Purification of Mammalian Filamin

SIMILARITY TO HIGH MOLECULAR WEIGHT ACTIN-BINDING PROTEIN IN MACROPHAGES, PLATELETS, FIBROBLASTS, AND OTHER TISSUES*

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We have purified the high molecular weight actin-binding protein, filamin from guinea pig vas deferens. We find this mammalian filamin is very similar to chicken gizzard filamin in subunit molecular weight, amino acid composition, actin-binding properties, immunological cross-reactivity, and the ability to be phosphorylated by cyclic AMP-dependent protein kinase. Anti-filamin antibodies cross-react with a high molecular weight macrophage actin-binding protein, and with a high molecular weight protein in platelets and fibroblasts. Furthermore like filamin, these proteins are also phosphorylated and cyclic AMP stimulates their phosphorylation. Anti-filamin antibodies do not cross-react with the erythrocyte membrane protein spectrin or with high molecular weight proteins in brain extracts. We conclude that filamin from avian and mammalian smooth muscle are very similar proteins and furthermore that many, but not all, non-muscle cells contain a protein closely related to filamin.

Extracts of many vertebrate and invertebrate cell types contain high molecular weight proteins that interact with actin (1-12). Several of these proteins have been purified and the purified proteins have been shown to be actin-binding proteins. Thus, the erythrocyte membrane protein spectrin has been purified by several groups (for review see Ref. 13) and has been shown to bind to actin (6, 7). Stossel and his associates have purified a high molecular weight actin-binding protein (ABP) from alveolar macrophages and leukemic leukocytes (8, 14-16). Filamin is another high molecular weight protein that has been isolated from chicken gizzard (9, 10, 17) and that has been shown to bind to F-actin (10, 18, 19). Filamin has also been identified immunochemically in cultured fibroblasts (9, 20).

The relatedness of these different high molecular weight actin-binding proteins is not clear. To investigate this question we have purified filamin from guinea pig vas deferens and chicken gizzard and compared these proteins with spectrin and the macrophage ABP. We find that both filamin and macrophage ABP have an identical electrophoretic mobility, that anti-filamin antibodies react with the macrophage ABP, and that the macrophage ABP, like filamin, can be phosphorylated in a CAMP-dependent manner. In contrast filamin and spectrin have different electrophoretic mobilities and do not show immunological cross-reactivity. Furthermore we have surveyed other mammalian tissues and find that platelets and fibroblasts contain substantial amounts of filamin and that this filamin can be phosphorylated. In contrast filamin is present in very low concentrations or absent from skeletal and cardiac muscle, from brain, and from erythrocytes.

EXPERIMENTAL PROCEDURES

Materials

Human platelets were obtained from Community Blood and Plasma Service, Baltimore, Md. Outdated human blood was from the National Institutes of Health Blood Bank. Sepharose Cl-4B and cyanogen bromide-activated Sepharose were from Pharmacia, Lyndhurst, N.J. [γ-32P]ATP and [35S] from New England Nuclear, and the IgG fraction of rabbit anti-goat IgG from Cappel Laboratories, Cockranville, Pa.

Methods

Analysis of Tissue Extracts

Preparation of Tissues—Human platelets were collected as follows. One unit of blood bank platelets (prepared from 500 ml of human blood) was used fresh on the day of collection. Residual erythrocytes were removed by centrifugation at 300 × g for 10 min and then platelets were sedimented at 2000 × g for 10 min. Platelets were washed once by resuspending in NaCl/P, to the original volume and resedimentation.

Human erythrocyte ghosts were prepared from outdated anticoagulated blood as follows. Erythrocytes were sedimented by centrifugation at 1500 × g for 5 min. The packed cells were then washed four times by resuspending in NaCl/P, to the original volume and centrifugation at 500 × g for 5 min. The erythrocytes were then lysed by resuspending packed cells in 20 volumes of a solution which contained 10 mm Tris-HCl, pH 7.4, and 1 mm MgCl2. After 30 min at 0°C the suspension was centrifuged at 20,000 × g for 10 min. This washing was repeated twice more and a final pellet of erythrocyte ghosts was recovered.

Rabbit alveolar macrophages were collected and purified as described by Stossel and Hartwig (8).

The abbreviations used are: ABP, actin-binding protein; NaCl/P, phosphate-buffered saline; SDF, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; anti-GP filamin antibody, anti-guinea pig filamin antibody.

