Cloning and Characterization of a Vertebrate Cellular Myosin Regulatory Light Chain Complementary DNA

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We have isolated two series of complementary DNAs (cDNAs) from a chicken gizzard cDNA library encoding two isoforms of phosphorylatable myosin regulatory light chain (RLC). One of the cDNAs encodes a previously isolated smooth muscle myosin RLC (also referred to as LC20-A); the other encodes a protein that shares 92% homology with the LC20-A isoform. The phosphorylatable threonine and serine residues at positions 18 and 19 of the two myosin RLC sequences are conserved. The two cDNAs are 81% homologous at the nucleotide level over the coding region; the 5′ and 3′ untranslated regions are divergent. Most of the DNA nonhomology in the coding region does not affect the protein sequence, indicating strong evolutionary conservation pressure to maintain the myosin RLC structure. Northern blot analysis using 3′ untranslated region probes reveals restrictive tissue specific expression of one myosin RLC isoform (LC20-A) in smooth muscle tissue and not in other tissues examined. In contrast, the novel myosin RLC isoform messenger RNA (mRNA) is uniformly expressed in all smooth and nonmuscle tissues examined and is designated as cellular myosin RLC for this reason. Our results indicate that cellular and smooth muscle myosin RLC isoforms are distinct and are encoded by separate genes. This report describes the cloning of a novel vertebrate cellular myosin RLC mRNA that differs from previously characterized smooth muscle RLC isoform mRNAs in both primary sequence and expression pattern. (*Circulation Research* 1990;67:933–940)

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Phosphorylatable myosin regulatory light chain (RLC, also known as MLC2) is a 20,000-Da protein that is associated with the amino-terminal globular head of the myosin molecule. Together with actin, myosin plays an important role in a wide range of contractile activities in both muscle and nonmuscle cells.[1] It is now generally accepted that interaction between actin and myosin in vertebrate smooth muscle cells and nonmuscle cells is regulated at the level of phosphorylation of myosin RLC.[2] In the absence of calcium, myosin RLC is not phosphorylated, and the myosin cannot interact with actin. Calcium activates a specific calmodulin-dependent myosin light chain kinase that phosphorylates myosin RLC, initiating myosin-actin interaction. Although most studies on the role of phosphorylation have concentrated on the regulation of actin-activated myosin ATPase activity, recent studies indicate that, in both smooth and nonmuscle cells, phosphorylation of myosin RLC seems to control the assembly of myosins into filaments.[3] Under conditions of physiological ionic strength and Mg-ATP concentration, myosins are assembled into filaments only when myosin RLC is phosphorylated. Myosin RLC is also phosphorylated by a number of enzymes, such as protein kinase C.[4] In addition, myosin RLC phosphorylation is stimulated by epidermal growth factor.[5] The implications of these phosphorylations are not yet clearly understood.

Structurally related myosin RLC isoforms that exhibit tissue-specific expression have been characterized from rat skeletal and cardiac muscle tissues by isolation of both complementary DNA (cDNA) and genomic clones.[6] These studies indicate that the structurally related skeletal muscle–specific and cardiac-specific myosin RLC isoforms are encoded by different genes. There is no evidence for alternate splicing of a single myosin RLC transcript giving rise to multiple isoforms, as has been shown for troponin T[7] and tropomyosin isoforms.[8,9] Characterization of a myosin RLC cDNA clone has been reported from rat

