Cloning and Expression of a Novel Acidic Calponin Isoform from Rat Aortic Vascular Smooth Muscle*

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The actin-binding protein calponin has been implicated in the regulation of smooth muscle contraction. We have isolated cDNA clones encoding a novel acidic calponin isoform from rat aortic vascular smooth muscle cells. The initial 273 residues of the deduced 330 amino acid polypeptide (M, 36,377) are highly homologous to basic smooth muscle calponin isoforms, but the remaining 57 residues at the carboxyl terminus comprise a unique and strongly acidic domain. The sequence of the acidic domain shows high homology (93.3% identity) to the partial sequence of HUMXT01244, an unidentified human hippocampal gene product (Adams, M., Dubnick, M., Kerlavgne, A. R., Moreno, R., Kelly, J. M., Uterback, T. R., Nagle, J. W., Fields, C., and Venter, J. C. (1992) Nature 355, 632-634). Transcripts encoding acidic calponin are expressed in cultured rat aortic vascular smooth muscle cells and in non-muscle and smooth muscle tissues of adult rat. Based on its calculated M, and the tissue distribution of its expression, acidic calponin is an excellent candidate for a previously detected non-muscle calponin homolog (Takeuchi, K., Takahashi, K., Abe, M., Nishida, W., Hwada, K., Nabeya, T., and Maruyama, K. (1991) J. Biochem. (Tokyo) 109, 311-316). Like basic calponin isoforms, acidic calponin synthesized in a bacterial expression system bound F-actin. However, unlike basic calponin, the acidic isoform did not interact with Ca\(^{2+}\)/calmodulin, indicating a functional distinction between the muscle and non-muscle forms.

Calponin is an actin- and tropomyosin-binding protein that has been implicated in actin-linked regulation of smooth muscle contraction by virtue of its reversible inhibition of smooth muscle actomyosin MgATPase (1, 2). Further support for the proposed regulatory function has been provided by the recent demonstration that calponin inhibits the myosin directed movement of actin filaments in vitro in a calcium-calmodulin-dependent manner (3). Multiple isoforms of calponin have been identified in avian and mammalian smooth muscle (4, 5), but the functional significance of the isoform diversity has yet to be elucidated. The identified isoforms appear sequentially during smooth muscle differentiation in vitro and disappear in the same order during the phenotypic modulation of cultured smooth muscle cells (4, 5). The disappearance of the calponin isoforms coincides with the phenotypic change in caldesmon (Cd) expression from the h-Cd to l-Cd isoform (5).

Like calponin, h-Cd is a thin filament associated protein of smooth muscle (6) that reversibly inhibits actomyosin interactions in vitro (Ref. 3 and reviewed in Refs. 7 and 8). Despite their apparent functional analogy, sequence analysis indicates that calponin and h-Cd are not related proteins (7-9) and recent studies suggest different mechanisms of inhibition (3). Furthermore, localization studies suggest that calponin and h-Cd bind to distinct thin filaments in smooth muscle cells (10). The expression of h-Cd is primarily confined to smooth muscle cells (11). By contrast, the l-Cd form has been shown to be widely expressed in non-muscle tissues (12). Amino acid sequences of the muscle and non-muscle Cd isoforms indicate that the primary difference between the two classes is the absence of a highly repetitive central domain in the low M, class (7, 8). Notably, the functional domains, including the F-actin binding/myosin ATPase inhibitory domains, are highly conserved (7, 8). Much current research is being directed toward resolving the physiologic role of the non-muscle isoform, with the accumulating evidence implicating l-Cd in regulation of non-muscle motility and/or F-actin filament organization (7).

