We describe the molecular cloning and characterization of a novel giant human cytoplasmic protein, trabeculin-α (M₆ = 614,000). Analysis of the deduced amino acid sequence reveals homologies with several putative functional domains, including a pair of α-actinin-like actin binding domains; regions of homology to plakins at either end of the giant polypeptide; 29 copies of a spectrin-like motif in the central region of the protein; two potential Ca²⁺-binding EF-hand motifs; and a Ser-rich region containing a repeated GSRX motif. With similarities to both plakins and spectrins, trabeculin-α appears to have evolved as a hybrid of these two families of proteins. The functionality of the actin binding domains located near the N terminus was confirmed with an F-actin binding assay using glutathione S-transferase fusion proteins comprising amino acids 9–486 of the deduced peptide. Northern and Western blotting and immunofluorescence studies suggest that trabeculin is ubiquitously expressed and is distributed throughout the cytoplasm, though the protein was found to be located to plasma membrane (12). The family currently comprises four principal members: desmoplakin, plectin, bullous pemphigoid antigen 1 (BPAG1), and envoplakin. All plakins share general structural features, with globular N- and C-terminal regions separated by a central rod domain. There is also extensive conservation of sequence and function between the members of the family. The C terminus of all plakins binds intermediate filaments and has conserved “helix/β-turn” motifs. The N terminus is responsible for the localization to plasma membrane sites and mediates interaction with other cytoskeletal filaments. All plakins share a N-terminal microtubule binding domain (though not all plakins have been observed to associate with microtubules) (12, 13), and plectin and BPAG1 have an actin-binding domain in this region (12). The central rod domain, finally, mediates homodimerization by forming an antiparallel α-helical coiled-coil structure with two plakin molecules (14). Thus, plakins appear to be asymmetric cross-linking proteins but may be able to achieve functional symmetry through dimerization. In addition, at least plectin and desmoplakin are phosphorylated, and in the latter case this seems to affect keratin binding (15, 16).

The spectrin family, which also includes fodrin, dystrophin, utrophin, and protein 4.1, is a group of actin-binding proteins that appear to have evolved from an α-actinin ancestral gene through a series of elongation and duplication events on the basic repeat (17, 18). As such, the presence of an actin binding domain (calponin homology) at the N terminus and of a calmodulin-like Ca²⁺-binding site near the C terminus, both present on α-actinin, is a hallmark of this protein family. The spectrin repeat motif folds into a triple-helix coiled-coil forming a flexible rod (19), which allows spectrin and fodrin (non-erythroid spectrin) to provide the plasma membrane with support and elasticity (20–22).

In this paper, we describe the molecular cloning and characterization of a novel human cytoplasmic giant protein, trabeculin-α. This protein binds F-actin, is ubiquitously expressed, and is distributed throughout the cytoplasm. It also shows strong similarities to both plakins and spectrins and appears to have evolved as a hybrid from the two families. Furthermore,..
the presence of similar yet distinct cDNAs in both humans and mice suggests that trabeculin may form a new subfamily of giant actin-binding/cytoskeletal cross-linking proteins.

**Experimental Procedures**

**Cell Culture**— The MRC-5 (human lung fibroblast), CCL-105 (human adrenal cortical carcinoma), and BSC-1 (African green monkey kidney epithelium) cell lines were purchased from ATCC, and the FS-2 (human foreskin fibroblast) and JMN (human melanoma) cell lines were kindly provided by the late Dr. Ruth Sager (Dana-Farber Cancer Institute) and Dr. Jim Rheinwald (Brigham and Women’s Hospital), respectively.

These cell lines were maintained in Dulbecco’s modified essential medium (DMEM) supplemented with 10% calf serum, 1 mM glutamine, 10 units/ml penicillin, and 0.1 mg/ml streptomycin. The C2Cl2 (murine myoblast) line was obtained from ATCC and cultured in DMEM supplemented with 20% fetal calf serum, 1 mM glutamine, 10 units/ml penicillin, and 0.1 mg/ml streptomycin. Preparation of myoblasts and differentiation into myotubes was carried out as described (23, 24); to induce myoblast fusion, the medium was changed to DMEM with 2% horse serum (differentiation medium, DM).

