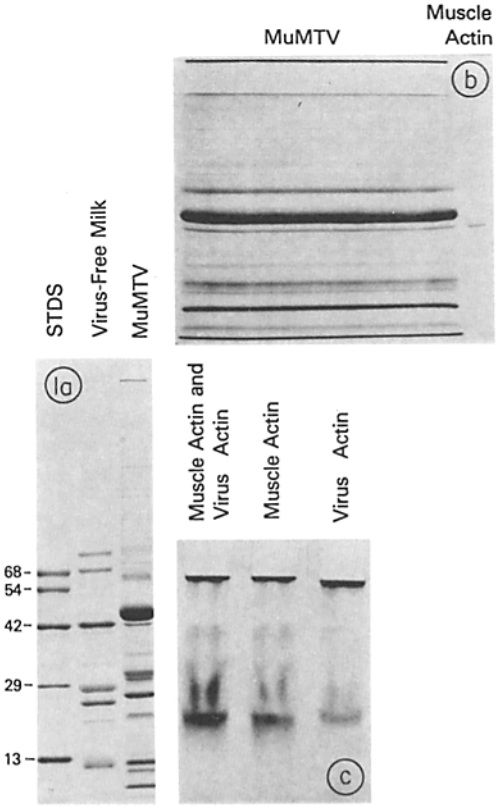


FIGURE 1 Electrophoretic evidence of the presence of an actin-like protein in purified MuMTV. (*a*) SDS-PAGE of molecular weight standards, purified MuMTV, and skimmed milk from virus-free mice. A band is present in both milk and virus preparations which migrates with purified chick skeletal muscle actin. (*b*) Preparative slab SDS gel of MuMTV used for the isolation of the 42,000-dalton viral protein. A similar preparation was used to isolate purified chick muscle actin. After elution of both proteins from the gels, they were rerun on an acid-urea gel. (*c*) Acid-urea gel of chick muscle actin, virus actin, and the two running in the same well. All three samples behaved identically.

As additional support for the presence of actin in MuMTV, the 42,000-dalton virus protein and purified chick skeletal muscle actin (27) were sequentially run on SDS-PAGE and acid-urea gel systems. Fig. 1 *b* shows a preparative SDS slab gel of MuMTV used for the isolation and elution of the presumptive virus actin band. Chick muscle actin was isolated from a similar preparation. Fig. 1 *c* shows that the isolated chick muscle actin and presumptive viral actin behave identically when run on the acid-urea gel system. This gel system separates proteins primarily on the basis of net charge and electrophoretic mobility in SDS is determined primarily on the basis of molecular weight. The processed viral and muscle actins also had the same electrophoretic mobility in an acid-urea gel system as purified chick muscle and platelet actins which had not been previously exposed to the SDS-PAGE and elution procedures (not shown).

Location of the Virus Actin

Because of the presence of a prominent band of 42,000-dalton protein in virus-free milk (Fig. 1 *a*), it was important to demonstrate that the putative virus actin was not an external contaminant of mouse milk which became nonspecifically bound to the virus particles during purification. Fig. 2 *a* shows densitometer tracings of autoradiographs of ^{125}I-labeled intact and disrupted virus. In the intact preparation, only the major external glycoprotein (GP55)[3] was labeled. In the disrupted preparation, several bands were labeled, including both actin and the major internal protein p28. These results show that the great majority of the 42,000-dalton protein is not accessible to labeling from the outside, and therefore, is not an external contaminant of the milk from which the virus was isolated. It was also important to determine that the source of actin in purified virus was not a small population of virus-free, actin-rich vesicles sloughed off from the cell surface during lactation, which might have co-purified with virus-containing vesicles. To this end, the protein composition of vesicles isolated from milk of virus-free mice was compared with that of virus isolated from milk of infected mice. The milks from the two groups of mice were subjected to identical procedures, and

[3] The molecular weight designations for MuMTV proteins are as described in references 21 and 25. GP55 is the major external protein and forms the surface spikes of the virion. p28 is the major internal nucleoid protein.

the corresponding regions of the second (isopycnic) gradients were removed for comparison. The vesicle preparation from virus-free milk contained only 14% the amount of protein per milliliter of milk when compared with the virus-containing preparation. When identical amounts of protein (25 μg) were examined for their electrophoretic mobility in SDS gels (Fig. 2*b*), the virus-free material did not contain either of the major viral polypeptides (GP55 or p28). The two preparations did have several other polypeptides in common, including a 42,000-dalton band. Quantitative densitometry of the two samples showed that this actin-like band comprised 4% of the virus-free vesicle material and 3% of the virus-containing material. These data indicate that a contaminating virus-free vesicle population present to the extent of 14% would account for 25% of the actin in the sample. Samples of purified virus and virus-free vesicles were examined by electron microscopy (Fig. 3*a–c*). Negative staining of both air-dried samples and samples prepared by the critical-point drying method shows virus-containing material to be morphologically distinguishable from virus-free vesicles. Morphometric analysis (33) of these preparations shows that virus-free vesicles contaminate purified virus preparations to the extent of ~ 12% (12.2 ± 6.3%). The morphological data, then, are consistent with the degree of contamination predicted from the protein content per milliliter of milk of the two preparations. Both sets of data indicate that most of the actin in the preparation is part of the virus-containing material.

