Identification of a New Protein Localized at Sites of Cell-Substrate Adhesion

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Abstract. A new protein found at sites of cell-substrate adhesion has been identified by analysis of a nonimmune rabbit serum. By indirect immunofluorescence this serum stains focal contacts (adhesion plaques) and the associated termini of actin filament bundles in cultured chicken cells. Western immunoblot analysis of total chick embryo fibroblast protein demonstrated an 82-kD polypeptide to be the major protein recognized by the unfractionated serum. This 82-kD protein is immunologically distinct from other known adhesion plaque proteins such as vinculin, talin, α-actinin, and fimbrin. Antibody affinity-purified against the electrophoretically isolated, nitrocellulose-bound 82-kD protein retained the ability to stain the area of the adhesion plaque, which confirms that the 82-kD protein is indeed a constituent of the focal contact. The 82-kD polypeptide has a basic isoelectric point relative to actin and fibronectin, and it appears to be very low in abundance. The 82-kD protein is ubiquitous in chicken embryo tissues. However, it appears to be more abundant in fibroblasts and smooth muscle than in brain or liver. Intermediate levels of the protein were detected in skeletal and cardiac muscle. The subcellular distribution of the 82-kD protein raises the possibility that this polypeptide is involved in linking actin filaments to the plasma membrane at sites of substrate attachment or regulating these dynamic interactions.

Actin is attached to the plasma membrane at sites where cells adhere to substrates or to each other (16, 20, 37). At these regions a cell establishes a transmembrane linkage between components of the extracellular milieu and the actin-rich cytoskeleton. Such areas where cells make close contact with a substrate or another cell have been referred to as adherens junctions. Collectively these junctions share much structural and biochemical homology and represent regions of the cell membrane specialized for interaction with actin filaments (14, 17, 35). As such, they provide systems in which the molecular composition and organization at sites of actin-membrane interaction can be studied.

A number of proteins are known to accumulate at the sites of actin-membrane association where cells are in close contact with the substrate. Vinculin (6, 15) and talin (5), for example, are assembled at these focal contacts or adhesion plaques. These two proteins interact with each other (7, 29), but they do not appear to be involved directly in linking actin to the plasma membrane (7, 13, 30, 33). Even though much progress has been made in identifying proteins localized at sites of actin-membrane-substrate interaction and characterizing their affinities for each other, clearly other components of this structure remain to be discovered before the molecular mechanism of the association is understood.

Perhaps not surprisingly, one feature shared by many of the proteins currently known to be localized at sites of actin-membrane-substrate interaction is that they are abundant proteins readily purified from muscle. It was from this fertile source that vinculin, talin, and α-actinin, three of the major components of fibroblast adhesion plaques, have been isolated. Recently, however, monoclonal antibody technology has enabled identification of components of adhesion plaques regardless of their abundance. For example, a glycoprotein complex (130 and 175 kd) localized in both the cleavage furrow and focal contacts has been described (32). Other components of focal adhesions have been discovered by use of a functional assay in which monoclonal antibodies that can disrupt or prohibit cell-substrate contacts were selected. Two of these monoclonal antibodies, referred to as CSAT (27) and JG22 (18), recognize the same glycoprotein antigen that is a receptor for fibronectin (1, 22). By use of these immunochemical reagents this fibronectin receptor complex has been localized to regions of cell-substrate contact (8, 12). Another monoclonal antibody, called anti-FC-1, recognizes a 60-kD glycoprotein component of focal contacts (28).

Here I report the discovery of a new protein that is localized at sites of cell-substrate interaction where actin is attached to the plasma membrane. This protein was identified by analysis of a nonimmune rabbit serum that contained specific antibodies directed against an 82-kD component of adhesion plaques.

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Materials and Methods

Cell Culture

Cultures of chicken embryo fibroblasts were prepared by trypsin treatment of skin samples from 11-d-old chicken embryos. The fibroblasts were subcultured in Dulbecco's modified Eagle's medium (DME) containing 10% fetal bovine serum and supplemented with penicillin-streptomycin. Pigmented retinal epithelial cells were derived from explants of retina from 11-d-old chicken embryos. These primary cultures were maintained in DME as above and were used within 4 d of plating.

Immunochemical Reagents

The F396 serum described here is a rabbit nonimmune serum. It was used at dilutions of 1:10 or 1:25 for indirect immunofluorescence and 1:100 for immunoblot experiments. The anti-vinculin antibody (designated C19) is a mouse monoclonal antibody raised against chicken vinculin by Ms. Linda Hertz and Dr. Keith Burridge (University of North Carolina, Chapel Hill, NC). Dr. Anthony Bretscher (Cornell University, Ithaca, NY) generously provided a sample of brush border fimbrin and a rabbit polyclonal anti-fimbrin antibody; the characterization of this antibody has been described previously (4). Rabbit polyclonal antibodies directed against vinculin and α-actinin used in immunoblot experiments were provided by Dr. Keith Burridge. The anti-talin antibody has been described previously (3). Rhodamine-phalloidin was obtained from Molecular Probes Inc. (Junction City, OR). Fluorochrome-labeled secondary antibodies were purchased from Cooper Biomedical, Inc. (Malvern, PA).

Indirect Immunofluorescence

Cells that had been plated for at least 48 h on 12-mm diam glass coverslips were used for the localization studies. The cells were fixed for 10 min in 3.7% formaldehyde in Dulbecco's phosphate-buffered saline, washed with Tris-buffered saline (TBS; 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.1% NaN_3), and permeabilized with 0.2% Triton X-100 in TBS for 5 min. The coverslips were then incubated with 30 μl of primary antibody at 37°C for 60 min and washed in TBS, followed by incubation with fluorochrome-labeled second antibody. The coverslips were then washed in TBS, rinsed briefly in deionized water, and mounted in a water-soluble polyvinyl alcohol, gelvatol (Monsanto Co., St. Louis, MO). Rhodamine-phalloidin was mixed with the second antibody for actin labeling.

