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Proteins Involved in the Attachment of Actin to the Plasma Membrane

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Proteins involved in the attachment of actin to the plasma membrane

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[Plates 1 and 2]

Proteins that may be involved in two types of actin–membrane association are discussed. The first set includes α-actinin, vinculin, fimbrin and a new cytoskeletal protein that are all concentrated in adhesion plaques, those regions of cultured fibroblasts where bundles of actin microfilaments terminate and where the plasma membrane comes close to the underlying substrate. The properties of non-muscle α-actinin suggest that it functions to cross-link actin filaments and thereby stabilize microfilament bundles rather than functioning in their attachment to the membrane. Fimbrin also appears to be involved in bundling of filaments rather than in attachment. In contrast, vinculin binds to the ends of actin filaments *in vitro* and is probably the best candidate for a role in the attachment of actin to membranes at the adhesion plaque. The discovery of a new protein, 215k, of unknown function, in the adhesion plaque suggests that many more proteins remain to be identified in this region.

Attachment of actin filaments to other regions of the plasma membrane is also considered and a protein is described that seems to be a spectrin homologue in brain and other tissues. The brain protein resembles erythrocyte spectrin in its physical properties, in binding actin, in being associated with cell membranes and in cross-reacting immunologically. We suggest that the brain protein and erythrocyte spectrin both belong to a family of related proteins (the spectrins) which function in the attachment of actin to membranes in many different cell types.

Introduction

An unresolved problem in cell motility concerns the question of how the force-generating proteins such as actin and myosin are associated with the plasma membrane. Although there are many examples in which actin filaments have been demonstrated to be associated with membranes, only in the red blood cell membrane and the microvillar membrane of intestinal epithelial cells is anything known about the molecules involved in actin–membrane attachment. These two systems, however, are often considered too specialized to be generally applicable to cells such as macrophages or fibroblasts. Supporting this view, evidence has been presented that the major actin-attachment protein in red blood cells, spectrin, is absent from other cell types (Hiller & Weber 1977). In cells such as fibroblasts it seems probable that actin may associate with the plasma membrane through a variety of different mechanisms and molecules, reflecting the different types of movement these cells display and different types of association that have been viewed by electron microscopy. For example, where microfilament bundles terminate at adhesion plaques, the attachment of actin to the plasma membrane may involve 'end-on' attachment, whereas much of the association of actin with the plasma membrane throughout the rest of the cell cortex may be through lateral associations to the membrane.

In this paper we shall consider proteins that may be involved in both types of actin–plasma-

membrane attachment. We shall begin by reviewing the properties of several proteins that have been identified in the adhesion plaques of cultured fibroblasts and that may have some role in the attachment of actin filaments to the membrane in this region. We shall also describe a new protein that we have recently found to be localized in adhesion plaques as well as in membrane ruffles. Secondly we shall describe the occurrence in many non-erythroid cells of a spectrin homologue, a protein that appears to function in the attachment of actin to the plasma membrane of many cell types.

α-Actinin

The first protein to be identified in the adhesion plaque was α-actinin. Immunofluorescence microscopy showed that this protein is localized periodically along stress fibres and concentrated at the ends of these structures where they terminate close to the plasma membrane (Lazarides & Burridge 1975). The localization of α-actinin in the adhesion plaque region led to the general idea that it might be involved in the attachment of actin to membranes in a manner analogous to its suggested role in the attachment of actin to the Z-line of striated muscle. This idea has not been supported by further evidence: in a study with isolated plasma membranes that were rich in actin, the α-actinin could be selectively extracted, leaving most of the actin still associated with the membranes (Burridge & McCullough 1980). The function of α-actinin in non-muscle cells has remained elusive and the protein has been neglected compared with many of the other cytoskeletal elements. This has been surprising in view of the large number of actin-binding proteins that have been purified from non-muscle cell types, many of which have molecular masses on sodium dodecyl sulphate (SDS) gels similar to that of α-actinin (*ca.* 100 kDa). Many of these proteins have been related to the formation or dissolution of actin gels, some being potent F-actin cross-linking proteins that are inhibited by calcium (Mimura & Asano 1978, 1979), whereas others have been shown to fragment actin filaments into short oligomers in the presence of calcium (Yin & Stossel 1979, 1980; Bretscher & Weber 1979, 1980*a*). It seemed important to determine whether any of these proteins corresponded to non-muscle α-actinin and so we have purified α-actinin from a non-muscle source and compared its properties with these other actin-binding proteins that have recently been described by others.

α-Actinin was purified from HeLa cells by using the purification scheme developed for purifying α-actinin from smooth muscle (Feramisco & Burridge 1980). Two forms with slightly different molecular masses on SDS–polyacrylamide gels could be separated by chromatography on hydroxyapatite (Burridge & Feramisco 1981, 1982). The two proteins appear very similar by all criteria and resemble muscle α-actinins in most of their properties, being dimeric, rod-shaped proteins with Stokes radii and *S* values close to those for muscle α-actinins. They cross-react immunologically and have similar, but not identical, peptide maps. Both proteins bind actin; this binding is diminished by tropomyosin at 37 °C, as is characteristic for muscle α-actinins. Based on these criteria we have concluded that these proteins are α-actinin. One major difference, however, is that these HeLa α-actinins are inhibited from cross-linking actin by *ca.* 1 μM calcium (Burridge & Feramisco 1981).

Examining the various actin-binding proteins in this molecular mass range that have been described by others, we have concluded that these proteins fall into two distinct classes (table 1) (Burridge & Feramisco 1981). One class corresponds to the non-muscle α-actinins. These proteins resemble closely the HeLa α-actinins in being proteins that will cross-link actin filaments in a manner inhibited by low free calcium concentrations. They are rod-shaped dimeric

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

proteins. The second class of proteins, including gelsolin (Yin & Stossel 1979, 1980) and villin (Bretscher & Weber 1979), are globular, monomeric proteins that will fragment actin filaments into short oligomers in the presence of calcium. Gelsolin appears to be widespread in vertebrate cell types (Yin *et al.* 1981), whereas villin appears to be confined to the brush border and may have derived from gelsolin, becoming specialized for its function in the microvillus.