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aortic smooth muscle cells in culture.[10] This rat aortic myosin RLC gene was found to be ubiquitously expressed in all muscle and nonmuscle tissues, leading to the suggestion that the smooth muscle and nonmuscle myosin RLC isoforms are identical and encoded by a single gene.[10] However, we have recently characterized a human smooth muscle–specific myosin RLC messenger RNA (mRNA) that is expressed specifically in smooth muscle tissues and not in other muscle tissues.[11] Analysis of myosin RLC isoforms, after immunoprecipitation and two-dimensional gel electrophoresis, indicates the presence of three isoforms in both rat aortic smooth muscle cells in culture[12,13] and human fibroblast cells.[11] These studies raised the possibility that the smooth muscle–specific and the nonmuscle myosin RLC isoforms are distinct and are encoded by different genes. In addition, it has been reported that porcine arterial smooth muscle tissue contains two isoforms of myosin RLC.[14,15] Moreover, evidence for a second, translationally regulated isoform of smooth muscle myosin RLC has recently been provided by the isolation of the corresponding cDNA clone from an embryonic chicken gizzard library.[16] Finally, a cDNA clone for a myosin heavy chain has recently been obtained from a chicken intestinal epithelial cell library; this gene was found to be expressed in all tissues examined.[17] Because this gene was expressed in both muscle and nonmuscle tissue, it was referred to as cellular myosin heavy chain. Taken together, these data indicate that smooth muscle and nonmuscle (cellular) myosin RLC proteins are distinct[14,15] and that myosin heavy chain genes for smooth muscle and cellular[17] isoforms are distinct.

We have previously reported the sequence of a full length cDNA for chicken smooth muscle myosin RLC (CsmRLC).[18] In the present study, we have characterized this clone as well as a novel cDNA, which together correspond to two distinct myosin RLC isoforms isolated from an adult chicken gizzard cDNA library. These two cDNA clones differ from the previously reported myosin RLC isoform cDNA clone isolated from embryonic gizzard[16] both in sequence and in number of nucleotides. Analysis of the expression of the two cDNAs described in this report in different muscle and nonmuscle tissues indicates that one of them (CsmRLC[18]) encodes a smooth muscle–specific myosin RLC isoform and that the second clone represents a novel myosin RLC isoform mRNA expressed in all tissues examined. Sequence analysis of these cDNA clones and blot hybridization to genomic DNA indicate that the two myosin RLC isoforms are encoded by different genes. To be consistent with nomenclature previously assigned to the ubiquitously expressed myosin heavy chain isolated from intestinal epithelium,[17] we refer to the novel myosin RLC isoform cDNA described in this report as chicken cellular myosin RLC (CceRLC).

Materials and Methods

Cloning and Sequencing

Approximately 4×10^4 plaque-forming units of an adult chicken gizzard cDNA library in a λgt10 vector were screened with a 927 nucleotide (nt) rat aortic myosin RLC cDNA probe.[10] Positive clones were obtained after 16–20-hour hybridization at 42° C in 50% formamide, 5× Denhardt's solution (0.1% polyvinylpyrrolidone, 0.1% Ficoll, and 0.1% bovine serum albumin), 5× saline–sodium phosphate–EDTA (SSPE) (20× SSPE contains 3.6 M NaCl, 0.2 M NaH_2PO_4 [pH 7.4], and 0.02 M EDTA), 0.1% sodium dodecyl sulfate (SDS), and 100 μg/ml sheared salmon sperm DNA and washing with 1× saline sodium citrate (SSC) (0.15 M NaCl and 0.015 M sodium citrate) at 55° C. The clones were plaque-purified, and *Eco*RI or *Bgl* II/*Hin*dIII inserts were cloned into M13 mp8 and M13 mp9 for sequencing using the dideoxy method.[19] One clone was identified as CsmRLC.[18] Another clone, consisting of a 380 nt *Eco*RI fragment homologous to CsmRLC, was used to rescreen the original gizzard cDNA library. Clone CceRLC-617, containing a partial coding region and 3′ untranslated region (utr), was isolated after overnight hybridization and washing in 0.1× SSC and 0.1% SDS at 55° C. A 380 nt *Pst* I fragment of clone CceRLC-617 containing only 3′utr was used to screen a second λgt10 adult chicken gizzard cDNA library made from poly(A^+) selected RNA[20] (constructed by Promega Biotec, Madison, Wis.). By use of the same hybridization and washing conditions as described above, clone CceRLC-677 was isolated; this clone contains the 5′utr, complete coding region, and partial 3′utr. *Bgl* II/*Hin*dIII restriction fragments of clones 617 and 677 were inserted into pUC19 for double-stranded sequencing using M13 universal and reverse sequencing primers (numbers 1212 and 1201, New England Biolabs, Beverly, Mass.). Additional sequence information was obtained using oligonucleotide primers synthesized using an automated DNA synthesizer (model 380A, Applied Biosystems, Inc., Foster City, Calif.).

