Journal of Supramolecular Structure 13:53–65 (1980)

The Association of α-Actinin With the Plasma Membrane

Keith Burridge and Lois McCullough

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

The role of α-actinin in the attachment of actin to plasma membranes has been investigated. Specific antibody staining of SDS gels has indicated that α-actinin is a major component in isolated plasma membranes prepared from three different cell types by two different procedures. Using specific extraction conditions, most of the α-actinin can be selectively extracted from the membranes with relatively little parallel release of actin. This selective dissociation of α-actinin from the plasma membrane leads us to conclude that α-actinin is present in these membrane preparations, because it is bound to actin, and that α-actinin does not form a direct link between actin and the membrane.

Key words: α-actinin, plasma membranes, actin attachment, immunoautoradiography on gels

During the last few years much has been learned about the proteins involved in the force generation required for cell movement, but the question of how force-generating elements such as actin are attached to cell membranes remains unanswered. One protein that has frequently been considered a possible candidate for linking actin to membranes is α-actinin. In muscle α-actinin is localized specifically at the Z-line, where it is generally considered to have some role in the attachment of actin filaments to these structures. The discovery of α-actinin in nonmuscle cells led to the suggestion that, by analogy, it might also be involved in the attachment of actin to membranes [1, 2]. Indirect evidence supporting this possibility has come from immunofluorescence studies, which have revealed α-actinin concentrated at the termini of the microfilament bundles in regions of cell–substrate and cell–cell contact [1]. Such regions in electron micrographs are often reminiscent of muscle Z-lines, showing both increased electron density and associated filaments [3]. Recently α-actinin has also been found in association with aggregated surface molecules that have been capped or patched [4], again consistent with a possible role in mediating actin attachment.

In this paper we examine the association of α-actinin with isolated plasma membranes. α-Actinin has been found as a major component in the plasma membranes prepared from several cell types. Evidence is obtained from the dissociation of α-actinin from these mem-

Abbreviations: ATP – adenosine triphosphate; EGTA – ethyleneglycol bis (β-aminoethylether)-N,N'-tetraacetic acid; EDTA – (ethylenedinitrilo)-tetraacetic acid; PBS – Dulbecco's phosphate-buffered saline; SDS – sodium dodecylsulfate.

Received January 16, 1980; accepted April 21, 1980.

0091-7419/80/1301-0053$02.60

branes that is more consistent with the theory that α-actinin has an indirect role in actin attachment whereby it may promote and stabilize the attachment by cross-linking adjacent filaments rather than by providing a direct link in the attachment itself.

MATERIALS AND METHODS

Cells

HeLa cells were grown suspended in Dulbecco's modified Eagle's medium (DME) supplemented with 10% calf serum. Mouse myeloma cells, P3 X63 Ag8, were a generous gift of Dr. G. Kohler and were grown suspended in DME with 9% fetal calf serum (FCS). Gerbil fibroma cells (IMR-33), which have a fibroblast morphology, were obtained from the American Type Culture Collection and were grown in 10 cm plastic Petri dishes in DME with 9% FCS.

Antibody Against α-Actinin

An antiserum was raised in rabbits against beef cardiac α-actinin, which was the generous gift of Dr. D. Goll in 1973. The protein was further purified by elution from preparative SDS polyacrylamide gels. The initial two injections were given ten days apart at multiple subcutaneous sites in Freund's complete adjuvant. These were followed by a series of four intravenous injections every 7–10 days, with the final two being injections of α-actinin that had not been purified from the preparative gels.

Reaction of Gels With Antibodies

Analytical SDS slab gels were run in the discontinuous buffer system of Laemmli [5]. The separating gels contained 10% acrylamide and 0.13% bisacrylamide or 7.5% acrylamide and 0.195% bisacrylamide; the stacking gels contained 5% acrylamide and 0.13% bisacrylamide.

Gels were treated with the crude antiserum against α-actinin diluted 1/30th in buffer A (0.15 MNaCl, 0.05 M Tris Cl, pH 7.5, 0.1% NaN_3), essentially as described previously [6, 7]. One major modification, however, was in the preparation of the second radioiodinated antibody. This was purified and iodinated as follows. An immunoadsorbant was prepared by covalently coupling rabbit IgG (purified by ammonium sulfate precipitation and chromatography on DEAE-cellulose) to Sepharose 4B, using cyanogen bromide to activate the Sepharose 4B [8]. After an overnight incubation with the rabbit IgG, the column was washed and any unreacted groups were blocked with 1 M ethanolamine, pH 8.5. The column was then washed with 0.1 M glycine-HCl, pH 2.3, to elute noncovalently bound protein. After washing in this buffer, the column was reequilibrated in buffer A, before incubating with goat anti-rabbit IgG (purchased from either Calbiochem or Miles). The column was washed with buffer A until the OD_{280} of the eluate returned to the background level, at which point the specific goat anti-rabbit IgG was eluted with 0.1 M glycine-HCl, pH 2.3. The eluted IgG was immediately neutralized with 1 M Tris buffer, pH 8.8, and then dialyzed against buffer A. For iodination a peak fraction of the eluted protein (~9 mg/ml) was dialyzed against 0.1 M sodium borate, pH 8.6, and was then reacted with 1 mCi of ^{125}I-labeled Bolton-Hunter reagent [9] (New England Nuclear) as described previously [7]. Typically activities of between 5×10^8 and 8×10^8 cpm/mg protein have been obtained. The iodinated antibody was separated from the iodinated low molecular weight reaction products on a small Sephadex G50 (fine) column, equilibrated in buffer A, and the iodinated protein was stored frozen together with 10 mg/ml of BSA. For reacting on gels the antibody was used at 10^7 cpm/ml in buffer A to which an addi-

tional 10 mg/ml human hemoglobin (Sigma) had been added. Reaction with gels was for about 12 hours with 3–4 day washings in buffer A both between the first and second antibodies and after the second antibody prior to staining the gel with Coomassie blue, drying, and autoradiography.

