Actin-Membrane Interaction in Fibroblasts:

What Proteins Are Involved in This Association?

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ABSTRACT   In this review we discuss some of the proteins for which a role in linking actin to the fibroblast plasma membrane has been suggested. We focus on the family of proteins related to erythrocyte spectrin, proteins that have generally been viewed as having an organization and a function in actin-membrane attachment similar to those of erythrocyte spectrin. Experiments in which we precipitated the nonerythrocyte spectrin within living fibroblasts have led us to question this supposed similarity of organization and function of the nonerythrocyte and erythrocyte spectrins. Intracellular precipitation of fibroblast spectrin does not affect the integrity of the major actin-containing structures, the stress fiber microfilament bundles. Unexpectedly, however, we found that the precipitation of spectrin results in a condensation and altered distribution of the vimentin class of intermediate filaments in most cells examined. Although fibroblast spectrin may have a role in the attachment of some of the cortical, submembranous actin, it is surprising how little the intracellular immunoprecipitation of the spectrin affects the cells. Several proteins have been found concentrated at the ends of stress fibers, where the actin filaments terminate at focal contacts. Two of these proteins, α-actinin and fimbrin, have properties that suggest that they are not involved in the attachment of the ends of the bundles to the membrane but are more probably involved in the organization and cross-linking of the filaments within the bundles. On the other hand, vinculin and talin are two proteins that interact with each other and may form part of a chain of attachments between the ends of the microfilament bundles and the focal contact membrane. Their role in this attachment, however, has not been established and further work is needed to examine their interaction with actin and to identify any other components with which they may interact, particularly in the plasma membrane.

Much has been learned about the structure and biochemistry of the filamentous elements that are the most prominent components of the cytomatrix of cells such as fibroblasts. Surprisingly little is known, however, about how any of the filament systems associate with cell membranes. In this brief overview we consider specifically how actin microfilaments associate with the fibroblast plasma membrane, focusing on the proteins that have been suggested to function in this association. Research in this area has been influenced greatly by two model systems, the erythrocyte plasma membrane and the brush border microvilli of the intestinal epithelium. Electron microscopy of microvilli has illustrated most clearly that actin filaments can interact with the plasma membrane both laterally through cross-bridges and end on at the tips of the filaments (60). Biochemical studies have shown that the major

component of the lateral cross-bridge is a 110-kdalton protein (57), and a 140-kdalton glycoprotein has been proposed as its receptor (18). Neither protein has a widespread distribution in other cells. The proteins linking the microvillar actin filaments at their tips to the plasma membrane continue to be elusive. Study of the erythrocyte plasma membrane has provided the most complete description in molecular terms of the proteins linking actin to a specific membrane (reviewed in reference 5). The primary proteins in this chain of attachment—spectrin, ankyrin, and the transmembrane glycoprotein band 3—were originally considered to be specialized proteins confined to the erythrocyte (see, for example, reference 5). During the last few years, however, proteins closely related to each of these erythrocyte components have been found in other cells as well (3, 4, 14, 15, 31, 32, 38, 45, 46,

62, 69). These findings have led to a view of the erythrocyte as a model that is more generally applicable to other cells than was formerly considered. We start by examining the family of proteins related to erythrocyte spectrin and present some of our recent findings that have led us to question the applicability of the erythrocyte model to other cells, particularly with regard to the role of a spectrin network beneath the membrane. We then consider the proteins that have been proposed to anchor microfilament bundles to the plasma membrane in the focal contact (adhesion plaque) regions.

Proteins Related to Spectrin

A family of proteins related to erythrocyte spectrin was discovered during the last two years by researchers in several laboratories (4, 14, 15, 31, 32, 38, 46, 62, 69) and has been given a variety of names: fodrin (51), CBPI (for calmodulin-binding protein I) (22), calspectin (46), and brain spectrin (4, 15) all refer to the same spectrin-related protein in brain tissue. TW 260/240 refers to a similar but distinct protein that is uniquely distributed in the terminal web of the intestinal epithelium (31). These proteins are structurally similar to erythrocyte spectrin, being made up of two nonidentical high molecular weight subunits (Table I) that in native form exist as tetramers $(\alpha\beta)_2$ (4, 31, 33, 34, 46). When viewed by electron microscopy they appear as rod-shaped elongated structures with the α and β chains intertwined and with a center of symmetry at the head-to-head junction of the two heterodimers (34). The TW 260/240 molecule is significantly longer (by ~25%) than the other molecules (31). These nonerythrocyte spectrins bind F-actin (4, 15, 22, 31, 32, 46, 51, 74), and two binding sites have been visualized, one at each end of the tetramer (33, 76). Nonerythrocyte spectrins also bind calmodulin (22, 31, 32, 46), and this binding site is located on the α chain close to the head-to-head dimer junction (one binding site per α chain, two per tetramer) (76). It has recently been proposed that a regulatory mechanism involving calmodulin and calcium controls the interaction between F-actin and brain spectrin (73). The brain protein binds to human inverted erythrocyte membranes depleted of

spectrin and actin and competes in this binding with erythrocyte spectrin (4, 15). This binding is specifically inhibited by ankyrin antibodies (15), indicating that the brain spectrin contains an ankyrin-binding site.