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Cultured fibroblasts were normal rat kidney fibroblasts originally obtained from Dr. E. Scdnick of the National Institutes of Health, subcloned in our laboratory, and grown under conditions previously described (20).

For the preparation of solid tissues such as skeletal muscle, cardiac muscle, vas deferens, and brain, guinea pigs of the Hartley or NIH strain weighing 300 to 600 g were lightly anesthetized with ether and killed by cardiac excision. Tissues were rinsed in NaCl/P., and then were minced. The minced tissues were solubilized by pulverizing the frozen tissue in a cooled stainless steel mortar, followed by homogenization in a Teflon-glass homogenizer in 2% SDS, 20 mM Tris-HCl, pH 8.9. The homogenate was adjusted to a final concentration of 2% SDS, 10% (v/v) glycerol, 0.1 M diithiobietral, and kept at 10°C, a solution of 6 M urea, 50 mM potassium phosphate, pH 7.3, 1 mM EDTA, 1 mM dithiothreitol, 0.02% bromphenol blue, and 20 mM Tris-HCl, pH 8.5. Samples were heated at 100°C for 2 min and then applied in SDS-polyacrylamide gels.

(ii) SDS-gel Electrophoresis Conditions. The electrophoresis was done on slab gels either with a continuous Tris/glycine buffer system (21) or with a discontinuous buffer system which was a slight modification of a system described by Laemmli (22, 23). Protein staining was done in a solution of 0.05% Coomassie brilliant blue in 50% (v/v) methanol, 10% (v/v) acetic acid. Destaining was done in 10% acetic acid. Autoradiography was done after vacuum drying the gel onto filter paper. The proteins used as standards for molecular weight estimation in gel electrophoresis were: chicken gizzard filamin, Mr = 250,000; heavy chain of rabbit skeletal muscle myosin, M, = 200,000; and a subunit of Escherichia coli RNA polymerase, Mr = 165,000 and 155,000; phosphorylase a, M, = 92,000; bovine serum albumin, Mr = 68,000; and ovalbumin, Mr = 42,000.

(iii) Immunoprecipitation of Tissue Extracts. Tissues were disrupted by homogenization (fibroblasts and vas deferens) or by brief (15 s) sonication (platelets and macrophages) in a solution containing 0.5 M sucrose, 50 mM potassium phosphate, pH 7.3, and 1 mM PMSF. The homogenate was centrifuged at 60,000 x g for 30 min and the supernatant was used for immunoprecipitation. The supernatant was adjusted to a final concentration of 2% SDS and 0.1 M diithiobietral.

For the preparation of tissue extracts for SDS-gel electrophoresis. The solid tissue was solubilized by pulverizing the frozen tissue in a cooled stainless steel mortar, followed by homogenization in a Teflon-glass homogenizer in 2% SDS, 20 mM Tris-HCl, pH 8.9. The homogenate was adjusted to a final concentration of 2% SDS, 10% (v/v) glycerol, 0.1 M diithiobietral, and kept at 10°C, a solution of 6 M urea, 50 mM potassium phosphate, pH 7.3, 1 mM EDTA, 1 mM dithiothreitol, and 0.02% bromphenol blue, and 20 mM Tris-HCl, pH 8.5. Samples were heated at 100°C for 2 min and then applied in SDS-polyacrylamide gels.

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Phosphorylation of Tissue Extract — (i) Preparation of Tissue Extracts. The procedure for determination of protein phosphorylation in tissue homogenates was similar to that of Casnelli and Greengard (24). Guinea pig vas deferens were collected in an ice cold solution of 0.44 M sucrose, 10 mM sodium acetate, pH 6.5, and 1 mM PMSF. Extraneous tissues and semen were removed, the tissue was rinsed with the above solution and then with a homogenization buffer: 4 mM EDTA, pH 7.3, 5 mM diithiobietral, and 1 mM PMSF. Vas deferens from 50 guinea pigs (8.8 g wet weight) were minced and homogenized in a glass Teflon homogenizer in 64 ml of homogenization buffer.