Ultrastructure of Virus-Producing Mammary Epithelium

Normal and tumor-derived mammary epithelium in vitro contain sheets of cells which retain the junctional complexes typical of epithelial cells in vivo, and a luminal surface that contains frequent and prominent microvilli (9). These microvilli

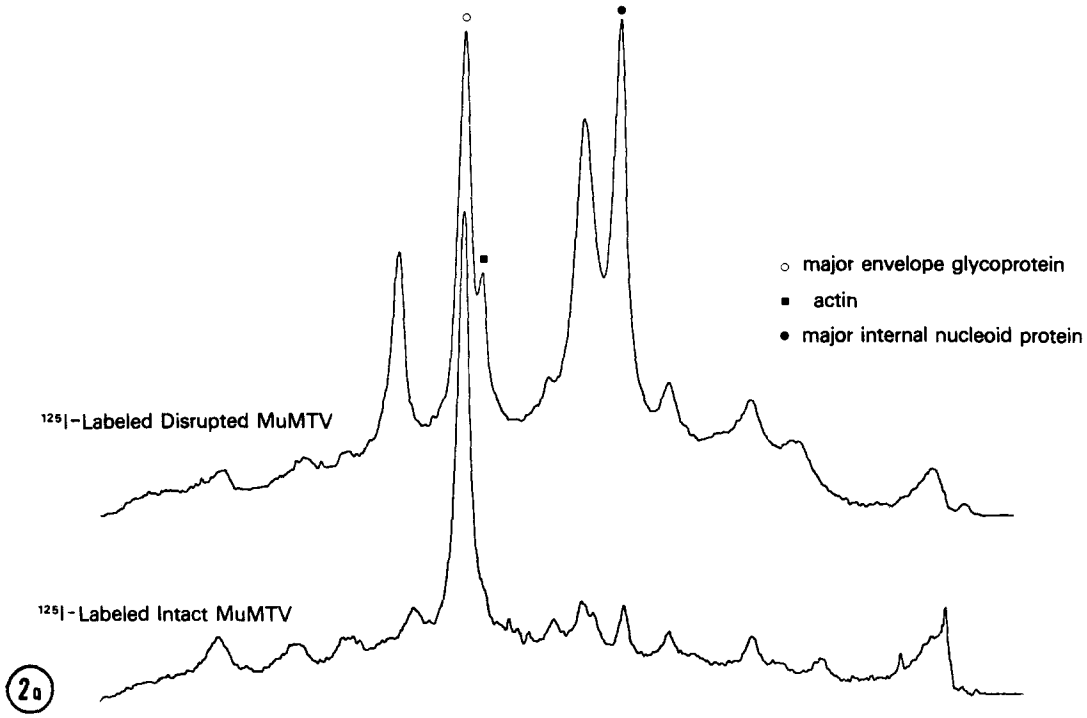


FIGURE 2 Evidence that the source of actin in purified virus preparations is not artifactual. (*a*) Densitometer tracings of autoradiographs of ^{125}I-labeled intact and disrupted virus run on SDS-polyacrylamide 10% gel. In the intact preparations, only the protein corresponding to the external glycoprotein of MuMTV (GP55) was labeled. The presumptive actin band, the internal nucleoid protein (p28), as well as other internal proteins were labeled only in the disrupted preparation.

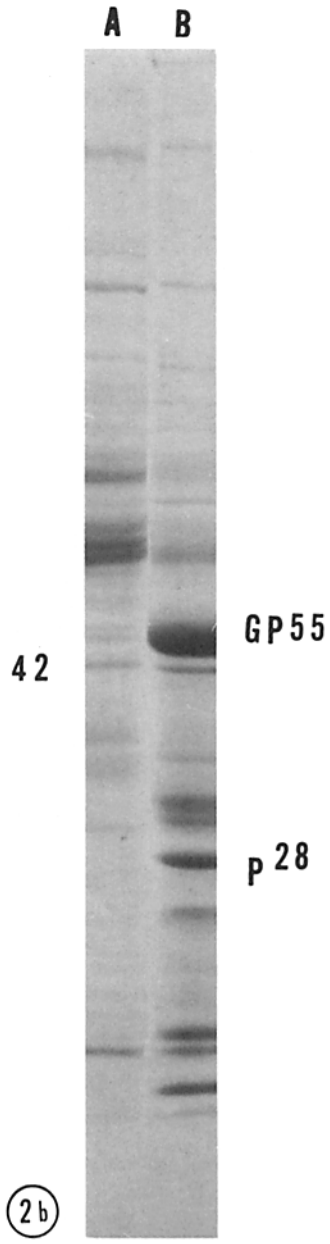


FIGURE 2 (*b*) SDS-polyacrylamide gels of (B) virus purified from milk and (A) virus-free vesicles purified in an identical fashion from virus-free milk. 25 γ protein was loaded onto each well. The virus-free vesicles do not contain p28 or GP55. However, the actin content of the two preparations is very similar. Because virus-free vesicles contaminate virus preparations to the extent of only 12% (Fig. 3), these vesicles cannot account for all the actin found in the purified virus preparations.