TABLE 1. CLASSIFICATION OF CALCIUM-SENSITIVE ACTIN-BINDING PROTEINS

1. non-muscle α-actinin class
   - HeLa α-actinins (Burridge & Feramisco 1981)
   - actinogelin (Mimura & Asano 1978, 1979)
   - *Dictyostelium* 95 kDa protein (Hellewell & Taylor 1979; Condeelis 1981)
   - *Acanthamoeba* 85 kDa protein (Pollard 1981)
2. gelsolin–villin class
   - gelsolin (Yin & Stossel 1979, 1980)
   - villin (Bretscher & Weber 1979, 1980*a*)
   - platelet 90 kDa protein (Wang & Bryan 1981)

What is the significance of the calcium inhibition of non-muscle α-actinins in their interaction with actin, and how might this relate to the concentration of α-actinin in the adhesion plaque region? Whereas in muscle there is a requirement for a stable actin lattice, in moving non-muscle cells the contractile elements must be capable of changing their relative configurations and of assembly and disassembly. An α-actinin that will cross-link actin filaments to form a stable gel in the absence of calcium, but which in response to a calcium flux will release the actin filaments, may be a distinct advantage during movement. It would seem likely that the response of α-actinin and gelsolin to calcium would be coordinated, possibly with α-actinin responding at lower calcium concentrations to release intact actin filaments and with gelsolin fragmenting the filaments into short oligomers at higher calcium concentrations. Such a scheme would permit a graded disassembly of a cross-linked actin matrix in response to a calcium flux. A direct comparison of the relative sensitivities of the two proteins to calcium will be needed to test this possibility.

The properties of the non-muscle α-actinins suggest that they are involved in forming stable actin gels in the absence of calcium. This may explain the concentration of α-actinin in the adhesion plaque, where it would function not in attaching but in stabilizing the bundle of actin filaments at their point of origin where tension is transmitted to the membrane. Adhesion plaques, however, are not permanent structures and the sensitivity of the α-actinin to calcium may have a role in the disassembly of these structures as cells move.

VINCULIN

A second protein to be localized at the adhesion plaque region of cultured fibroblasts is vinculin (Geiger 1979; Burridge & Feramisco 1980). Immunoelectron microscopy has also revealed vinculin at regions of concentrated actin–membrane attachment in other cell types (Geiger *et al.* 1980) and the vinculin has been shown to be closer to the plasma membrane than the α-actinin. This localization has led to the suggestion that vinculin may be a protein involved in actin membrane attachment. Evidence against vinculin's being involved as a general membrane attachment protein has come from studies of isolated plasma membranes that were rich

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

in actin but in which vinculin was in very low concentration or absent (Burridge & Feramisco 1982). This does not, however, rule out a possible function for vinculin in the attachment of whole bundles of filaments such as are found in cultured fibroblasts but which were absent from the isolated plasma membranes we investigated.

Vinculin has been shown to interact with actin by several groups but its mode of interaction is complex and the interpretations differ. Jockusch & Isenberg (1981) have shown that high

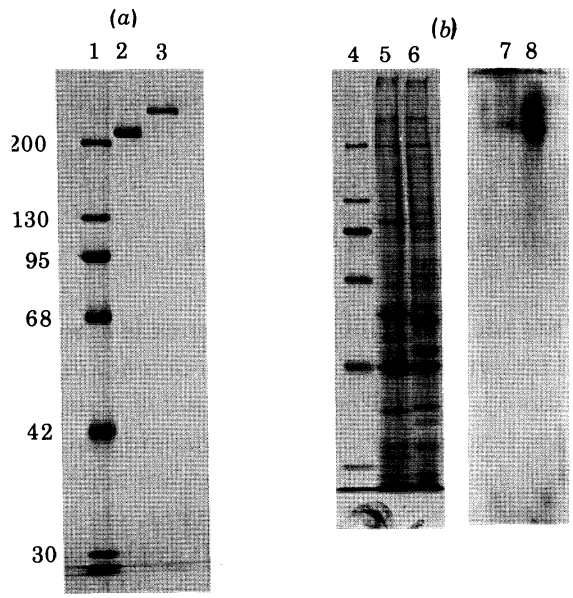

FIGURE 1. SDS–polyacrylamide gel analysis of the 215k protein. (*a*) A 10% polyacrylamide gel analysis of standard protein molecular mass markers (lane 1, myosin, β-galactocidase phosphorylase, bovine serum albumin, ovalbumin and carbonic anhydrase), purified 215k (lane 2), and purified chicken gizzard filamin (lane 3). Numbers on the left are molecular masses in kilodaltons. (*b*) A 10% polyacrylamide gel of molecular mass standards (lane 4), whole gerbil fibroma cells (lane 5) and whole chick-embryo fibroblasts (lane 6), and a corresponding immunoautoradiograph of a parallel gel slice (lanes 7 and 8) which had been reacted first with rabbit antiserum against 215k followed by ^{125}I-labelled goat anti-rabbit IgG. Note the band at about 215 kDa that reacts with the antibody in both cell types, and that in chick embryo fibroblasts a higher molecular mass band is also seen. Gels and immunoautoradiography have been described previously (Burridge 1976).

concentrations of vinculin will induce the bundling of actin filaments into almost paracrystalline arrays. Because vinculin is found in cells where bundles of actin filaments terminate, this interaction of vinculin with actin *in vitro* could be important in the formation of bundles in the cell. On the other hand, the work of Wilkins & Lin (1982) and our own work (Burridge & Feramisco 1982) indicates that vinculin has a high-affinity binding site on actin filaments to which it will bind with a very low vinculin:actin monomer ratio. This is consistent with vinculin's binding to the end of the actin filaments. If further work confirms this interaction of vinculin with the ends of actin filaments, then this would also support the possibility of vinculin's functioning as an 'end-on' attachment protein for actin filaments at the adhesion plaque. Clearly it will be important to identify the other proteins with which vinculin interacts.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