RNA Isolation and Blot Analysis

Tissues were dissected from freshly killed chickens, frozen in liquid nitrogen, and homogenized in 4 M guanidine thiocyanate to extract total RNA as described previously.[21] Total RNA (10 μg) was electrophoresed as described[22] and transferred to GeneScreen Plus (E.I. duPont de Nemours & Co., Inc., Wilmington, Del.) using buffers and conditions recommended by the manufacturer. Northern blots were probed with ^{32}P nick-translated DNA fragments (New England Nuclear, Boston) corresponding to the 3′utr from the two myosin RLC isoforms. Hybridization was carried out for 16–20 hours at 42° C in 50% formamide, 1% SDS, 1 M NaCl, and 10% dextran sulfate. Blots were washed at low stringency with 2× SSC and 0.1% SDS at 50° C. Autoradiography was performed using Kodak XAR-5 film at −70° C with Cronex Lightening Plus intensifying screens (duPont).

Genomic Southern Blot

Purified chicken gizzard genomic DNA (10 μg) was digested with *Bam*HI, *Bgl* II, *Eco*RI, *Hin*dIII,

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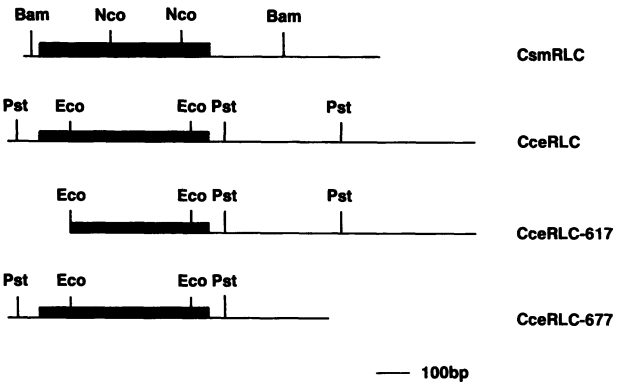

FIGURE 1. *Myosin regulatory light chain complementary DNA (cDNA) clones. Partial restriction maps are presented for two clones of the novel cellular (Cce) regulatory light chain (RLC) isoform cDNA: clone CceRLC-677 is 939 nucleotides (nt) long, and CceRLC-617 is 1,191 nt long. The composite 1,378 nt CceRLC cDNA was obtained from the overlap of clones 617 and 677. Also shown is the 1,075 nt cDNA for the chicken smooth muscle (Csm) myosin RLC.[18] The coding regions are represented by a solid box; the untranslated regions, by a thin line.*

and *Xho* I restriction enzymes (New England Biolabs) and fractionated by electrophoresis on an 0.8% agarose gel.[22] DNA was transferred to GeneScreen Plus (duPont) and hybridized to ^{32}P-labeled fragments corresponding to coding regions from CsmRLC and CceRLC isoforms. Hybridization conditions were as described for RNA blots. The Southern blot was washed in 2× SSC and 0.1% SDS at 50° C and autoradiographed at −70° C with an intensifying screen. The same blot was used for rehybridization after removing the first probe by shaking the blot in 0.4N NaOH at 42° C for 30 minutes and in 0.1× SSC and 0.2 M Tris-HCl (pH 7.5) at 42° C for 30 minutes.

