Human β-Filamin Is a New Protein That Interacts with the Cytoplasmic Tail of Glycoprotein Ibα*

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We have cloned and sequenced a 9.4-kilobase cDNA specifying a new 280-kDa protein interacting with the cytoplasmic tail of glycoprotein (Gp) Ibα and showing considerable homology to actin-binding protein 280 (ABP-280) and chicken retinal filamin. We term this protein human β-filamin. The gene for β-filamin localizes to chromosome 3p14.3-p21.1. β-Filamin mRNA expression was observed in many tissues and in cultured human umbilical vein endothelial cells (HUVECs); only minimal expression was detected in platelets and the megakaryocytic cell line CHRF-288. Like ABP-280, β-filamin contains an NH2-terminal actin-binding domain, a backbone of 24 tandem repeats, and two “hinge” regions. A polyclonal antibody to the unique β-filamin first hinge sequence identifies a strong 280-kDa band in HUVECs but only a weak band in platelets, and stains normal human endothelial cells in culture and in situ. We have confirmed the interaction of β-filamin and GpIbo in platelet and HUVEC lysates. In addition, using two-hybrid analysis with deletion mutants, we have localized the binding domain for GpIbα in β-filamin to residues 1862-2148, an area homologous to the GpIbα binding domain in ABP-280. β-Filamin is a new member of the filamin family that may have significance for GpIbα function in endothelial cells and platelets.

Glycoprotein (Gp) Ibα, GpIβ, GpIX, and GpV are all members of the leucine-rich glycoprotein superfamily and form a complex in the platelet membrane (1). All four polypeptides of the GpIb complex are also present in human endothelial cells. We term this new protein human β-filamin, reserving the term α-filamin for ABP-280.

EXPERIMENTAL PROCEDURES

Two-hybrid Library Screening and Full-length cDNA Cloning—A DNA fragment coding the cytoplasmic tail of GpIbα between residues 1633 and 1976 (11) was amplified from human genomic DNA (because the GpIbα gene has no introns in this area), directionally cloned in-frame into the yeast expression vector pAS2–1 (CLONTECH), and confirmed by sequencing. This vector expresses a fusion protein consisting of the cytoplasmic tail of GpIbα and the yeast GAL4 DNA-binding domain.

cDNA libraries (CLONTECH) derived from human bone marrow (in pGAD10) and human placenta (in pACT2) were screened in the yeast two-hybrid system. Yeast strain CG-1945 was transformed sequentially with the GpIbα tail vector and the library vectors. Transformants were plated on SD/Trp-/Leu-/His- plates, and incubated at 30 °C until colonies appeared. β-Galactosidase activity of each colony was determined qualitatively in a filter-lift assay. In order to exclude false positives, positive clones were re-introduced into yeast together with either the GpIbα bait vector, the pAS2–1 original vector or pLAM5*, a vector encoding a human lamin cDNA in the two-hybrid DNA-binding domain vector pGBT9. To obtain the remaining 5′ cDNA, a human placenta 5′-STRETCH cDNA library in agt10 (CLONTECH) was screened as described under “Results.”

Isolated cDNA inserts were transferred to the sequencing vector pBluescript (Stratagene, La Jolla, CA), and both strands were sequenced using universal primers or synthetic oligonucleotides. Sequencing was performed using Dydeoxy Terminator Cycle Sequencing kits and a model 377 DNA sequencing system (Applied Biosystems, Foster City, CA).

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**Sequence Analysis**—We used the BLAST computer program to search the GenBank data base for expressed sequence tags (ESTs) and sequence-tagged sites (STSs) containing sequences identical to those in β-filamin cDNA. The UNIGENE program was then used to group ESTs and STSs.

**Northern Blot Hybridization and RT-PCR**—A human multiple tissue blot was purchased from CLONTECH, and a blot of HUVECs and CHRF-288 cell poly(A)RNA was kindly supplied by Dr. Barbara Konkle, Cardeza Foundation, Jefferson Medical College. β-Filamin mRNA expression was detected with a 2.6-kb probe from the 5’ end of cDNA clone THC-106 (Fig. 1). ABP-280 mRNA expression was detected with a 2.7-kb (at 5065–303 end) ABP-280 cDNA clone isolated from the bone marrow library. To confirm the quality of the blots, we used β-actin as a probe.