Salt Extraction and Ammonium Sulfate Fractionation — The homogenate was centrifuged at 60,000 x g for 30 min and the supernatant discarded. The pellet was then suspended in 64 ml of 0.6 M KCl, 0.05 mM potassium phosphate, pH 7.3, 1 mM EDTA, 1 mM diithiobietral, 1 mM PMSF and homogenized intermittently for 10 min at 0°C in a Teflon-glass homogenizer. This extract was centrifuged 60,000 x g for 30 min and the precipitate discarded. The salt extract was dialyzed 2.5 h against 3 liters of 0.1 M KCl, 0.05 mM potassium phosphate, pH 7.3, 1 mM EDTA, 1 mM diithiobietral (Solution A). The precipitate that formed was removed by centrifugation at 60,000 x g for 30 min. The filamin was precipitated from the supernatant by addition of ammonium sulfate to a final concentration of 35%. A pH of 7.0 to 7.5 was maintained with 1 M KH2PO4. The pellet was collected by centrifugation at 25,000 x g for 20 min and then resuspended in 16 ml of Solution A. After dialysis for 1 h against Solution A and centrifugation at 60,000 x g for 30 min to remove insoluble material, the ammonium sulfate precipitation was repeated. The second ammonium sulfate pellet was solubilized in 5 ml of Solution A and then the KCl concentration adjusted to 0.6 M with 3 M KCl. Insoluble residue was then removed by centrifugation at 60,000 x g x 30 min and dialyzed against 0.6 M NaCl/P. and 1 mM diithiobietral.

Chromatography on Sepharose 4B — The sample was chromatographed on a column (1.6 x 100 cm) of Sepharose CL-4B equilibrated with 0.6 M KCl, 0.05 mM potassium phosphate, pH 7.3, 1 mM EDTA, and 1 mM diithiobietral. The elution profile was similar to that of the chicken gizzard extract (10) and filamin was eluted as a broad peak between 90 and 110 ml.

DEAE-cellulose Chromatography — The pooled fraction from the Sepharose column were dialyzed 2 h against 50 mM potassium phosphate, pH 7.3, 1 mM EDTA, 1 mM diithiobietral and then applied to a column of 1 ml of DEAE-cellulose. The column was washed with 10 ml of pH 7.3, 10 mM sodium phosphate, pH 7.3, with 50 mM of the salt extract eluted as the major peak between 0.1 M and 0.3 M KCl. The elution of guinea pig vas deferens filamin required somewhat higher KCl concentrations than that of the chicken gizzard filamin chromatographed under identical conditions. The pooled eluate from this column contained 4.5 mg of pure filamin.

Preparation of Anti-filamin Antibody

Immunization — Prior to immunization, guinea pig vas deferens filamin was further purified by preparative SDS-gel electrophoresis. A goat was immunized with 100 mg of vas deferens filamin every 3 weeks for three immunizations. The first and second injections included Freund’s complete adjuvant; the third contained incomplete adjuvant. Serum was collected 2 weeks after the last injection.

Purification of Anti-filamin Antibody— γ Globulin was precipitated from serum by adding ammonium sulfate to 30% saturation. The globulin was washed three times with a solution of 45% saturated ammonium sulfate in NaCl/P., and then solubilized in NaCl/P. containing 1 mM PMSF, dialyzed against NaCl/P., to remove the ammonium sulfate, and centrifuged at 12,000 x g for 10 min to remove any remaining precipitate.

Samples of the guinea pig filamin in 0.5 M NaCl and 0.1 M sodium borate, pH 8.0, were covalently bound to cyanogen bromide-activated Sepharose. After removal of nonbound material the Sepharose was packed in columns and equilibrated with NaCl/P. Samples of anti-filamin globulin were then applied and incubated with the immobilized filamin column for 2 h at 4°C. Unbound globulin was removed by washing with NaCl/P. and by two cycles of washing with a
Actin-binding Assay

Analytical Methods

RESULTS

Purification of Guinea Pig Vas Deferens Filamin

An initial objective of these studies was to prepare antisera to mammalian filamin. We therefore purified filamin from mammalian smooth muscle. Following a procedure similar to that reported for the purification of chicken gizzard filamin (10) we have been able to obtain highly purified guinea pig vas deferens filamin (Fig. 1). As shown in Fig. 1, Lane 1, guinea pig vas deferens contains substantial amounts of filamin (upper arrow). Sequential steps of high salt extraction (Lane 2), ammonium sulfate fractionation (Lane 3), gel filtration chromatography (Lane 4), and ion exchange chromatography (Lane 5) resulted in a preparation of highly purified filamin. Starting from 8.8 g of fresh tissue the procedure yielded 4.5 mg of purified protein.