A class of non-muscle calponin isoforms, comparable with l-Cd, has yet to be conclusively demonstrated. However, immunoblotting analysis has revealed that antibodies specific to the avian smooth muscle calponin isoform cross-react with a protein with molecular mass of 36-kDa in various bovine non-muscle cells (12, 13). Furthermore, Takeuchi et al. (13) have reported that immunoreactive forms of calponin are associated with the actin cytoskeleton and co-localize with l-Cd in various non-muscle tissues. In this work, we report a rat cDNA sequence encoding a novel protein that appears to be an excellent candidate for non-muscle calponin. The encoded protein exhibits extensive homology to calponin, but contains an additional highly acidic domain. RNA blot analysis reveals that the acidic calponin isoform is expressed in both smooth muscle and non-muscle adult rat tissues, as well as in passaged cultured rat aortic vascular smooth muscle cells (VSMC). Analysis of acidic calponin synthesized in a bacterial expression system reveals that the protein binds F-actin, but does not interact with calmodulin either in the presence or absence of calcium.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases and DNA modifying enzymes were purchased from New England Biolabs (Beverly, MA). [\(^{32}\)P]NCTP

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U06755.

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* The abbreviations used are: Cd, caldesmon; VSMC, vascular smooth muscle cells; IPTG, isopropyl-1-thio-\(\beta\)-D-galactopyranoside; PAGE, polyacrylamide gel electrophoresis; BiTrrs, 2-(bis[2-hydroxyethyl]amino)-2-(hydroxymethyl)-propane-1,3-diol.

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and [α-32P]CTP were from DuPont NEN. Kits for ribonuclease protection and in vitro transcription were from Ambion (Dallas, TX).

Oligonucleotide primers—An oligonucleotided cDNA library constructed in AZAPII vector (Stratagene, La Jolla, CA) derived from poly(A)+ RNA of platelet-derived growth factor-treated (20 ng/ml, BB homodimer, 3 h) cultured rat aortic VSMC (14) was screened with a cDNA probe corresponding to the coding region of avian calponin α (9). The avian calponin cDNA fragment, labeled with [α-32P]CTP by random oligonucleotide priming (15), was generated by reverse transcription-polymerase chain reaction (reverse transcription PCR) from total avian gizzard smooth muscle RNA, following protocols described in Ref. 16. The following sequences were used as oligonucleotide primers.

**Sense:** ACTTACTACATAGTGCAACAGCTTCAAC
**Antisense:** TGGGATCCGGGCGTTATTGAGTGT

With the exception of the underlined nucleotides, the primers correspond to the avian calponin α cDNA sequence (9). Substitutions were made to engineer restriction sites to facilitate subsequent cloning.

Screening of the VSMC library resulted in the identification of eight clones, which on the basis of the insert size fell into two categories. A representative of each class, pRAC1 and pRAC2, was isolated, subcloned, and sequenced. DNA sequencing was carried out on both strands using BioSystem model 373A DNA sequencer.

**Cell Culture**—VSMC were isolated from the thoracic aortas of 200–300-g male Sprague-Dawley rats by enzymatic dissociation (17). Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated calf serum, 100 unit/ml penicillin, and 100 μg/ml streptomycin and serially passaged before reaching confluence. Experiments shown employed passages 12–15.

**RNA Preparation and Blot Hybridization**—Total RNA was extracted from VSMC and from adult rat tissues by the guanidinium method. Final washes for all blots were in 0.1 M SSC.

**Molecular Cloning and Primary Structure of Rat Acidic Calponin**—Two of the eight cDNA clones identified by screening a rat aortic VSMC cDNA library with an avian calponin α cDNA probe were isolated, subcloned, and sequenced. The two cDNA clones, designated pRAC1 and pRAC2, contained identical open reading frames of 330 amino acids (Fig. 1). The cDNA clones, designated pRAC1 and pRAC2, contained identical open reading frames of 330 amino acids (Fig. 1). The sequence AUUUA, which has been shown to be a point in the 3′ end of the cDNA clone beginning at base 1903.