There is some disagreement in the published reports concerning the properties of the various nonerythrocyte spectrins and, in particular, their relationship with erythrocyte spectrin. The apparent discrepancies seem to have arisen largely because of differences between the avian and mammalian systems. As pointed out most clearly by Glenney and colleagues (29, 30, 32), the situation in chicken tissues appears relatively simple. On the basis of both immunological cross-reactivity and peptide mapping (Table I), the three characterized proteins, erythrocyte spectrin, brain spectrin (fodrin), and the intestinal component TW 260/240, all appear to have a common α chain of 240 kdaltons. On the other hand, each protein possesses a distinct β chain that confers specificity to the molecule (29, 30). Using specific β-chain antibodies, Glenney and Glenney (29, 30) have demonstrated that the brain type of protein is present in most of the nonerythroid tissues examined, whereas the erythrocyte type is present in heart and skeletal muscle. The TW 260/240 protein is found exclusively in the small and large intestinal epithelium. They (29, 30) also have shown that more than one type of spectrin can be found in the same cell. For example, both fodrin (the brain spectrin type) and TW 260/240 are found in intestinal epithelial cells. During embryogenesis these two proteins are expressed at different times; the brain type is detected in 13-d-old chicken embryo intestines, whereas TW 260/240 appears later, on day 17 or 18. Similar findings have been made in other chicken tissues by Lazarides and Nelson, who have studied the erythrocyte and brain forms of spectrin. During myogenesis in vitro, primary myoblasts express the brain type (called $\alpha\gamma$ spectrin by these authors), and after myoblast fusion the differentiating myotubes express predominantly the erythrocyte type ($\alpha\beta$ spectrin in their nomenclature) (63). They also have shown the coexistence of these two types of spectrin in the cerebellum (48).

The situation in mammals is more complex. The erythrocyte and brain (fodrin) forms of spectrin differ not only in their β chains but also in their α chains (Table I). (As yet no data are available on the biochemistry of the mammalian intestinal form of spectrin corresponding to the avian TW 260/240, although antibodies against the brain form [fodrin] have been used to study the organization of the cross-reactive proteins in mammalian brush borders [43].) The α chain of the mammalian brain spectrin (fodrin) resembles the α chain of avian erythrocyte spectrin more than it does the α chain of mammalian erythrocyte spectrin (possessing, for example, a similar calmodulin-binding site [29, 32, 46]), and herein lies the clue to the apparent complexity. Mammalian erythrocytes have diverged and the spectrin has become more specialized. Avian erythrocytes are more primitive and their spectrin is closer to what is presumably the more primitive and general form of spectrin, the brain type (fodrin). Appreciating the evolutionary relationship between these proteins, it is not surprising that the mammalian nonerythrocyte spectrin (fodrin) is more closely related to avian erythrocyte spectrin than to mammalian erythrocyte spectrin. Nevertheless, mammalian brain and erythrocyte spectrins do share many structural and functional features: they have similar morphologies (4, 33, 34, 46) and very similar amino acid compositions (33); both have an ankyrin-binding site (4, 15), and functional

TABLE I

Polypeptide Composition of Avian and Mammalian Spectrin Molecules

Avian	
(common 240-kdalton α chain [all bind calmodulin], different β chains)	
Erythrocyte spectrin	$\beta = 220$ kdalton ($\beta' = 230$ kdalton)
Brain spectrin (fodrin)	$\beta = 235$ kdalton (γ spectrin)
Intestinal TW 260/240	$\beta = 260$ kdalton

Mammalian	
(different α and β chains for each spectrin type)	
Erythrocyte spectrin	$\alpha = 240$ kdalton (does not bind calmodulin)
	$\beta = 220$ kdalton
Brain spectrin (fodrin)	$\alpha = 240$ kdalton (binds calmodulin)
	$\beta = 235$ kdalton
Intestinal TW 260/240	$\alpha = ?$
	$\beta = ?$

The β' 230-kdalton chain of avian erythrocyte spectrin refers to the precursor form of the β chain (62); γ spectrin is the equivalent of the β chain of avian brain spectrin in the nomenclature proposed in reference 62.

hybrid molecules will assemble from the separated respective α and β chains (3, 21).

Lateral Association of Actin with the Plasma Membrane: What Role Does Nonerythrocyte Spectrin Play?