Immunoblot Analyses

Western immunoblots were performed by modification of the procedures developed by Towbin and co-workers (36). Proteins were subjected to electrophoresis on 10% SDS polyacrylamide gels (25) containing 0.13% bisacrylamide. After electrophoretic transfer of the proteins to nitrocellulose, the nitrocellulose strips were incubated on a rotary shaker for 60 min in blocking buffer (TBS containing 2.5% BSA, 0.2% gelatin, and 0.05% Tween 20 [2]), followed by 90 min in the primary antibody diluted in blocking buffer containing an additional 5% normal horse serum (horse serum buffer). After being washed in blocking buffer minus BSA, the nitrocellulose strips were incubated for 60 min with radioiodinated (23), affinity-purified goat anti-rabbit IgG (10^5–10^6 cpm/ml) in horse serum buffer. All manipulations were performed at room temperature. After a final wash, the blots were dried and exposed to x-ray film with an intensification screen at −70°C.

Antigen Characterization

The molecular mass estimations for the F396 antigen were made by performing linear regression analyses on the relative mobilities of standard proteins (BSA, M_r 66,200; phosphorylase B, M_r 92,500; β-galactosidase, M_r 116,250) from BioRad Laboratories (Richmond, CA) subjected to electrophoresis in 10% SDS polyacrylamide gels. Four independent immunoblot experiments having internal standards were analyzed to estimate the molecular mass of the F396 antigen. By this approach the apparent molecular mass of the F396 antigen was determined to be 82,000 ± 2,500 D.

Two dimensional gels were performed according to the method of O'Farrell (31). Ampholines, pH 6–8 (LKB Instruments Inc., Bromma, Sweden), were used in the first dimension isoelectric focusing gels. To prepare the sample, a 60-mm dish of confluent chick embryo fibroblasts was harvested into 400 μl of isoelectric focusing gel sample buffer. 30–50 μl of sample were used per gel. The second dimension SDS gels were analyzed by the immunoblot method described above.

Affinity Purification of Antibody

Affinity-purified antibody against the 82-kD polypeptide was prepared by a modification of the method of Cox, Schenk, and Olmsted (10). The 82-kD protein was partially purified from a low ionic strength extract of chicken gizzard smooth muscle followed by ammonium sulfate fractionation and ion-exchange chromatography. The column fractions were assayed for the presence of the 82-kD polypeptide by the immunoblot method. Although no protein that strictly correlated with the immunoreactive component was detected by Coomassie Blue staining, the fractions containing the 82-kD antigen were unambiguously identified by this immunochemical approach. These fractions were pooled and subjected to preparative SDS PAGE. The proteins were transferred to nitrocellulose; narrow strips of nitrocellulose from the left and right sides of the preparative gel replica were excised and stained with Ponceau S. After destaining, these marker strips were aligned with the remainder of the blot, and the location of the 82-kD antigen was determined by its relationship to the relative mobilities of more abundant proteins in the preparation. A 4–5-mm band of nitrocellulose in the region thought to contain the 82-kD protein was excised from the preparative gel replica. A small sample from the strip was reserved and used to confirm the presence of the F396 antigen by immunoblot. A control strip of nitrocellulose of equivalent dimensions was excised from a region of the nitrocellulose above the position of the 82-kD protein; this region was selected to eliminate the possibility that a proteolytic product of the 82-kD protein would be adsorbed to the control region of the blot. The control strip of nitrocellulose and the strip containing the 82-kD polypeptide were treated equivalently from this point, but for ease of description I will discuss only the 82-kD region. The strip of nitrocellulose containing the 82-kD protein was incubated for 2 or more hours at 4°C on a rocker platform with blocking buffer. At the end of this treatment the strip was cut into small pieces ~4-mm square. These pieces were incubated overnight at 4°C on a rocker platform with the F396 serum diluted 1:10 in horse serum buffer. After this treatment, the pieces were washed extensively (five times for 10 min in 50 ml, each time) in blocking buffer minus BSA. After washing, the nitrocellulose pieces were transferred to a 5-ml disposable syringe and the bound antibodies were eluted with 4 ml glycine-HCl, pH 2.3. The nitrocellulose strips were exposed to the elution buffer for precisely 2 min with gentle agitation before the contents of the syringe were dispensed into a tube containing Tris-HCl, pH 9.0 sufficient to neutralize the glycine-HCl. BSA (100 mg/ml in water) was added immediately to a final concentration of 1 mg/ml. The eluted material was then concentrated ~ 8–10-fold in an Amicon Corp. (Danvers, MA) centricon concentrator device. The nitrocellulose strips were washed in TBS to neutralize the pH and were then stored at 4°C for future use.

Preparation of Tissue Samples

Selected tissues were dissected from an 18-d-old chick embryo. The samples were weighed and homogenized rapidly in 5 vol of deionized water containing 1 mM phenylmethylsulfonyl fluoride. To this mixture 5 vol of boiling Laemmli sample buffer (25) was added, and the samples were again homogenized briefly. The samples were then passed through a 26-gauge syringe needle to shear the DNA and were boiled for 4 min. For SDS PAGE, 10 μl of each sample was used per lane.

Results

The F396 Antigen Is Localized at Sites of Cell-Substrate and Cell-Cell Adhesion

During a routine screening of rabbit preimmune sera by indirect immunofluorescence, I identified one rabbit (F396) whose unfractionated serum recognized sites of actin-membrane interaction. Specifically, the F396 serum recognized a component of fibroblast focal contacts (adhesion plaques) as well as material associated with regions of cell-cell contact in pigmented retinal epithelial cells.