A new protein of the adhesion plaque

Many of the cytoskeletal proteins in non-muscle cells are closely related to those found in smooth muscle, but in smooth muscle they are found in much greater quantities, which makes them more amenable for purification and analysis. In the expectation that many cytoskeletal proteins remain to be identified, we have pursued the purification and characterization of various unidentified proteins from smooth muscle. We have recently purified a protein from smooth muscle which has a molecular mass on SDS–polyacrylamide gels of 215 kDa (which we shall refer to as 215k). The purified protein is shown in figure 1. The purification will be described elsewhere (Connell & Burridge, in prep.). Antibodies have been raised against native 215k and used to locate the protein in various cultured fibroblasts. Some of the results are illustrated in figure 2, plate 1. The most prominent distribution of this protein is in the adhesion plaques, a distribution similar to that for vinculin. To confirm that the antibodies are not against vinculin, various immunological controls have been performed. The antibody against 215k does not cross-react with vinculin by Ouchterlony double diffusion or by immunoprecipitation but does label specifically a band at 215 kDa when used to stain gels of whole-cell extracts (figure 1). In chick-embryo fibroblasts a higher molecular mass polypeptide is also labelled by this antibody. The significance of this is uncertain at the moment. Besides a localization in the adhesion plaque regions of these cells, the protein is also localized in the ruffling membranes and leading lamellae of cultured fibroblasts (figure 2). These distributions are not seen with antibodies against vinculin.

Preliminary evidence indicates that 215k interacts with actin, causing an increase in apparent viscosity of the actin–215k mixture with increasing concentrations of 215k. Compared with various other actin-binding proteins (e.g. α-actinin, filamin) it is a very modest 'gelation' factor and this leads us to query the significance of its interaction with actin, given that so many proteins do interact with actin.

Much of the interest in vinculin has been generated by its location in the adhesion plaque, a location that makes it a candidate for a role in actin membrane attachment. Finding another protein with a similar distribution emphasizes the complexity of the adhesion plaque structure and the fact that many cytoskeletal proteins may function in the organization of the microfilament bundles in such regions. The interactions of these various proteins with each other and with actin remain to be determined. The distribution of 215k is more extensive than vinculin, being found also in the leading lamellae and in ruffling membranes – regions of active motility. This distribution is more reminiscent of fimbrin (Bretscher & Weber 1980*b*), a protein involved in the bundling of actin filaments in microvilli, but which is also found in ruffling membranes and in adhesion plaques. It suggests that the function of 215k in the cytoskeleton is not confined to the adhesion plaque region but that it may also function in the organization or attachment of actin filaments to membranes elsewhere in the cell.

A spectrin homologue in non-erythroid cells

Investigating the association of α-actinin and actin with isolated HeLa cell plasma membranes several years ago we noted a high molecular doublet on SDS–polyacrylamide gels that could be eluted from these membranes under low ionic strength conditions, which also eluted actin. The HeLa plasma membranes were inadequate as a source for purifying this protein,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

although we were able to demonstrate that it bound actin and was distinct from myosin and filamin. Subsequently we noticed that brain was very rich in this or a similar doublet of polypeptides and we developed a purification for this protein from brain. During the course of this work the same protein was purified from brain by Levine & Willard (1981), who named it fodrin.

The purification and characterization of this protein will be described in detail elsewhere (Burridge, Kelly & Mangeat, submitted). In brief, the protein is eluted from a crude membrane fraction from brain homogenates, concentrated by fractionation with ammonium sulphate and chromatographed successively on DEAE-cellulose, Sepharose C1-2B and phosphocellulose. The purified protein is illustrated in figure 3 after electrophoresis on 10% polyacrylamide gels. The protein consists of a high molecular mass doublet of polypeptides with molecular masses of about 240 and 235 kDa, respectively. The heavier polypeptide migrates close to the α-subunit of human erythrocyte spectrin (*ca.* 240 kDa). Two minor bands can be detected in these preparations with molecular masses between 150 and 160 kDa. These probably represent proteolytic breakdown products because they reveal immunological cross-reaction with the high molecular mass doublet but are not detected immunologically in SDS–polyacrylamide gels of whole cells (figure 3). Like spectrin, the brain protein will bind to F-actin and cross-link F-actin to cause a marked increase in actin viscosity (Burridge, Kelly & Mangeat, submitted). This brain protein and spectrin are not identical and SDS–polyacrylamide gels of partial proteolytic digestions show rather few peptides in common (data not shown).

This high molecular mass doublet from brain was used to immunize rabbits and the resulting antibody has been used to study the antigenic relation of this protein to other high molecular mass proteins and its distribution in various different cells. Antibodies against this brain protein show cross-reaction with spectrin from red blood cells (figure 3) but not with filamin, another high molecular mass actin-binding protein. The cross-reaction with spectrin is not strong and initially was not detected by us until autoradiographs were exposed such that we judged the panel corresponding to the brain protein to be overexposed. A doublet of similar high molecular mass has been detected immunologically in gels of whole fibroblasts (chick embryo, gerbil fibroma) and of crude preparations of HeLa cell plasma membranes (figure 3).

The same antibody has been used to examine the distribution of this protein in cultured fibroblasts (figure 4, plate 2). Unlike most other actin-binding proteins this brain protein is not localized along the length of the microfilament bundles or at their ends but rather has a distribution over the whole cell. There is frequently a concentration around the nucleus but not over it, and the protein appears concentrated in ruffling membranes. It should be noted, however, that it is usually not more prominent in the adhesion plaque regions than in the rest of the cell. With antibodies against this same protein, Levine & Willard (1981) have shown that in cross sections of tissues the protein is localized at or very close to the plasma membrane. With thin sections through various tissues we have confirmed this finding.