Plasma Membrane Preparation

Plasma membranes were prepared from HeLa cells and the cultured myeloma cells by the Brunette and Till procedure [10], with a modification that has been described previously [11] to decrease nuclear contamination. This procedure was also used to isolate plasma membranes from the substrate-adherent gerbil fibroma cells after these were suspended by treatment with PBS containing EDTA. The procedure of Thom et al [12] was also used to isolate plasma membranes from the gerbil fibroma cells.

Electron Microscopy

Isolated membranes were fixed with 2% glutaraldehyde and 2% tannic acid in PBS at 4°C for 1 h, then centrifuged into a pellet. The pellet was washed three times in PBS and post-fixed in 1% OsO_4 in PBS for 30 min at 20°C. The pellets were then stained with saturated aqueous uranylacetate for 20 min, dehydrated in graded alcohols over 15 min, and embedded in Epon 812. Thin sections were prepared and stained with lead citrate.

Membrane fractions were prepared for negative staining by placing a 10 μl sample on a Formvar carbon-coated grid for 2–3 min and then removing excess buffer. The grid was stained with 2% aqueous uranylacetate for 30 sec and air-dried. Membrane fractions were decorated with heavy meromyosin after the method of Ishikawa et al [13].

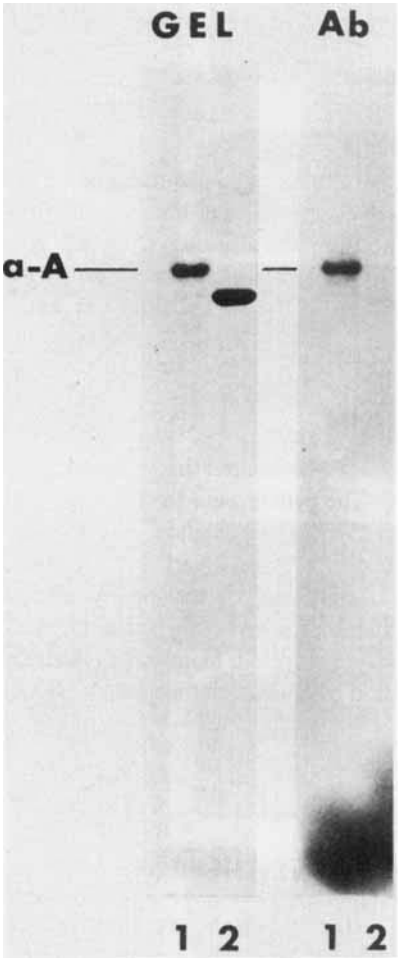
RESULTS

Characterization of the α-Actinin Antibody

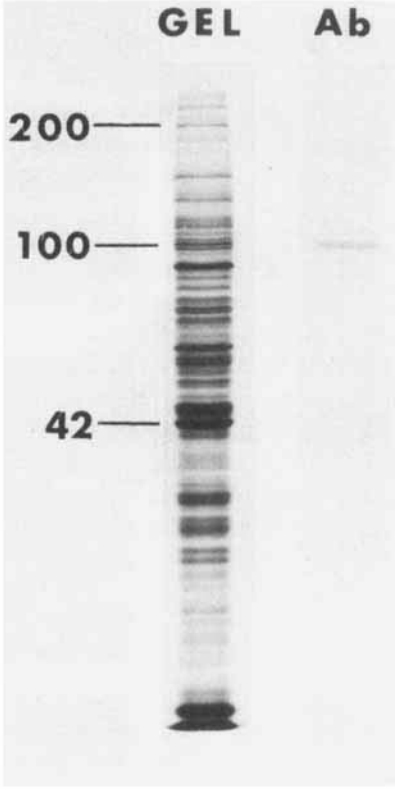
The antibody used in this study was similar to one we have used previously [1], and like that one gave specific staining of myofibril Z-lines in indirect immunofluorescence (data not shown). We have tested this antibody further by using it to stain SDS gels of both pure proteins and complex mixtures such as cell lysates. In Figure 1 purified chicken gizzard α-actinin and chicken breast phosphorylase were electrophoresed in parallel gel slots. The proteins were compared by conventional staining with Coomassie blue, and the gel was also reacted with the anti-α-actinin antibody. This labeled specifically the α-actinin gel band but not that corresponding to phosphorylase. Figure 2 shows an SDS gel of whole HeLa cells stained for protein and a parallel autoradiograph of a similar gel reacted with the same antibody. A single band at 100,000 molecular weight is labeled with this antibody. On some gels with enhanced resolution this band could be resolved clearly as a doublet, as was reported previously [1], and this was not affected by the presence of protease inhibitors during the gel sample preparation.

α-Actinin as a Component of Isolated Plasma Membranes

The Brunette and Till [10] procedure has been used to isolate plasma membranes from several different cell types. A typical preparation of plasma membranes isolated in this way from HeLa cells is shown in Figure 3. The inset shows a low-magnification phase micrograph of the membranes in suspension. They can be seen to consist of sheets, envelopes, or rolls of plasma membrane. When a section through a pellet of these membranes is examined by transmission electron microscopy, little contamination by other organelles can



1



2

Fig. 1. Immunological detection of α-actinin in an SDS gel. Purified chicken gizzard α-actinin and chicken breast phosphorylase were electrophoresed in adjacent slots of a 10% polyacrylamide SDS slab gel. A photograph is shown of the Coomassie blue stained dried-down gel, together with the corresponding autoradiograph of the same gel after it had been reacted first with anti-α-actinin and then with ^{125}I-labeled goat anti-rabbit IgG. The anti-α-actinin antibody labels only the band corresponding to the α-actinin and not the phosphorylase.