Comparison of Guinea Pig Vas Deferens Filamin and Chicken Gizzard Filamin

Having purified a mammalian filamin we compared its properties with chicken gizzard filamin. As shown in Fig. 2, extracts of guinea pig vas deferens (Lane A) are very similar to extracts of chicken gizzard (Lane B) when analyzed by SDS-polyacrylamide gel electrophoresis. In particular the electrophoretic mobility of the filamin in the two tissues is comparable. Furthermore purified chicken gizzard filamin and guinea pig vas deferens filamin co-electrophorese and mixtures of the two proteins cannot be separated by SDS-polyacrylamide gel electrophoresis (data not shown). In addition their amino acid compositions are very similar (Table I).

To determine whether mammalian filamin was an actin-binding protein we examined its interaction with skeletal muscle F-actin (Fig. 3). In the absence of F-actin at 30 mM KCl vas deferens filamin was not sedimented by centrifugation at 100,000 x g for 30 min (Fig. 3, Group A). However if F-actin and filamin were combined in the presence of 30 mM solution containing 0.5 mM NaCl and 0.1 mM sodium borate, pH 8.6; followed by washing with a solution containing 0.5 mM NaCl and 0.1 mM sodium acetate, pH 4.8. The bound antibody was then dissociated from the column with a solution of 0.5 mM NaCl, 0.2 mM acetic acid. The eluted protein was immediately neutralized with 2 mM Tris-Cl, pH 8.0 (0.5 ml to each 1 ml of eluted protein). The purified antibody was concentrated by ammonium sulfate precipitation and frozen in aliquots at concentrations of 2 mg/ml.

Induction of Anti-filamin Antibody—Purified anti-filamin antibody was labeled with 125I as follows: 4 mCi of 125I in 0.1 M NaOH were neutralized with excess potassium phosphate buffer, pH 7.5, and incubated for 45 s with a solution containing 1.2 mg of the affinity-purified anti-guinea pig filamin antibody and 5 mg of chloramine-T. The reaction was carried in 1 ml of NaCl/P, The iodination was stopped by adding 0.5 ml of 40 mg/ml of sodium metabisulfite. Carrier sodium iodide was added (50 µl of 1 M solution) and the antibody was freed from free iodide on a column of Bio-Gel P2 which had been prewashed with NaCl/P, containing 1 mg/ml of bovine serum albumin. The labeled antibody (1.5 x 10^8 cpm/µg of protein) was diluted in 10 ml of a solution of 1 mg/ml of bovine serum albumin and 1 mM PMSF in NaCl/P, and frozen.

Gel Localization of Filamin with 125I-Anti-filamin Antibody—A method of reacting 125I-labeled antibodies with proteins directly in acrylamide gels was previously described by Burridge (25). We introduced several changes in the method in order to increase its sensitivity and to decrease nonspecific binding of the antibody. Coomassie blue stain was removed from the gel with a solution of 25% isopropanol alcohol, 10% acetic acid. The gel was then incubated in 0.1 M NaCl, 0.05 M sodium phosphate buffer, pH 8.0, and 0.1% sodium azide in order to neutralize the acetic acid. It was then soaked in 1 h in 1 mg/ml of bovine serum albumin, 1 mg/ml of γ-globulin prepared from a nonimmunized goat, 0.1% sodium azide, and 1 mM PMSF in NaCl/P. The gel was then incubated for an additional 24 h in this same solution in which 1 µg/ml of labeled antibody (2 x 10^6 cpm/ml) was included. Nonspecifically bound antibody was then removed by several washes: three washes for 1 h each in NaCl/P, one wash for 2 h in NaCl/P plus 1% Triton X-100, and one wash for 6 h with NaCl/P plus 1% Triton X-100 and 3 mM urea. Sodium azide (0.1%) was included in the washing solutions. The gel was then stained, dried, and autoradiographed. The inclusion of carrier γ-globulin with the labeled antibody greatly decreased nonspecific binding of the antibody to proteins on the gel.

Other Proteins

Chicken gizzard filamin was purified as previously described (10) with previously reported modifications (19).

Rabbit skeletal muscle F-actin was purified from an acetone powder of rabbit skeletal muscle according to the procedure of Spudich and Watt (26).