For RT-PCR, total RNA from platelets, HUVECs, or the HEL and CHRF-288 cell lines were isolated using Triazol (Life Technologies, Inc.). Each first-strand cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase (Seikagaku America, Rockville, MD) with oligo(dt). PCR was performed using ExTaq polymerase (Takara, Madison, WI) with a sense primer (5’TGATAAGGGCAAAAAGGC-3’) (nt 7065–7082) and an antisense primer (5’GCCACCTGTATTGTGACAC-3’) (nt 7459–7506). These sequences were chosen to amplify β-filamin and not ABP-280. The 442-bp cDNA fragment obtained was sequenced in order to confirm its origin from β-filamin mRNA.

**Procedure for Yeast Two-hybrid and Western Blotting**—Because the first “hinge” region in β-filamin differs completely from that in ABP-280 (Fig. 4), we synthesized a peptide (TIDGETVAVEAPVNCPPG) corresponding to that sequence and coupled it to activated keyhole limpet hemocyanin or to activated bovine serum albumin (BSA) (Pierce). The keyhole limpet hemocyanin peptide was used as immunogen. Rabbits were injected every 2–3 weeks, and serum titers were measured on the BSA-peptide-coated ELISA plates. IgG fractions were isolated from serum using protein G-Superose (Amersham Pharmacia Biotech) and affinity-purified on BSA-peptide Sepharose columns.

HUVECs were harvested from human umbilical cords and cultured essentially as described (2). HUVECs were washed with PBS three times before detachment in lifting buffer (0.01 M phosphate, 0.15 M NaCl, 5 mM NaHCO₃, 10 mM EDTA, 5 mM EGTA, 0.1% BSA, 1 mM phenylmethylsulfonyl fluoride, pH 7.2). HUVECs were centrifuged at 250 g for 10 min, washed with lifting buffer, and incubated for 30 min at 4 °C with a lysis buffer consisting of 50 mM Tris-HCl, 5 mM EDTA, 5 mM EGTA, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, pH 7.2. HUVECs were centrifuged for 1 h with either 100 μl of a 10 μg/ml solution in 0.1 M NaHCO₃/Na₂CO₃, pH 9.6, of either purified anti-β-filamin mAb Ib1 (12) (kindly supplied by Dr. Zaverio Ruggeri, Scripps Research Institute, La Jolla, CA) or normal mouse IgG (Pel-Freeze, Rogers, AR). After washing three times with PBS, the wells were blocked with 3% BSA-PBS. Platelets and HUVECs were lysed with lysis buffer at concentrations of 10⁶/ml for 10 min, washed with PBS, and centrifuged at 100,000 g for 90 min, in order to remove cytoskeleton actin filaments (3). Wells were incubated with serial dilutions of either platelet or HUVEC supernatant for 4 h at room temperature and then with fresh supernatant overnight at 4 °C. The wells were washed six times and incubated for 1 h with either 100 μl of a 10 μg/ml solution in 3% BSA-PBS of anti-β-filamin polyclonal IgG (13), affinity-purified polyclonal anti-β-filamin IgG, or pre-immune rabbit IgG. The wells were then washed six times with PBS, incubated with 100 μl of phosphatase-labeled goat anti-rabbit IgG (Kirkegaard and Perry, Gaithersburg, MD), washed six times, and developed using 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium phosphate substrate (Kirkegaard and Perry). Experiments were done in duplicate.

**Identification of the β-Filamin Domain Binding to the Cytosplasmic Tail of GpIbα**—Deletion mutants in the vector pACT2 were made using cloning sites of the vector and internal restriction sites of β-filamin (Aprl at nt 5708, NcoI at nt 6571 and nt 7462, and EcoRI at nt 7341) (Fig. 10). Because all inserts except clone 1 lacked stop codons in their sequence, expression of clones 2–6, which required use of vector stop codons, resulted in products containing 10 additional amino acids. Each mutant was co-transformed into yeast with the GpIbα bait vector and processed for quantitation of β-galactosidase activity using a liquid culture assay with α-nitrophenyl-β-D-galactoside (Sigma) as substrate. β-Galactosidase units were calculated as recommended by the supplier (CLONTECH).