Discussion

This paper has been concerned with proteins that may link actin to the plasma membrane in two different locations. The first part of the paper focuses upon the adhesion plaque, describing proteins from cells such as fibroblasts that are localized there; the second part concerns a protein found in many cells that resembles spectrin, the protein responsible for actin attachment to the erythrocyte membrane.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

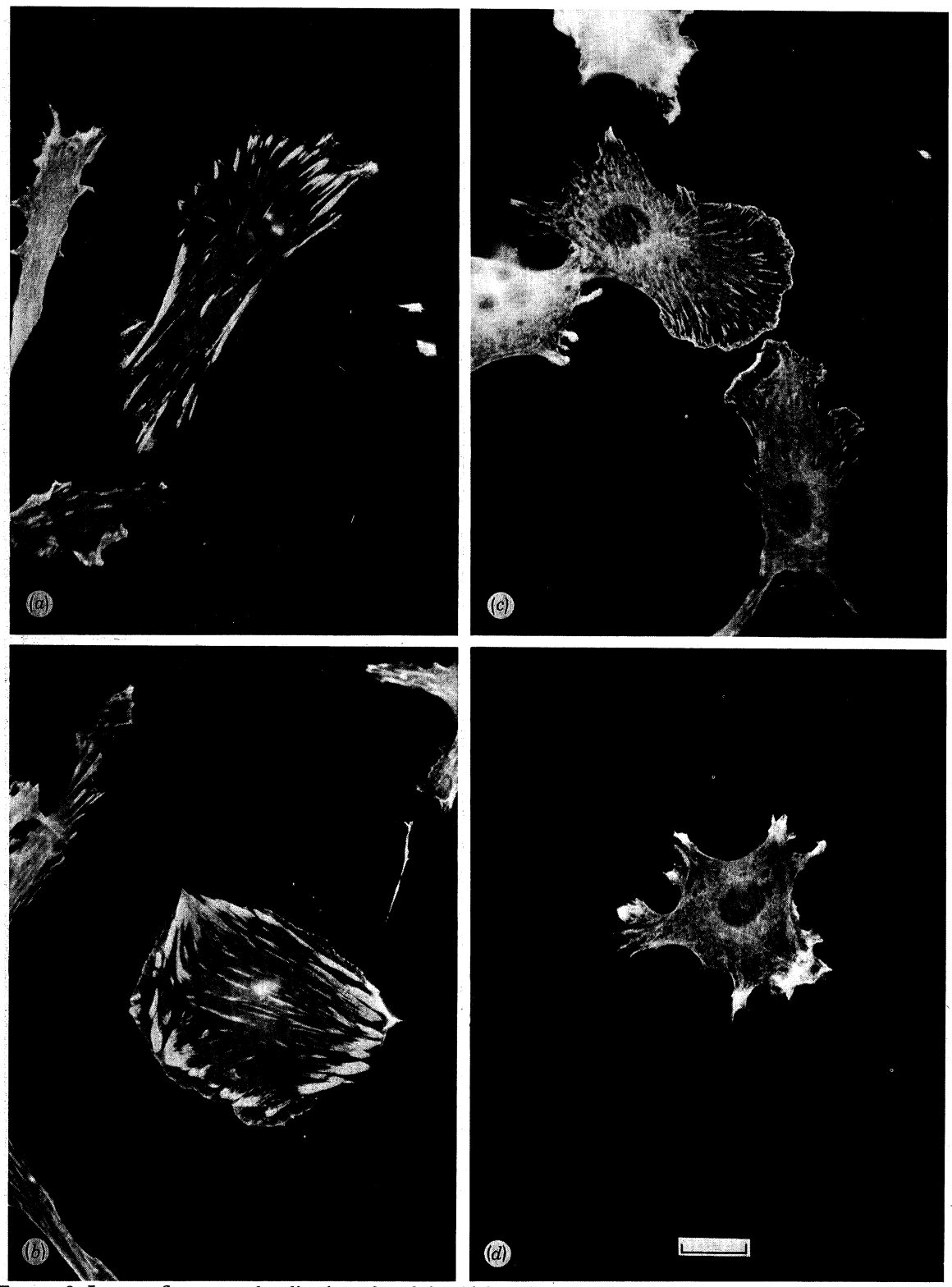

FIGURE 2. Immunofluorescent localization of 215k in chick embryo fibroblasts. (*a–c*) Well spread chick-embryo fibroblasts. Note the prominent focal distribution of this antigen. In (*c*) the leading lamellae of the cells are also labelled. (*d*) A respreading fibroblast in which the 215k antigen is prominent in the ruffling membranes. Bar = 16 μm.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