Actin-binding Assay

Guinea pig vas deferens filamin (10 µg) was combined with rabbit skeletal muscle F-actin (50 µg) in a 100 µl of a solution containing 10 mM potassium phosphate, pH 6.0, 1 mM ATP, 2 mM MgCl₂, 0.1 mM dithiothreitol, and KCl at either 20, 150, or 600 mM final concentration. Samples were incubated 10 min at 23° and then centrifuged at 100,000 x g for 30 min. The pellet was dissolved in SDS-gel mixture (see "SDS-Gel Electrophoresis"). The supernatant was precipitated by addition of trichloroacetic acid to 10% final concentration, trichloroacetic acid was then neutralized and the precipitated protein dissolved in gel mixture. Aliquots (25%) of pellet and supernatant were then heated 100°C for 2 min and submitted to SDS-gel electrophoresis.

Analytical Methods

Protein was estimated with the Lowry reagent (27) using bovine serum albumin as a standard. Amino acid analysis was done as previously described (10).
TABLE I

| Amino acid   | Guinea pig vas deferens filamin (10) | Chicken gizzard filamin (10) | Rabbit macrophage ABP (15) | mol/100 mol amino acid |
|--------------|-------------------------------------|------------------------------|---------------------------|-----------------------|
| Aspartic acid| 9.2                                 | 7.6                          | 8.7                       |                       |
| Threonine    | 6.4                                 | 5.7                          | 6.2                       |                       |
| Serine       | 6.8                                 | 6.4                          | 6.8                       |                       |
| Glutamic acid| 10.8                                | 9.4                          | 11.4                      |                       |
| Proline      | 8.7                                 | 8.4                          | 7.1                       |                       |
| Glycine      | 12.9                                | 12.9                         | 11.8                      |                       |
| Alanine      | 7.7                                 | 9.2                          | 7.4                       |                       |
| Valine       | 9.5                                 | 11.5                         | 8.5                       |                       |
| Methionine   | 1.1                                 | 1.0                          | 1.3                       |                       |
| Isoleucine   | 4.1                                 | 3.1                          | 4.4                       |                       |
| Leucine      | 6.9                                 | 5.7                          | 6.2                       |                       |
| Tyrosine     | 2.7                                 | 1.9                          | 3.1                       |                       |
| Phenylalanine| 2.2                                 | 2.7                          | 3.2                       |                       |
| Lysine       | 6.4                                 | 5.2                          | 6.0                       |                       |
| Histidine    | 2.1                                 | 2.2                          | 2.2                       |                       |
| Arginine     | 3.5                                 | 5.5                          | 4.1                       |                       |

KCl, all the filamin sedimented with the actin (Fig. 3, Group B). If the KCl concentration was increased to 150 mM KCl (Fig. 3, Group C) or 600 mM KCl (Fig. 3, Group D) less filamin sedimented with the actin. Thus, like chicken gizzard filamin, mammalian filamin binds to F-actin and this interaction can be reduced by high concentrations of KCl (10, 18).

Immunologic Similarity of Mammalian and Avian Filamins —Having purified guinea pig vas deferens filamin and demonstrated its similarity to chicken gizzard filamin, we prepared an antibody to guinea pig filamin. The anti-GP filamin antibody was purified by affinity chromatography and

![FIG. 2. SDS-gel electrophoresis of extracts of cells and tissues. Coomassie blue-stained pattern obtained after electrophoresis of extracts of cells and tissues on a 4 to 18% gradient acrylamide gel with a discontinuous buffer system. Extracts prepared as described under “Experimental Procedures.” Lane A, guinea pig vas deferens (25 μg); Lane B, chicken gizzard (25 μg); Lane C, rabbit alveolar macrophages (20 μg); Lane D, human erythrocyte ghosts (25 μg); Lane E, human platelets (50 μg); Lane F, cultured rat fibroblasts (40 μg); Lane G, guinea pig brain (cerebrum) (60 μg); Lane H, guinea pig skeletal muscle (50 μg); Lane I, guinea pig cardiac muscle (ventricular) (40 μg). Numbers on left indicate molecular weight of marker proteins x 10^3. F, M, and A stand for filamin, heavy chain of myosin, and actin, respectively.](http://www.jbc.org/)