have several structural features that are similar to those of the intestinal epithelium, although they are not arrayed in so orderly a fashion (18, 19, 28, 29). Fig. 4*a* shows the luminal half of a mammary tumor epithelial cell which is actively producing virus. Virus nucleoids are visible both at the cell surface and at the ends of microvilli. Sites of interaction of virus nucleoids with the surface membrane are characterized by spikes extending from the external leaflet of the surface membrane (consisting of GP55), and by connections spanning the 100 Å electronlucent space between the nucleoid and the inner leaflet of the surface membrane (Fig. 6*a*, *b*). The cytoplasm extending about 1 μm into the cell from the luminal surface (cortex) is largely free of organelles, except for clusters of intracytoplasmic A particles (26), and small, smooth-surfaced vesicles with which the A particles appear to be intimately associated. These A particles are immunologically related to mature MuMTV, but it has not been established whether or not they represent precursor nucleoids or nucleoids previously assembled, which for some reason cannot be extruded from the cell (26). The major constituent of this cortical region appears to be actin, as demonstrated by its ability to bind myosin subfragment 1 (Fig. 4*b*). The actin extends into microvilli where it forms a core of 6- to 8-nm filaments (Figs. 4–7). In microvilli which do not contain virus particles, and in which the section passes through the microvillus tip (Fig. 5*a*), the filaments can be seen to be embedded in a dense material associated with the membrane. In favorable sections, cross-links appear to connect some filaments to the surface membrane along the length of the microvilli (Fig. 5*a–c*). These structural features are similar to those observed by Mukherjee and Staehelin (19), and by Mooseker and Tilney (18, 29) in intestinal microvilli. In microvilli which do contain a virus particle (Fig. 6*a*, *b*), the actin filaments appear to be embedded in the virus nucleoid itself, although it is also possible that the filaments circumvent the nucleoid and embed on the presumptive viral membrane.

Examination of chick embryo fibroblasts transformed by Rous sarcoma virus in which actin has been identified by its ability to bind to deoxyribonuclease I (14, 32) shows that these cells have extensive filopodia and cytoplasmic extensions, although these projections are more pleiomorphic than the microvilli of mammary epithelium. Fig. 7 is a section through several such processes, includ-