**RESULTS**

**Isolation of β-Filamin cDNA**—Using as bait the cytoplasmic tail of GpIbα, we first screened a human bone marrow two-hybrid library. Of 4 × 10⁶ clones screened, we isolated six His/LacZ clones, each with the same size ~2.7-kb insert. The sequence of one of these clones was identical to the sequence of ABP-280 (6). Because ABP-280 cDNA has an EcoRI site within this sequence, we compared the cleavage patterns of the remaining five clones with that of the sequenced clone, and found that they to be identical.

We then screened a human placental two-hybrid library. Of 5 × 10⁶ clones screened, we isolated eight His/LacZ clones. To confirm their positive nature, we re-introduced these clones into yeast together with either the GpIbα bait vector, the pAS2–1 original vector or pLAM5* (a vector containing human lamin, used as a control). Transactivation of HIS3 and lacZ was observed only in the presence of the GpIbα bait vector.
One of those clones, THC-106 (Fig. 1), containing a 4.7-kb insert terminating in poly(A) was sequenced. The other seven clones also contained 4.7-kb inserts and showed the same restriction enzyme digestion pattern as THC-106. The sequence of THC-106 suggested that this was a partial cDNA encoding a new filamin homolog.

To obtain the remaining 5' sequence, a human placental cDNA library in λgt10 was screened, using initially a probe (probe 1, Fig. 1) derived from THC-106, resulting in the isolation of clones FPC-1 and FPC-3. In order to screen further 5', we constructed a primer based on the sequence of an EST clone (EST52361, TIGR/ATCC, Rockville, MD) that had been submitted as filamin-like cDNA. We designed a sense primer (5'-CA-CCGTGAAGTGTGTAGG-3') according to this EST sequence and an antisense primer (5'-GACAATGGCTCTTTTGGG-3') according to the two-hybrid cDNA clone (THC-106). Using these primers, the cDNA fragment T23 was amplified from HUVEC cDNA and cloned in pGEM-T Easy vector (Promega, Madison, WI). By sequencing, we confirmed that the sequence of the 3' end was identical to that of THC-106. We then used the 5' half of T23 as probe 2, and isolated clones FPC-5 and FPC-7. Although the sequence of FPC-7 encodes the expected first methionine, based on homology to ABP-280, no in-frame termination codon was found upstream of this methionine. In order to identify the most 5' sequence, we used probe 3 derived from clone FPC-7 and isolated three clones, FPC-14, FPC-15, and FPC-17, spanning up to nucleotides 6, 8, and 31, respectively, of FPC-7. THC-106 and the seven phage clones shown in Fig. 1 were overlapping and spanned 9.4 kb.

As shown in Fig. 2, the cDNA sequence predicts a polypeptide consisting of 2602 amino acids. The methionine designated as the initiation codon was chosen for several reasons. First, the clone FPC-7 was isolated from a library designed to include the most 5' upstream sequences of cDNAs and four different clones we isolated reached up to nearly the same upstream site. Second, when we attempted to amplify the most 5' sequence of the β-filamin cDNA by PCR from a human placental library (in λgt10), using a sense primer derived from the phage arm and an antisense primer derived from β-filamin (nt 312–329), we obtained a PCR product that was 8 nucleotides shorter than FPC-7 (data not shown). Third, the predicted amino acid sequence NH2-terminal to this methionine has no homology with other actin-binding proteins, whereas the sequence COOH-terminal to this methionine contains an actin-binding domain signature at residues 18–27. Moreover, as we show, the molecular mass of β-filamin determined by Western blotting is about 280 kDa, consistent with the 278-kDa molecular mass predicted from our cDNA sequence. However, the proposed initiation site is not flanked by an initiation consensus sequence (CCA/GCC) (14). Thus, to confirm the initiation site it will be necessary to obtain β-filamin genomic sequence.