Results

Identification of Myosin RLC Isoform cDNAs

A putative rat aortic myosin RLC probe[10] was used to screen an adult chicken gizzard cDNA library prepared in λgt10. We have previously reported the isolation of a cDNA clone[18] whose coding region predicted a myosin RLC that is identical to that obtained by sequencing purified adult chicken gizzard myosin RLC protein (LC20-A). This smooth muscle myosin RLC cDNA clone is designated CsmRLC in Figure 1. Additional cDNA clones were isolated and found to have homologous DNA sequences to LC20-A. The first clone that was isolated contained a 380 nt *Eco*RI insert, which was then used to screen a second cDNA library from adult chicken gizzard. Two overlapping cDNA clones, which together span the coding and untranslated regions of the cDNA, are shown in Figure 1. Clone CceRLC-617 begins at the first *Eco*RI site in the coding region and extends past the polyadenylation site. Clone CceRLC-677 begins in the 5′utr immediately upstream from a *Pst* I site, continues past the *Eco*RI sites in the coding region, and ends just upstream from the second *Pst* I site of clone CceRLC-617.

Sequence of a Novel Chicken Myosin RLC Isoform cDNA

The DNA sequence information, obtained from the overlap of the two CceRLC partial cDNA clones is given in Figure 2. The combined sequence for the CceRLC cDNA clone contains a 92 nt 5′utr, a 519 nt coding region (including the ATG initiation codon and TGA termination codon), and a 767 nt 3′utr, including the poly(A) tail. The amino acid sequence encoded by the CceRLC cDNA is given above the nucleotide sequence. Also shown in Figure 2 is the sequence for CsmRLC cDNA[18] isolated from adult gizzard as well as a smooth muscle myosin RLC isoform cDNA obtained from embryonic chicken gizzard (LC20-B1).[16] Only nucleotide differences are shown in the figure. The CceRLC cDNA is 1,378 nt long, compared with 1,075 nt for the CsmRLC clone and 899 nt for LC20-B1. Most of this difference is due to a long 3′utr in the CceRLC cDNA. Although the DNA homology between the CceRLC and CsmRLC cDNAs is greater than 80% over the coding region, most of the differences are silent and do not affect the protein sequence (shown in Figure 3). In contrast, the CceRLC and LC20-B1 cDNA coding regions differ by only three nucleotides. Except for an identical 33 nt stretch in the 3′utr of CceRLC and LC20-B1 cDNAs, both the 5′utr and 3′utr are divergent for all three cDNAs examined. Note also the presence of AATAAA polyadenylation signals at nucleotide positions 828 and 1,357 in CceRLC and 827 in LC20-B1 cDNAs.

Comparison of Myosin RLC Sequences From Different Species

A comparison of the chicken myosin RLC isoform protein sequences with other myosin RLCs is shown in Figure 3. There are three clusters of differences between CceRLC and other myosin RLC protein sequences: one at amino acid position 4–7, a second at position 69–73, and a third at position 114–121. It is interesting to note that at these locations the CceRLC encoded protein most closely resembles the chicken embryonic gizzard LC20-B1 (overall homology >99%) and rat aortic myosin RLC (overall homology 95%), whereas the chicken smooth muscle and human smooth muscle myosin RLC sequences are identical at all three positions. The functional significance of the changes in the amino acids at positions 4–7, 69–73, and 114–121 in the myosin RLC structure is, however, not known.