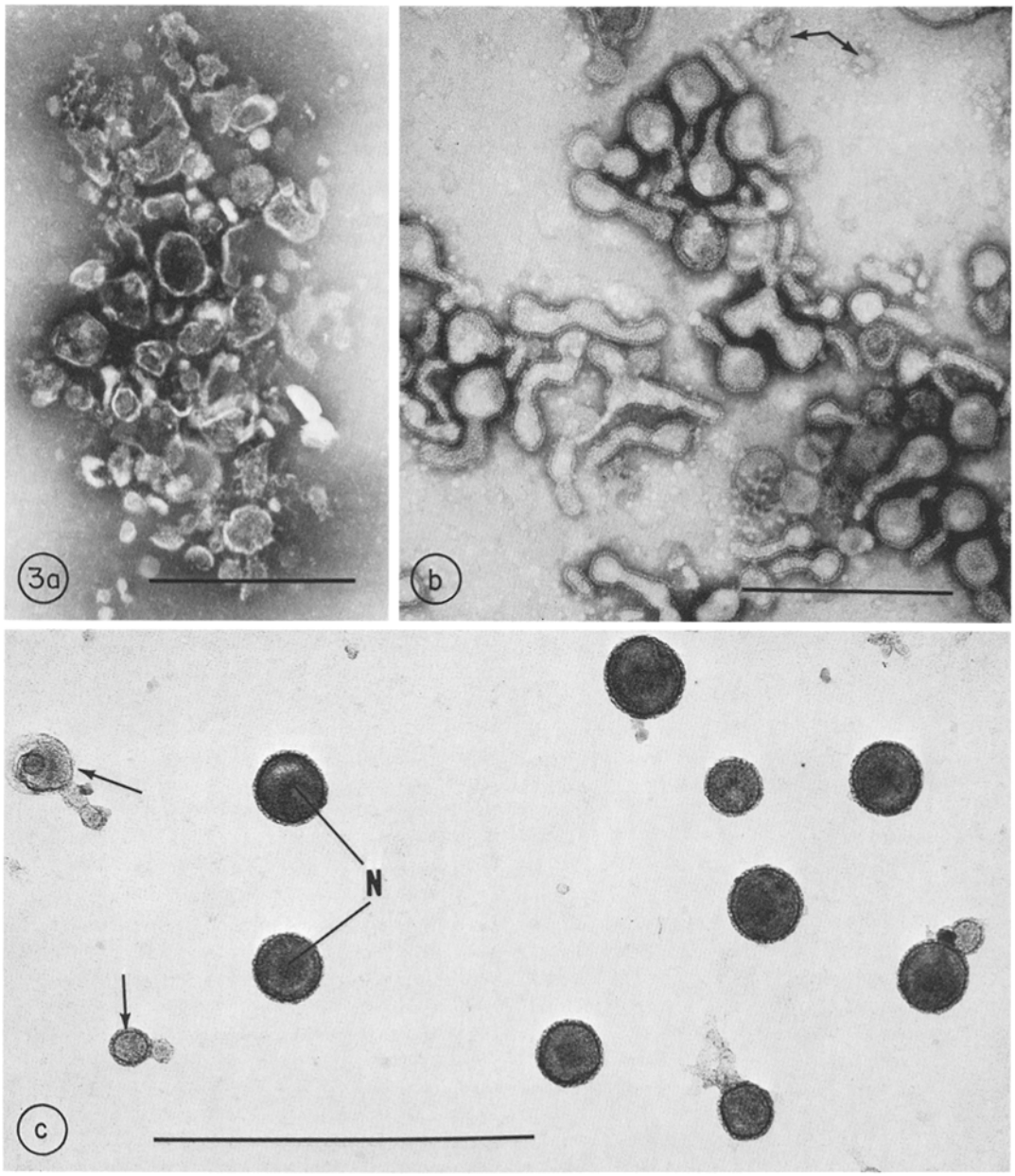


FIGURE 3 Air-dried, negatively stained preparations (1% phosphotungstic acid [PTA]) of (*a*) virus-free vesicles purified from the milk of virus-free animals, and (*b*) virus purified from the milk of infected animals. Virus-containing particles can be distinguished from virus-free vesicles by the presence of external spikes representing GP55, and by the head-tail morphology characteristic of unfixed virus stained with PTA. (*c*) Critical-point dried, purified, fixed virus stained with 1% PTA. Virus-containing particles are distinguishable by the presence of surface spikes and dense acentric nucleoid (*N*). Virus-free particles in both *b* and *c* (arrows) represent ~ 12% (12 ± 6.2%) of the purified virus preparations. *a*, *b*, × 60,000; *c*, × 110,000. Unless otherwise indicated, bar, 0.5 μm.

ing one which contains a viral particle. Fibrillar material is visible in these projections, although well-organized filament bundles are not demonstrable. Where filaments are visible within these processes, they have the characteristic width of actin-containing microfilaments.