![FIG. 3. Co-sedimentation of actin and guinea pig vas deferens filamin, effect of KCl. Filamin alone or filamin plus rabbit skeletal muscle F-actin were incubated as described under “Experimental Procedures” in varying concentrations of KCl. Samples were centrifuged 100,000 × g for 30 min at 23°C and then pellet (PEL) and supernatant (SUP) were solubilized in SDS solution and applied to a 5% polyacrylamide gel. Electrophoresis was with a continuous buffer system. Proteins were detected by Coomassie blue staining. A, filamin alone; B, filamin and F-actin, 50 mM KCl; C, filamin and F-actin, 150 mM KCl; D, filamin and F-actin, 600 mM KCl; F-arrow, filamin; A-arrow, actin.](http://www.jbc.org/)
the specificity of the antibody checked by immunoprecipitation (Fig. 4). As shown in Fig. 4, Group A, anti-GP filamin antibody quantitatively immunoprecipitated the filamin from an extract of guinea pig vas deferens (Fig. 4, Group A). Furthermore no other vas deferens proteins were immunoprecipitated by the antibody. The specificity of the anti-GP filamin antibody is also demonstrated by the gel localization technique (Fig. 5, Group A). When acrylamide gels containing an extract of guinea pig vas deferens were exposed to $^{125}$I-anti-GP filamin, the filamin band was the only protein to bind anti-filamin antibody.

We next tested the cross-reactivity of guinea pig vas deferens filamin and chicken gizzard filamin (Fig. 6). As shown in Fig. 6, anti-GP filamin antibody immunoprecipitated both

![Fig. 4. Immunoprecipitation of tissue extracts with anti-guinea pig filamin antibodies. Extracts of various cells and tissues were incubated with either pre-immune globulin or anti-guinea pig filamin antibody as described under "Experimental Procedures." The whole extract (Lane 1), material immunoprecipitated with pre-immune globulin (Lane 2), or material immunoprecipitated with anti-guinea pig filamin antibody (Lane 3) were then subjected to SDS-gel electrophoresis on 5% acrylamide gels with a continuous buffer system. Proteins were detected by Coomassie blue staining. Group A, extract from guinea pig vas deferens; Group B, extract from rabbit alveolar macrophage; Group C, extract from human platelets; Group D, extract from cultured rat fibroblasts; F-arrow, filamin; M-arrow, myosin heavy chain; H-arrow, immunoglobulin heavy chain; A-arrow, actin; L-arrow, immunoglobulin light chain.](http://www.jbc.org/)

![Fig. 5. Gel localization of extracts of cells and tissues with $^{125}$I-anti-guinea pig filamin. Extracts of cells and tissues prepared as described under "Experimental Procedures" were electrophoresed on 5% polyacrylamide gels in a continuous buffer system. Gels were then either stained with Coomassie blue (Protein) or with $^{125}$I-anti-guinea pig filamin antibody followed by autoradiography ($^{125}$I) as described under "Experimental Procedures." Group A, extract of guinea pig vas deferens (25 μg); Group B, extract of rabbit alveolar macrophage (20 μg); Group C, extract of human erythrocyte ghosts (20 μg); Group D, extract of human platelet (10 μg); Group E, extract of cultured rat fibroblast (20 μg); Group F, extract of guinea pig brain (cerebrum) (60 μg); arrows, the location of filamin, myosin, and actin.](http://www.jbc.org/)
FIG. 6. Cross-reaction of guinea pig vas deferens filamin and chicken gizzard filamin. Group A, purified guinea pig filamin (10 μg) was incubated with preimmune globulin (Lane 1), anti-guinea pig filamin antibody (Lane 2), or anti-chicken gizzard filamin antibody (Lane 3). Group B, purified chicken gizzard filamin (10 μg) was incubated with preimmune globulin (Lane 1), anti-guinea pig filamin (Lane 2), or anti-chicken gizzard filamin antibody (Lane 3). Incubations and immunoprecipitations were carried out as described under "Experimental Procedures." Immunoprecipitates were subjected to SDS-gel electrophoresis on 5% polyacrylamide gels in a continuous buffer system followed by Coomassie blue staining. Arrows indicate filamin, immunoglobulin heavy chain, and immunoglobulin light chain.