The human erythrocyte, although clearly very specialized, provides a model in which erythrocyte spectrin is viewed as a submembranous network linking short actin oligomers to the membrane via another protein, ankyrin (5). The discovery of proteins closely related to erythrocyte spectrin in nonerythrocytes, coupled with the submembranous localization of these proteins (31, 48, 51, 62, 63, 69), has resulted in the hypothesis that the brain or fodrin type of spectrin present in most nonerythroid cells and tissues is identical in function and organization. This idea has been supported by the discovery of an ankyrinlike protein in brain tissue (3), which suggests the possibility that a mechanism of attachment similar to that in erythrocytes exists. It seems unlikely, however, that all spectrin-related proteins bind to the membrane via similar ankyrinlike molecules. For example, no proteins cross-reacting with antibodies to ankyrin were detected in a membrane fraction of HeLa cells that contained a specific HeLa spectrin-binding site (55). In cells containing two different types of spectrin, such as in cells in the cerebellum (48) and the intestinal epithelium (29, 30), two different modes of membrane attachment must exist to explain the differential localization of these two proteins. Although the structural and biochemical similarities between erythrocyte and nonerythrocyte spectrins suggest a function for the nonerythrocyte spectrin in the attachment of actin to membranes, it is desirable to approach this function directly. It would be helpful to have mutant cells defective in spectrin, but because these are not available we have attempted to generate cells in which the spectrin is temporarily defective by microinjecting the cells with specific antibodies against the brain spectrin (56). Using either a monoclonal antibody (IgM) or an affinity-purified polyclonal antibody raised against brain spectrin, we have achieved precipitation of the nonerythrocyte spectrin in fibroblasts and epithelial cells following microinjection (56). A cell in which spectrin has been precipitated by microinjection of specific antibodies is shown in Fig. 1 C. As judged by immunofluorescence microscopy, all of the spectrin in these cells can be caused to precipitate by either antibody, although the polyclonal antibody results in more compact aggregates that align in fibroblasts more prominently along the microfilament bundles (56). The spectrin-antibody precipitates are cleared into vesicular structures (presumably lysosomes), but the rate of clearance varies considerably among cell types. In some cells this occurs within 3 h, whereas in others there is no significant clearance of the precipitates even after 24 h.

Cells in which the spectrin has been precipitated permit us to examine the function of spectrin, and we can ask how such a drastic rearrangement of this protein affects the behavior of the cell. Surprisingly, after spectrin precipitation, we have not detected any effect on cell shape, cell movement, or cell division. We have examined the effect of this precipitation on other elements of the cytomatrix. Again we were surprised to observe no effect on the bundles of microfilaments (stress fibers). This result suggests that, if lateral associations between the microfilament bundles and the plasma membrane involve spectrin, they are not required for the integrity of these bundles. These results also led us to conclude that membrane anchorage of the microfilament bundles at their ends (not mediated by spectrin but by other proteins, such as vinculin and talin) is the predominant factor in the stabilization of these structures. No effect of intracellular spectrin precipitation was observed on the distribution of microtubules or coated vesicles. An unexpected effect was seen, however, on the distribution of the vimentin class of intermediate filaments in most of the cell types examined (see below). After the intracellular immunoprecipitation of nonerythrocyte spectrin, certain other proteins (e.g., the 36-kdalton protein substrate of the Src kinase) continue to have a distribution very similar to that of the nonaggregated spectrin (56). If the nonerythrocyte spectrin is part of a submembranous network, it would seem to be just one element in this network and an element that is nonessential given that its aggregation does not affect the distribution of other elements within, presumably, the same network.

Erythrocyte spectrin has been shown to restrict the lateral mobility of certain erythrocyte membrane proteins (26, 37). In view of the reported redistribution of nonerythrocyte spectrin during the "capping" of cell surface components (52, 61), the question has been raised as to whether nonerythrocyte spectrin serves a similar function in other cells. In experiments so far, the precipitation of spectrin within fibroblasts has not been found to affect the lateral mobility of certain surface antigens, nor to affect antibody-induced redistribution of β-2 microglobulin and other specific surface glycoproteins on fibroblasts (P. Mangeat et al., manuscript in preparation). Because of the technical difficulty of microinjecting lymphocytes, we have not yet examined the effect of spectrin precipitation on capping in these cells.

Another difference between the situation in erythrocytes and other cells may well be in how close to the membrane the spectrin is found. The submembranous network of spectrin in erythrocytes is very close to the lipid bilayer. Nonerythrocyte spectrin has been shown by immunofluorescence microscopy to be close to the plasma membrane in cross-sectional profiles of a number of different cell types (31, 48, 51, 62, 63, 69), but the level of resolution in these studies has been insufficient to determine how close to the bilayer this protein is located. Detailed electron microscopy has only been performed on the intestinal epithelium (35, 43), and it is interesting that here the nonerythrocyte spectrin is found extending quite deep into the cytoplasm. Should this be found also in other cells, it would suggest that this protein has functions other than or in addition to actin-membrane attachment.

Compared with large bundles of microfilaments, actin in the cell cortex is difficult to visualize by light microscopy using immunofluorescence or the fluorescent probe for F-actin, 7-nitrobenz-2-oxa-1,3-diazole (NBD)-phallacidin[1] (2). As a result, our experiments do not address directly the question of whether nonerythrocyte spectrin has a role in the association of this cortical actin with the plasma membrane. Nevertheless, if nonerythrocyte spectrin does function in this association, we are surprised that so few effects are seen in cell behavior after the drastic reorganization of spectrin in cells that have been microinjected with specific antibodies.