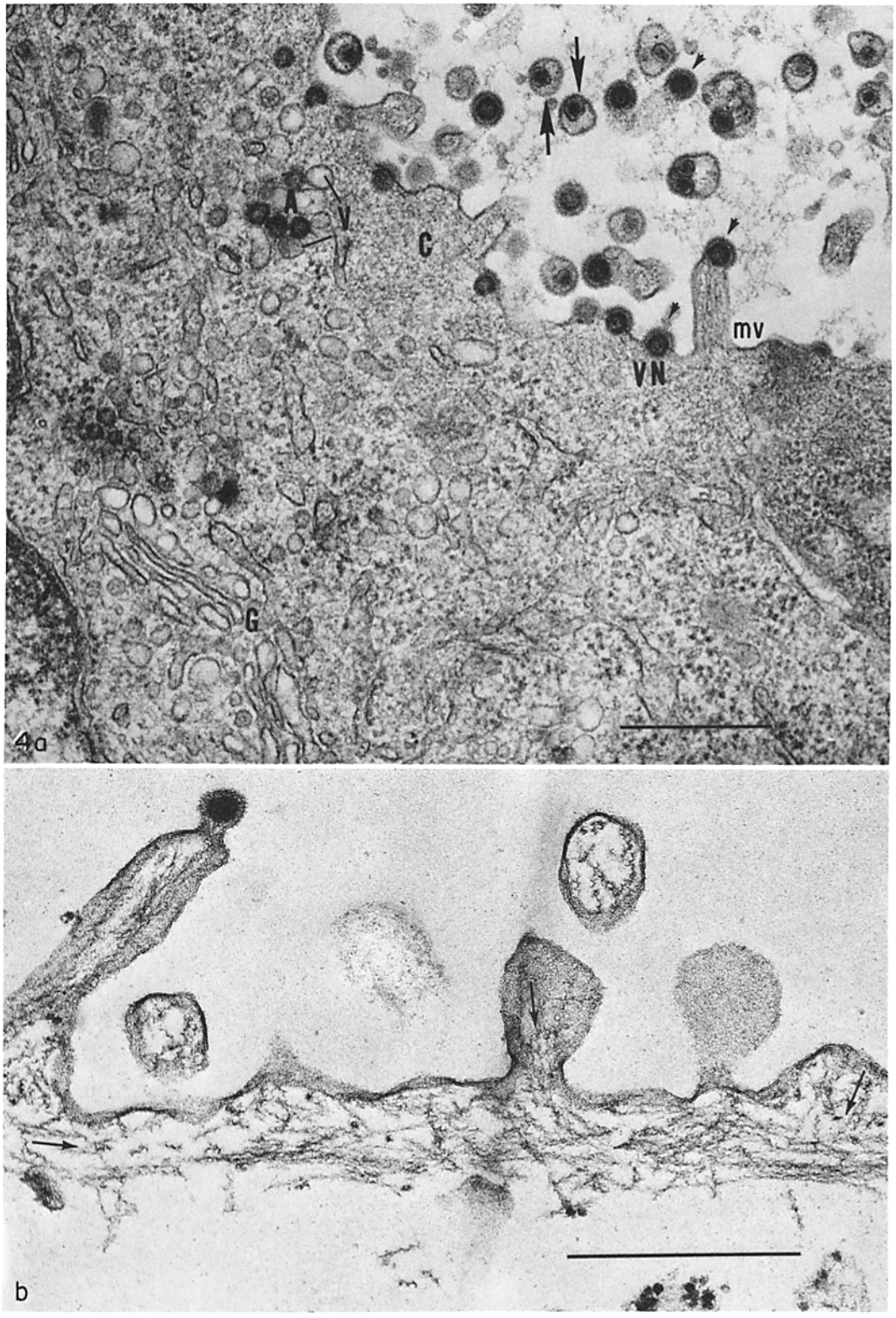
DISCUSSION

Our electrophoretic and labeling data strongly suggest that actin is a bona fide internal component of MuMTV, a B-type tumor virus secreted by an epithelial cell. This confirms the report of Wang et al. (32), identifying actin in several C-type oncornaviruses and paramyxoviruses by virtue of its electrophoretic mobility in SDS gels and its affinity for deoxyribonuclease I. Mammalian cells contain a great deal of actin in their subsurface cytoplasm (8). Therefore, to assess the significance of the observations presented in this paper, it is important to determine whether the actin found in released viruses is only passively incorporated into the virus particle as it assembles at the surface membrane, or whether the actin plays an active role in the extrusion and release of the particle. The ultrastructure of the mammary epithelial cells which produce MuMTV reveals a very striking association between the maturing virus particles and the actin filaments present in the microvilli from which these viruses are released. This relationship provides strong, if circumstantial, evidence that actin plays an active role in the extrusion of this virus.

The mammary tumor epithelial cells are particularly useful for the study of the ultrastructure of virus production because the viruses are released from prominent, well-organized microvilli. Mammary epithelial microvilli share several structural similarities with intestinal microvilli (18, 19, 28, 29), including the presence of a core of actin filaments which appears to embed terminally in a plaque of dense material associated with the microvillus tip. This dense material is thought to contain α-actinin in intestinal microvilli because of its ability to react with anti-α-actinin (22; preliminary report). In several grazing sections, we have also observed cross-link material which appears to connect some microfilaments laterally to the microvillus membrane along its length. Attempts to induce the microvillar actin filaments to form a paracrystalline arrangement in the presence of high Mg^{++}, in order to measure reliably the periodicity of the cross-links as has been done for intestinal microvilli (18), have been unsuccessful so far. Experiments with intestinal microvilli have demonstrated that the presence of a stable microfilament bundle is required for the formation of microvilli, and suggest that the growth of microvilli is the result of the polymerization of the actin filament bundle, which is stabilized in part by both terminal and periodic lateral interactions with the protruding surface membrane (18, 28, 29). Because of the similarities in the ultrastructure of mammary and intestinal microvilli, the mammary cell microvilli may be produced in a similar manner.

In virus-producing cells, viral nucleoids are present at the tips of many, but not all, microvilli. The microfilaments in these virus-containing microvilli seem either to embed in the material associated with the virus nucleoid, or to circumvent the nucleoid and embed in the presumptive virus membrane. The membrane at the tip of these microvilli is highly modified by the virus, displaying spikes that extend from the outer membrane leaflet and periodic connections that traverse the 150-Å electronlucent space between the nucleoid and the internal leaflet of the surface membrane (Fig. 6*a*, *b*). No plaque of dense material is visible at the tips of these microvilli. If such material (α-actinin?) is required for the attachment of microfilaments to membranes and to other structures, as is suggested by the work of several investigators (18, 22, 23, 28), it may be associated with the virus nucleoid in virus-containing microvilli, or it may be present in a more diffuse form on the presumptive virus membrane. We have detected a small amount of a protein which co-migrates with purified α-actinin on SDS-PAGE, and comprises $<0.5\%$ of the virus protein. We are currently trying to confirm the presence of α-actinin immunologically.