Homology between β-filamin, CRF, and ABP-280—β-filamin shows strong homology to CRF (15) and human ABP-280 (6) over its entire sequence, with the exception of the first hinge region, and with other actin-binding proteins, including spectrins, α-actinin and dystrophins, in its amino terminus (residues 1–275). β-filamin, ABP-280, and CRF all contain 24 internally homologous repeats (Fig. 3); β-filamin and ABP-280 contain two hinge regions, whereas CRF contains only the more COOH-terminal of the two hinge regions. Furthermore, al-
though the second hinge regions of β-filamin and ABP-280 are 44% identical, the first hinge regions of the two proteins are entirely dissimilar (Fig. 4). Excluding the first hinge region, the amino acid sequence of β-filamin is 70% identical with ABP-280, but 83% identical with CRF, whereas ABP-280 is 66% identical with CRF. The partial sequence of ABPL (the chromosome 7 homolog) is 68% identical with CRF and resembles CRF in lacking a first hinge; the available sequence does not extend sufficiently 3′ to identify the second hinge region (8).

Chromosomal Localization of β-Filamin—During analysis of β-filamin sequence data, we observed that an STS marker, WI-8718, was part of the gene. This marker has been linked to microsatellite marker D3S1295 in 3p14.3-p21.1 by analysis of the GeneBridge 4 Radiation Hybrid Panel at the Whitehead Institute. The β-filamin gene maps between D3S1313 and D3S1295.

Tissue and Cellular mRNA Expression of β-Filamin—Northern blot analysis revealed that β-filamin (Fig. 5A), like ABP-280 (Fig. 5B), is constitutively expressed in many adult human tissues. However, there appear to be some differences in the tissue-specific expression of the two species (for example, kidney and pancreas versus heart and lung). Two different-sized β-filamin mRNA transcripts were detected (Fig. 5A), one somewhat larger than 9.5 kb and one somewhat smaller than 9.5 kb, the latter almost identical in size to the ABP-280 transcript.

Information is available on the World Wide Web (http://www-genome.wi.mit.edu).
sequenced and were identical to the expected line, as well as in HUVECs. The 442-bp RT-PCR products were cytocytic cell line CHRF-288 and the erythroleukemic HEL cell mRNA expression was observed in platelets, the megakaryocyte punc
tate staining of von Willebrand factor is shown in Fig. 8c. 280 shows a similar pattern (data not shown). In contrast, the appears in a more linear distribution within the cytoplasm; ABP-280 and b-filamin mRNA is expressed in megakaryocytes (and can be found in platelets), but that expression in megakaryocytes is considerably less than in endothelial cells.

Detection of b-Filamin Protein—By Western blotting with the antibody to b-filamin’s unique first hinge, we detected substantial amounts of 280-kDa protein in HUVECs, but only ABP-280 mRNA in CHRF-288. As shown in Fig. 6, by RT-PCR, b-filamin mRNA expression was observed in platelets, the megakaryocytic cell line CHRF-288 and the erythroleukemic HEL cell line, as well as in HUVECs. The 442-bp RT-PCR products were sequenced and were identical to the expected b-filamin sequence. In order to confirm that these 442-bp products did not arise from genomic DNA, we amplified genomic DNA using the same primers and observed an approximately 5.5-kb product. Sequence of this product demonstrated the presence of at least two introns (data not shown). From these results, we conclude that b-filamin mRNA is expressed in megakaryocytes (and can be found in platelets), but that expression in megakaryocytes is considerably less than in endothelial cells.

Immunohistochemistry—As shown in Fig. 8a, b-filamin is detectable in normal human dermal vessels; the vessel shown here is a venule. The pattern is the same as that seen for GpIbα (data not shown). As shown in Fig. 8b, by confocal microscopy b-filamin in cultured HUVECs is localized primarily on the cytoplasmic aspect of the cell and nuclear membranes, but also appears in a more linear distribution within the cytoplasm; ABP-280 shows a similar pattern (data not shown). In contrast, the punctate staining of von Willebrand factor is shown in Fig. 8c.