Chick embryo fibroblasts were prepared for indirect immunofluorescence and were double-labeled with a monoclonal anti-vinculin antibody and the F396 preimmune serum. The result of such labeling is shown in Fig. 1. The anti-vinculin antibody staining revealed the location of the adhesion plaques within these cells (Fig. 1, *B* and *E*). As de-

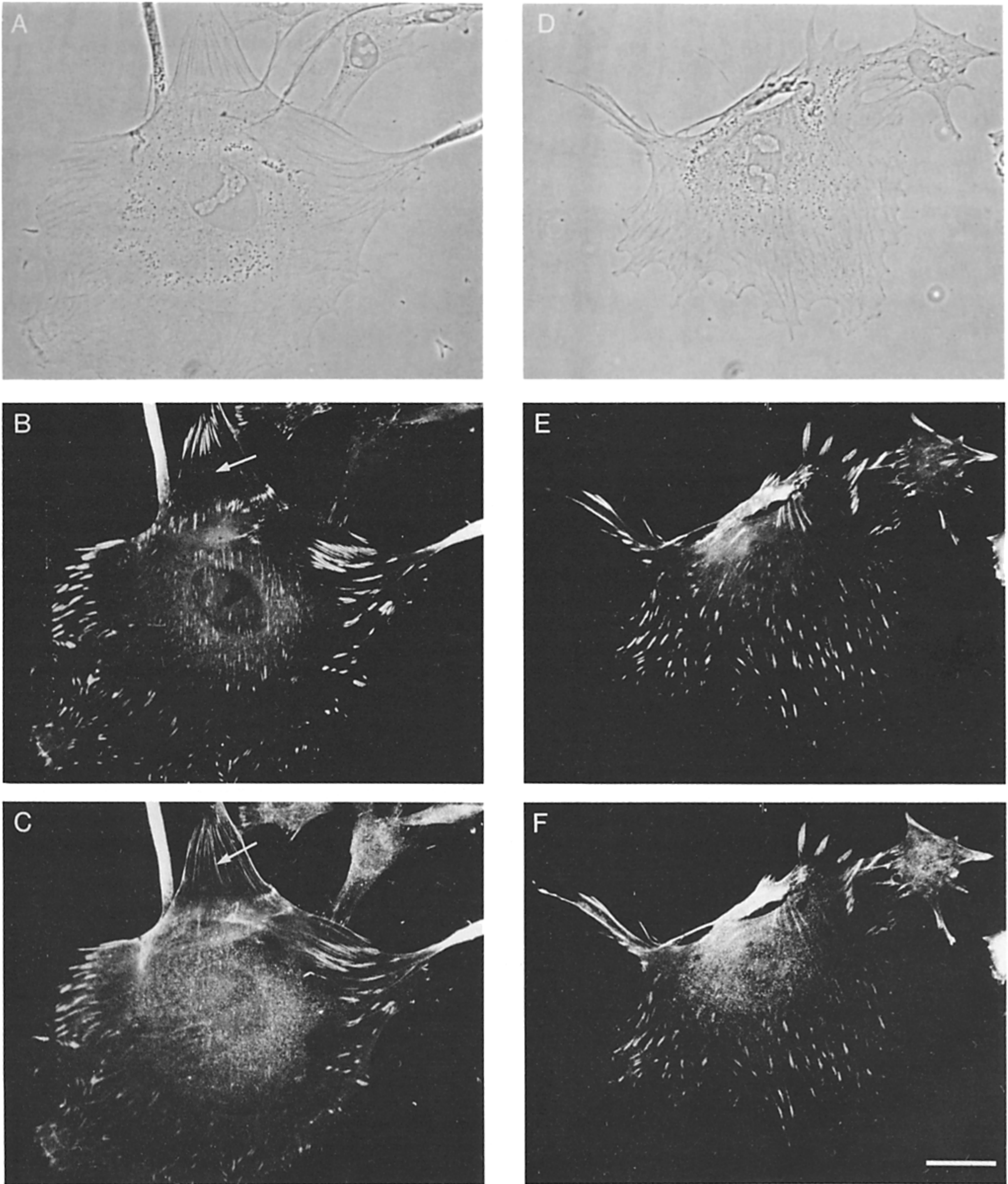
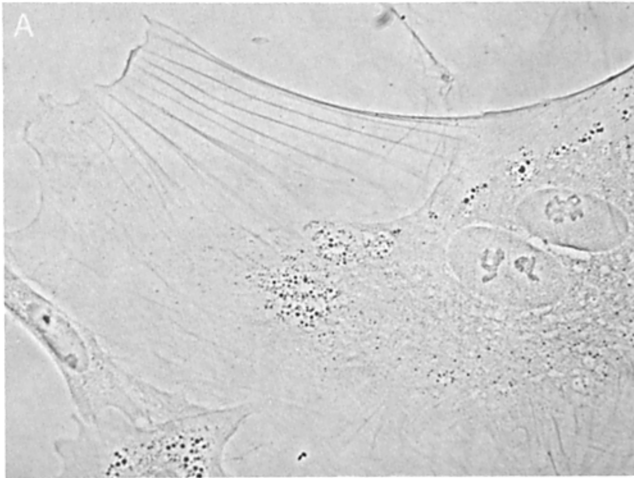
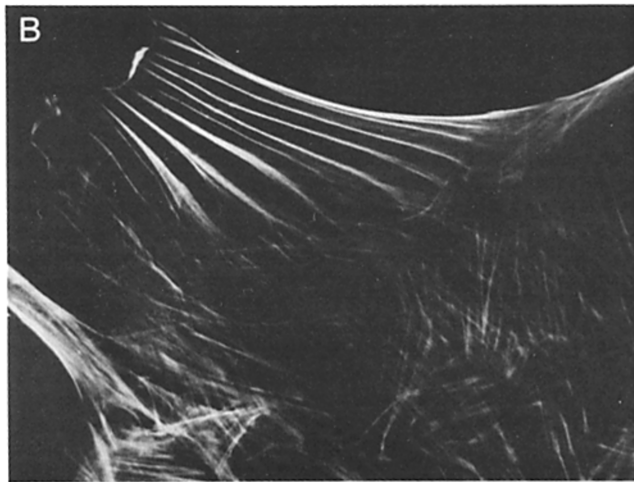