**RESULTS**

**Molecular Cloning and Primary Structure of Rat Acidic Calponin**—Two of the eight cDNA clones identified by screening a rat aortic VSMC cDNA library with an avian calponin α cDNA probe were isolated, subcloned, and sequenced. The two cDNA clones, designated pRAC1 and pRAC2, contained identical open reading frames of 330 amino acids (Fig. 1). The untranslated regions of the two were also identical, with the exception of an additional 199 bases at its 3′ end and 20 additional bases at the 5′ end of the pRAC1 clone (Fig. 1). In both clones, the translation initiation site was assigned to the first ATG triplet, which appeared 52 bases downstream of an in-frame nonsense codon. Both sequences contained a single polyadenylation signal beginning 495 bases downstream of the stop codon. The sequence AUUUA, which has been shown to be a mediator of mRNA degradation (24), was found in the larger clone beginning at base 1903.

The deduced polypeptide, which has a calculated molecular weight of 36,377 and an isoelectric point of 5.2, is a unique acidic isoform of calponin. Its assignment as a calponin isoform is based on the high homology between the first 273 amino acids and comparable portions of smooth muscle calponin isoforms (Fig. 2, Table I), which notably are all characteristically basic. This portion of the acidic calponin sequence is likewise strongly basic (calculated isoelectric point of 8.9) and contains three tandem repeats of 29 amino acids (Fig. 1b), a motif that is characteristic of calponin isoforms (9). The unique acidity of the rat VSMC calponin isoform is conferred by the 57 residues at the carboxyl terminus which have a predicted isoelectric point of 3.7. Found within the acidic domain is a novel structural motif, characterized by a four spacing periodicity of tyrosine residues, with each tyrosine preceded by a negatively charged amino acid (Fig. 1c).

**Acidic Calponin** — The sequence of the GenBank/EMBL data bank identified six other sequences with significant homologies to rat acidic calponin (Table I). Five are proteins that have comparable homologies to basic calponin isoforms. These include three species variants of SM22α, a smooth muscle protein of unknown function (26), and two other functionally unknown proteins, rat neuronal protein 25, and S112748, a gene product overexpressed in human Werner syndrome Schwann cell fibroblasts (28). The only sequence that showed significant homology to the acidic domain was the human HUMXT01244 gene product (29).
Acidic Calponin

**Fig. 1. Structure of cloned cDNAs encoding acidic calponin.** a, nucleotide sequence of full-length inserts of pRAC1 and pRAC2. Numbering corresponds to pRAC1. The sequence of pRAC2 begins at nucleotide 20 and terminates at base 1733. The underlined regions at the 5' and 3' ends indicate sequences found only in pRAC1. A polyadenylation signal site between residues 1708 and 1713 is shown in parentheses. The boxed sequence identifies the nucleotides encoding the 61-residue region which overlaps with HUMX071244. Underlined in the boxed region are the repeating tyrosines. The Patl and Xhol sites used for subcloning are identified and underlined. b, 29-residue repeat motif of amino acid sequence characteristic of calponin isoforms. Identical residues are enclosed within boxes. c, repeat motif characterized by 4 spacing periodicity of tyrosine residues with each tyrosine preceded by an acidic residue. d, schematic representation of organization of pRAC1 sequence. Thin bars correspond to sequence found only in pRAC1, but not pRAC2. Thick bars correspond to the untranslated region found in both pRAC1 and pRAC2. The rectangle indicates open reading frame. Assignments of calponin and acidic domains are based on sequence homology data (see Fig. 2). The arrow shows the Patl-Xhol fragment used as an antisense probe (see Fig. 5).
Fig. 2. Amino acid sequence similarities to basic calponin isoforms and to HUMXT01244. A, sequence alignment between acidic calponin and previously identified basic calponin isoforms. The first 273 amino acids of the deduced sequence of acidic calponin (RAC) are shown. In the aligned sequences, underlined regions indicate residues identical to RAC. Nonidentical residues are shown. Periods denote missing residues. Note only homologous portions of sequences are shown; divergent residues at the carboxyl terminus are not included. Other abbreviations: RH1, rat calponin h1 (25); MH1 and MH2, mouse uterine calponin h1 and h2 (GenBank Z19542 and Z19543); PH1 and PH2, pig smooth muscle calponin h1 and h2 (GenBank Z19538 and Z19539); Cha and Chb, avian calponin α and calponin β (9). B, sequence alignment between acidic calponin and translated amino acid sequence deduced from the longest open reading frame of the partial cDNA sequence of HUMXT01244 (28). Identical residues are boxed.