Whatever the particulars turn out to be for the organization of actin filaments in virus-containing microvilli, the association of virus nucleoids with the surface membrane, followed by their ultimate release from prominent microvilli which require, for their existence, the polymerization of an actin filament bundle, strongly suggests that the extrusion of MuMTV before its release requires an interaction with actin either by the virus nucleoid or by the presumptive virus membrane. This is well illustrated by Fig. 6*a*, which shows particles both at the cell surface and at the microvillis tip. If the usual route for the release of MuMTV from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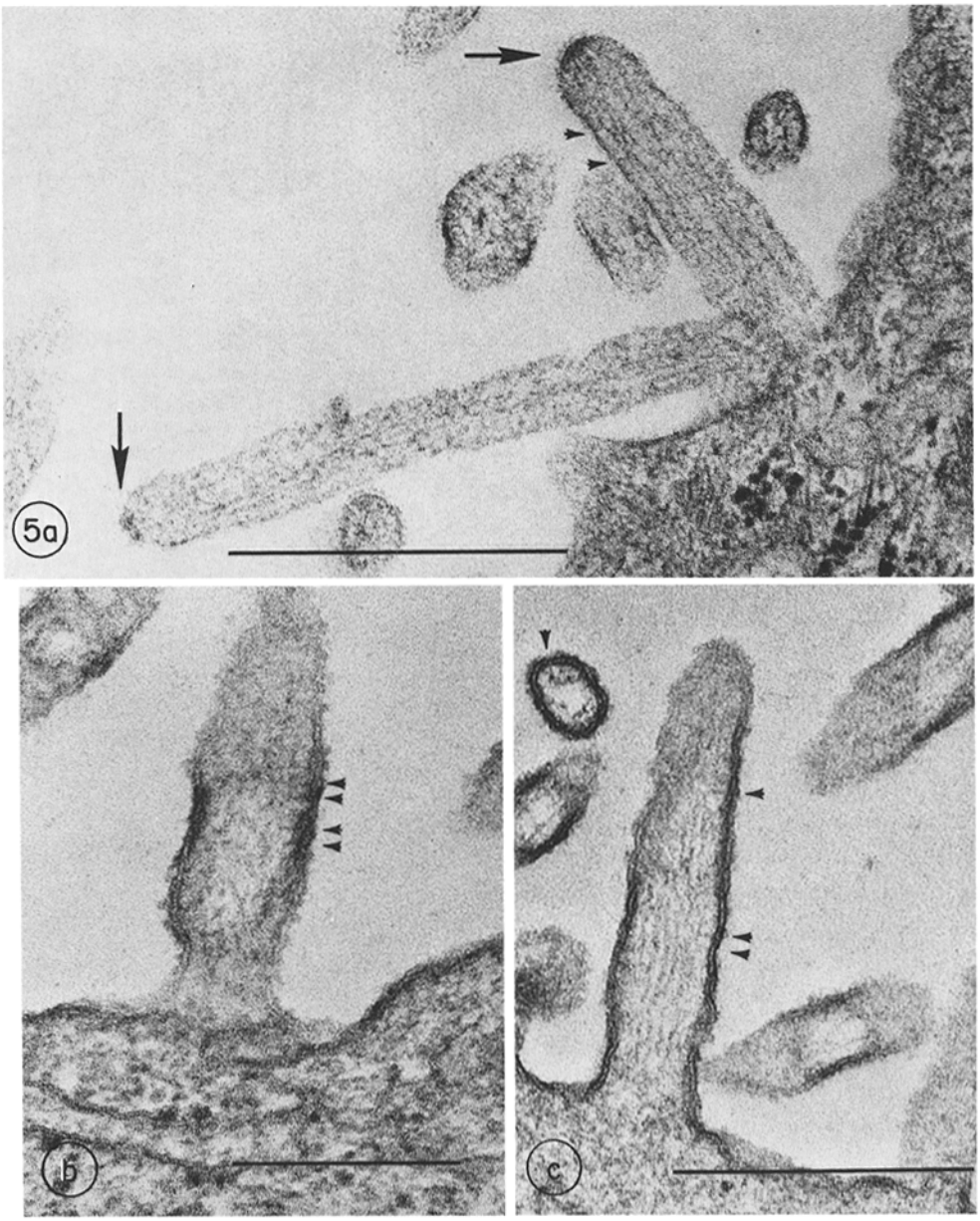

FIGURE 5 Virus-free microvilli from mammary tumor cells in culture. Virus-free microvilli contain a bundle of filaments which appears to distally terminate into a discrete patch of dense material at the microvillus tip (*a*, large arrows). Lateral cross-links (small arrowheads) between microfilaments and microvillus membrane are visible in favorable longitudinal and cross sections of microvilli (*a–c*). (*a*) × 90,000, (*b*) × 150,000, (*c*) × 80,000. Bar on (*b*), 0.2 μm.

FIGURE 4 Cell from an RIII mammary tumor, producing MuMTV from the luminal surface. (*a*) Viral nucleoids (VN) are visible at the cell surface and at the ends of microvilli (*mv*). Surface membrane patches associated with VN have external spikes (small arrowheads) not found on the rest of the surface membrane. Note organelle-free cortical region (*C*), clusters of intracytoplasmic A particles (*A*) which appear to be associated with small vesicles (*V*), and prominent Golgi (*G*). Large arrowheads mark virus particles with an acentric nucleoid which distinguishes mature B-type virus from mature C particles. × 48,000. (*b*) Luminal surface of a BALB/cfC3H-derived mammary tumor cell in culture. Cell was treated with Triton X-100 and myosin subfragment 1 before fixation, as described in Materials and Methods. Cortical network of filaments reacts with myosin subfragment 1 to produce characteristic arrowhead pattern diagnostic of actin-containing filaments. Decorated filaments extend into virus-containing microvilli. Arrows indicate that arrowheads point away from the membrane. × 75,000.