Chicken Myosin RLC Isoforms Exhibit Differential Expression

Duplicate Northern blots of RNA isolated from several chicken tissues were hybridized with divergent 3′utr probes specific for myosin RLC isoforms. The blots were washed under low stringency condi-

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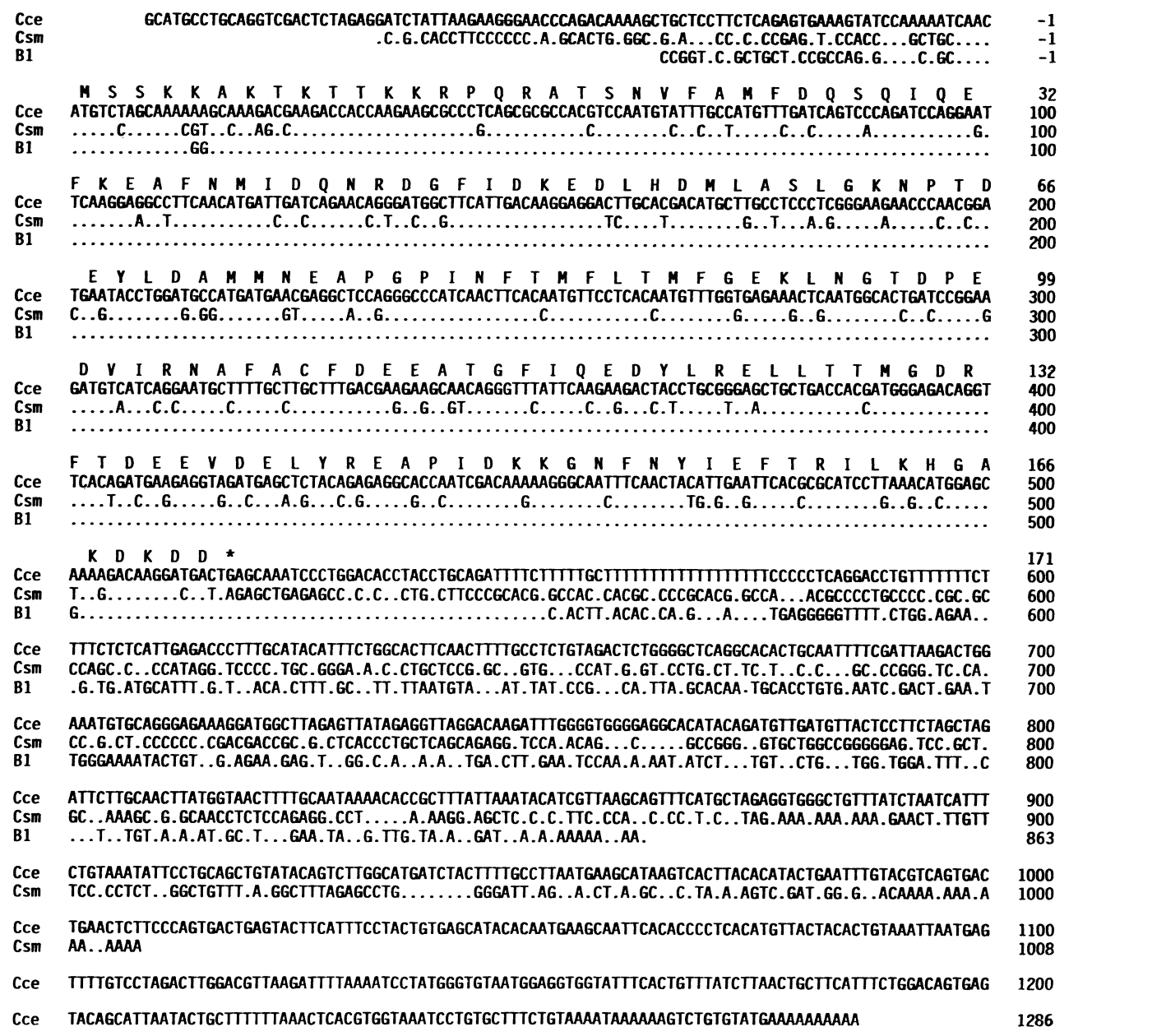