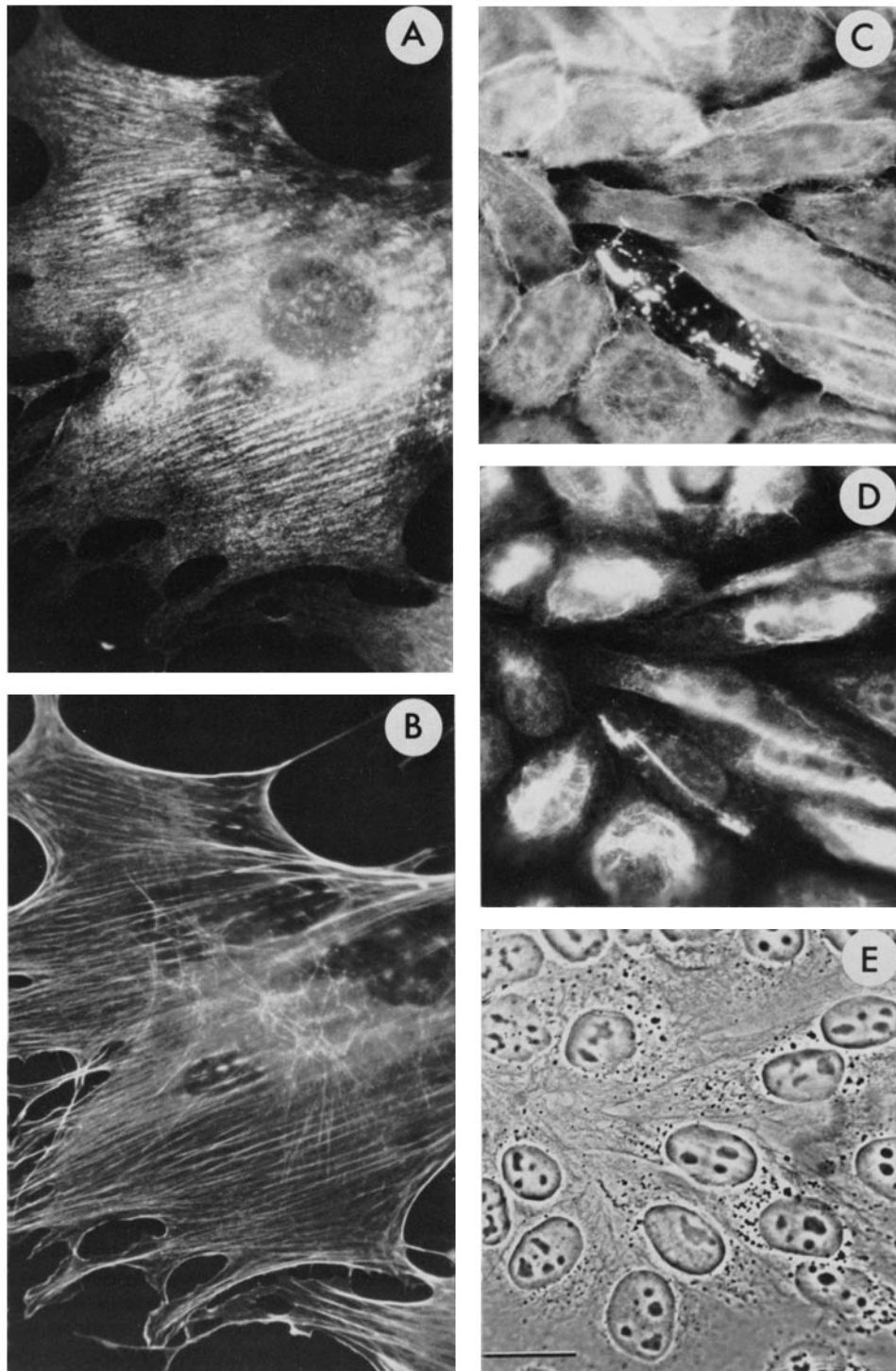[1] *Abbreviation used in this paper:* NBD, 7-nitrobenz-2-oxa-1,3-diazole.

FIGURE 1 Nonerythrocyte spectrin localization in cultured cells and precipitation in a live cell induced by microinjection of anti-brain spectrin antibody. Cells were grown on glass coverslips, fixed, permeabilized, and processed for indirect immunofluorescence as described previously (15). A and B show a well-spread mouse fibroblast (C3H 10T$_{1/2}$) stained with affinity-purified rabbit anti-brain spectrin antibody followed by rhodamine-labeled goat anti-rabbit IgG antibody (A) and with NBD-phallacidin (B) to reveal the distribution of actin (2). Note the characteristic pattern of nonerythrocyte spectrin, which tends to be excluded from the actin microfilament bundles. C–E show HeLa cells, one of which was microinjected with concentrated affinity-purified rabbit anti-brain spectrin 3 h before fixation. After fixation and permeabilization, the cells were stained both with the affinity-purified rabbit anti-brain spectrin antibody (C) (diluted 1:100) and with a mouse monoclonal antibody (JLB7, reference 53), which stains a component of the vimentin-type intermediate filaments (D). The same cells are visualized in phase contrast (E). In C, the microinjected cell is easily recognized because the spectrin has been quantitatively precipitated and cleared from its normal distribution. (Compare this pattern with that of the neighboring cells, which were not injected.) When the pattern of the vimentin intermediate filaments is examined in D, it can be seen that there has been a drastic change in their distribution in the injected cell. Note that there is considerable alignment of the condensed intermediate filaments with the spectrin precipitates. For further details, see reference 56. Bar, 30 µm.