**Northern Blot Analysis**— Multiple tissue Northern blots were purchased from CLONTECH and used according to the manufacturer’s instructions. Total RNA from primary myoblasts and differentiated myotubes was prepared using RNAzol. Northern blots were prepared and probed as described previously (23). The trabeculin probe used was about 3 kilobase pairs in length and spanned the region from 996 to 4082 bp of the trabeculin-cDNA, corresponding to amino acids 9–1036. Generation of Trabeculin Antibodies— GST fusion proteins of trabeculin fragments 4HP6 (amino acids 2933–3558) and 4HP12 (amino acids 1868–2300) were generated by subcloning of the relevant fragment into vector pGEX3T (CLONTECH). Purification of GST fusion proteins from Escherichia coli was carried out as described previously (25). Briefly, the transformed bacterial cell culture (DH5α) was grown overnight in 1 L broth supplemented with 50 μg/ml ampicillin (LB-Amp), diluted 1:10 in fresh LB-Amp, and further grown for 1 h at 37 °C. Protein production was induced by adding 1 mM isopropyl-1-thio-β-D-galactopyranoside for 2 h at 37 °C. Cells were collected and resuspended in PBS before sonication and centrifugation at 10,000 ×g for 20 min. The supernatant was incubated with glutathione-agarose beads to displace the cross-linking agent, dithiobis(succinimidyl propionate) at 0.2 mg/ml for 5 min at room temperature, and washed in PBS and microtubule stabilizing buffer (MTSB: 100 mM Pipes, 1 mM EGTA, 4% polyethylene glycol 8000). Cells were then permeabilized with 0.5% Triton X-100, washed twice in PBS, and fixed in ice-cold methanol for 5 min. The anti-α-tubulin monoclonal antibody (Sigma) was applied at 1:1000 dilution for 1 h, followed by incubation with secondary rhodamine-conjugated anti-mouse antibody (Jackson ImmunoResearch). 100 ng/ml for 30 min at 37 °C, and then washing and mounting in glycerol/gelatin (Sigma).

Cells were examined using an LSM410 confocal laser scanning microscope (Carl Zeiss, Germany) equipped with an external argon-krypton laser (568 nm). Optical sections of 512 × 512 pixels were digitally recorded in the 2x line-averaging mode. Images were processed for reproduction using the Photoshop software (Adobe Systems, Mountainview, CA).

**Actin Filaments Co-sedimentation Assay**— An actin binding assay system (Cytoskeleton, Inc.) was used to examine the ability of GST-TrABD, a GST fusion protein comprising trabeculin’s α-actinin-like sequences, to associate with F-actin. Recombinant proteins were centrifuged (20 min, 28 pounds/square inch; ~100,000 × g) in a Beckman Airfuge to remove aggregates. F-actin was prepared by resuspension in General Actin Buffer (supplied with the kit) and polymerization at room temperature for 1 h. The tests proteins (>20 μl) were mixed with F-actin (23 μl) and incubated at room temperature for 30 min. Filaments were pelleted by centrifugation at 150,000 × g for 1.5 h at 24 °C. Supernatants and pellets were separated, and the proteins present in each were analyzed by SDS-PAGE and Coomasie Blue staining. Actin-binding proteins and F-actin were present in the pellet, whereas proteins that did not associate with actin remained in the supernatant.

**Cytoskeletal Disruption**— Disruption of microtubules was induced in CCL-105 cells, with 2 μg/ml nocodazole (Sigma) applied for 2 h at 37 °C, and disruption of actin fibers with 1 μM cytochalasin D (Sigma) under the same conditions. Cells were then washed, fixed, and labeled as described before.

**Results**

**Molecular Cloning of Trabeculin-α—** Human trabeculin-α was cloned from the HL1131b human prostate cDNA library (23, 26). Sixteen contiguous partial cDNAs were identified that enabled assembly of the full-length trabeculin-α cDNA structure (Fig. 1A); the complete trabeculin-α cDNA sequence has been deposited in GenBank™ (accession number AF141968). The total sequence identified in our screening was 17,749 bp, including a 16,122 bp open reading frame, a 973-bp 5′ untranslated region, and a 654-bp 3′ untranslated region. The deduced amino acid sequence of the open reading frame reveals a protein of 5373 amino acids, with a predicted Mr of 614,000. Fig. 1B shows the predicted amino acid sequence of human trabeculin-α. Fluorescence in situ hybridization was performed to map the human chromosomal location of the trabeculin-α gene. A 6112-bp fragment of trabeculin-α (bp 8161–14272, amino acids 2397–4433) hybridized to a single locus, on chromosome 3 region p33-p34.2 (not shown).