Figure 1. Distribution of the F396 antigen in chick embryo fibroblasts. Chick embryo fibroblasts were double-labeled for indirect immunofluorescence with anti-vinculin antibody and the serum from rabbit F396. (*A* and *D*) Phase-contrast micrographs of the chick embryo fibroblasts. (*B* and *E*) Localization of vinculin by immunofluorescence. Vinculin is concentrated at the termini of stress fibers where the cells are in close contact with the substrate. (*C* and *F*) The immunofluorescent staining pattern obtained with the F396 serum. The serum recognizes a component colocalized with vinculin in the adhesion plaques. In regions of the cell periphery where the stress fibers are well developed, the F396 antigen can be found extending along the actin filament bundles beyond the confines of the focal contact (see, for example, the area noted by the arrow). Bar, 20 μm.





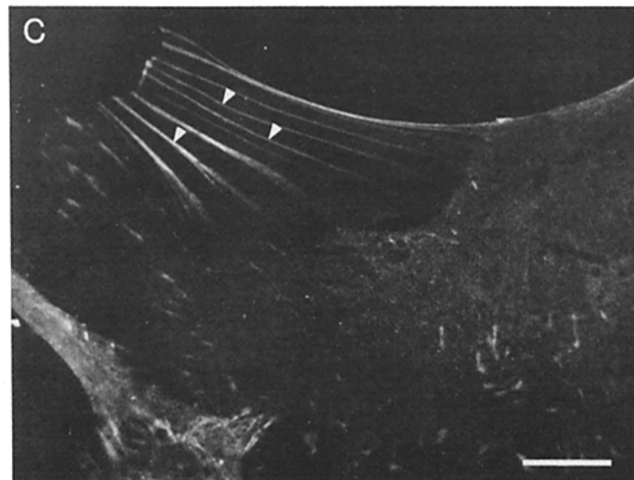
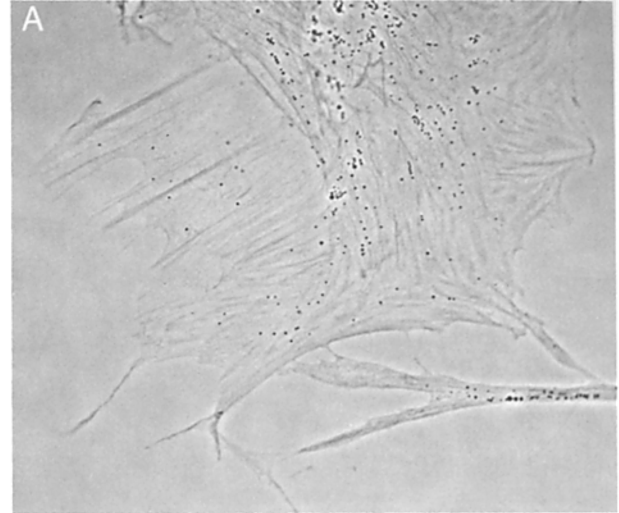
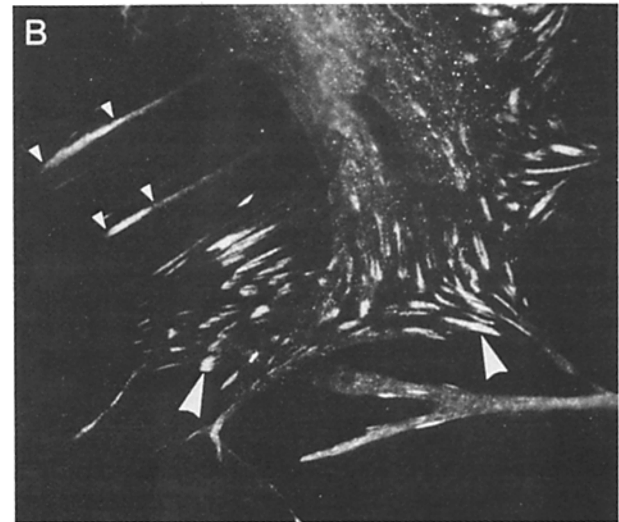


Figure 2. Localization of actin and the F396 antigen. A high magnification view of an area of a chick embryo fibroblast rich in stress fibers. (*A*) Phase contrast. (*B*) Actin distribution as revealed by rhodamine-phalloidin. (*C*) Distribution of the F396 antigen. Where bundles of actin filaments are prominent (*arrows*) the F396 antigen is localized at the focal contact where actin filaments terminate and is also detected along the filament bundle. As the tight actin bundles begin to splay out, the F396 antigen staining also dissipates. Bar, 20 μm.