An Interaction between Nonerythrocyte Spectrin and the Vimentin Class of Intermediate Filaments

The intracellular immunoprecipitation of spectrin within fibroblasts did not affect the large bundles of microfilaments but, contrary to our expectations, did affect the distribution of the vimentin class of intermediate filaments in most of the cell types examined (56). Fig. 1 C shows a HeLa cell in which the spectrin has been precipitated by specific antibody microinjection. The injected cell is surrounded by several cells that were not injected, and in these the typical distribution of spectrin in HeLa cells is seen. The spectrin in the injected cell has been induced to form compact aggregates, some of which (those appearing vesicular) may have entered lysosomes. The distribution of the vimentin type of intermediate filaments in the same cells is shown in Fig. 1 D, and it can be seen that the vimentin filaments in the injected cell are much more condensed than in the other cells. Much of the aggregated, precipitated spectrin is coincident with the condensed intermediate filaments. We observed this phenomenon in a variety of different cell types (56). The intermediate filaments, although distorted and condensed as a result of the spectrin precipitation, usually did not collapse to form coils around the nucleus, as occurs when microtubules are disrupted by drugs such as colchicine. This perturbation of the vimentin filaments was not induced by microinjection of irrelevant antibodies, nor was it induced by microinjection of a monoclonal antibody (IgM) against spectrin into a cell with which the monoclonal antibody did not cross-react (56). These results suggest that there is an interaction (direct or indirect) between nonerythrocyte spectrin and the vimentin class of intermediate filaments. That these two structures are associated is supported by experiments in which the intermediate filaments were induced to coil up around the nucleus by treatment with colcemid. In these cells the spectrin was observed to redistribute with an increased concentration over the coiled intermediate filaments (50, 56). The electron microscope data of Hirokawa et al. (43) also provide evidence that there is an association between the spectrin and intermediate filaments of the brush border terminal web. Finally, the desmin class of intermediate filaments has been found in skeletal muscles to colocalize with spectrin in the regions where myofibrils associate with the sarcolemma (costameres) (19, 40, 67). Thus, an association between nonerythrocyte spectrin and intermediate filaments may be a general phenomenon and it could be that the nonerythrocyte spectrins play a role in the attachment of intermediate filaments to the plasma membrane. The interaction of nonerythrocyte spectrins with the different types of intermediate filaments will have to be pursued biochemically to determine whether it is direct or indirect. This interaction is consistent with a theme of this supplement that the various filamentous elements are structurally associated within the cytomatrix. An association between intermediate filaments of the vimentin type and microtubules has long been recognized in electron micrographs and now there is evidence of an association between intermediate filaments and nonerythrocyte spectrin, a component normally considered part of the microfilament system.

Proteins in Focal Contacts

Several proteins (α-actinin, fimbrin, vinculin, talin, and a 200-kdalton protein [see Table II]) have been found concentrated at the cytoplasmic face of fibroblast focal contacts

Table II

Proteins Concentrated at the Ends of Actin Microfilament Bundles at Focal Contacts

Protein	Molecular weight on SDS gels	Native structure	References
α-Actinin	100,000	dimer	12, 13, 49
Fimbrin	68,000	monomer	6, 7
Vinculin	130,000	monomer	27
Talin	215,000	monomer	9 and unpublished data
200-kdalton protein	200,000	?	54

(adhesion plaques), those regions where bundles of microfilaments terminate and where the cells adhere most tightly to the underlying substrate. Except in the case of fimbrin, it has been proposed at different times that all the proteins function in actin-membrane attachment. That this was not suggested for fimbrin is probably because fimbrin was first isolated from the brush border microvilli of intestinal epithelial cells, where it was recognized as a component of the core bundle of filaments (7). Subsequently, the purified protein was shown to cross-link actin filaments into bundles in vitro (6, 36). Although fimbrin from other sources has not been studied, it has generally been assumed that in other situations it is also an actin filament "bundling" protein. The distributions, relative to each other and to actin, of three of the other proteins, α-actinin, vinculin, and talin, are shown in Fig. 2.