HUMXT01244 is an “expressed sequence tag,” or partial cDNA sequence, of an unidentified human hippocampal protein (29). The final 60 of the 71 amino acids contained within the longest open reading frame of HUMXT01244 have extremely high homology (93.3 and 82.2% on the amino acid and DNA levels, respectively) to the carboxyl terminus of rat acidic calponin (Fig. 2b). The 61 residues of acidic calponin which overlap with HUMXT01244 include the unique acidic carboxyl-terminal domain of acidic calponin (Figs. 1a and 2b). The 61 residues of acidic calponin which overlap with HUMXT01244 include the unique acidic domain (Figs. 1a and 2b) not found in smooth muscle calponins. HUMXT01244 was the only sequence in the database which contained the repeating tyrosine motif found in acidic calponin. The high degree of homology strongly suggests that HUMXT01244 represents the partial sequence of the human equivalent of the acidic calponin. Notably, HUMXT01244 is expressed in human hippocampus, suggesting that acidic calponin is a non-muscle calponin homolog.

Expression of Rat Acidic Calponin—Blot analysis of RNA isolated from cultured rat aortic VSMC revealed that the full-length insert of pRAC1 identified transcripts that migrated between approximately 1.8 and 2 kilobases (Fig. 3, lane 1). At short exposure times, an apparent doublet could be resolved (data not shown). The doublet was also evidenced using a specific 183-base pair probe spanning the sequence that encodes the unique acidic carboxyl-terminal domain of acidic calponin (Fig. 3, lane 2). The apparent doublet suggests that both the full-length and the 183-base pair specific probe hybridize to at least two structurally related but distinct transcripts. The mobility of the detected bands eliminates the 1.3-kilobase transcript that encodes the basic calponin h1 isoform (25) as a potential candidate. It is likely that the multiple bands reflect either mRNAs encoding other unidentified calponin isoforms or heterogeneity in the untranslated regions of transcripts encoding acidic calponin. Evidence for such heterogeneity is suggested by the different lengths of the untranslated regions in the pRAC1 and pRAC2 clones and the presence of the mRNA degradative sig-
Acidic Calponin

The deduced amino acid sequence of rat acidic calponin was queried against GenBank®/EMBL databank (593) for homology using the Genetics Computer Group (Vol. 7.2) sequence comparison program on the Vax 4000-300 computer.

Physically, the probe was hybridized with the radiolabeled 390-nucleotide probe spanning residues 850–1180 of pRAC1 and containing 60 nucleotides corresponding to the pBluescript polylinker. Bands of mobility with 330 nucleotides represented fully protected species. Note lower mobility bands in aortic RNA digests, indicative of partial protection. Lane 5 shows control RNase digestion of probe hybridized with 10 μg of yeast tRNA. Lanes 6 and 7 show different loadings of full length probe. Molecular weight markers are shown on the extreme left and right. The asterisk indicates mobility of bromphenol blue marker dye.

The full-length pRAC1 probe also identified transcripts migrating between 1.8 and 2 kilobases in a wide range of adult rat tissues. The most intense hybridization signals were detected in RNA samples from kidney, lung, brain, and stomach. Less intense signals were also detected in RNA from aorta, heart, and intestine. In RNA isolated from testis and thymus, the hybridization signal was barely detectable. In some tissues, lower mobility bands were also evident.