Since trabeculin is a novel protein, we performed a Northern blot analysis to determine the tissue distribution of its transcripts in humans. Fig. 1C shows the Northern blot for several human tissues. The probe used in this analysis was a 3-kilobase pair fragment of the trabeculin-α cDNA (from 996 to 4082 bp). All tissues shown had detectable levels of trabeculin mRNAs, with the highest expression in muscle (heart and skeletal), prostate, gastrointestinal tract (intestine and colon), and gonads, and lowest expression in brain, spleen, thymus, and
liver, placenta, and lung.

The Structural Features of Trabeculin-α—Our analysis of the structural motifs present in trabeculin-α revealed several interesting features, summarized in Fig. 2A. Near the N terminus of the protein (amino acids 45–257) are tandem regions with marked sequence homology to the α-actinin-like actin binding domain of spectrins and some plakins.

Proceeding toward the C terminus, trabeculin-α displays a
region of approximately 900 amino acids that strongly resembles a region common to all plakins and found to be the microtubule binding domain (MTBD) of BPAG1 (13); and the C-terminal region of trabeculin-α contains a 76 amino acid region with strong homology (52% identity, 69% homology) to another plakin, GAR22 (27). These two plakin-like motifs, together with the presence of an actin binding domain, suggest that trabeculin-α may interact with various cytoskeletal proteins.

Perhaps the most striking feature of trabeculin-α resides in its central region: a 29-fold repeat of a 110–120 amino acid stretch, bearing considerable identity to the spectrin-like repeats found in human dystrophin and other spectrins (17, 28) (Fig. 2B). Secondary structure prediction based on the sequence information suggest that each repeat is made up of three α-helices, as for the spectrin-type repeat. Similarities with the spectrin-like repeat also include a very conserved tryptophan residue at position 18 in the alignment followed by a hydrophobic residue, conservation of positively and negatively charged residues (Fig. 2B, in red and purple) at given positions throughout the alignment, a leucine at the end of each repeat, and abundant prolines and glycines (Fig. 2B, in yellow) in the loop regions.

There are also multiple regions in trabeculin-α that may be able to transduce information for intracellular signaling pathways as follows: two tandem calcium-binding EF-hand motifs (amino acids 5000–5100), similar in sequence and relative position to those in the C terminus of dystrophin and other spectrins (17, 29); and several tyrosine residues that have surrounding sequences consistent with their being tyrosine kinase substrates. Finally, the extreme C terminus of trabecul-
lin-α contains a serine-rich region (amino acids 5350–5500 are 28% serine), including a GRXX repeat motif also present in the same relative position (40–50 amino acids to the C terminus) in plecin and desmoplakin.

**Trabeculin Homologues and Family**—Several structural homologues of human trabeculin-α have been reported, including, notably, Kakapo from *Drosophila melanogaster* (30–32) and its Caenorhabditis elegans counterpart, CeKak (30); and the mouse ACF7 gene (GenBank™ accession number AF150755), which shares 88% overall identity with the human trabeculin-α clone reported here. Alignments of the deduced protein sequences for two of the best conserved regions, the putative actin binding domains and the GAR22 homology, are shown in Fig. 2, C and D. A 1157-bp partial human ACF7 cDNA has also been reported (33) which ends with an 886-bp stretch identical to a region near the N terminus of our trabeculin-α cDNA, starting right around the beginning of the putative actin binding domain.

Another partial human cDNA clone was reported recently (34) that displays 77% identity with a region near the C terminus of trabeculin-α, over its 1054 deduced amino acid sequence. This region includes conserved EF-hand motifs, GAR22 homology region, and GRXX repeats (Fig. 3). Correspondingly, we also found over a dozen mouse ESTs with strong similarities to mouse ACF7, although clearly of non-identical sequence. For example, the longest of these ESTs (GenBank™ accession number AA014416) encodes the last 134 amino acids of a mouse trabeculin homologue with 75% overall identity with mouse ACF7. It is notable that these clones display a conserved GRXX repeat but not the general serine-rich C terminus (Fig. 3). Moreover, B13 immunoprecipitates these proteins specifically from cell lysates. These data suggest that the high molecular weight proteins are in fact trabeculins and that the B13 antibody does not significantly cross-react with other proteins.