Fig. 2. Detection of α-actinin in a gel of whole HeLa cells by reaction with specific antibody. Whole HeLa cells were dissolved and electrophoresed in SDS. The photograph of a gel slice stained with Coomassie blue is shown, together with the autoradiograph of a parallel gel slice that was reacted first with anti-α-actinin and then with the iodinated second antibody, as described in Materials and Methods. A single band that can sometimes be resolved into a tight doublet is labeled with a molecular weight of approximately 100,000.

be observed. One face of the membrane appears "fuzzy" with particulate and filamentous material attached, and we presume this to be the cytoplasmic face. Occasionally, discrete filaments can be seen to emerge from this submembranous cortex, and these filaments will bind HMM, confirming that they are actin. In some regions structures with the appearance and dimensions of coated vesicles or coated pits can also be seen and have been indicated.

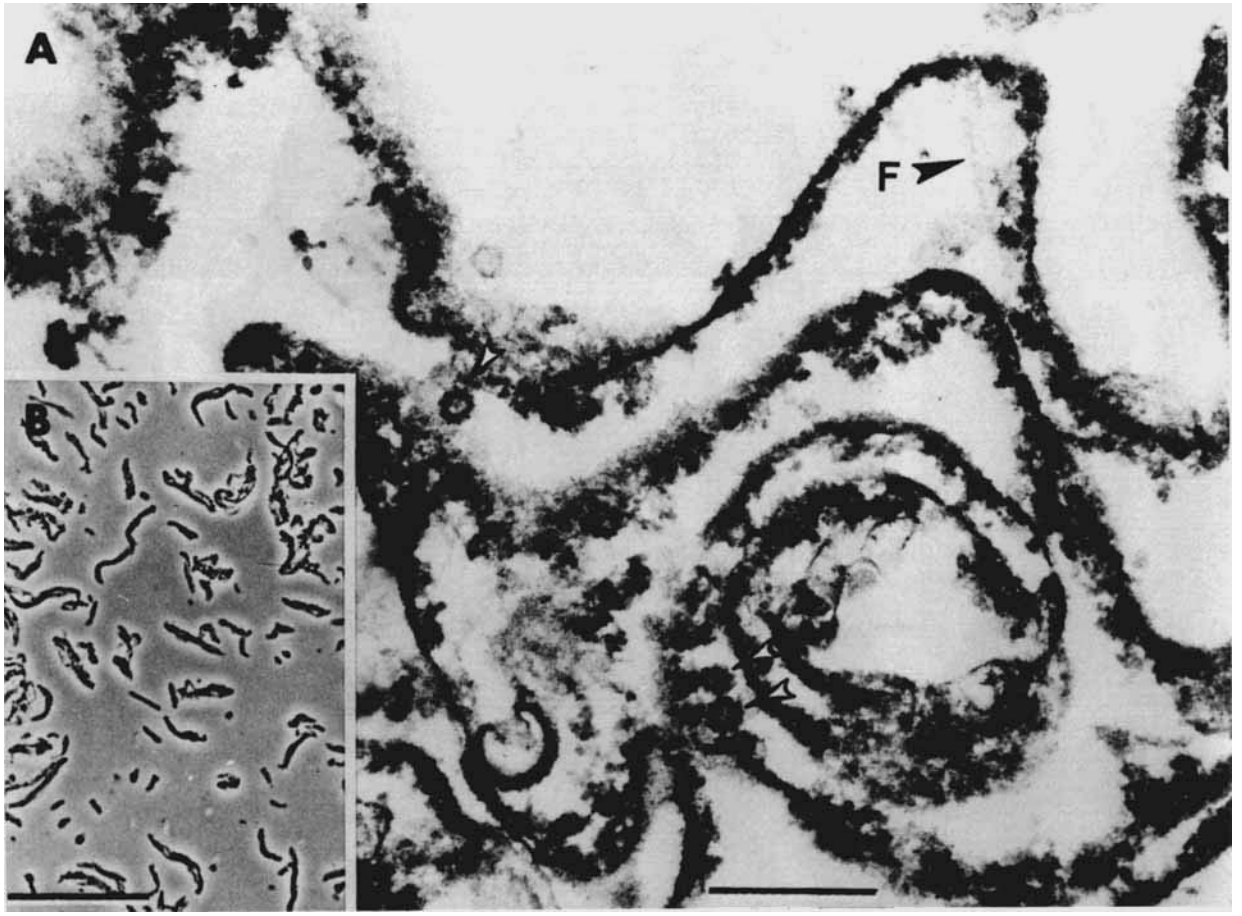
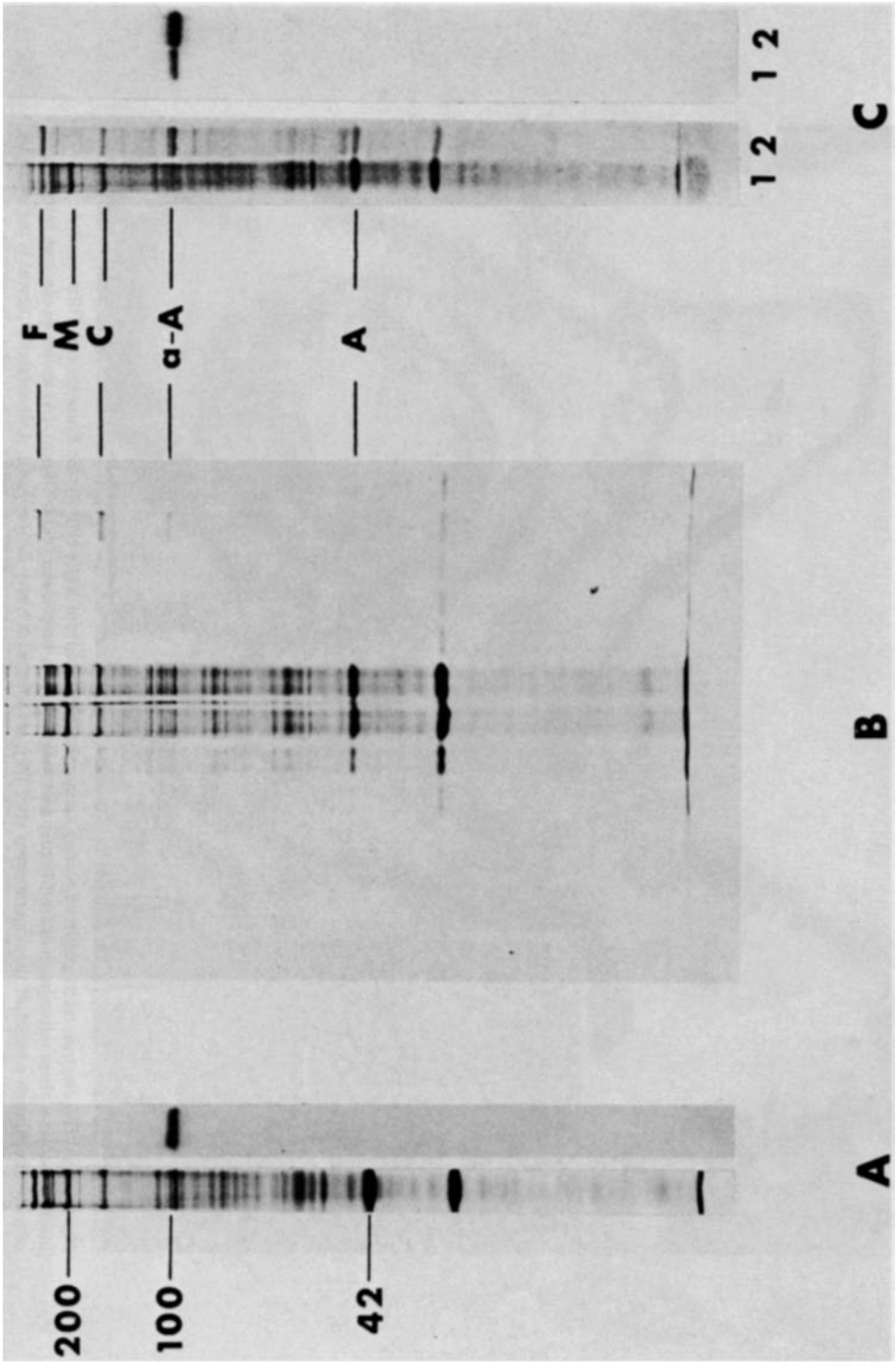