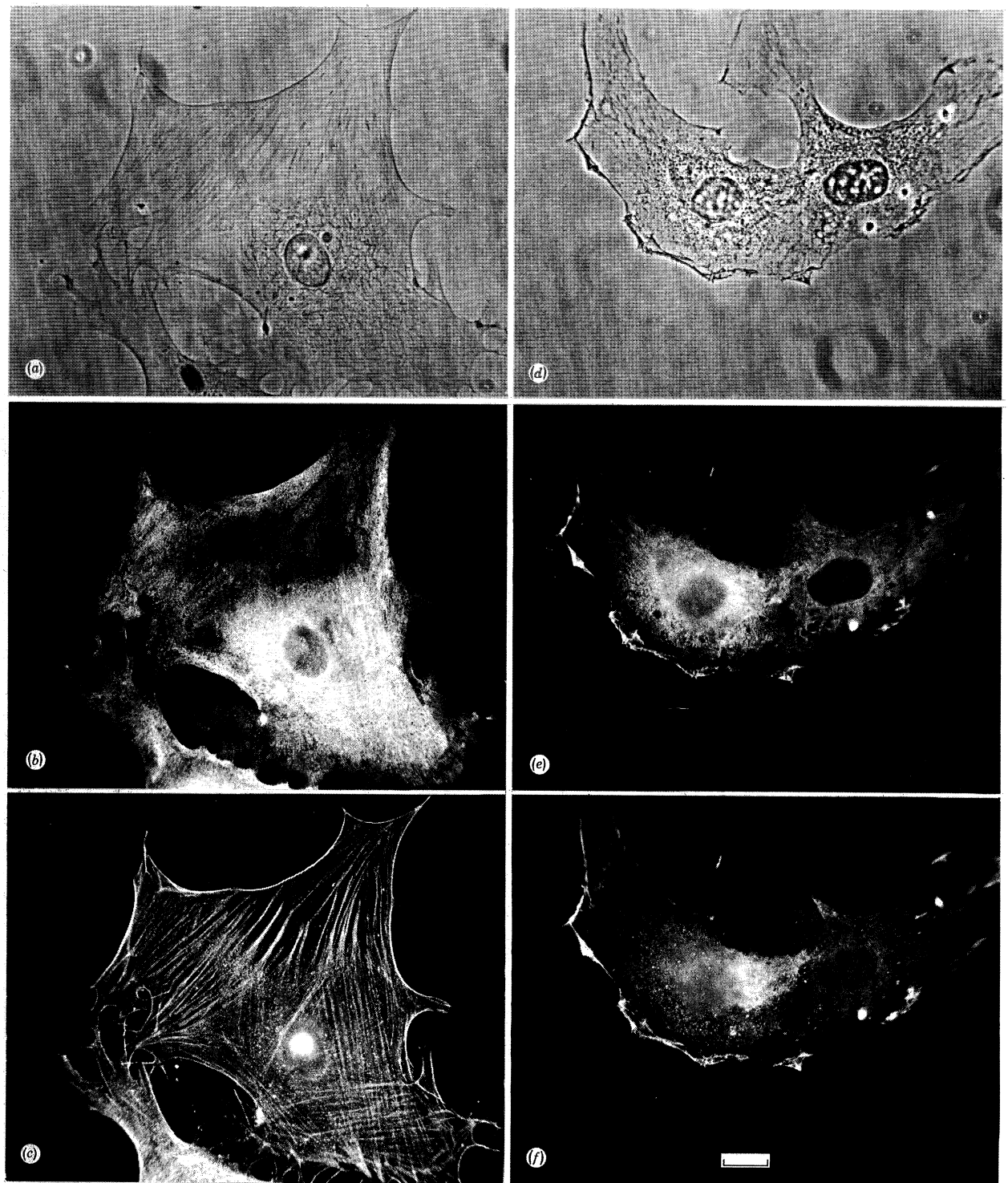

FIGURE 4. Immunofluorescent localization of the brain spectrin antigen in cultured fibroblasts. (*a–c*) The same gerbil fibroma cell (CCL 146); (*d–f*) the same respreading 10T½ mouse fibroblasts. (*a, d*) Phase micrographs; (*b, e*) the distribution of the rabbit anti-brain spectrin as revealed by fluorescein-labelled goat anti-rabbit IgG; (*c, f*) the distribution of actin in the same cells as revealed by mouse monoclonal antibodies against actin, with the use of goat anti-mouse immunoglobulin labelled with rhodamine. The monoclonal anti-actin was the generous gift of Dr J. Lin. Note that in the well spread gerbil fibroma cell the brain spectrin antigen has a rather uniform mottled distribution over the whole cell but is concentrated around the nucleus. There is no co-localization with the actin microfilament bundles; indeed where some of the microfilament bundles terminate there is an apparent reduction in the staining of the brain spectrin antigen. In the respreading 10T½ cells there are no microfilament bundles. The most prominent distribution of both the actin and the brain spectrin antigen is in the ruffling membranes. Bar = 16 μm.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Adhesion plaques are regions where bundles of actin microfilaments terminate in cells such as cultured fibroblasts and where the plasma membrane comes close to the underlying substrate. As a region in which there is a concentration of actin filaments terminating at or close to the plasma membrane, the adhesion plaque provides a model for examining actin–membrane interaction. Various actin-binding proteins have been found to be localized in this region, including α-actinin, fimbrin, vinculin and a new protein described here briefly, which we have

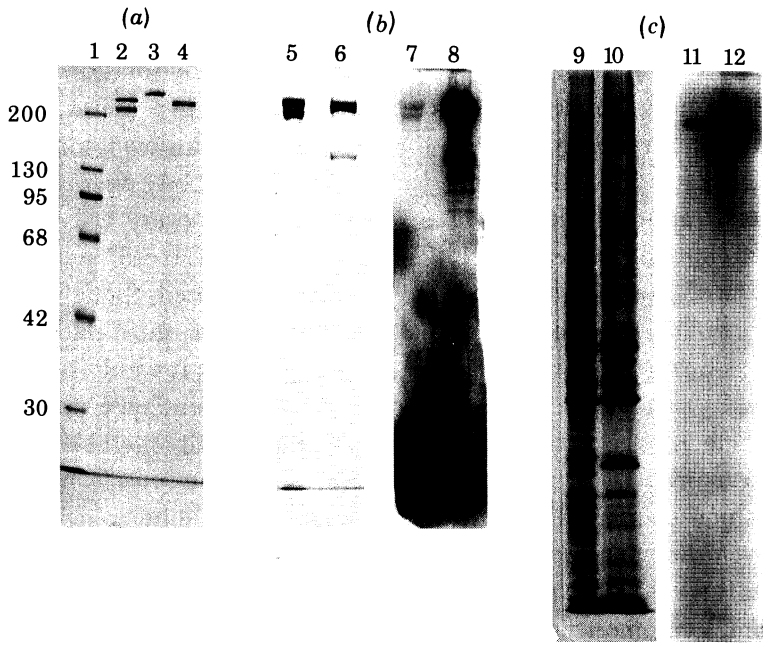

FIGURE 3. SDS–polyacrylamide gel analysis of brain spectrin. (*a*) A 10% polyacrylamide SDS gel of pure proteins: lane 1, molecular mass markers (values in kilodaltons); lane 2, erythrocyte spectrin; lane 3, filamin; lane 4, brain spectrin. (*b*) A 7.5% polyacrylamide gel and corresponding immunoautoradiograph after reaction of a parallel gel slice first with rabbit antiserum against brain spectrin and secondly with ^{125}I-labelled goat anti-rabbit IgG. Lane 5 contains erythrocyte spectrin, lane 6 brain spectrin, and lanes 7 and 8 are the corresponding immunoautoradiographs. (*c*) A 10% polyacrylamide gel of whole chick-embryo fibroblasts (lane 9) and crude HeLa cell plasma membranes (lane 10) and the corresponding immunoautoradiograph after reaction with the antiserum against brain spectrin (lanes 11 and 12).

referred to as 215k. Although initially suggested as a possible attachment protein, the properties of non-muscle α-actinin indicate that it functions in the cell to cross-link actin filaments to form stable actin filament assemblies. The sensitivity of non-muscle α-actinin to calcium suggests a way in which these actin filament networks or bundles may be disassembled. Fimbrin, a protein first isolated from brush-border microvilli, is another protein that appears to be involved in bundling rather than in attachment (Bretscher & Weber 1980). In contrast to α-actinin and fimbrin, vinculin has been shown to have an affinity for the ends of actin filaments (Wilkins & Lin 1982; Burridge & Feramisco 1982) and as such appears to be the best candidate of the known proteins in the adhesion plaque to be involved in actin anchorage in this location. Our recent discovery of a new protein, 215k, in the adhesion plaque emphasizes the complexity of this structure as well as the fact that probably many other proteins remain to be identified in this region of the cell. The potential role of 215k in the attachment of actin filaments and in the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

organization of the microfilament bundle remains to be determined. It will be important to determine which of these proteins interact with each other and which also interact with membrane components in the adhesion plaque.