We performed an immunofluorescence analysis of various cell types using the B13 anti-trabeculin antibody. A representative selection of these data are shown in Fig. 4, C—F. These figures show the staining pattern of anti-trabeculin in the CCL-105, BSC-1, JMN, and FS-2 cell lines. As a negative control, staining with the preimmune serum was performed, and only minimal background staining was observed (data not shown). In these and the more than 20 other cell types we examined (not shown), the subcellular distribution of anti-trabeculin staining had the following salient features: 1) distribution throughout the cytoplasm, 2) exclusion from the nucleus, and 3) appearance as a filamentous network, in some cells displaying a fiber-like staining pattern reminiscent of actin stress fibers. Occasionally, we observed a punctate or globular staining that might be associated with localization to vesicular structures like the Golgi apparatus or endoplasmic reticulum (not shown).

**Trabeculin Is Distributed Throughout the Cytoplasm and Does Not Form Distinct or Focal Structures**—We raised an antibody (B13) to the spectrin homology region of trabeculin to perform immunofluorescence studies of the subcellular localization of the protein. Since this region of trabeculin reveals close homology to many other proteins, it was critical to establish the specificity of this antibody, before the subsequent analyses. Fig. 4A shows that in Western analysis, the B13 anti-trabeculin antibody recognizes the trabeculin portions of the bacterially expressed GST-trabeculin fusion proteins to which it was raised. The B13 antiserum recognizes two high molecular weight proteins on Western blots of cultured CCL-105 or MRC-5 cells (Fig. 4B). These proteins are of the same relative molecular weight (app. 83 kDa) or of mouse brain lysate (not shown). Moreover, B13 immunoprecipitates these proteins specifically from cell lysates. These data suggest that the high molecular weight proteins are in fact trabeculins and that the B13 anti-trabeculin antibody does not significantly cross-react with other proteins.

We then tested the ability of GST-TrABD to bind F-actin in a sedimentation binding assay (24). Fig. 5A shows that GST-TrABD efficiently binds F-actin through its N-terminal actinin-like domains since the N terminus of trabeculin-α contains a domain that has significant homology to the α-actinin-γ domain. We constructed a GST-TrABD fusion protein of the putative N-terminal actin binding domain (ABD) of trabeculin-α (amino acids 9–486), GST-TrABD (82 kDa by SDS-PAGE). We then tested the ability of GST-TrABD to bind F-actin in a sedimentation binding assay (24). Fig. 5A shows that GST-TrABD efficiently binds F-actin filaments. In this experiment, GST-TrABD, positive (α-actinin) or negative (BSA) control proteins were incubated with F-actin,

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2 C. Leung, D. Sun, M. Zheng, D. Knowles, and R. K. H. Liem, EMBL accession number AF150755.
Fig. 4, A, the B13 anti-trabeculin antibody is reactive against the fusion proteins to which it was raised. Two GST fusion proteins (4HP6 and 4HP12) of trabeculin were generated, mixed together, and used as immunogens in rabbits. The resulting immune serum (B13) was then used at 1 μg/ml in Western blot analysis. Samples of the GST-trabeculin were either left intact (Int) or cleaved using Factor Xa (Xa) or thrombin (Th) to separate GST from the trabeculin fragment. The samples were resolved by SDS-PAGE, transferred to polyvinylidene difluoride membrane, and probed with B13 anti-trabeculin. B13 anti-trabeculin recognizes both fusion proteins before and after cleavage, thus the relevant epitope is not in the GST portion of the fusion. B, the B13 anti-trabeculin antibody immunoprecipitates (IP) and recognizes two high molecular weight proteins from cell lysates. Cell lysates were prepared from CCL-105 or MRC-5 tumor cells. Immunoprecipitation was performed with either preimmune serum (PI) or immune serum B13 (I). Proteins from immunoprecipitations or post-nuclear lysate (lys) were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes for Western analysis with 1 μg/ml B13 anti-trabeculin. C–F, immunofluorescence analysis of trabeculin subcellular localization. Cells were prepared as described under “Experimental Procedures.” Staining was carried out using the B13 anti-trabeculin antibody, with appropriate preimmune serum-staining controls (not shown). C, CCL-105, human adrenal cortex carcinoma; D, BSC-1, African green monkey kidney epithelia; E, JMN, human mesothelioma; F, FS-2, human foreskin fibroblasts. Bar in E = 10 μm; bar in F = 25 μm.

and the reactions were centrifuged to separate binding from non-binding proteins. Both GST-TrABD and α-actinin, but not BSA, co-precipitated with F-actin. We verified separately that GST does not bind actin (not shown).