Fig. 3. Light and electron micrographs of plasma membranes purified from HeLa cells by the Brunette and Till procedure. The inset (B) shows a phase micrograph of freshly prepared membranes in suspension (bar = 50 μm). (A) shows an electron micrograph of a section through a pellet of these membranes after their reaction with heavy meromyosin. A decorated filament is indicated by the arrow marked F. Three structures resembling coated vesicles or coated pits have also been marked with arrows (bar = 0.5 μm).



4

Routinely such membranes were purified further by centrifugation over a sucrose gradient or by placing in dense sucrose beneath a sucrose gradient and then by centrifugation up the gradient. Reducing agents (either dithiothreitol or β-mercaptoethanol) were included in the gradients to reduce any disulphide bonds that had been formed by the presence of zinc in the membrane isolation. The membranes band at a density of about 1.17 g/ml. When membranes that have been spun up a gradient are analyzed by SDS gel electrophoresis a complex pattern of bands is seen (Fig. 4A). When such a gel is analyzed by indirect immunoautoradiography with the antibody against α-actinin, a single band (or tight doublet of bands) is labeled with a molecular weight of about 100,000 (Fig. 4A). This labeled band corresponds to a band in the Coomassie blue stained gel and comigrates with purified rat skeletal muscle α-actinin, but it has a slightly faster mobility when compared with the α-actinin from chicken gizzard. We have tentatively identified several of the other prominent bands in the gels of these plasma membrane preparations as cytoskeletal elements, including actin, myosin, and filamin, either by their comigration with the purified proteins or by their reaction with the corresponding antibodies (data not shown).

When preparations of the HeLa plasma membranes were placed above continuous sucrose gradients and subjected to prolonged centrifugation, then SDS gel analysis of fractions across the gradient (Fig. 4B) indicated that several prominent proteins partially dissociate from the plasma membranes and remain at the top of the gradient. We have identified one of these proteins as α-actinin by its reaction with the anti-α-actinin antibody (Fig. 4C). In this gel equal volumes of a fraction at the top of the gradient and one corresponding to the position of the peak of the membranes were compared. More α-actinin is detected in the fraction at the top of the gradient. Usually, however, the membranes were collected in about three fractions, whereas only a single fraction at the top of the gradient was enriched for α-actinin.