The blot hybridization results strongly suggest that unlike the smooth muscle-specific basic calponin isoforms (9, 25), acidic calponin is expressed in both smooth muscle and non-muscle tissues and in cultured VSMC. However, unambiguous interpretation is precluded by potential cross-hybridization of the full-length probe with transcripts encoding other calponin isoforms. For this reason, and because an acidic, non-muscle isoform of calponin is unprecedented, it was deemed necessary to rigorously demonstrate expression of corresponding transcripts in adult rat tissues. Toward this end, two independent methods, RNase protection assays and reverse transcription PCR, were employed to test for expression of acidic calponin in adult rat brain and aorta.

RNase protection assays were performed using a radiolabeled antisense riboprobe which spanned nucleotides 850–1180 of the pRAC1 sequence. Complete protection of the probe from RNase digestion was obtained with both brain and aortic RNA (Fig. 4), providing strong evidence for expression of acidic calponin transcripts in both tissues. The probe used for the protection is notable in that it spans the sequence encoding 46 of the 61 residues which overlap HUMXT01244 (see Figs. 1a and 2b). In the lane corresponding to brain RNA (Fig. 4), the only major band observed corresponds to the fully protected probe. The apparent absence of lower mobility bands, which would be indicative of partial protection of the probe by structurally related transcripts, suggests that the mRNA encoding acidic calponin is the only analog of HUMXT01244 expressed in rat brain. In contrast to the findings with rat brain, two intense bands with lower mobility were observed with aortic RNA. The two bands may represent partial protection of the probe by structurally related transcripts expressed in aorta. Because the probe spans part of the sequence homologous to basic calponins (see Figs. 1a and 2a), these are likely candidates.

To test further for expression of transcripts encoding acidic calponin in adult rat aorta and brain, as well as in cultured VSMC, cDNAs were generated by reverse transcription and amplified by PCR. As discussed under “Experimental Procedures,” the sense and antisense primers used for this analysis contained, respectively, 28 and 30 nucleotides corresponding to the 5' and 3' ends of the coding region of the rat calponin cDNA sequence. The major PCR product from cDNAs generated from

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**Table I**

Proteins with sequence homology to rat acidic calponin

<table>
<thead>
<tr>
<th>Protein</th>
<th>Identity</th>
<th>Residues a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat calponin h1 (25)</td>
<td>72.2</td>
<td>1-273</td>
</tr>
<tr>
<td>Mouse calponin h1 b</td>
<td>72.2</td>
<td>1-273</td>
</tr>
<tr>
<td>Pig calponin h1 c</td>
<td>71.7</td>
<td>1-273</td>
</tr>
<tr>
<td>Mouse calponin h2 d</td>
<td>68.9</td>
<td>1-273</td>
</tr>
<tr>
<td>Pig calponin h2 e</td>
<td>66.2</td>
<td>1-273</td>
</tr>
<tr>
<td>Avian calponin α (9)</td>
<td>68.3</td>
<td>1-273</td>
</tr>
<tr>
<td>Avian calponin β (9)</td>
<td>72.8</td>
<td>1-233</td>
</tr>
<tr>
<td>Rat SM22 (27)</td>
<td>59.7</td>
<td>51-190</td>
</tr>
<tr>
<td>Human SM22 (28)</td>
<td>42.1</td>
<td>1-197</td>
</tr>
<tr>
<td>Avian SM22a (27)</td>
<td>38.4</td>
<td>16-219</td>
</tr>
<tr>
<td>Rat neuronal protein 25 f</td>
<td>38.3</td>
<td>19-215</td>
</tr>
<tr>
<td>Human S112748 (28)</td>
<td>40.7</td>
<td>22-236</td>
</tr>
<tr>
<td>Human XT01244 (29)</td>
<td>93.3</td>
<td>60 of 71 deduced from partial sequence</td>
</tr>
</tbody>
</table>

a Numbers refer to deduced amino acid sequence of respective protein.

b Mouse calponin h1: GenBank Z19542.

c Pig calponin h1: GenBank Z19538.

d Mouse calponin h2: GenBank Z19543.

e Pig calponin h2: GenBank Z19539.

f Rat neuronal protein 25: GenBank M84725.