Effects of Nocodazole and Cytochalasin D—We also tested whether an association of GST-TrABD with actin could be detected in a cellular context. Due to the large size and modular nature of trabeculin, an approach where association is detected by co-immunoprecipitation was not feasible. Therefore, we examined the pattern of trabeculin distribution in the absence and presence of drugs that disrupt the cytoskeleton. We stained for actin, tubulin, and trabeculin in CCL-105 cells treated with either cytochalasin D, which disrupts microfilaments (35), or nocodazole, a disruptor of microtubules (36). Fig. 5B shows that cytochalasin D effectively disrupts actin-containing microfilaments, causing a transition from prominently filamentous to punctate actin staining, with no remaining evidence of linear structures. Cytochalasin D has no effect on the organization of microtubules, as observed with tubulin staining. In contrast, nocodazole disrupts tubulin-containing microtubules but does not change microfilament organization. We stained cells for trabeculin in the absence or presence of either of these drugs. In untreated cells, the ubiquitous, fine filament structure of trabeculin was evident. In cells treated with nocodazole, no change in the distribution of trabeculin staining was observed. However, cytochalasin D treatment resulted in a marked (although not complete) disruption of trabeculin fine filament structures and the appearance of punctate aggregates of trabeculin. These results suggest that trabeculin is at least in part associated with actin filaments.

Myoblast to Myotube Transition Involves an Up-regulation in Trabeculin Levels—Since trabeculin appears to be an actin-binding protein and myogenesis involves a major increase in actin expression, we examined the levels of trabeculin in myoblasts undergoing differentiation to myotubes. C2C12 cells were placed in differentiation medium (DM) and monitored over a 6-day period for morphology (not shown) and trabeculin expression. Fig. 6A shows that trabeculin mRNA levels increase steadily from Day 0 (100% myoblasts) to Day 5–6 (fused myotubes). In contrast, myosin levels, reflecting the differentiation to functional muscle, only increase toward the end of the fusion process, when the cells develop their contractile apparatus.

Fig. 6B illustrates the difference in trabeculin expression levels between myoblasts and myotubes. A mixture of myoblasts and myotubes were stained with the B13 anti-trabeculin antibody. As above, trabeculin is distributed throughout the cytoplasm and is excluded from the nuclei in all cells. There is a striking difference, however, between the levels of staining in the fused myotubes compared with the individual myoblasts. Taken together with the Northern blot data of Fig. 6A, these results suggest that myoblast fusion is accompanied by an up-regulation of trabeculin levels.

DISCUSSION

The data presented above describe the molecular cloning and initial analysis of a novel human cytoskeletal protein, trabeculin-α. The domain structure of trabeculin-α is complex, with
multiple potential sites for protein-protein interactions. The region of homology (tandem repeat) to the ABD of \( \alpha \)-actinin suggested that trabeculin-\( \alpha \) may bind F-actin (37), which we have confirmed. Similar ABD motifs are found near the N termini of all members of the spectrin superfamily and of some plakins (plectin and BPAG1n). The actin binding domain of trabeculin-\( \alpha \), however, is a lot more similar to that of the plakins (80% identity) than to that of spectrins (50% identity). Trabeculin-\( \alpha \) also bears two regions of specific homology to members of the plakin family (12, 27), including an extended region common to all plakins and found to be the MTBD of BPAG1 (13). This putative MTBD may carry particular significance, because it was recently shown that overexpression of BPAG1n3 confers resistance to microtubule-disrupting agents (13). Such agents include Taxol and other vinca alkaloids widely used as chemotherapeutic agents in the treatment of cancer, and microtubule stabilization by plakins (or trabeculins) may therefore represent a mechanism of resistance to those drugs.