Fig. 4. SDS gel analysis of plasma membranes isolated from HeLa cells. (A) Plasma membranes isolated by the Brunette and Till procedure were further purified by flotation up a 30–55% sucrose density gradient (containing 50 mM $NaPO_4$, pH 7.0, 0.1% β-mercaptoethanol). Centrifugation at 40,000 rpm in a Beckman SW50.1 rotor caused the membranes to float up to their buoyant density of about 1.17 g/ml. About 20 μg of protein from the peak membrane fraction was electrophoresed in one slot of a 10% polyacrylamide gel. A photograph of the gel stained with Coomassie blue is shown, together with a corresponding autoradiograph after reacting a parallel gel slice with anti-α-actinin followed by ^{125}I-labeled second antibody. The antibody reacts with a tight doublet of bands with a molecular weight of about 100,000. (B) Plasma membranes purified by the Brunette and Till procedure were resuspended in PBS + 0.2% β-mercaptoethanol and layered above a linear gradient of 30–55% sucrose containing 50 mM $NaPO_4$, pH 7.0, 0.1% β-mercaptoethanol, with an underlying cushion of 60% sucrose. The gradient was centrifuged in a Beckman SW27.1 rotor at 25,000 rpm for 15 h. Equal volume fractions were collected, and 25 μl of each fraction (except the bottom two which were not analyzed) were electrophoresed in successive slots of a 10% polyacrylamide SDS gel. The top fraction of the gradient is on the right of the figure. (C) The identification of α-actinin in two fractions of a sucrose gradient in which plasma membranes had been sedimented. A gradient such as that shown in B was run, and 25 μl of the peak membrane fraction and a fraction at the top of the gradient (fraction 14, where 15 were collected) were electrophoresed in adjacent slots (1 and 2, respectively) of a 10% polyacrylamide gel. The photograph of the Coomassie blue stained, dried-down gel and the corresponding autoradiograph after the same slice had been reacted first with anti-α-actinin and then with the second ^{125}I-labeled antibody are shown. More α-actinin is detected in the fraction at the top of the sucrose gradient than that corresponding to the peak membrane fraction. The positions of migration of the purified proteins filamin (F), myosin (M), clathrin (C), α-actinin (α-A), and actin (A) are indicated for the gels shown in B and C. It should be noted that gels electrophoresed in A, B, and C were run on different occasions, and slight differences in protein migration can be detected.

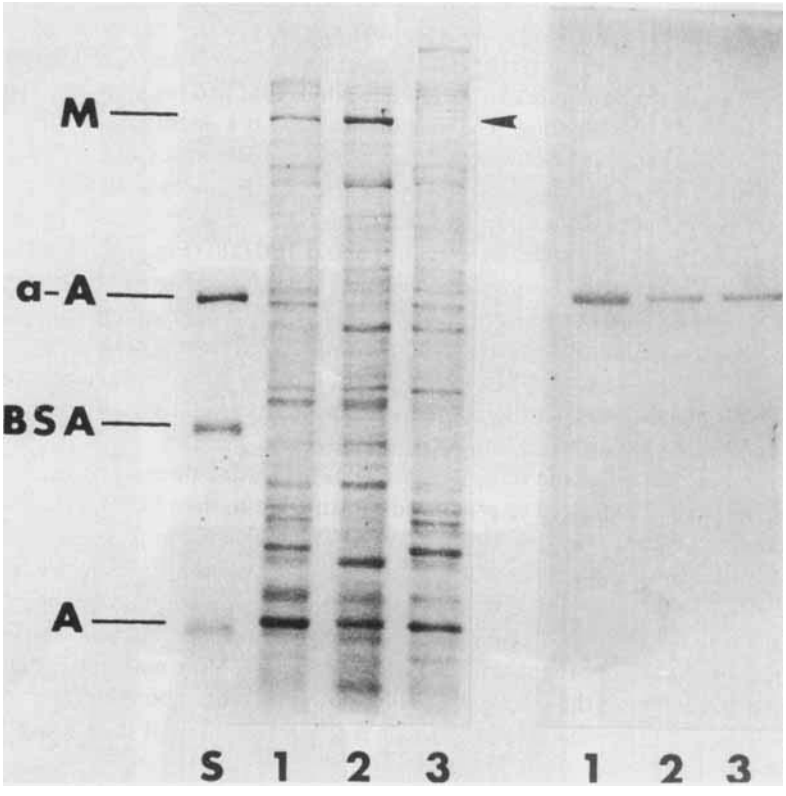


Fig. 5. SDS gel analysis of plasma membranes before and after incubation with extraction buffers. 0.2 ml of HeLa cell plasma membranes isolated by the Brunette and Till procedure were extracted at 4°C with 4 ml of either 2mM Tris HCl, 0.2 mM EDTA, 0.1% β-mercaptoethanol, pH 8.8, or 0.6 M NaCl, 0.1 M $NaPO_4$, 1 mM $MgCl_2$, 4 mM ATP, 0.1% β-mercaptoethanol, pH 6.5. After 1.5 h the membranes were sedimented at 27,000g for 20 min. The pellets were dissolved in SDS and compared with a sample of the starting membranes on a 7.5% polyacrylamide gel. The first sample (1) was the starting membranes, the second (2) had been extracted with the low ionic strength buffer, the third (3) had been extracted with the high ionic strength ATP-containing buffer. A photograph of a dried-down gel stained with Coomassie blue is shown. Approximately 40 μg of protein of the 3 membrane samples were electrophoresed in parallel slots next to a sample of standards (S), which contained the purified proteins α-actinin, BSA, and ovalbumin. The positions of migration of the proteins myosin (M), α-actinin (α-A), BSA and actin (A) are indicated next to the gel. The position of the myosin heavy chain is also marked with an arrow next to the third membrane sample, indicating its absence or marked extraction from this sample. An autoradiograph is also shown of a parallel gel of the three membrane samples after it was reacted with the anti-α-actinin antibody followed by the ^{125}I-labeled second antibody.

Plasma membranes have been isolated from a myeloma cell line and from gerbil fibroma cells (cells with a fibroblastic morphology) by the Brunette and Till procedure [10], and from the gerbil fibroma cells also by the procedure of Thom et al [12]. In each case SDS gels of these membranes have revealed a prominent band by Coomassie blue staining at 100,000, which has reacted with the α-actinin antibody. Human red blood cell membranes isolated by hypotonic lysis and centrifugation were also analyzed for α-actinin. Of all the plasma membranes examined, these alone did not yield a band at 100,000 molecular weight that reacted with the anti-α-actinin antibody (data not shown).