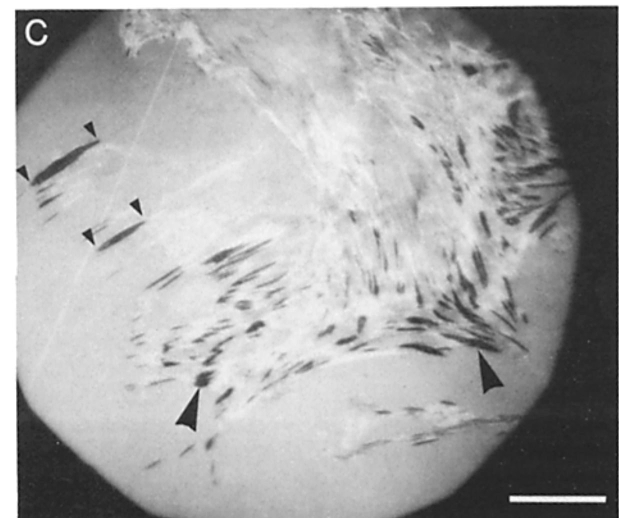


Figure 3. The F396 antigen is found at focal contacts as defined by interference reflection microscopy. (*A*) Phase-contrast micrograph of a region of a chicken embryo fibroblast. (*B*) Indirect immunofluorescence with the F396 serum. (*C*) Interference reflection microscopy. The large arrowheads in *B* and *C* designate regions of coincidence between the interference reflection and fluorescence patterns. However, note that there are some instances when the fluorescence

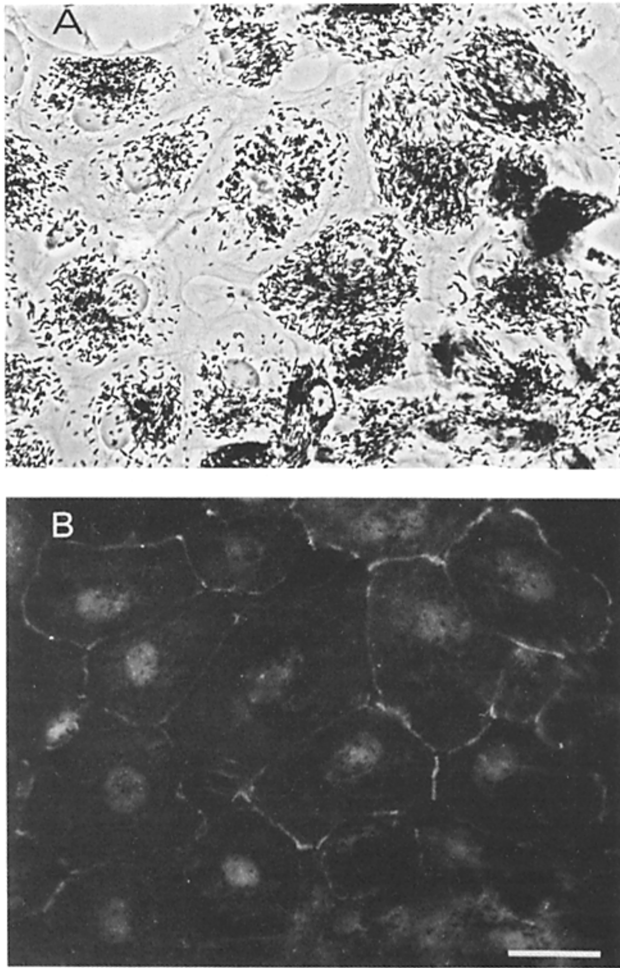


Figure 4. The F396 antigen is localized at regions of cell–cell contact in epithelial cells. (*A*) Phase-contrast micrograph of chick embryo pigmented retinal epithelial cells. (*B*) Indirect immunofluorescence with the F396 serum. A circumferential band of staining in regions of close cell–cell contact is observed above the level of the substrate. Adhesion plaques in these cells are also labeled with the F396 serum; however, they are not visible in this plane of focus. The peripheral membrane staining is specific for regions where cell–cell contact exists; for example, single cells show no such staining. Bar, 20 μm.

scribed above, adhesion plaques are regions of close cell–substrate contact where bundles of actin filaments (stress fibers) terminate near the plasma membrane. The F396 serum, like the anti–vinculin antibody, recognized a component of the adhesion plaque (Fig. 1, *C* and *F*).

One apparent difference in the distribution of vinculin and the F396 antigen in these cells is that the vinculin was restricted to the adhesion plaque, whereas the F396 antigen frequently appeared to extend beyond this region along the actin–filament bundles. Some examples of this distribution are seen in Fig. 1 *B* and at higher magnification in a different cell in Fig. 2. In Fig. 2 the chick embryo fibroblast was double-labeled for indirect immunofluorescence with rhodamine-phalloidin and the F396 serum. Some prominent stress fibers were evident by phase-contrast optics (Fig. 2 *A*), and the actin content of these phase-dense filaments was confirmed by the rhodamine-phalloidin staining (Fig. 2 *B*). The F396 antigen was not restricted to the end of the actin filament bundle, as one would expect for a strictly-defined adhesion plaque constituent (Fig. 2 *C*). Rather, when an actin filament bundle was large and well-defined, the F396 antigen was seen to extend along the stress fiber for a few microns beyond the domain of the focal contact. The distribution of the F396 antigen relative to the adhesion plaque was also analyzed directly by use of interference reflection microscopy. In Fig. 3, the distribution of the F396 antigen was visualized by indirect immunofluorescence (Fig. 3 *B*), and the staining was observed to correlate directly with the location of focal contacts as defined by the intereference reflection image (Fig. 3 *C*). With this approach, too, there are many instances in which the immunoreaction extends beyond the domain of the focal contact (see the figure legend for specific examples).

No staining of adhesion plaques by the F396 serum was observed if the cells were not first permeabilized with an agent such as Triton X-100, indicating that the antigenic determinant, at least, is intracellular. Moreover, incubation of living cells with the F396 serum did not appear to perturb the cells' ability to adhere to the substrate.

In addition to being localized at focal contacts, the F396 antigen was also found at sites where epithelial cells are associated with each other. Specifically, the F396 serum stained a peripheral ring of cell–cell contacts in pigmented retinal epithelial cells (Fig. 4). This staining was found above the substrate and may correspond to the zonula adherens, another region specialized for the attachment of bundles of microfilaments to the membrane. An electron microscopic analysis will be necessary to determine unequivocally whether the F396 antigen is located in the junctional complexes. No staining of cell surface protrusions such as microspikes has been detected in either the epithelial cells or the fibroblasts.