FIGURE 2. *Nucleotide and derived amino acid sequence comparison between chicken cellular (Cce) myosin regulatory light chain, chicken smooth muscle (Csm) regulatory light chain,[18] and embryonic chicken gizzard regulatory light chain isoform (B1).[16] The nucleotide sequence of Cce regulatory light chain was obtained from the overlapping clones 617 and 677. Only nucleotide differences are shown in the sequence comparison. The single letter amino acid sequence for Cce regulatory light chain is given above the complementary DNA sequence. The numbers for the Cce regulatory light chain amino acid sequence as well as the complementary DNA nucleotide sequences are given to the right of the figure.*

tions to detect any related mRNAs. The probe for CsmRLC was a 325 bp *Alu* I fragment from the 3′utr of CsmRLC cDNA; the probe for the CceRLC cDNA was a 373 nt *Pst* I fragment from the 3′utr of CceRLC-617 (Figure 1).

The results of the Northern blots are shown in Figure 4. Figure 4A shows the Northern blot analysis using the CsmRLC *Alu* I 3′utr fragment as a probe. High level expression of a 13S RNA, approximately 1,100 nt, is detected in smooth muscle tissue (lanes 1 and 3, containing RNA from gizzard and aorta, respectively). No signal was observed for RNAs from other tissues (lanes 2 and 4, containing brain and kidney RNA, respectively), even after prolonged exposure (>120 hours, not shown). Apparent hybridization to higher molecular weight RNAs in the blot in Figure 4A may be due to the incomplete denaturation of the RNA sample because purified polyadenylated RNA from chicken smooth muscle showed hybridization to the 13S species alone with the 3′utr *Alu* I fragment used as a probe (data not shown). The apparent hybridization of the labeled CsmRLC *Alu* I

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CceRLC    MSSKKAKTKTTKKRPQRATSNVFAMFDQSQIQEFKEAFNMIDQNRD   45
LC20-A    ----R--A-------------------------------------
LC20-B1   ----R--A-------------------------------------
RamRLC    ----R--A-------------------------------------
HsmRLC    ----R--A-------------------------------------

CceRLC    GFIDKEDLHDMLASLGKNPTDEYLDAMMNEAPGPINFTMFLTMFGE   91
LC20-A    --------------M---------EG--S------------------
LC20-B1   ------------------------------------------------
RamRLC    --------------M-------------------------------
HsmRLC    ------------------------EG--S-----Y-----------

CceRLC    KLNGTDPEDVIRNAFACFDEEATGFIQEDYLRELLTTMGDRFTDEE  137
LC20-A    ----------------------S---H--H-----------------
LC20-B1   ------------------------------------------------
RamRLC    ----------------------I-T---------------------
HsmRLC    ----------------------SS---H--H-----------------

CceRLC    VDELYREAPIDKKGNFNYIEFTRILKHGAKDKDD               171
LC20-A    ---M-------------V---------------
LC20-B1   ---------------------------------
RamRLC    ---------------------------------
HsmRLC    ---M-------------V---------------
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FIGURE 3. *Comparison of amino acid sequence for myosin regulatory light chain (RLC) proteins in different species. Cce, chicken cellular; LC-20A, chicken adult smooth muscle; LC20-B1, chicken embryonic smooth muscle; Ram, rat aortic; Hsm, putative human smooth muscle.*

3′utr fragment to higher molecular weight RNAs in the Northern blot may also be due to the low stringency washing conditions (see "Materials and Methods").

In contrast, the CceRLC 3′utr probe (Figure 4B) shows the low level expression of a 15S RNA species, approximately 1,400 nt, in all tissues examined, including gizzard, brain, aorta, and kidney. Note that the exposure time of Figure 4A was 18 hours; that of Figure 4B was 120 hours (washing conditions were identical).