The second part of this paper concerns a high molecular mass actin-binding protein that appears to be involved in the attachment of actin to the plasma membrane throughout much of the cell cortex. This same protein was described recently by Levine & Willard (1981), who named it fodrin; it has many similarities to spectrin from erythrocytes. It probably corresponds also to the protein recently detected immunologically in non-erythroid cells with antibodies against spectrin (Goodman *et al.* 1981). The purified brain protein and spectrin both consist of high molecular mass doublets on SDS–polyacrylamide gels. In solution both proteins are extremely large, asymmetric molecules. Both are actin-binding proteins that are found in association with cell membranes from which they can be eluted at low ionic strength. Spectrin and the brain protein cross-react immunologically and the antibody against the brain protein demonstrates a cross-reacting high molecular mass doublet in every cell type that we have examined (chick embryo fibroblasts, gerbil fibroma cells, HeLa cells and smooth muscle). Based on these similarities we suggest that these proteins are related, though clearly not identical, having slightly different molecular masses and differing in their native partial peptide maps. If the proteins belong to the same family of polypeptides it would seem appropriate to refer to them all by the same name (spectrin) prefaced by the tissue type rather than coining a new name for each one isolated. The myosin family of polypeptides might serve as an example, where initially new names were coined for the equivalent non-muscle proteins, but where subsequent work demonstrated sufficient structural and functional homologies to justify calling them all myosin, even though some do not show immunological cross-reaction. Similarly with this class of proteins it would seem probable that they are related structurally and functionally, having evolved from a common precursor, with erythrocyte spectrin possibly being the most specialized form.

The similarity of the non-erythroid spectrin to spectrin from red blood cells argues for a parallel function in actin attachment to membranes in non-erythroid cells. The close association of the non-erythroid spectrins with membranes and the fact that they bind actin supports this idea strongly. It will be important in future work to determine how much of the actin in non-erythroid cells is linked to the membrane through spectrin and how much through other proteins. It will also be important to determine whether spectrin is linked to these non-erythroid cell membranes through ankyrin as it is in the red blood cell. Here it should be noted that ankyrin has been detected immunologically in other cell types (Bennett 1979), suggesting that many of the lessons learnt from red blood cells regarding the pathway of actin membrane attachment may be applicable to other cells too (reviewed by Branton *et al.* 1981). Finally, it will be important to determine the factors that regulate the attachment of actin to spectrin in non-erythroid cells and the role that this mode of actin–membrane attachment plays in the movement of cellular membranes generally.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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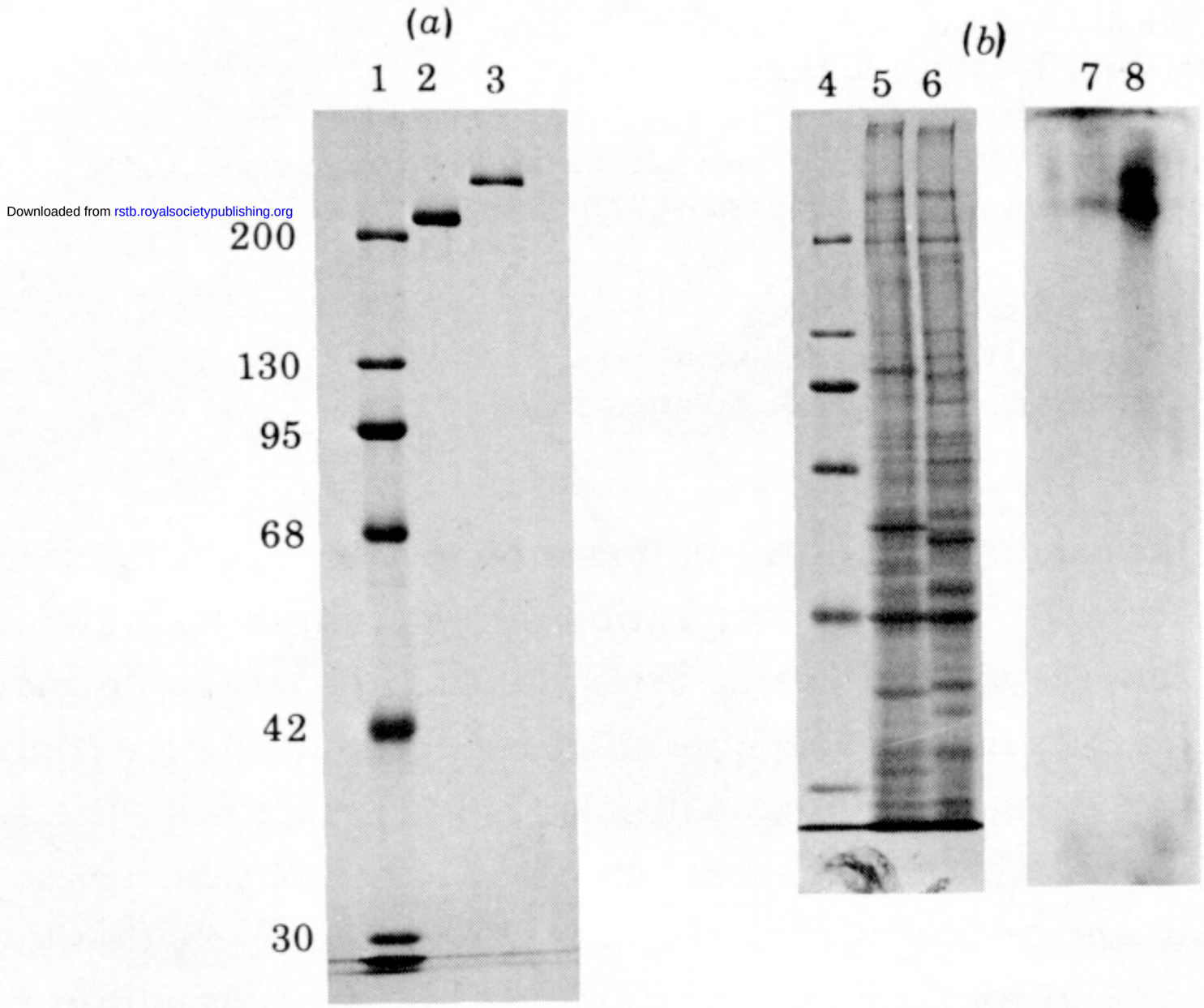