Association between GpIbα and b-Filamin—We used an antigen-capture ELISA assay to detect the association between GpIbα and b-filamin in 100,000 × g supernatants of Triton extracts of platelets and HUVECs. The anti-GpIbα mAb Ib1 captured GpIbα (Fig. 9, open bars) from both platelet and HUVEC lysates in a dose-dependent manner; the lesser amount captured from HUVECs is consistent with the lower level of GpIbα protein expression in HUVECs than in platelets (2). The anti-b-filamin polyclonal antibody detected b-filamin associated with the GpIbα captured by mAb Ib1 (Fig. 9, diagonally striped bars). The solid bars depict controls using pre-immune rabbit IgG in place of the primary antibodies. Because the antibodies used for the platelet and EC supernatants were identical, we compared the absorbance ratios of captured b-filamin to GpIbα for platelets and ECs. The b-filamin:GpIbα ratios for the 1:2 dilutions (after subtracting the pre-immune rabbit IgG control) were 0.08 and 1.34 for platelets and ECs, respectively, suggesting that a much larger fraction of EC GpIbα than platelet GpIbα is bound to b-filamin. When normal mouse IgG was used for coating the ELISA wells, no b-filamin was captured (data not shown).

Localization of the Domain of b-Filamin Binding to the Cytoplasmic Tail of GpIbα—To determine the domain of b-filamin interacting with the cytoplasmic tail of GpIbα, we performed two-hybrid analysis. Six constructs were prepared in pACT2, as indicated in Fig. 10. Each construct expressed a fusion protein consisting of the yeast GAL4-activation domain and one cDNA fragment. After co-transformation of each construct with the GpIbα bait vector, we analyzed expression of HIS3 and lacZ by growth selection and -galactosidase assay, respectively. As shown in Fig. 10, fusion constructs 1, 2, and 3 were positive in both assays, whereas construct 4, lacking residues 1862–2148, was negative. These results indicate that the

Fig. 6. RT-PCR amplification of b-filamin mRNA. The 442-bp cDNA fragments were obtained by RT-PCR using b-filamin-specific primers.

Fig. 7. Analysis of protein expression in HUVECs and platelets by Western blotting. Numbers to the left indicate molecular sizes of prestained standards.

Fig. 8. Immunofluorescence microscopy. a, normal human dermis stained for b-filamin; b, and c, cultured HUVECs stained for b-filamin (b) or vWF (c).
DISCUSSION

The term “filamin” was introduced by Singer’s laboratory (17) to describe a 250-kDa protein isolated from chicken gizzard, normally present as a dimer (18), that is capable of inducing actin polymerization (19). Filamins have been isolated from a number of organs and cell types from chicken, rabbit, and other species (20–24), but most biochemical studies have been performed using filamins isolated from chicken gizzard, rabbit macrophage or human uterus. In 1990, Gorlin et al. (3) reported the sequence of a human endothelial cell cDNA specifying a protein which they termed actin-binding protein 280, or ABP-280. This protein is probably identical to the actin-binding protein purified from human uterus, based on the limited amino acid sequence (25) and on cross-reactivity with monoclonal antibodies (6), and which exists as a 560-kDa dimer (6). Because of its 280-kDa size, its ability to dimerize, and its ability to induce actin polymerization, ABP-280 has often been referred to as human non-muscle filamin, although it is present in smooth (uterine) muscle (6, 25) and in skeletal muscle (Ref. 8, this paper, Fig. 5). ABP-280 contains a 274-residue amino-terminal actin-binding domain, followed by 24 internally homologous repeats among which are intercalated two hinge regions, one of 32 residues between repeats 15–16 and one of 34 residues between repeats 23–24 (these figures are based on our sequence alignment of ABP-280, β-filamin, and CRF). Gorlin et al. (6) localized the site of dimerization of ABP-280 to repeat 24. Electron microscopic studies of human uterine actin-binding protein by these investigators suggested that the first hinge region might allow the ABP-280 dimer to form a “Y,” with the COOH-terminal halves of the protein forming a rigid dimeric structure and the NH2-terminal halves, containing the actin-binding domains, forming the separated ends of the Y. The two hinge regions were found to be sites of calpain cleavage, resulting in the formation of 185-, 90-, and 10-kDa fragments (6, 26).