Plakins are responsible for cross-linking other cytoskeletal proteins and for anchoring actin microfilaments and intermediate filaments to proteins that are integral to the plasma membrane (13, 38). Plectin was described as a regulator and reinforcer of the cytoskeleton (39) and, like trabeculin, is ubiquitously expressed and distributed throughout the cytoplasm (40). On the other hand, GAR proteins are highly up-regulated in growth-arrested cells and are known to be involved in cytoskeletal organization (41, 42). Correspondingly, we found that trabeculin protein levels are strongly increased upon differentiation of myoblasts to myotubes, an event associated with the loss of cell proliferation properties and the buildup of sarcomeric structures. The cleavage of GAR proteins during apoptosis contributes to cytoskeleton destabilization; although it remains to be seen whether trabeculin is also subject to prote-
binding domain of trabeculin-α is more similar to that of the plakins that carry it (plectin and BPAG1n) than to that of spectrins. Taken together, these structural observations suggest that this novel protein may be a “hybrid” with plakin-like N (ABD and MTBD) and C termini (GAR22 and Ser-rich/GSRX repeat) and a spectrin-like rod domain plus adjacent sequence (including the calmodulin homology) in the middle.

During our cloning and analysis of human trabeculin-α, the D. melanogaster protein Kakapo was described in three studies reporting its cloning and functional analysis (30–32). Kakapo (45) is a 5437-amino acid protein with sequence and structural similarities to trabeculin-α. Similarities include putative actin-binding motifs near its N terminus, two plakin homology regions (common MTBD and Gas2/GAR22), and Ca
²⁺-binding EF-hand motifs. Both proteins also have the critical spectrin-type α-helical repeats (22 in Kakapo, 29 in trabeculin) forming the major part of their sequence. Functional analysis of Kakapo in Drosophila embryos (30–32) suggested a role for Kakapo in anchoring muscle cells to the epidermis. The model proposed in these studies places Kakapo at the termini of microtubules in the epidermal cells, linking these and the cortical actin cytoskeleton to integrins in the epidermal basolateral membrane (the well conserved Gas2/GAR22 homology domain seems important for this interaction, as mutants in this region fail to differentiate properly). In turn, these integrins are suggested to interact with extracellular matrix proteins that form bridges with the muscle cells.

Despite their very similar domain structures, trabeculin-α does not appear to be the functional homologue of Drosophila’s Kakapo in the human system; trabeculin does not significantly localize to focal complexes and is distributed throughout the cytoplasm. In contrast, Kakapo is found exclusively at the cell periphery. Moreover, trabeculin is apparently not associated with the microtubule system since the disruption of these filaments does not cause a redistribution of trabeculin. Rather, the identification of two distinct trabeculin/ACF7 genes in human and mouse, and of alternatively spliced isoforms at least in mouse, points to the existence of a family of trabeculins whose members may have characteristic functions and distribution. We propose the nomenclature trabeculin-α for the human gene reported here and its mouse homologue, ACF7 (GenBank™ accession number AF150755)² with its splice variants (46), and trabeculin-β for the human gene cloned by Nagase et al. (34) and its mouse counterpart defined by EST AA014416 and others (GenBank™). It is not clear from simple sequence analysis whether Kakapo from Drosophila and CeKak from C. elegans (30) may truly be assigned to either of these classes, and it should be noted that our searches revealed only one putative trabeculin homologue in the completely sequenced genome of C. elegans.

The existence of different trabeculin isoforms may also explain some of the unexpected observations reported herein. First, Western blotting of CCL-105 and MRC-5 cells post-nuclear protein preparations revealed two distinct anti-trabeculin-reactive bands, and these may be related to trabeculins α and β. Second, some of the anti-trabeculin-reactive filamentous structures seemed to resist cytochalasin D treatment, even though the actin-containing filaments were completely destroyed; it is possible that some trabeculin isoforms are not associated with actin microfilaments. Indeed, some of the mouse ACF7 isoforms reported by Bernier et al. (46) lack the N-terminal α-actinin-like actin binding domains; similarly, an isoform of the plakin BPAG1, BPAG1n3 (13), and an alternative transcript of Kakapo, “Kakapo form B” (30), have been described which lack part of this domain and have impaired actin binding ability. (It should also be noted that our anti-