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**Fig. 3. Blot analysis of acidic calponin mRNA in cultured rat aortic VSMC and in adult rat tissues.** Lanes 1 and 2 contain 10 μg of RNA isolated from proliferating cultures of rat aortic VSMC. Lanes 3–11 contain 10 μg of RNA isolated from the indicated tissues of adult Sprague-Dawley rat. Equal loading was confirmed by ethidium bromide staining. Blots were hybridized with the full-length pRAC1 probe (lanes 1 and 3–11) and with the 183-base pair cDNA probe spanning residues 1039–1221 of pRAC1 sequence (region boxed in Fig. 1a) (lane 2). Exposure time for blot shown in lane 2 was reduced (12 versus 24 h) to enhance visualization of doublet.

**Fig. 4. Detection of acidic calponin transcripts in adult rat aorta and brain by RNase protection assays.** RNA from adult rat aorta (10 μg, lane 2) and brain (2.5 and 5 μg, lanes 3 and 4, respectively) were hybridized with the radiolabeled 390-nucleotide probe spanning residues 850–1180 of pRAC1 and containing 60 nucleotides corresponding to the pBluescript polylinker. Bands of mobility with 330 nucleotides represent fully protected species. Note lower mobility bands in aortic RNA digests, indicative of partial protection. Lane 5 shows control RNase digestion of probe hybridized with 10 μg of yeast tRNA. Lanes 6 and 7 show different loadings of full length probe. Molecular weight markers are shown on the extreme left and right. The asterisk indicates mobility of bromphenol blue marker dye.
RNA isolated from cultured VSMC and from adult rat aortic and brain tissues had the predicted mobility of 990 base pairs (Fig. 5). As observed, the product was not evidenced when reverse transcription was precluded by omission of enzyme. The 990-base pair product generated from each type of RNA was directionally cloned into pET-3xa vector, and the sequence of each was analyzed. The sequences of the amplified products from all three types of RNA corresponded to the coding region of acidic calponin (data not shown). The results provide conclusive evidence that transcripts encoding acidic calponin are expressed in both rat aorta and brain tissue, as well as in the cultured VSMC.

Functional Properties of Acidic Calponin—Directional cloning of the rat acidic calponin coding region into the pET-3xa expression plasmid provided a system for synthesizing sufficient quantities of the protein for actin- and calmodulin-binding assays. IPTG-induced expression of acidic calponin in transformed E. coli was demonstrated by the abundant presence of a protein with molecular mass of approximately 36 kDa (Fig. 6, lane c), which cross-reacted with a monoclonal antibody specific for avian gizzard calponin (data not shown). Acidic calponin was partially purified from the supernatant fraction of cell lysates by anion exchange column chromatography as described under “Experimental Procedures.”

In the absence of F-actin, acidic calponin remained entirely in the supernatant during airfuge centrifugation (Fig. 6, lane d). By contrast, when F-actin was present, greater than 50% of total acidic calponin co-sedimented with actin during airfuge centrifugation (Fig. 6, lane g). With increasing concentrations of F-actin, larger fractions of acidic calponin co-sedimented (data not shown). Note that all of the contaminating bacterial lysate proteins remained in the supernatant in the presence or absence of actin (Fig. 6, lanes d and f). The results demonstrate that acidic calponin binds F-actin.