α-Actinin

α-Actinin was the first of these proteins to be found concentrated at the ends of microfilament bundles, but unlike vinculin or talin it is also distributed periodically along their lengths (49) (Fig. 2, C and E). In the chicken embryo fibroblasts of Fig. 2, the periodic distribution of α-actinin along the microfilament bundles is less apparent than it is in many, typically larger mammalian cells (see, for example, references 39, 49, 71, 78). Compared with the distributions of vinculin and talin, α-actinin is relatively less concentrated at the ends of microfilament bundles. Nevertheless, because of its location at this site of actin-membrane attachment as well as its location in the Z lines of striated muscle sarcomeres (where it was generally assumed to be involved in the attachment of actin filaments to these structures), it was suggested as a possible linker between microfilaments and membranes in nonmuscle cells (49). In a further examination of this possibility, isolated plasma membranes from a variety of different cell types were found to contain α-actinin, but the α-actinin could be extracted from these membranes under conditions that left much of the actin still associated with them (16). From this it was concluded that α-actinin was not linking the actin to these membranes. The properties of purified nonmuscle α-actinin have been studied (12, 13). Like muscle α-actinin, the nonmuscle form has been found to cross-link actin filaments but with the significant difference that the nonmuscle α-actinin is inhibited by calcium in the micromolar concentration range (12, 13). Whereas a stable actin lattice cross-linked by α-actinin is probably a necessity for effective muscle contraction, the nonmuscle cytomatrix is a dynamic structure. In nonmuscle cells, having the cross-
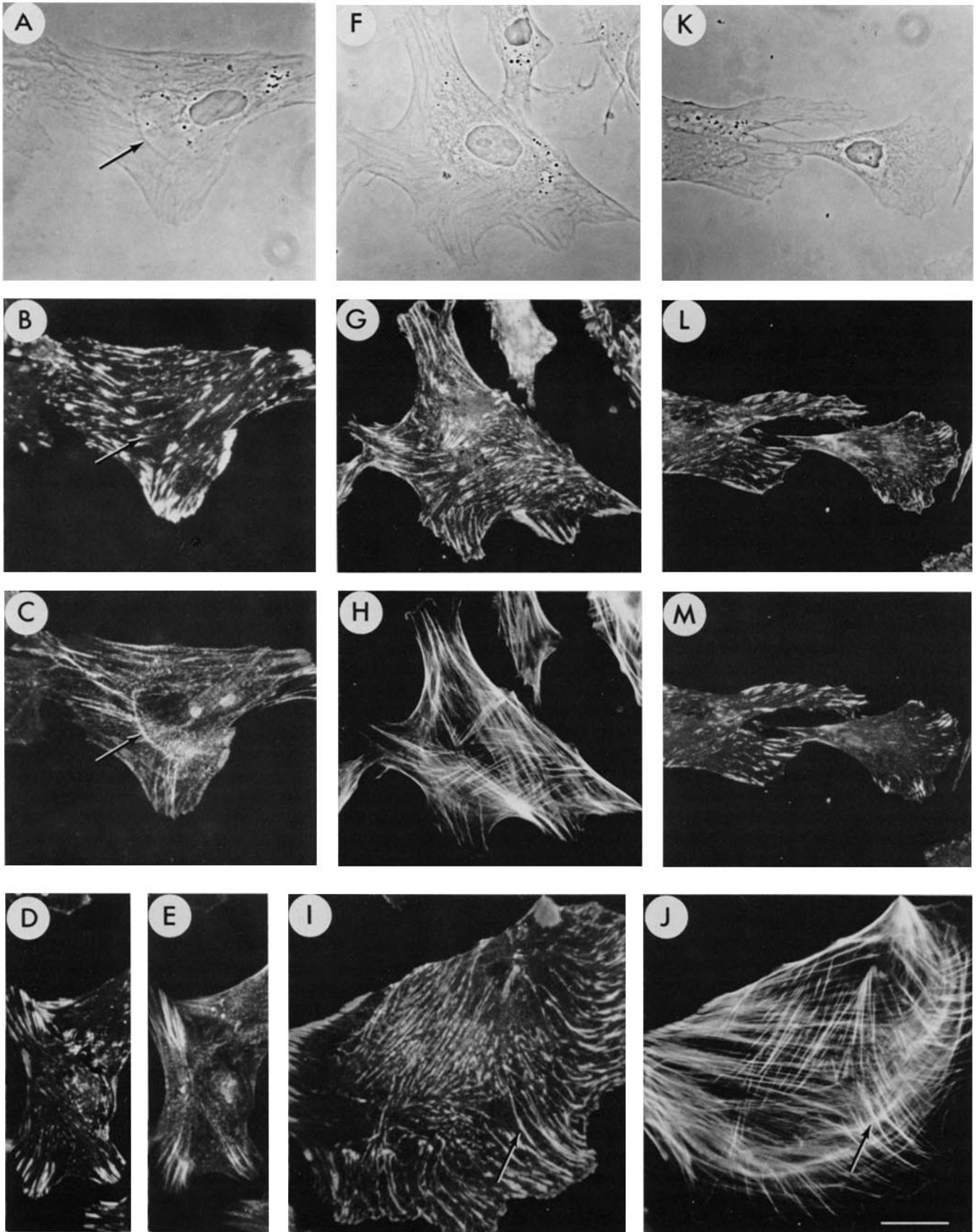
FIGURE 2   Distribution of actin, α-actinin, vinculin, and talin in embryonic chicken heart fibroblasts. Cell culture and conditions for immunofluorescence have been described previously (9, 15). A–C show a cell in phase contrast (A) and double-label stained with an antivinculin mouse monoclonal antibody (B) and a rabbit antibody against beef heart α-actinin (C). D and E show another comparison of the distribution of vinculin (D) and α-actinin (E) in the same cell. F–H show a cell in phase contrast (F) and double-

linking of actin filaments by α-actinin regulated by calcium would confer on the system a means of changing the actin lattice in a finely controlled manner. Proteins that appear to be nonmuscle α-actinin by almost every criteria have been isolated (and studied in detail) from a variety of different cell types and organisms, including Ehrlich ascites cells (a protein called actinogelin; 58, 59), blood platelets (70), *Dictyostelium discoideum* (*Dictyostelium* 95-kdalton protein; 17, 24), and *Acanthamoeba* (68). In each case, these nonmuscle α-actinins have properties in vitro that suggest a function in the cross-linking of actin filaments in a calcium-sensitive manner rather than in the linking of actin to the plasma membrane.