FIG. 6. A, Northern analysis of trabeculin expression during myoblast differentiation. Myoblasts were differentiated into multinucleated myotubes over 6 days in differentiation medium (DM). mRNA was prepared from cells harvested at daily intervals, and Northern analysis using the indicated probes was performed. Trabeculin protein is more abundant in differentiated myotubes. B, immunofluorescent staining of mixed myotubes (cultured in DM for 6 days) using anti-trabe-

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trabeculin polyclonal B13 antiserum was raised against the spectrin repeat region. Since such spectrin repeats are likely to be present in both trabeculin-α and -β subfamilies, our antiserum would likely label both gene products and their variants. Third, the divergence in the 5′-ends of human trabeculin-α and ACF7 cDNAs may result from the alternative splicing of a common primary transcript or by the activity of alternative promoters, with the N terminus of trabeculin-α being replaced by another sequence in the ACF7 transcript.

These possibilities were also evoked by Byers et al. (33), and both a multiplicity of alternatively spliced transcripts and the presence of multiple promoters appear to be commonplace among plakins (12, 13). As mentioned earlier, three isoforms of the murine ACF7 have also been described, which apparently result from differential splicing of exons near the N terminus, encompassing part of the α-actinin-like actin-binding motifs (46).

Similarly, the common sequence between the two human clones of trabeculin-α (the full-length sequence reported here and the partial, N-terminal human ACF7 clone of Byers et al. (33)) starts at an Asp residue near the beginning of the α-actinin-like actin binding domain homology. It may be noteworthy that chromosome 1 region p33-p34.2, where the trabeculin gene resides, encompasses part of the α-actinin gene, a likely target for phosphorylation. Phosphorylation may result in profound defects in the organization and maintenance of the actin cytoskeleton. In this light, it is noteworthy that chromosome 1 region p33-p34.2, where the trabeculin locus is found, is often deleted in human dystrophies. (Byers et al. (33) also localized ACF7/trabeculin-α to human chromosome 1.) With only a portion of dystrophies directly attributable to dystrophin defects, trabeculin may be viewed as a candidate gene for these disorders.

In summary, we have described the novel human cytoskeletal giant protein, trabeculin-α, a member of a new family of actin-binding proteins. Trabeculins are widely expressed and are distributed throughout the cytoplasm of all cells examined. Trabeculin-α can bind actin filaments and possibly other cytoskeletal target molecules, possibly contributing to their spatial separation by virtue of the rigid structure formed by the multiple spectrin-type repeats in its center. In addition, trabeculin-α contains several putative sites that may be involved in the transduction of signals to intracellular pathways. Expression of trabeculin may also be linked to the differentiation/growth status of muscle cells, as its expression levels are greatly increased upon myogenic differentiation. Further study of this new giant actin-binding protein family may lend insight into novel aspects of cellular architecture.