The binding of acidic calponin to F-actin was not influenced by the addition of calmodulin (1 mg/ml) in the presence of 1 mM calcium (Fig. 6, lane i). By contrast, calmodulin has been shown to inhibit the binding of chicken gizzard smooth muscle calponin to actin in the presence of calcium (30, 31). The inhibition is due to competition of the Ca\(^{2+}\)/calmodulin complex with actin for calponin binding (31). To test if acidic calponin binds Ca\(^{2+}\)/calmodulin, partially purified calponin was applied to a calmodulin affinity column in the presence of 1 mM Ca\(^{2+}\). Spectrophotometric measurements of OD\(_{280}\) and subsequent SDS-PAGE analysis revealed that none of the applied protein was bound by the column (data not shown). The apparent absence of interaction between acidic calponin with calmodulin indicates a functional distinction between acidic and basic calponins, potentially indicative of different mechanisms regulating their actin binding affinities in vivo.

**DISCUSSION**

This study reports the cloning of a novel acidic isoform of calponin from rat aortic VSMC. This protein is structurally related to previously identified smooth muscle calponin isoforms (9, 25), but is distinguished by the presence of a highly acidic domain at its carboxyl terminus. Acidic calponin is expressed in non-muscle, as well as smooth muscle adult rat tissues, and is strongly expressed in passaged cultured rat aortic VSMC. By contrast, the basic smooth muscle calponin isoforms are predominantly smooth muscle specific (9), and their expression is strongly down-regulated with de-differen-
Acidic Calponin

Acidic calponin diversity is preceded by the reported detection of multiple electrophoretically distinct basic calponin protein forms in various smooth muscles (4, 5). The expression of these forms was reported to be smooth muscle specific and tightly linked to smooth muscle development (4, 5). Based on sequences of mouse and pig cDNA clones, at least two of the detected basic isoforms, the h1 and h2 forms, are encoded by distinct transcripts. The calponin h1 form, which has also been identified in rat aorta (25), is the equivalent of avian calponin α (9), the predominant calponin isoform found in chicken smooth muscle (9). The functional significance of the expression of the distinct calponin h2 form remains to be clarified. The diversity of calponin isoform expression is further complicated by the identification of an acidic variant that is expressed in non-muscle, as well as smooth muscle tissues. The considerable divergence between the sequences of acidic calponin and either the h1 and h2 forms suggests that distinct genes encode each of the three forms.

The identification of a unique calponin isoform expressed in non-muscle tissues is consistent with well documented evidence for distinct cytoplasmic (non-muscle) isoforms of other smooth muscle contractile proteins, including myosin (33, 34), actin (35, 36), and caldesmon (CaD) (7, 8). The molecular mass of acidic calponin is in good agreement with that of a putative calponin homolog identified in various non-muscle cells, including bovine brain (12) and platelets (13). This protein, which has an apparent molecular mass of approximately 36 kDa, was detected by immunoblotting using gizzard calponin polyclonal antibodies (12, 13). Based on its size, high degree of homology to gizzard calponin, and distribution of expression, acidic calponin appears to be an excellent candidate for the non-muscle calponin homolog. Consistent with this assignment, expression of transcripts encoding acidic calponin in adult rat brain tissue was demonstrated by two independent techniques. Furthermore, these results strongly suggest that the hippocampal gene, HUMXT01244, encodes the human equivalent of acidic calponin. As noted above, the portion of the amino acid sequence deduced from the partial HUMXT01244 cDNA sequence shows extremely high homology to the carboxyl terminal acidic domain of calponin. A fundamental question concerns the physiologic role of acidic calponin in non-muscle cells. Previous immunofluorescence studies using antibodies directed against avian gizzard calponin indicated that the distribution of calponin homologs in non-muscle cells mimicked that of 1-CaD, the cytoplasmic CaD isoform (13). Like 1-CaD, the immunoreactive forms of calponin localized to stress fibers of cultured bovine aortic VSMC and mouse fibroblasts and were associated with the actin cytoskeleton in bovine platelets (13). The precise role of 1-CaD in non-muscle cells has yet to be resolved; however, recent studies from a number of laboratories provide provocative evidence implicating 1-CaD in regulation of the organization of the actin cytoskeleton and/or non-muscle motility (7). Particularly intriguing are recent results from Matsumura and co-workers (37, 38) demonstrating that mitotic-specific phosphorylation by the cdc2 kinase causes dissociation of 1-CaD from actin microfilaments. The phosphorylation-induced dissociation of 1-CaD is likely to be fundamental to the reorganization of the actin cytoskeleton during mitosis and potentially plays a role in regulating the actomyosin interactions underlying cytokinesis. Co-localization of acidic calponin with 1-CaD in non-muscle cells would provide suggestive evidence that these proteins have analogous roles.