Extraction of α-Actinin From Isolated Plasma Membranes

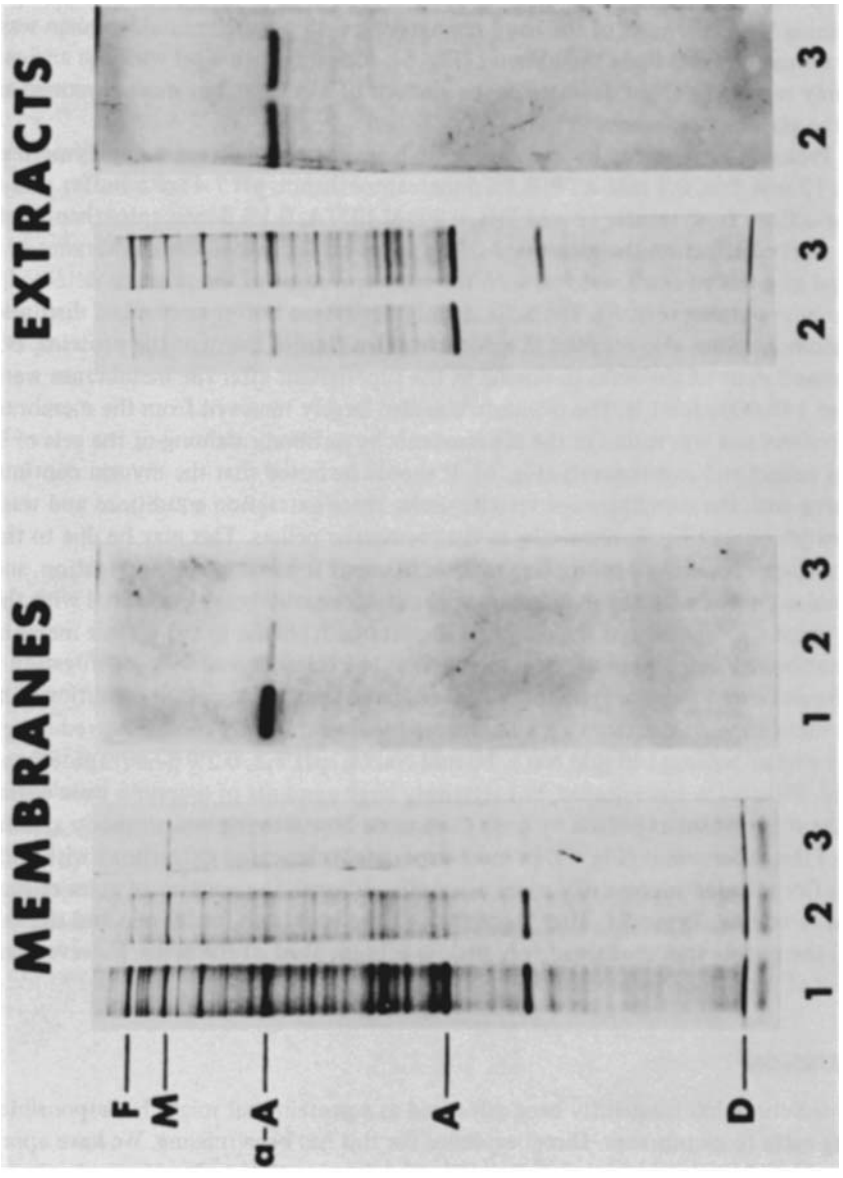
The nature of the association of α-actinin with the plasma membranes was investigated by extraction of the HeLa membranes with various buffers. For the extraction experiments, the membranes were taken either from the sucrose gradients or after washing the membranes from the Brunette and Till preparation in PBS containing 0.2% β-mercaptoethanol. When the membranes were extracted with a myosin-extraction buffer (high salt-containing MgATP), most of the band comigrating with purified muscle myosin was selectively extracted from these membranes (Fig. 5). Reacting such a gel with the anti-α-actinin antibody revealed a slight decrease in the amount of α-actinin, but most remained associated with the plasma membranes.

Prolonged extraction by dialysis for 36 h against either an actin depolymerization buffer (2 mM Tris, 0.2 mM ATP, 0.1% β-mercaptoethanol, pH 7.4) or a buffer that will extract α-actinin from muscle (2 mM Tris, 0.2 mM EDTA, 0.1% β-mercaptoethanol, pH 8.8) had a marked effect on the membranes. The latter buffer caused the membranes to vesiculate and give rise to small vesicles, with the release of most of the proteins detected by Coomassie blue staining (Fig. 6). The actin depolymerization buffer caused less disruption of the membranes but also resulted in extensive extraction of many of the proteins. Both buffers caused most of the actin to remain in the supernatant after the membranes were pelleted at 145,000g for 1 h. The α-actinin was also largely removed from the membranes by both buffers and was found in the supernatants by antibody staining of the gels of the resulting pellets and supernatants (Fig. 6). It should be noted that the myosin continued to sediment with the membranes or vesicles under these extraction conditions and was one of the few prominent bands remaining in the membrane pellets. This may be due to the low ionic strength conditions promoting myosin filament formation and aggregation, such that it would sediment with the membranes without necessarily being associated with them.

Since a proportion of the α-actinin dissociated from the initial plasma membrane preparations by suspension of these membranes in PBS, followed by centrifugation of the membranes over a sucrose gradient, we investigated these dissociation conditions further. When membranes taken from a gradient were incubated in PBS containing reducing agents or in a similar buffer (140 mM NaCl, 10 mM $NaPO_4$, pH 7.2, 0.2% β-mercaptoethanol) for 30 min, little actin was released, but relatively large amounts of α-actinin were extracted into the supernatant as judged by both Coomassie blue staining and antibody staining of a gel of the supernatant (Fig. 7). In most experiments repeated extractions with PBS or this buffer released successively more α-actinin, although the amount of actin released continued to be low. Typically, after three such extractions (two for 30 min and the third overnight) the membranes contained very little α-actinin. Most of the actin, however, was still associated with these membranes (Fig. 7).