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REFERENCES
1. Schmidt, A., and Hall, M. N. (1998) Annu. Rev. Cell Dev. Biol. 14, 305–338
2. Hall, A. (1994) Annu. Rev. Cell Biol. 10, 31–54
3. Hall, A. (1998) Science 279, 509–514
4. Squire, J. M. (1997) Curr. Opin. Struct. Biol. 7, 247–257
5. Burnidge, K., Fath, K., Kelly, T., Nuckolls, G., and Turner, C. (1998) Annu. Rev. Cell Dev. Biol. 4, 487–510
6. Lo, S. H., and Chen, L. B. (1994) Cancer Metastasis Rev. 13, 9–24
7. Yamada, K. M., and Geiger, B. (1997) Curr. Opin. Cell Biol. 9, 76–85
8. Lamarche, N., Tapon, N., Stowers, L., Burbelo, P. D., Aspenstrom, P., Bridges, T., Chant, J., and Hall, A. (1996) Cell 87, 519–529
9. Nobes, C. D., and Hall, A. (1995) Cell 81, 53–62
10. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
11. Ridley, A. J., Paterson, H., Johnston, C. L., Diekmann, D., and Hall, A. (1992) Cell 70, 410–419
12. Ruhrberg, C., and Watt, F. M. (1997) Curr. Opin. Genet. & Dev. 7, 392–397
13. Yang, Y., Bauer, C., Strasser, G., Wollman, R., Julien, J. P., and Fuchs, E. (1998) Cell 99, 229–239
14. Green, K. J., Virata, M. L., Elgart, G. W., Stanley, J. R., and Parry, D. A. (1992) Int. J. Biol. Macromol. 14, 145–153
15. Foisner, R., Maleca, N., Dressel, N., Stadler, C., and Wiche, G. (1996) Mol. Biol. Cell 7, 273–288
16. Stappenbeck, T. S., Lamb, J. A., Corcoran, C. M., and Green, K. J. (1994) J. Biol. Chem. 269, 29351–29354
17. Faschul, K., Schmidt, C. F., Branton, D., and Block, S. M. (1992) Biophys. J. 63, 784–793
18. Bennett, V., and Gilligan, D. M. (1993) Annu. Rev. Cell Biol. 9, 27–66
19. Dahl, S. C., Geib, R. W., Fox, M. T., Edidin, M., and Branton, D. (1994) J. Cell Biol. 125, 1057–1065
20. Lo, S. H., An, Q., Bao, S., Wong, W. K., Liu, Y., Janmey, P. A., Hartwig, J. H., and Chen, L. B. (1994) J. Biol. Chem. 269, 22310–22319
21. Lo, S. H., Janmey, P. A., Hartwig, J. H., and Chen, L. B. (1994) J. Cell Biol. 125, 1067–1075
22. Smith, D. B., and Johnson, K. S. (1988) Genes Dev. 2, 31–40
23. Davis, S., Lu, M. L., Lo, S. H., Lin, S., Butler, J. A., Druker, B. J., Roberts, A. B., and Thomas, M. A. (1991) Science 252, 712–715
24. Zucman-Rossi, J., Leggeix, P., and Thomas, G. (1996) Genomics 38, 247–254
25. Koenig, M., and Kunkel, L. M. (1990) J. Biol. Chem. 265, 4560–4566
26. Kawasaki, H., and Kretsingr, R. H. (1995) Protein Profile 2, 297–490
27. Gregory, S. L., and Brown, N. H. (1998) J. Cell Biol. 143, 1271–1280
28. Prokop, A., Uhler, J., Roote, J., and Bate, M. (1998) J. Cell Biol. 143, 1293–1294
29. Strumpf, D., and Volk, T. (1998) J. Cell Biol. 143, 1259–1270
30. Byers, T. J., Bees, A. H., McNally, E. M., and Kunkel, L. M. (1995) FEBS Lett. 368, 500–504
31. Nagase, T., Ishikawa, K., Suyama, M., Kikuno, R., Miyajima, N., Tanaka, A., Kotani, H., Homura, N., and Obara, O. (1990) DNA Res. 5, 277–286
32. Cooper, J. A. (1987) J. Biol. Chem. 262, 1473–1478
33. Presley, J. F., Cole, N. B., Schroer, T. A., Hirschberg, K., Zaal, K. J., and Klahr, B. A. (1997) J. Biol. Chem. 272, 706–778
34. Nanase, T., Ishikawa, K., Suyama, M., Kikuno, R., Miyajima, N., Tanaka, A., Kotani, H., Homura, N., and Obara, O. (1990) DNA Res. 5, 277–286
35. Prokop, A., Uhler, J., Roote, J., and Bate, M. (1998) J. Cell Biol. 143, 1271–1280
36. Strumpf, D., and Volk, T. (1998) J. Cell Biol. 143, 1259–1270
37. Byers, T. J., Bees, A. H., McNally, E. M., and Kunkel, L. M. (1995) FEBS Lett. 368, 500–504
38. Nagase, T., Ishikawa, K., Suyama, M., Kikuno, R., Miyajima, N., Tanaka, A., Kotani, H., Homura, N., and Obara, O. (1990) DNA Res. 5, 277–286
39. Cooper, J. A. (1987) J. Biol. Chem. 262, 1473–1478
40. Presley, J. F., Cole, N. B., Schroer, T. A., Hirschberg, K., Zaal, K. J., and Klahr, B. A. (1997) J. Biol. Chem. 272, 706–778
41. Nanase, T., Ishikawa, K., Suyama, M., Kikuno, R., Miyajima, N., Tanaka, A., Kotani, H., Homura, N., and Obara, O. (1990) DNA Res. 5, 277–286
42. Nanase, T., Ishikawa, K., Suyama, M., Kikuno, R., Miyajima, N., Tanaka, A., Kotani, H., Homura, N., and Obara, O. (1990) DNA Res. 5, 277–286