Acidic calponin is also expressed in adult smooth muscle tissues, as evidenced by the detection of corresponding transcripts in RNA from adult rat aorta. A large body of literature exists documenting expression of non-muscle forms of other contractile proteins in adult smooth muscle (33–36), but the physiologic significance is little understood. Previous immunocytochemical studies suggest that actin thin filaments in smooth muscle cells are distributed in two distinct domains, one associated with the “contractile actomyosin (muscle) domain” and the other with the “cytoskeletal actin intermediate filament (non-muscle) domain” (39). Future immunolocalization studies are needed to test whether basic and acidic calponins are segregated in the two distinct domains. Such studies may reconcile discrepancies between various laboratories concerning the localization of calponin in smooth muscle (10).

The assignment of acidic calponin as a component of the actin cytoskeleton is consistent with preliminary functional analysis. Like avian gizzard calponin, acidic calponin generated in a bacterial expression system bound F-actin. The functional conservation is consistent with the sequence homology between these proteins. In particular, 32 of the 38 amino acids in the assigned actin binding domain of gizzard calponin (40) (residues 145–182) are identical in acidic calponin. However, unlike gizzard calponin, we found no evidence for binding of acidic calponin to calmodulin, despite high sequence conservation (67 of 93 residues are identical, 10 are highly conserved substitutions) in the region assigned as the calmodulin binding domain of gizzard calponin (residues 52–144) (40).

The physiologic role of calmodulin binding by calponin is highly controversial (41). Although calponin inhibition of actomyosin interaction is reversed in the presence of Ca\(^{2+}\)/calmodulin (3, 30, 31), the estimated Kds of the calponin–calmodulin interaction is much too low to be physiologically relevant (41). Furthermore, a recent study by Wills et al. (41) suggests that the calponin–calmodulin interaction is nonspecific and that calponin interacts in a similar Ca\(^{2+}\)-dependent, but nonspecific, manner with various other Ca\(^{2+}\)-binding proteins. These authors argue that promiscuous interactions between calponin and numerous Ca\(^{2+}\)-binding proteins, including S100, may be an important means of regulating smooth muscle contractility (41). With respect to the mechanism underlying the nonspecific interactions, it is clearly of interest to determine if a mutant form of acidic calponin, lacking the acidic domain, binds to calmodulin and various other acidic Ca\(^{2+}\)-binding proteins. These studies may also lend insight into the functional significance of the highly acidic carboxyl-terminal domain.

The discovery of a non-muscle isoform of calponin opens many avenues for future research. Interactions between actin and non-muscle myosin I and II isoforms have been implicated in various non-muscle motile phenomena (42, 43), with abundant evidence demonstrating that non-muscle myosin II is the motor protein involved in cytokinesis (43, 44). However, little is known about the mechanisms regulating actomyosin interactions in non-muscle cells. Similarly, the information concerning regulatory mechanisms dictating the organization of the actin cytoskeleton is incomplete. Insight into the potential involvement of acidic calponin in regulating non-muscle motility and/or cytoskeletal organization may be gained from further functional analysis. Properties yet to be examined include the affinity of acidic calponin for muscle and non-muscle isoforms of tropomyosins, as well as the effect of phosphorylation on actin binding affinity. Most important, future studies need to determine if acidic calponin inhibits actomyosin interactions in vitro. These future functional analyses, together with immunolocalization studies, will be instrumental in elucidating the physiologic role of acidic calponin.

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