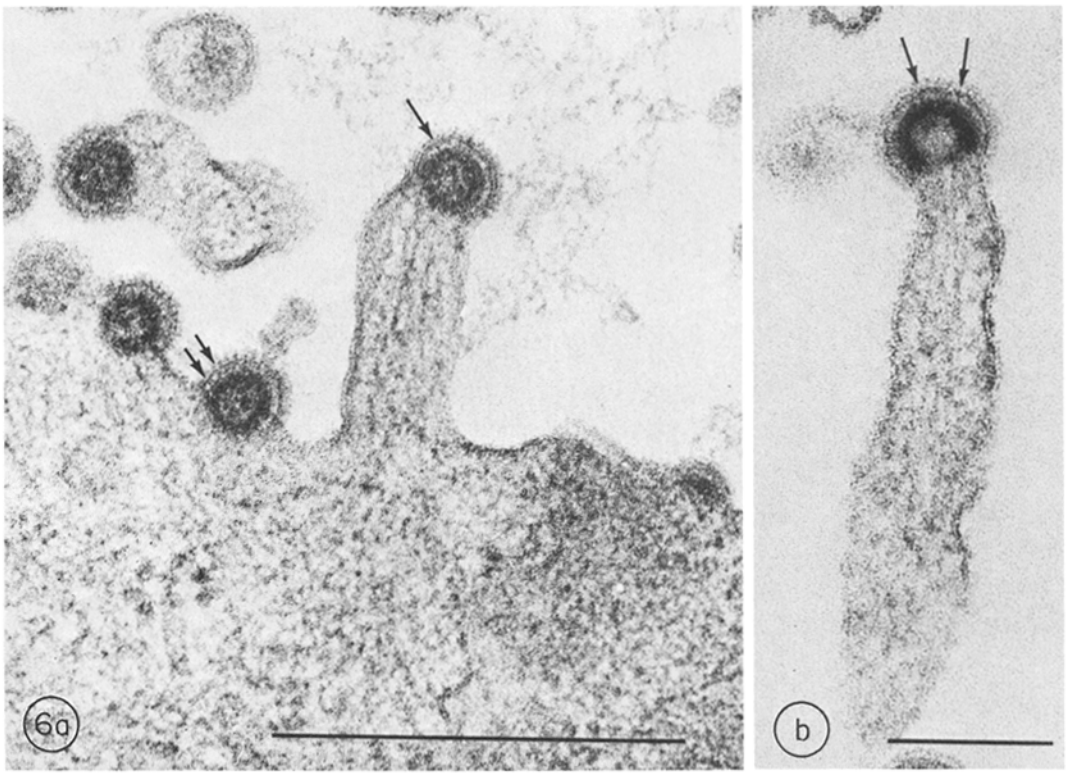

FIGURE 6 Virus-containing microvilli from mammary tumor cell in culture. Microfilament bundle terminates either in material associated with viral nucleoid or with the presumptive viral membrane. Space between the virus nucleoid and the surface membrane at the microvilli tip appears to be free of the dense material typical of the virus-free microvilli, and to be electron-lucent except for the dense strands which appear to connect the nucleoid with the microvilli tip (arrows). (*a*) × 110,000, (*b*) × 115,000. Bar on (b), 0.2 μm.

the cell is via microvilli, it is difficult to see how the nucleoid or presumptive viral membrane could avoid interacting with actin during its extrusion. That the microvillus is the only route of MuMTV release is difficult to prove directly. Examination of large numbers of micrographs of sectioned material and of replicas of critical-point dried cells (Sheffield, unpublished observations) can only indicate tht microvilli are a very common route of release. Attempts to disrupt microvilli by using cytochalasin B to assess the affect of a reduction in the number of microvilli on virus release gave ambiguous results. Although the average number of microvilli per micrometer of cell surface decreased by 30% after a 10- and 15-h exposure to 5 or 10 μg/ml of cytochalasin B, the ultrastructure of the remaining microvilli appeared normal. This suggested an effect of the drug on microvillus turnover, but not structure. Virus particles released during the 10-h exposure to the drug were decreased by ~30%,[4] although the numbers of unreleased virus particles still present at the cell surface and at the ends of microvilli were also reduced. Because the sites of action of cytochalasin B are not well understood and may include an effect on viral synthesis itself, a more precise antagonist of microvillus turnover will have to be found to answer whether or not MuMTV can be released from nonmicrovillar regions of the plasma membrane if microvilli are not available.

A further point of interest in these studies concerns whether maturing nucleoids induce the formation of microvilli at sites where they have been committed to assemble, or whether the site at which a microvillus is about to be formed determines where the nucleoid will assemble. Again, cause and effect relationships are difficult to prove. What we can say is that there does not

[4] As determined by radioimmunoassay (24).