The relationship of filamin to macrophage actin-binding protein (ABP) – Stossel and Hartwig have reported that extracts of macrophages contain a major protein (ABP) with an electrophoretic mobility slower than the heavy chain of myosin. As shown in Fig. 2, Lane C, upper band, the electrophoretic mobility of ABP is very similar to that of filamin. Furthermore, the amino acid composition of guinea pig vas deferens filamin is very similar to that reported by Stossel and Hartwig for rabbit alveolar macrophage ABP. We also used the antibody to guinea pig vas deferens filamin to investigate the similarity of filamin to ABP. As shown in Fig. 4, Group B, anti-GP filamin antibody specifically immunoprecipitated all the ABP from extracts of macrophages. Furthermore, no other macrophage proteins were immunoprecipitated. An alternative demonstration of the cross-reactivity of ABP and filamin is shown by the gel localization technique. As shown in Fig. 5, Group B, ABP binds 125I-anti-GP filamin antibodies and no other proteins in the macrophage extract cross-react with filamin. Thus macrophage ABP clearly cross-reacts with anti-GP filamin antibodies. Since filamin is a phosphoprotein we wished to determine whether macrophage ABP can be phosphorylated. As shown in Fig. 7, Group B, incubation of extracts of macrophages with [γ-32P]ATP leads to 32P labeling of ABP, and cAMP markedly stimulated this phosphorylation (Fig. 7, Group B, Lanes 2 and 3).

Relationship of filamin to human erythrocyte spectrin – Erythrocyte membranes contain spectrin, a high molecular weight actin-binding protein. Spectrin is composed of two high molecular weight polypeptides. As shown in Fig. 2, Lane D, these two high molecular weight polypeptides (the major Coomassie blue-stained bands between 250,000 and 200,000) have an electrophoretic mobility greater than filamin. Furthermore, gel localization of extracts of erythrocyte ghosts...
with anti-GP filamin antibody revealed no binding of anti-filamin antibodies to spectrin (Fig. 5, Group C). No other proteins in erythrocyte ghosts reacted with anti-GP filamin antibodies. Thus filamin and spectrin are not immunologically related.

Relationship of Filamin to Platelet High Molecular Weight Proteins—Human platelets contain three major proteins with electrophoretic mobilities between 250,000 and 300,000 (Fig. 2, Lane E). The lower of these bands has the same mobility as myosin heavy chain and is presumably platelet myosin. The upper of these bands has an electrophoretic mobility identical to filamin (Fig. 2, Lane E). To determine whether human platelets contain filamin, extracts of platelets were immunoprecipitated with anti-GP filamin antibodies. As shown in Fig. 4, Group C, anti-GP filamin antibodies quantitatively and specifically immunoprecipitated the uppermost of the platelet high molecular weight proteins, indicating this protein is filamin. The gel localization technique confirmed this observation (Fig. 5, Group D). Furthermore as in other tissues, no other proteins reacted with the anti-filamin antibody.

Since filamin and the related ABP are phosphoproteins, and since cAMP probably plays an important role in platelet function, it was of interest to determine whether platelet filamin could be phosphorylated. As shown in Fig. 7, Group C, extracts of platelets incubated with $\gamma^{32}P$-ATP phosphorylated filamin and cAMP greatly stimulated this phosphorylation (compare Fig. 7, Group C, Lanes 2 and 3). Furthermore this protein binds $\gamma^{32}P$-anti-GP filamin and is the only fibroblast protein to do so (Fig. 5, Group E).

Comparison of Filamin and Spectrin—Several lines of evidence suggest that filamin and spectrin are unrelated proteins. Wang et al. (9) have demonstrated the differences in electrophoretic mobility of chicken gizzard filamin and human spectrin and we have observed similar differences with mammalian smooth muscle and non-muscle filamins. Furthermore, anti-filamin antibodies do not cross-react with human erythrocyte spectrin. Also studies with anti-spectrin antibodies (28, 29) have failed to detect spectrin in tissues rich in filamin such as platelets and fibroblasts. Therefore, the evidence seems to suggest that although filamin and spectrin are both high molecular weight proteins that interact with actin, they are unrelated molecules.

Variation in Filamin Distribution—The content of filamin in different cell types and tissues is quite variable. Thus, filamin is a major constituent of smooth muscle and of platelets and is present in substantial amounts in macrophages and fibroblasts. However, filamin is either completely absent from or present in very low amounts in other tissues such as brain, skeletal and cardiac muscle and erythrocytes.
Since the function of filamin in cells is unclear, the significance of these differences is not yet understood. Any conclusion on the role of filamin in cell function will have to take into account this marked tissue specificity.

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