In 1993, Barry and co-workers (15) cloned and sequenced chicken retinal filamin and found it to be highly homologous to ABP-280, with the major exception that the CRF sequence lacked the more NH2-terminal of the two hinge regions present in ABP-280 (15). If one assumes that CRF and chicken gizzard filamin are identical and that human ABP-280 and rabbit actin-binding protein are also identical, this difference in structure may account for the observations that 1) chicken gizzard filamin produces parallel actin bundles (27) whereas human ABP-280 induces the formation of branched actin polymers (28), and that 2) chicken gizzard filamin is cleaved by calpain into only two fragments: 240 and 10 kDa (27).

Other reports suggest the existence in a single species of more than one filamin. For example, Gomer and Lazarides (29, 30) described an apparent switch in filamin types during chick skeletal muscle development, and Mangeat and Burridge (31) described the presence of two filamin types in HeLa cells. In addition, two other proteins showing homology with CRF and ABP-280 have been isolated from chicken gizzard, the 450-kDa “fucin” (32), and the 260-kDa “ABP-260” (33). Partial cDNA sequence is available for each, although in neither case is sequence available for the areas corresponding to the hinge regions.

In this paper, we describe a new 280-kDa protein, β-filamin, highly homologous to ABP-280 and CRF. β-Filamin contains the same NH2-terminal actin-binding signatures present in the other two proteins as well as in α-actinin (34), β-spectrin (35), and dystrophin (36). Following the actin-binding domain, β-filamin shares with ABP-280 and CRF a structure (not present in α-actinin, β-spectrin, and dystrophin) consisting of 24 internally homologous repeats (Figs. 2 and 3). However, β-filamin differs from the other two sequenced filamins in the region of the first of the two hinges; its first hinge sequence is totally distinct from that of ABP-280 (Fig. 4), whereas the reported...
CRF sequence lacks a first hinge completely. β-Filamin does not contain in its first hinge the calpain cleavage sequence PQQ, TAY present in the first hinge of ABP-280 (6) and is only 44% identical in the second hinge region, suggesting that the two proteins may have different calpain cleavage patterns, a possibility currently under investigation in our laboratory.

Three other human sequences have been reported that are closely related to ABP-280 and β-filamin.

1) In 1993, Leedman et al. (9), using immunoglobulins from Graves’ disease patients, reported the cloning from a human thyroid library of a cDNA specifying a 195-amino acid protein homologous to the COOH terminus of ABP-280. This predicted protein has been termed TABP or thyroid autoantigen. It was suggested that TABP may function in concert with ABP in the thyroid to link integral membrane glycoproteins, such as the thyroid-stimulating hormone receptor, to cytoskeletal actin and thus play a role in signal transduction. However, TABP protein has not been identified in thyroid tissue, and further characterization of TABP has not been done. TABP and β-filamin are probably transcribed from the same gene, because the sequence of TABP and of the corresponding region of β-filamin are almost identical, and the genetic locus of TABP has been identified as chromosome 3 (UniGene accession number Hs. 81008).

2) Very recently, Zhang and co-workers (10), using the cytoplasmic loop region of presenilin-1 as bait in a two-hybrid screened, isolated cDNA fragments encoding the 358 COOH-terminal amino acids of ABP-280, as well as an unknown 291-amino acid sequence showing 69% identity with ABP-280, which they termed “filamin homolog 1” (Fh1). They mapped the Fh1 gene to chromosome 3. The sequence they reported is identical to the COOH-terminal 291 amino acids of β-filamin except for two residues (L2312M and G2382E, using the β-filamin residue numbers), making it very likely that it represents the COOH-terminal region (most of repeat 22, repeat 23, the second hinge, and repeat 24) of β-filamin.

3) Maestrini et al. (8) reported two partial cDNA sequences, mapping to chromosome 7, that are homologous to repeats 4–6 and 15–19 of ABP-280. The second of these cDNAs contains the region around the first hinge but lacks any first hinge sequence (Fig. 4). By Northern blotting, an mRNA similar in size to ABP-280 was identified exclusively in skeletal and cardiac muscle. We have confirmed by RT-PCR the existence of such a species and its limited tissue distribution.3 We are currently attempting to isolate and sequence a larger region of this protein, particularly the area that spans the second hinge region, to establish the relationship between this entity and the other filamins.