Unlike talin and vinculin, α-actinin is a prominent component of microfilament "arcs" (see arrows in Fig. 2, *A* and *C*) that have been described in migrating fibroblasts (42, 75), in respreading cells (75), and in fibroblasts that are capping aggregated surface components (41). These arcs superficially resemble the stress fiber type of microfilament bundle, but unlike stress fibers they are highly motile; they form at the cell periphery or leading edge of the cell and are swept back centripetally below the dorsal surface. An arc is visible in the cell in Fig. 2*A* (arrow) and is stained strongly with antibodies against α-actinin (Fig. 2*C*) but is not seen when the same cell is stained with an antibody against vinculin (Fig. 2*B*). Similarly, in the cell in Fig. 2*J*, in which filamentous actin has been visualized with NBD-phallacidin, an arc is visible (arrow), but no staining of this arc is detected when the same cell is stained with antibodies against talin (Fig. 2*I*). The absence of vinculin and talin from these moving arcs of microfilaments is interesting and it will be important to determine whether these proteins continue to be absent when aggregated cell surface components are being cleared from the cell by centripetal motion of the arcs (41).

Vinculin

The protein that has generated the most interest with respect to a possible role in attachment at the focal contacts is vinculin (27, 28). The typical distribution of vinculin in fibroblasts is shown in Fig. 2, *B*, *D*, and *M*. Vinculin is found not only in focal contacts of fibroblasts but also at various other sites of actin-membrane interaction, such as underlying cell surface fibronectin (11) and at the adherens junctions of both epithelial cells and cardiac muscle (28). Vinculin has also been identified in the circumferential regions (costameres) of attachment between skeletal muscle myofibrils and the surrounding sarcolemmal membrane (19, 67). The suggestion that vinculin functions in attachment gained support from several studies showing that preparations of vinculin interact with actin (13, 44, 77). Here, however, there have been inconsistencies in the results reported by different groups and controversy over the interpretation of the results. Some data have indicated that vinculin causes actin filaments to bundle (44), whereas other data indicate that it binds to one end of

an actin filament (the "barbed" end as determined by the "arrowheads" formed when actin filaments bind heavy meromyosin) (77). In spite of the apparent discrepancies, the interaction of vinculin with actin has generally been accepted as positive support for the idea that vinculin has an attachment role. However, Evans and Robson (23) have reported that a component responsible for one of vinculin's supposed effects on actin (the decrease in F-actin viscosity) can be separated from vinculin by chromatography on a cation exchange column. We have confirmed these results (B. M. Paschal and K. Burridge, unpublished observation) as have others (J. J. Otto, personal communication; J. A. Wilkins and S. Lin, personal communication). The discrepancies between the findings of different groups investigating vinculin's interaction with actin may well be due to different contaminants in vinculin preparations. Certainly some of the interaction with actin previously attributed to vinculin is due to another protein and it becomes most important to determine whether vinculin interacts with actin at all. It also will be important to characterize thoroughly the "contaminant(s)" in vinculin preparations since these may have some of the functions previously ascribed to vinculin.

Regardless of whether vinculin interacts directly with actin, it may play a role in the attachment of bundles of microfilaments to the plasma membrane. Just as in the attachment of actin to the erythrocyte membrane, in which a chain of proteins mediates this association, so too, in regions such as focal contacts, there may be a chain of components between the ends of the filaments and proteins in the lipid bilayer. Vinculin may be one link in this chain. Experiments by Avnur et al. (1) have shown that vinculin can remain associated with the focal contact regions in cells that have had their dorsal surfaces sheared off and the actin disassembled. This association of vinculin with the plasma membrane in the absence of actin indicates the presence of vinculin-binding proteins associated with the focal contact regions that are independent of actin. However, this association does depend on rather special buffer conditions, and under many conditions in which fibroblasts are permeabilized, vinculin is readily extracted from the focal contacts. Similarly, vinculin was not detected in plasma membrane preparations isolated from cultured HeLa cells (13).