Molecular Identification of the F396 Antigen

In an attempt to identify the protein recognized by the F396 serum in indirect immunofluorescence experiments, Western immunoblot analysis of the unfractionated serum was performed. By this approach, the F396 serum was shown to recognize most prominently a protein with a molecular mass of 82,000 D (Fig. 5). The 82-kD F396 antigen does not appear to be related to any other known component of the adhesion plaque. Specifically I have investigated whether the F396 serum recognizes purified vinculin, talin, α-actinin, or their proteolytic products by the immunoblot method (Fig. 5). The F396 serum did not recognize any of these proteins purified from chicken smooth muscle, nor did it recognize the 80-kD polypeptide that is a frequent contaminant of α-actinin preparations or brush border fimbrin (data not shown). Moreover, previously characterized antibodies prepared against these known adhesion plaque proteins (3, 4, 6) did not recognize the 82-kD protein when they were used in

staining extends beyond the domain of the focal contact. The pairs of small arrowheads indicate some examples of this situation. The small arrowheads bracket focal contacts as delimited by interference reflection optics. In *B*, it is clear in some cases that the fluorescence is not confined to this region but rather extends along the actin filament bundle beyond the strictly defined domain of the adhesion plaque. Bar, 20 μm.

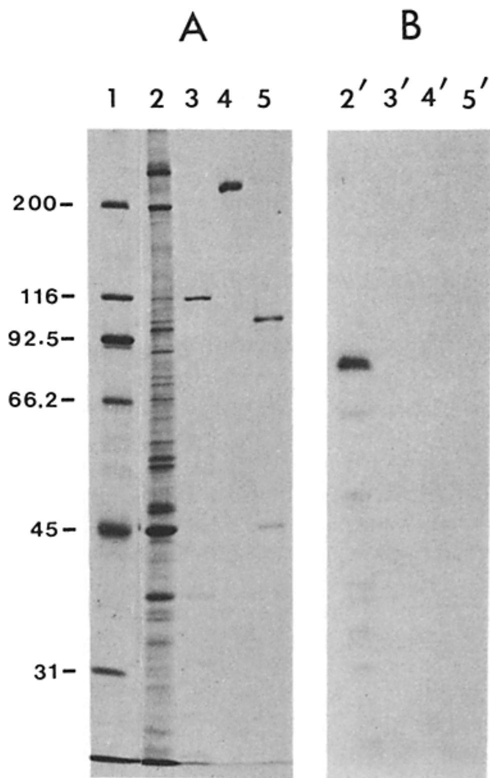


Figure 5. Immunoblot analysis of the F396 serum. (*A*) A 10% SDS polyacrylamide gel of molecular mass standards (lane *1*), total chick embryo fibroblast protein (lane *2*), vinculin (lane *3*), talin (lane *4*), and α-actinin (lane *5*). (*B*) An equivalent gel was transferred to nitrocellulose and probed with the serum followed by radioiodinated goat anti-rabbit IgG. A polypeptide of ~82,000 D is recognized most prominently by the antibody (lane *2′*). Some minor lower molecular weight species are also detected. The antibody does not cross-react with vinculin, talin, or α-actinin purified from chicken smooth muscle.

immunoblots of total chick embryo fibroblast protein (data not shown).

To ascertain whether the 82-kD polypeptide recognized by the F396 serum in the immunoblot was in fact the same material recognized in immunofluorescence experiments and therefore localized at sites of actin–membrane interaction, affinity-purified antibody was prepared. As shown in Fig. 6, the affinity-purified anti-82-kD antibody retained the ability to recognize a component of the adhesion plaque. It is interesting to note that the affinity-purified antibody appeared to be more specific for the adhesion plaque, having lost the capacity to stain the perinuclear components seen with the unfractionated F396 serum (e.g., compare the staining in Fig. 6 with that in Fig. 1).

The F396 antigen was further characterized by performing immunoblots on total chick embryo fibroblast proteins resolved on two-dimensional gels (Fig. 7). By this approach the 82-kD F396 antigen was shown to migrate to a position in the gel that indicated that it has a basic isoelectric point relative to actin (Fig. 7 *B*).

Tissue Distribution of the 82-kD Protein

By immunoblot analysis of 18-d-old chick embryo tissues (Fig. 8), the 82-kD protein was found to be ubiquitous. However, the level of the 82-kD protein detected by the antibody varied depending on the tissue source. Comparison of wet weight equivalent tissue samples indicated that the F396 antigen was most abundant in smooth muscle sources such as gizzard/stomach and intestine. Fibroblasts, the cell type in which the protein was originally localized, exhibited levels of the antigen comparable to those found in the smooth muscle sources. Skeletal and cardiac muscle showed intermediate levels of the 82-kD protein. The lowest levels of the 82-kD polypeptide were observed in samples from brain and liver.