DISCUSSION

α-Actinin has frequently been advanced as a protein that might be responsible for linking actin to membranes. Direct evidence for this has been missing. We have approached this problem by asking whether α-actinin is associated with the plasma membrane and by investigating the nature of the association we have found. Using specific antibody staining of SDS gels as an assay for α-actinin, we have demonstrated this protein in isolated plasma membranes prepared from three different cell types using two different preparative procedures. Only with red blood cell membranes did we not find evidence for the presence of α-actinin. It should be noted that in a previous report α-actinin was also tentatively identi-



6

fied in plasma membrane preparations isolated from sarcoma 180 ascites cells by comigration of a band in SDS gels with purified muscle α-actinin [14]. To quantitate the amount of α-actinin in association with the plasma membranes, we developed a radioimmunoassay, but this was not successfully applied to the plasma membranes because the nonmuscle α-actinin only partially cross-reacted with the muscle α-actinin standards against which the antibody had been raised.

To determine how the α-actinin is associated with the membranes, we have tried a series of extractions with different buffers. The principle of selective extraction is well illustrated for myosin (Fig. 5), which could be extracted from the membranes by brief incubation in a buffer that will dissociate actin–myosin interactions. The release of myosin from the membranes was not accompanied by an equivalent actin extraction, demonstrating that the association of myosin with these membranes was via actin and not the other way around, as was suggested some years ago [15]. Could the same approach be applied to actin and α-actinin to determine whether the α-actinin mediates actin attachment to the membranes or vice versa?

An initial indication that some α-actinin could be preferentially released from the membrane without an equivalent loss of actin came from experiments in which freshly prepared membranes were either washed with isotonic phosphate-buffered saline containing reducing agents or were resuspended in this phosphate buffered saline and sedimented in a sucrose density gradient. This was best illustrated by the analysis of fractions across a sucrose gradient. Here it was found that a significant portion of the α-actinin did not sediment with the plasma membranes but rather remained at the top of the gradient (Fig. 4B, C). The amount of actin in these fractions at the top of the gradient varied between experiments but frequently was lower than the amount of α-actinin, indicating that at least a fraction of the α-actinin will dissociate from these membranes without an equivalent loss of actin.

The HeLa plasma membranes were extracted with several different buffers. Two of these were of particular interest at the outset: one an actin depolymerizing buffer and the other a solution that will extract α-actinin from muscle. Both these extraction buffers removed many proteins from the membranes and effectively stripped the membranes of both actin and α-actinin, such that it was not possible with these to determine whether the actin was bound via the α-actinin, or vice versa. Since some of the α-actinin appeared to dissociate

---

Fig. 6. SDS gel analysis of plasma membranes after extraction with low ionic strength buffers. HeLa cell plasma membranes were isolated by the Brunette and Till procedure and were extracted by dialysis against either 2 mM Tris Cl, 0.2 mM ATP, 0.1% β-mercaptoethanol, pH 7.4 (sample 2), or 2 mM Tris, 0.2 mM EDTA, 0.1% β-mercaptoethanol, pH 8.8 (sample 3). After 36 h of dialysis the membranes were sedimented at 145,000g for 1 h, and the supernatants and pellets were compared by electrophoresis in SDS on a 10% polyacrylamide gel. A photograph of the stained gel of the pellets (membranes) is shown on the left, together with its corresponding autoradiograph after reacting the gel with the anti-α-actinin antibody, followed by the second iodinated antibody. On the right a photograph of the stained gel of the extract supernatants is shown together with their corresponding autoradiograph after reaction with the same antibodies. Sample 1 is the starting membrane preparations, samples 2 and 3 were the pellets and supernatants after dialysis against the buffers described above. Equal volumes were loaded in the gel slots, not equal protein concentrations. Each pellet was dissolved in 1 ml of gel sample buffer and 25 μl was loaded in the respective gel slots. Similarly, equal volumes of the supernatants were electrophoresed. The autoradiograph indicates very little α-actinin remaining in these membranes after extraction, and most can be seen in the gels of the supernatants. The autoradiograph of the supernatant gels reveals some minor bands reacting with the α-actinin antibody, which probably correspond to minor proteolytic degradation products, since during the dialysis no protease inhibitors were included.