A protein very similar to vinculin has been purified from chicken gizzard smooth muscle (25, 72). Antibodies raised against this 150,000-dalton protein ("metavinculin") cross-react with vinculin and label the focal contacts of fibroblasts. In immunoblots of SDS gels of whole gizzards, two bands (at 150,000 and 130,000 daltons) are labeled by antibodies against both vinculin and metavinculin (25, 72), but the corresponding immunoblots of fibroblasts only label the vinculin band at 130,000 daltons (25, 72). The significance of metavinculin in smooth muscle has not been determined, but vinculin and metavinculin have been shown to be the products of different genes (25).

label stained with an affinity-purified rabbit antibody against talin (*G*) and with NBD-phallacidin (*H*) to reveal the distribution of actin in the same cell. *I* and *J* show another comparison of the distribution of talin (*I*) and actin (*J*) in the same cell. *K–M* show cells in phase contrast (*K*) and double-label stained with an affinity-purified rabbit antibody against talin (*L*) and a mouse monoclonal antibody against vinculin (*M*). Note that in *L* (with anti-talin) the margin of the cell on the right is brighter than the staining of the same cell in *M* with antivinculin. In *A–C*, *I*, and *J*, the arrows indicate microfilament arcs (see text). These can be seen to contain actin (*J*) and α-actinin (*C*) but not vinculin (*B*) or talin (*I*). Bar, 30 μm.

Talin

We have identified another component of focal contacts (9, 10, 14) and shown that this 215,000-dalton protein, talin, interacts with vinculin (15a). The distribution of talin is illustrated in Fig. 2, G, I, and L. It is very similar to that of vinculin, as can be seen by comparing the two distributions in the same cell (Fig. 2, L and M). Both proteins are concentrated in focal contacts and show fibrillar distributions that align simultaneously with underlying stress fibers and with fibronectin on the cell surface (9-11). Talin, however, appears to be relatively more concentrated at cell margins and ruffling membranes. Comparing the distributions of talin and vinculin in Fig. 2, L and M, it can be seen that talin is more prominent than vinculin in the leading edge of the cell on the right.

Because of their codistribution in regions such as focal contacts, we have asked whether vinculin and talin interact. Using several different assays we have found that the two proteins bind to each other with a dissociation constant of about 10^{-8} M (15a). Talin has been identified independently as a vinculin-binding protein by Otto (66), who developed a gel overlay method using radioiodinated vinculin to detect components that bind vinculin. It could well be that vinculin and talin are two elements in the chain of attachment of microfilament bundles to the plasma membrane, but until more evidence is accumulated the function of these two proteins must remain uncertain. Experiments are in progress to examine any interaction of the complex of the two proteins with actin and again to look for additional components with which they may interact, particularly in the plasma membrane.

A 200-kdalton protein that may be related to talin has been described by Maher and Singer (54). Whereas talin was initially isolated from chicken gizzards, this protein was isolated from preparations of cardiac muscle fascia adherens. Antibodies against this 200-kdalton protein stain the focal contacts of chicken embryo fibroblasts. Although this protein may be related to talin, preliminary evidence indicates a lack of antigenic cross-reaction. In future work it will be important to compare these two proteins in detail to determine whether they are the same or whether they are different but both members of one family.

Focal Contact Membrane Components

When considering the organization at focal contacts, most workers have directed their efforts at identifying proteins associated with the ends of the microfilament bundles. Much less attention has been given to specific proteins or glycoproteins in the membrane at focal contacts. Two groups of researchers have raised monoclonal antibodies that label focal contacts from the exterior (20, 65). Another interesting glycoprotein that has been localized to focal contacts is the glycoprotein D of herpes simplex virus (64) in cells infected with this virus. As yet, little biochemistry has been done on any of these components, but it will be interesting to find out whether any of them interact with the cytoskeletal proteins described above, in particular with vinculin or talin.

Conclusion

In this brief overview we have considered some of the proteins for which roles in linking actin to the plasma membrane in fibroblasts have been suggested. There are other components that we have not discussed, for example, the laminin receptor, connectin, which has been shown to bind to actin (8). Connectin is certainly a strong candidate for an actin-membrane attachment protein, but further work still needs to be done to show that connectin is transmembrane and that it is the cytoplasmic portion of the protein that binds actin. This is critical inasmuch as various extracellular proteins, such as fibronectin (47), are known to bind actin.

With the discovery of the family of nonerythrocyte spectrins, much has been made of the erythrocyte as a model for how actin may be attached to the plasma membrane of other cells. Nonerythrocyte spectrins may be involved in the attachment of some actin to the plasma membrane, but our experiments in which spectrin is precipitated inside cells by specific antibody microinjection have led us to question the validity of the erythrocyte as a general model. The behavior of cells in tissue culture appears remarkably unaffected by this drastic perturbation of spectrin organization, giving us few clues to the function of nonerythrocyte spectrin in cells such as fibroblasts. Spectrin does not seem to be important in the attachment of bundles of microfilaments to the plasma membrane at focal contacts. Considerable progress has been made in identifying components that are concentrated in these areas and some, such as α-actinin and fimbrin, would seem to be primarily involved in cross-linking actin filaments and in the formation or stabilization of the microfilament bundles. Other proteins, such as vinculin and talin, may be involved in attachment, but this has not been established. A major advance has been the demonstration that these two proteins interact with each other. It is now most important to clarify their interaction with actin and to identify any other components with which they may interact, particularly in the plasma membrane.

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