Discussion

This paper reports the identification of an 82-kD protein that is localized at adhesion plaques. The distribution of the 82-kD protein as determined by immunoblot analysis of chick embryo tissues is consistent with the finding that it is seen by indirect immunofluorescence at sites of actin–membrane

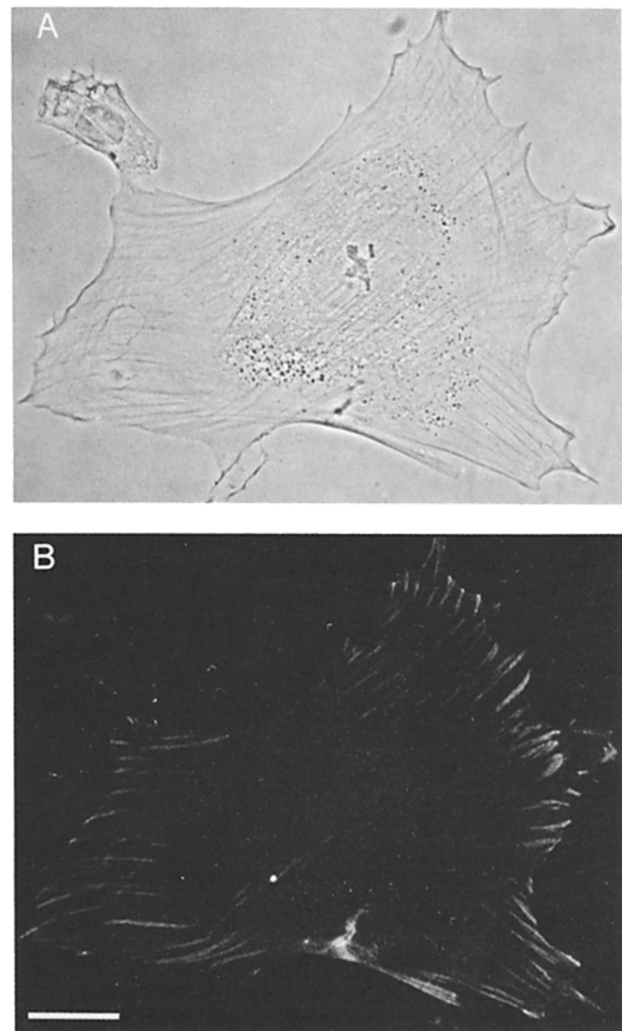


Figure 6. The 82-kD protein is a component of adhesion plaques. The F396 serum was affinity-purified against the electrophoretically isolated 82-kD protein immobilized on nitrocellulose. A chicken embryo fibroblast stained by indirect immunofluorescence with the affinity-purified antibody is shown here in phase-contrast (*A*) and fluorescence (*B*) optics. The anti-82-kD Ig recognizes a component of the adhesion plaques. Bar, 20 μm.

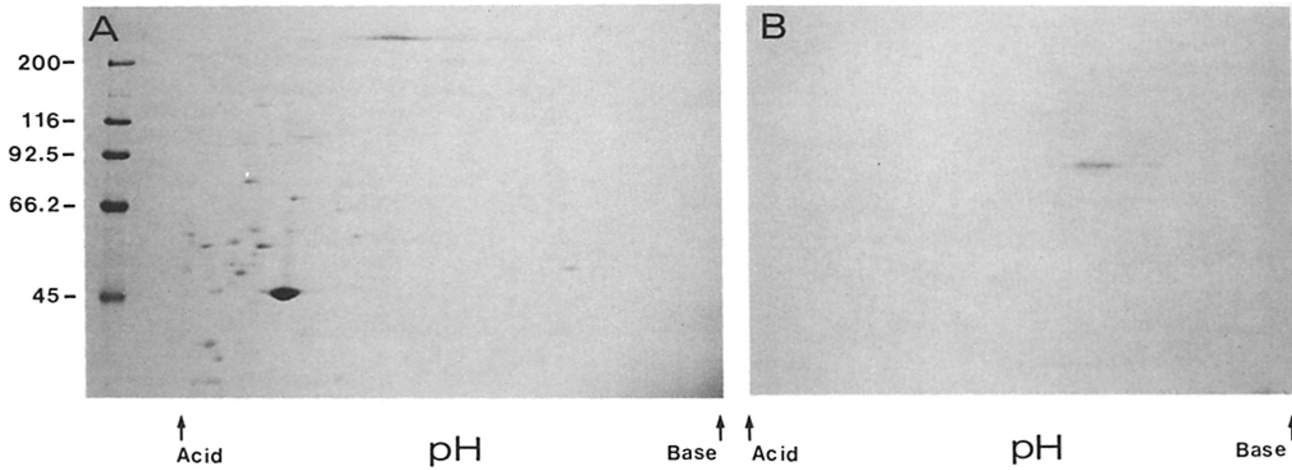


Figure 7. Two-dimensional gel analysis of the 82-kD F396 antigen. Chick embryo fibroblast proteins were resolved by two-dimensional gel electrophoresis. The Coomassie Blue-stained second dimension SDS gel is shown in *A*. The major protein that co-migrates with the 45-kD standard is actin; the abundant protein above the 200-kD standard is fibronectin. To determine the position of the 82-kD protein, a duplicate of the gel shown in *A* was analyzed by the immunoblot method using the F396 nonimmune serum followed by ^{125}I-goat anti-rabbit IgG. The resulting autoradiograph is shown in *B*. The 82-kD polypeptide recognized by the F396 serum has migrated to a position to the right of actin and fibronectin, indicating that it has a more basic isoelectric point. A small, more basic satellite spot is barely detectable at this exposure. No Coomassie Blue-stained protein that co-migrates with the immunoreactive material is detectable.

interaction. In general, the 82-kD polypeptide appears to be most abundant in fibroblasts and smooth muscle sources where sites of actin-membrane interaction are prominent and least abundant in brain and liver, two sources having few organized regions of filament-membrane interaction. Of course, quantitative interpretation of such immunochemical analysis of antigen distribution relies on the validity of the assumption that the antibody recognizes various tissue isoforms of the antigen equally well. What can be concluded without ambiguity is that the 82-kD protein is detected in all the tissues examined. The ubiquitous occurrence of the F396 antigen suggests that it may be of general importance.

The 82-kD polypeptide is not an abundant protein, especially when compared with other adhesion plaque proteins (e.g., vinculin, talin, or α-actinin) isolated from smooth muscle. The low abundance of the 82-kD protein raises the possibility that it represents a regulatory component as opposed to a structural element that requires a higher copy number for function. It is most likely that some regulatory proteins are associated with the adhesion plaque since actin-membrane-substrate interactions are dynamic. However, to date, the mechanism of regulation is completely unknown.