FIGURE 1. SDS–polyacrylamide gel analysis of the 215k protein. (*a*) A 10% polyacrylamide gel analysis of standard protein molecular mass markers (lane 1, myosin, β-galactocidase phosphorylase, bovine serum albumin, ovalbumin and carbonic anhydrase), purified 215k (lane 2), and purified chicken gizzard filamin (lane 3). Numbers on the left are molecular masses in kilodaltons. (*b*) A 10% polyacrylamide gel of molecular mass standards (lane 4), whole gerbil fibroma cells (lane 5) and whole chick-embryo fibroblasts (lane 6), and a corresponding immunoautoradiograph of a parallel gel slice (lanes 7 and 8) which had been reacted first with rabbit antiserum against 215k followed by ^{125}I-labelled goat anti-rabbit IgG. Note the band at about 215 kDa that reacts with the antibody in both cell types, and that in chick embryo fibroblasts a higher molecular mass band is also seen. Gels and immunoautoradiography have been described previously (Burridge 1976).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 2. Immunofluorescent localization of 215k in chick embryo fibroblasts. (*a–c*) Well spread chick-embryo fibroblasts. Note the prominent focal distribution of this antigen. In (*c*) the leading lamellae of the cells are also labelled. (*d*) A respreading fibroblast in which the 215k antigen is prominent in the ruffling membranes. Bar = 16 μm.