Filamin-type proteins are not limited to vertebrates. A closely related actin-binding protein has been identified in Dicyostelium discoideum (37). This 120-kDa protein, referred to as ABP-120, consists of an NH2-terminal actin-binding domain followed by six filamin-like 100 residue repeats, but lacks any hinge regions, and exists as a 240-kDa homodimer (37). The three-dimensional structure of repeat 4 has been determined by NMR spectroscopy and consists of seven β-sheets arranged in an immunoglobulin-like fold (38). This is the only reported structural analysis of a filamin repeat. ABP-120 appears to modulate pseudopod extension in D. discoideum (39).

These studies and the present report define a group of filamin-related proteins, which we suggest be termed the filamin superfamily, on the basis of homology with chicken filamin. Within this superfamily, we suggest naming well characterized members by species and by order of discovery and full characterization. Thus, ABP-280 would be termed human α-filamin and our protein would be called human β-filamin. The term “human γ-filamin” should probably be reserved for the chromosome 7 product.

The filamins appear to function as promoters of actin polymerization and to connect cell membrane constituents to the actin cytoskeleton. The former function has been demonstrated for filamins from several species (40). The latter function has been implied by several immunohistochemical localization studies in chickens (29, 32) but investigated in detail only for human ABP-280 (α-filamin). The first membrane protein shown to interact with ABP-280 (α-filamin) was GpIbα (3, 26, 41). The interaction appears to involve residues 536–568 of the 96-residue cytoplasmic tail of GpIbα (4, 5) and an area within residues 1850–2136 (from the end of repeat 16 to the beginning of repeat 20) of ABP-280 (α-filamin) (16). The interaction of β-filamin with the cytoplasmic tail of GpIbα involves an area within residues 1862–2148 (from the middle of repeat 17 to the middle of repeat 20). Therefore, on the basis of the homology between these two filamins, it is likely that the GpIbα binding sequence is somewhere between the middle of repeat 17 and the beginning of repeat 20.

In addition to its interaction with GpIbα, ABP-280 (α-filamin) has been shown to associate with other membrane proteins, including the high affinity IgG receptor FcγRI (42), the β2-integrin CD18 subunit (43), presenilin 1 (10), tissue factor (44), and the calcium-dependent serine endoprotease furin (45), as well as with the cytoplasmic protein SEK-1 (46). Although the binding sites for FcγRI and β2-integrin are not known, the regions of ABP-280 (α-filamin) binding to SEK-1 and furin are contained within residues 2282–2454 (repeats 21–23) and residues 1490–1607 (repeats 13–14), respectively. Furthermore, our data and the data of Zhang et al. (10) identify repeats 17–20 and 22–24 of β-filamin as distinct binding regions for the cytoplasmic tails of GpIbα and presenilin-1, respectively. Thus, despite their strong internal homology, sufficient unique information resides in different repeats to create specific binding sites for a number of proteins. We are currently investigating the interactions of β-filamin with other cellular constituents.

The functions of the filamins are not fully known, but ABP-280 has been demonstrated to play a crucial role in cellular cytoskeletal and membrane organization (47), and may also be critical for membrane expression of some receptors (48). We have published evidence that ECs synthesize and express on their membranes all four components of the GpIb/IX/V complex, although the membrane density of these components in ECs is substantially lower than in platelets (2). β-Filamin is expressed in only trace amounts in platelets, and we observed only small amounts of GpIbα bound to β-filamin, consistent with the observations of Ezzell et al. that as much as 90% of platelet GpIbα is bound to ABP-280 (α-filamin) (26). However, substantial amounts of both β-filamin and ABP-280 (α-filamin) are present in ECs, and in ECs we detected relatively large amounts of β-filamin bound to GpIbα. The homology between β-filamin and ABP-280, as well as our findings and those of Zhang et al. (10), suggest that these two proteins share a number of functions. On the other hand, the differences in structure, particularly in the hinge regions, suggest that β-filamin may have unique effects on membrane-cytoskeletal interactions, an area under active investigation in our laboratory.

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3 T. Takafuta and S. S. Shapiro, unpublished data.