Although a number of proteins localized at adhesion plaques have been characterized, the specific mechanism by which the actin-rich cytoskeleton is linked to the plasma membrane at these sites has not been established. To put the 82-kD protein in some perspective, a highly schematic (and over-

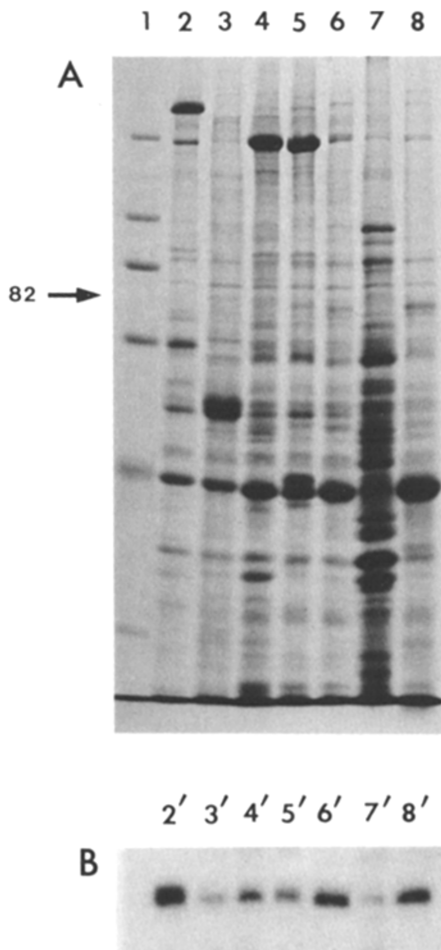


Figure 8. Tissue distribution of the 82-kD component of adherens junctions. (*A*) A 10% SDS polyacrylamide gel showing the polypeptide compositions of a variety of chicken embryo tissues. Lane *1*, molecular mass standards; lane *2*, chick embryo fibroblasts from skin; lane *3*, brain; lane *4*, heart; lane *5*, breast; lane *6*, intestine; lane *7*, liver; lane *8*, gizzard/stomach. (*B*) Autoradiograph of the relevant region of an equivalent gel transferred to nitrocellulose and incubated with the F396 serum followed by ^{125}I-goat anti-rabbit IgG. The immunoblot shows that the 82-kD F396 antigen is found in all tissues, however, most prominently in the smooth muscle-like sources (fibroblast, lane *2′*; intestine, lane *6′*; and gizzard/stomach, lane *8′*). Cardiac (lane *4′*) and skeletal (lane *5′*) muscle contain moderate levels of the 82-kD protein, while brain (lane *2′*) and liver (lane *7′*) exhibit the lowest level of immunoreactive material.

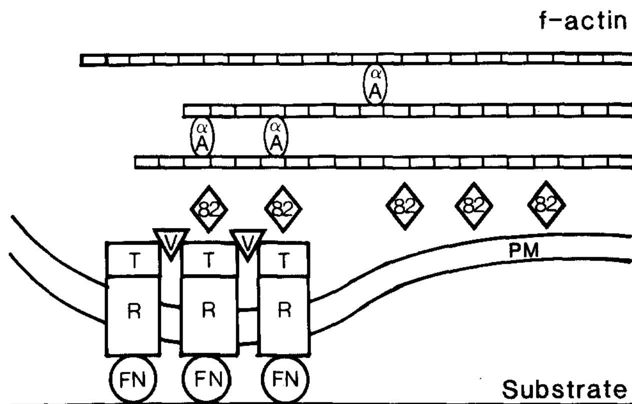


Figure 9. Schematic representation of some of the components localized at sites of actin–membrane–substrate interaction. The diagram shows a simplified outline of the molecular architecture postulated to exist at sites of actin–membrane interaction where a cell associates with the extracellular matrix protein fibronectin. The components that touch each other in the diagram have been shown to interact with each other in biochemical studies. The 82-kD F396 antigen is found in the adhesion plaque proper and also extends along the actin filament outside the focal contact. (See text for more detailed discussion.) *PM*, plasma membrane; *FN*, fibronectin; *R*, fibronectin receptor; *T*, talin; *V*, vinculin; *α-A*, α-actinin; *82*, the 82-kD F396 antigen described in this paper.

simplified) model of the molecular organization at sites of actin–membrane–substrate interaction is shown in Fig. 9. As indicated in the diagram, a cell can associate with an extracellular matrix component such as fibronectin at (or near) the focal contact (9, 34). The fibronectin receptor is a transmembrane glycoprotein complex (19, 24) that has an extracellular binding site for fibronectin (22) in addition to a domain that interacts with the vinculin-binding protein, talin (21). The transmembrane linkage of talin with the extracellular matrix via the fibronectin receptor provides one mechanism by which cytoplasmic components can become functionally coupled to extracellular information. (However, the interactions between a cell and a substrate are clearly much more complex and heterogeneous than outlined here.) Filamentous actin that is anchored to the plasma membrane at the adhesion plaque is associated with a number of actin-binding proteins with α-actinin accumulating at the termini of the filaments (26). The nature of the structural connection between actin filaments and the proteins localized at the adhesion plaque is not clear, but it has been suggested that an association between α-actinin and vinculin could bridge the gap (11). The 82-kD polypeptide described in this paper is localized with vinculin and talin at the adhesion plaque as well as along actin filament bundles adjacent to the region of cell–substrate attachment. The specific function of the 82-kD protein remains to be determined; however, its cytoplasmic distribution illustrates that it is in a position to function in attachment of actin filaments to the plasma membrane or, perhaps, to regulate this membrane–cytoskeletal association.

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