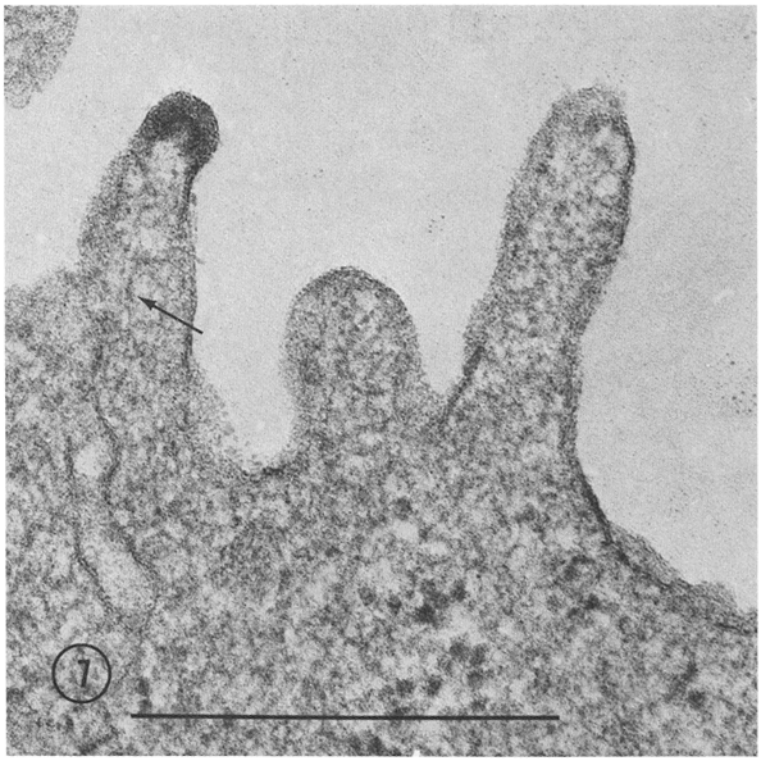

FIGURE 7 Portion of an RSV-CEF cell with surface protrusions which, in favorable section planes, contain recognizable microfilaments (arrow). These processes are quite pleiomorphic and do not demonstrate the well-organized microfilament bundle seen in mammary cell microvilli. A maturing virus particle is seen at the end of one process. × 110,000.

appear to be any large change in the distribution and frequency of microvilli in tumor cells which are producing virus, as compared with other tumor cells in the same preparation which are not producing virus (3.5 ± 1.2 microvilli per micrometer surface; Damsky and Sheffield, unpublished observations). This would imply that the nucleoid does not induce microvillus formation, but follows the lead of the microvillus and assembles at a site which has already been determined as a site for future microvillus growth. We can also say that virions are never found partway up an existing, completely formed microvillus. They are always found at the tips of these processes. This would imply that the signal for the virion to assemble at a particular site closely follows the determination of that site as a potential location for microvillus growth. These observations are consistent with the idea that the process of virus nucleoid extrusion has been closely coupled to the normal process of microvillus formation.

It is possible to suggest that the prominence of microvilli in mammary epithelium has led to an association of this B-type virus and actin which is peculiar to this cell type, and not typical of other cells which produce C-type enveloped virus. The presence of actin has been recently detected in a wide variety of purified C-type virus preparations. It has been determined in these studies that the actin is associated with the virus nucleoid (32). Most fibroblastic cells produce virus from shorter, more pleiomorphic cytoplasmic projects in which organized actin filament bundles are not easily visualized. Our examination of Rous sarcoma virus-transformed chick embryo fibroblasts shows that fibrillar material, although not organized filament bundles, can be observed in the projections from which virus are released, as well as in other surface filopodia and microvilli which these cells produce. Distinct filaments are sometimes visible in these processes which measure 6–8 nm in width and are most likely actin. Images as shown in Fig. 7 are consistent with the suggestion that the interaction of actin with viral components during as-

sembly or extrusion may be a general phenomenon in the release of enveloped virus.

The observations on MuMTV presented in this paper demonstrate the need to reevaluate current thinking on virus release which assumes a self-assembly mechanism for the final stages of virus formation and release, independent of a contractile mechanism. We suggest (*a*) that an interaction between actin and material associated with maturing virus particles is required for enveloped virus to be released from their host cell, and (*b*) at least some of the actin found in purified virus preparations is derived from host cell microfilaments. Conclusive demonstration of a requirement for actin in the final stages of virus assembly will require isolation of assembly mutants, preferably temperature-sensitive, which may have an altered protein that is not capable of interacting with actin. Assembly of such a virus should start at the surface, but its extrusion should be restricted at the nonpermissive temperature. Temperature-sensitive assembly mutants have been found in virus-producing avian (11) and murine (34) systems. The specific lesions involved in many of the mutant clones have not been reported, but some may involve a defect in viral interaction with host contractile proteins.

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