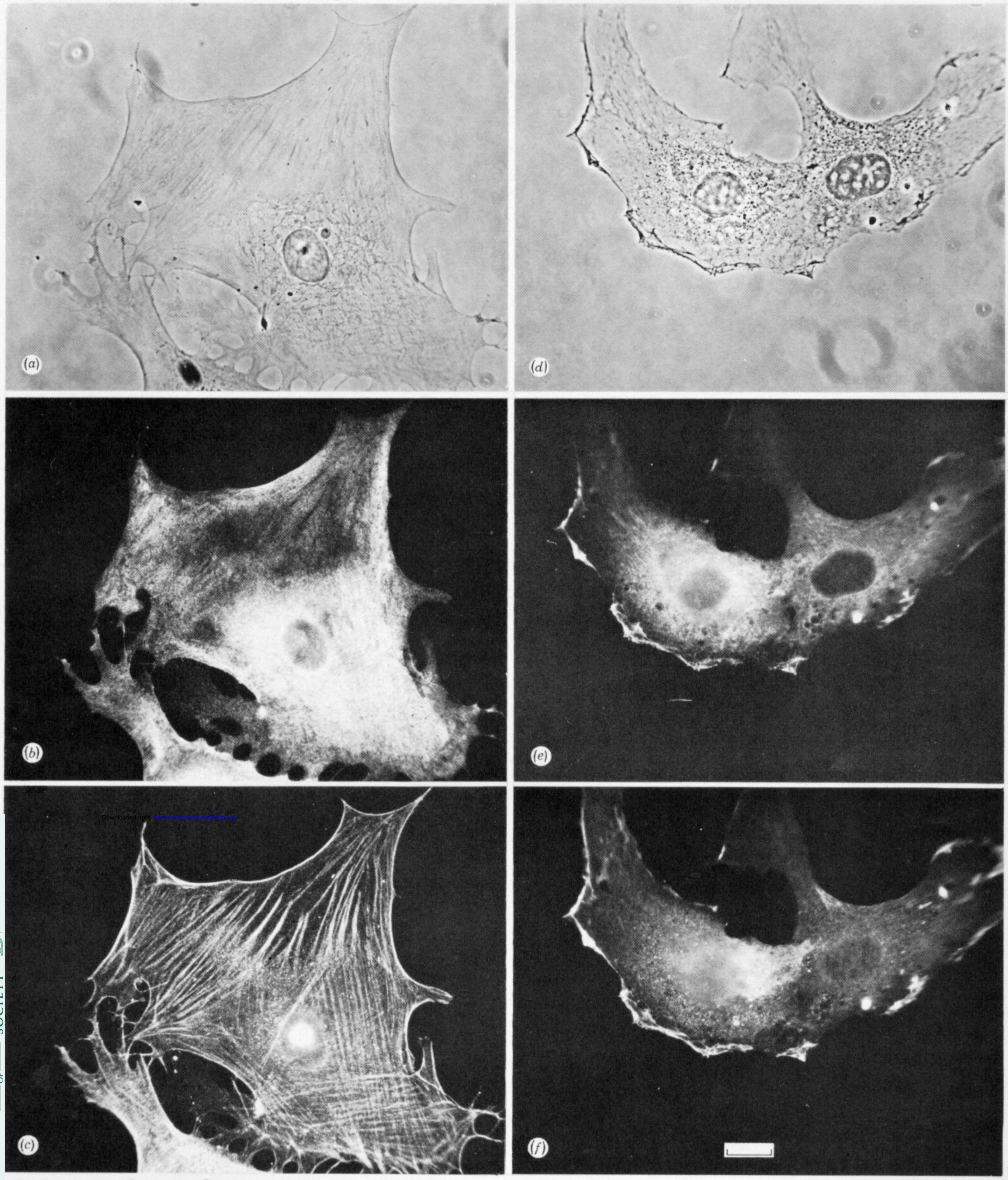

FIGURE 4. Immunofluorescent localization of the brain spectrin antigen in cultured fibroblasts. (*a–c*) The same gerbil fibroma cell (CCL 146); (*d–f*) the same respreading 10T½ mouse fibroblasts. (*a*, *d*) Phase micrographs; (*b*, *e*) the distribution of the rabbit anti-brain spectrin as revealed by fluorescein-labelled goat anti-rabbit IgG; (*c*, *f*) the distribution of actin in the same cells as revealed by mouse monoclonal antibodies against actin, with the use of goat anti-mouse immunoglobulin labelled with rhodamine. The monoclonal anti-actin was the generous gift of Dr J. Lin. Note that in the well spread gerbil fibroma cell the brain spectrin antigen has a rather uniform mottled distribution over the whole cell but is concentrated around the nucleus. There is no co-localization with the actin microfilament bundles; indeed where some of the microfilament bundles terminate there is an apparent reduction in the staining of the brain spectrin antigen. In the respreading 10T½ cells there are no microfilament bundles. The most prominent distribution of both the actin and the brain spectrin antigen is in the ruffling membranes. Bar = 16 μm.

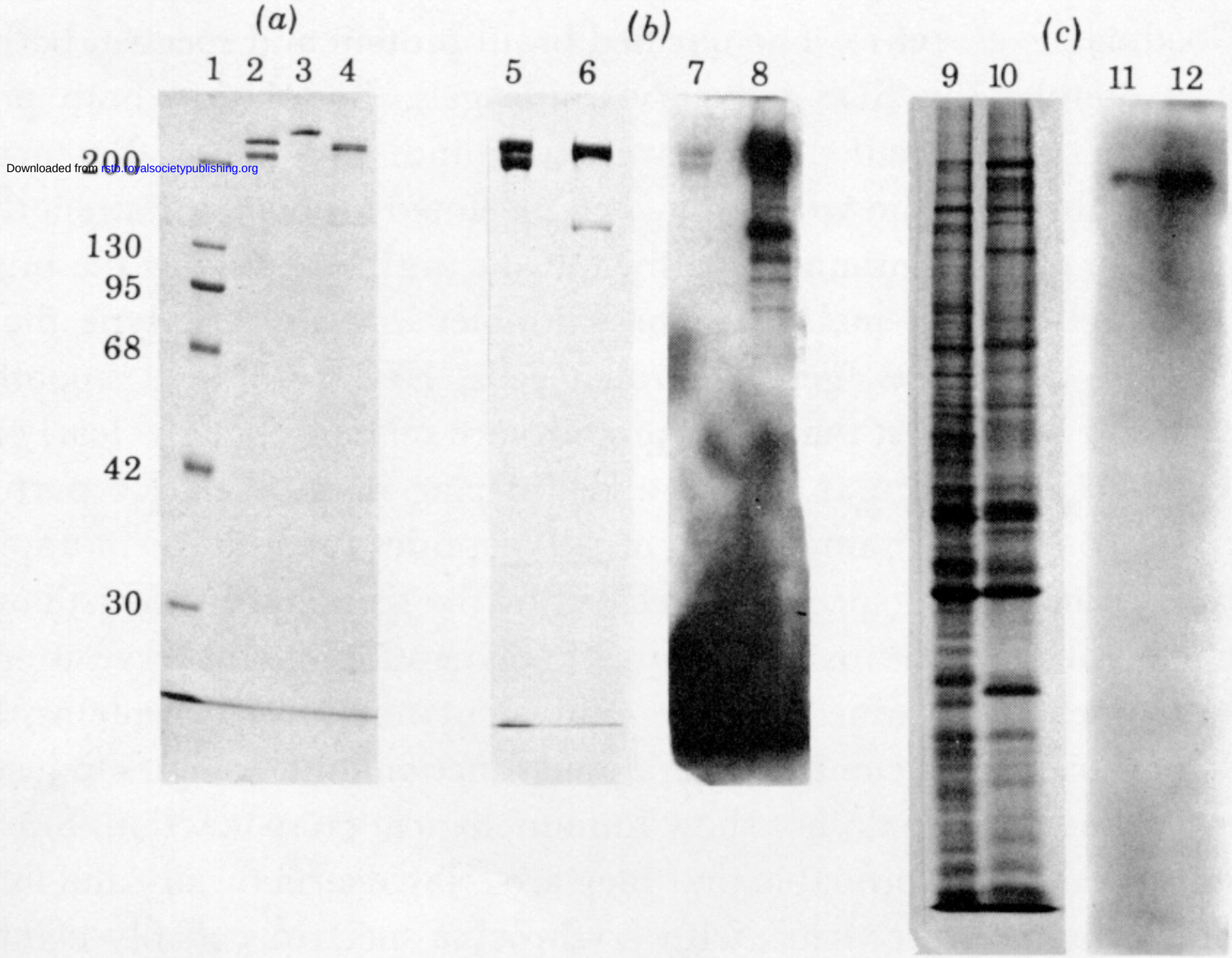

FIGURE 3. SDS–polyacrylamide gel analysis of brain spectrin. (*a*) A 10 % polyacrylamide SDS gel of pure proteins: lane 1, molecular mass markers (values in kilodaltons); lane 2, erythrocyte spectrin; lane 3, filamin; lane 4, brain spectrin. (*b*) A 7.5 % polyacrylamide gel and corresponding immunoautoradiograph after reaction of a parallel gel slice first with rabbit antiserum against brain spectrin and secondly with ^{125}I-labelled goat anti-rabbit IgG. Lane 5 contains erythrocyte spectrin, lane 6 brain spectrin, and lanes 7 and 8 are the corresponding immunoautoradiographs. (*c*) A 10 % polyacrylamide gel of whole chick-embryo fibroblasts (lane 9) and crude HeLa cell plasma membranes (lane 10) and the corresponding immunoautoradiograph after reaction with the antiserum against brain spectrin (lanes 11 and 12).