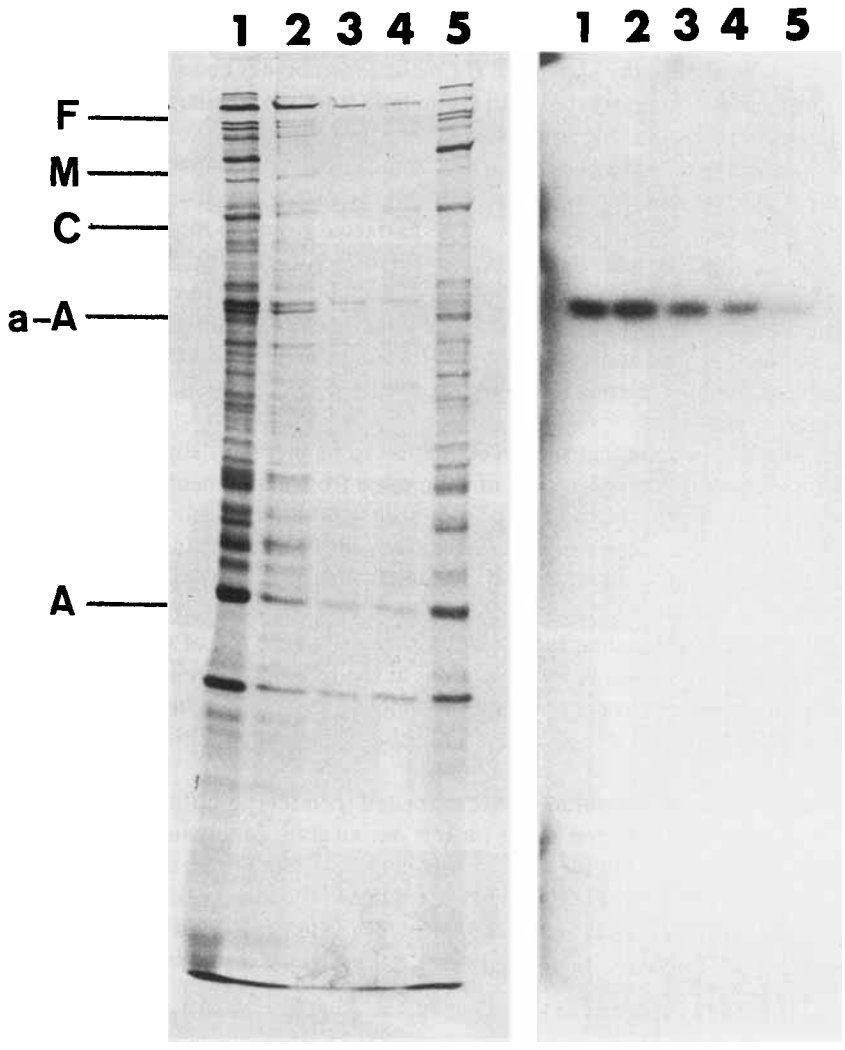


Fig. 7. SDS gel analysis of plasma membranes showing the progressive extraction of α-actinin. HeLa plasma membranes were taken from a sucrose gradient and resuspended in 0.14 M NaCl, 10 mM $NaPO_4$, pH 7.2, 0.2% β-mercaptoethanol. The starting plasma membranes were analyzed in gel track 1. After 30 min in this extraction buffer the membranes were centrifuged for 10 min at 10,000g and the supernatant was analyzed on the gel (track 2). The membrane pellet was resuspended in the same buffer and after 30 min was repelleted. The supernatant was analyzed in track 3 of the gel. The membrane pellet was again resuspended in the same buffer, incubated overnight, and then centrifuged as before. The final supernatant and pellets were analyzed on the gel (tracks 4 and 5, respectively). For each fraction an equivalent volume was loaded on this 10% gel. The left panel shows the gel stained with Coomassie blue, and the right panel shows an autoradiograph of the same gel after incubating first with α-actinin antibody and then with the ^{125}I-labeled second antibody. On the left side the positions of migration of standard proteins are shown: filamin (F), myosin (M), clathrin (C), α-actinin (α-A), and actin (A). Note the prominent α-actinin band in the starting membranes and its progressive extraction into the successive supernatants. Note, too, that only a trace of the protein is detected in the final membranes. Although some actin is extracted, however, it continues to be prominent in the final membrane pellet.

after resuspension in PBS followed by centrifugation in a sucrose gradient, we pursued this dissociation further. We found that if membranes taken from a sucrose gradient were incubated in PBS or a similar buffer (140 mM NaCl, 10 mM $NaPO_4$, pH 7.2, 0.2% β-mercaptoethanol) then there was usually some further selective release of the α-actinin without a parallel dissociation of the actin. The amount of α-actinin released varied in different experiments, but if such incubations were repeated several times usually very little α-actinin could be detected in the membrane preparations, although the amount of actin with the membranes remained substantial (Fig. 7). This result leads us to conclude that most of the α-actinin cannot be linking actin to the membrane but rather is present in the membrane preparations because it is bound to actin. It is possible that further extractions with this buffer would remove all the α-actinin while still leaving much of the actin associated with these membranes. At present, however, it cannot be ruled out that the trace of α-actinin remaining is functioning as a linker protein for actin attachment, but until this is demonstrated we favor an indirect role for α-actinin in the attachment of actin to membranes that is based on its ability to cross-link actin filaments. This property would tend to promote and stabilize actin attachment if an equilibrium exists between free actin filaments and those bound to attachment proteins at the membrane.

ACKNOWLEDGMENTS

The authors thank Dr. J. D. Watson for his support of this work. We thank Anne Bushnell for help with the electron microscopy and Ted Lukralle for photographic assistance. Madeline Szadkowski patiently typed several versions of this manuscript. This work was supported by a Cancer Center Grant from the National Cancer Institute to the Cold Spring Harbor Laboratory (CA 13106), by a grant from the NIH (GM26298-01), and by a grant from the Muscular Dystrophy Association.

REFERENCES

1. Lazarides E, Burridge K: Cell 6:289, 1975.
2. Schollmeyer JE, Furcht LT, Goll DE, Robson RM, Stromer MH: In Goldman R, Pollard T, Rosenbaum J (eds): "Cell Motility." New York: Cold Spring Harbor Laboratory, 1976, p 361.
3. Heaysman JEM, Pegrum SM: Exp Cell Res 78:71, 1973.
4. Geiger B, Singer SJ: Cell 16:213, 1979.
5. Laemmli UK: Nature 227:680, 1970.
6. Burridge K: Proc Natl Acad Sci USA 73:4457, 1976.
7. Burridge K: Meth Enzymol 50:54, 1978.
8. Porath J, Axen R, Emback S: Nature 215:1491, 1967.
9. Bolton AE, Hunter WM: Biochem J 133:529, 1973.
10. Brunette DM, Till JE: J Membr Biol 5:215, 1971.
11. Gruenstein E, Rich A, Weihing RR: J Cell Biol 64:223, 1975.
12. Thom D, Powell AJ, Lloyd CW, Rees DA: Biochem J 168:187, 1977.
13. Ishikawa H, Bischoff R, Holtzer H: J Cell Biol 43:312, 1969.
14. Moore PB, Ownby CL, Carraway KL: Exp Cell Res 115:331, 1978.
15. Willingham MC, Ostlund RE, Pastan I: Proc Natl Acad Sci USA 71:4144, 1974.