Hybridization of CsmRLC and CceRLC cDNAs to Genomic DNA

Genomic DNA was isolated from chicken gizzard smooth muscle tissue, digested with several restriction enzymes subjected to agarose gel electrophoresis, and transferred (blotted) to a GeneScreen membrane (duPont). The blot was prepared, hybridized, and washed as described in "Materials and Methods." The same blot was hybridized to coding region probes for CsmRLC and CceRLC sequentially after removal of the first probe. Figure 5A shows the banding pattern of chicken genomic DNA probed with a CsmRLC coding region probe. Major bands of 6.6, 9.4, 9.4, 23, and approximately 8 kilobases (kb) are detected in lanes 1–5, respectively. Additional minor bands are also visible in some lanes. Figure 5B shows the same blot hybridized to a CceRLC coding region probe. The major bands at 6.6 kb (lane 1), 9.4 kb (lane 2), and approximately 8 kb (lane 5) are shared between both probes. Several bands (present in Figure 5A) are not found in the CceRLC probed Southern blot (Figure 5B), including the 9.4-kb band (lane 3) and the 23-kb band (lane 4). New bands appear at approximately 3 kb (lane 2), 4 and 2 kb (lane 3), and 8 and 3 kb (lane 4), as well as several additional faint bands in the CceRLC probed Southern blot (Figure 5B).

Discussion

We have previously reported the cloning of a smooth muscle–specific myosin RLC cDNA from chicken gizzard.[18] This report describes the cloning of a novel cDNA encoding an isoform of vertebrate myosin RLC as well as the differential expression of myosin RLC isoform mRNAs in several muscle and nonmuscle tissues.

The data presented in this report provide further evidence that smooth muscle and nonmuscle (cellular) myosin RLCs are distinct and are encoded by separate genes. The cDNA clone described in this work, designated CceRLC, encodes a 171 amino acid RLC whose protein sequence is highly homologous (92%)

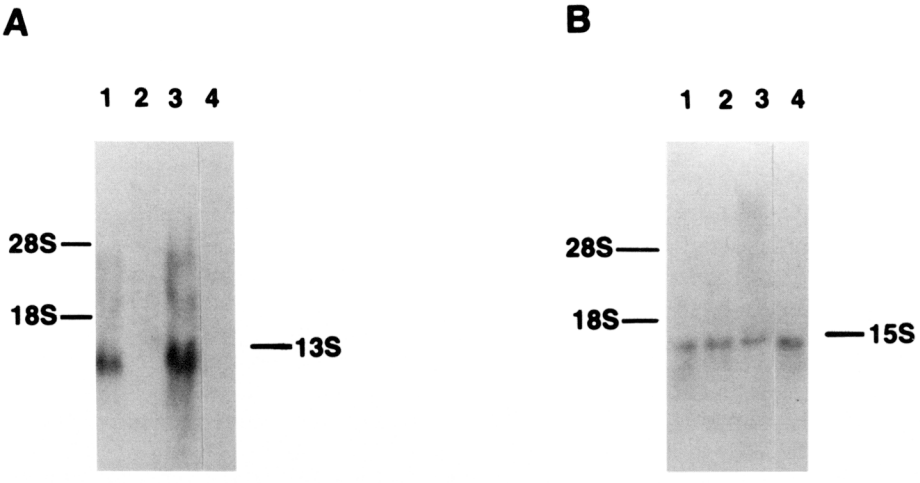

FIGURE 4. *Northern blots showing expression of chicken myosin regulatory light chain isoform RNAs in various chicken tissues. Total cellular RNA (20 μg) was size-fractionated, transferred to a gene screen, and hybridized to ^{32}P-labeled untranslated region probes for chicken smooth muscle regulatory light chain* (panel A) *or chicken cellular regulatory light chain* (panel B). *Positions of 28S and 18S ribosomal RNAs are indicated on the left of each blot. Size of hybridizing RNA is given to the right of each blot. Samples are gizzard (lane 1), brain (lane 2), aorta (lane 3), and kidney (lane 4). Exposure time was 18 hours in panel A and 120 hours in panel B. Hybridization and washing conditions are as described in "Materials and Methods."*

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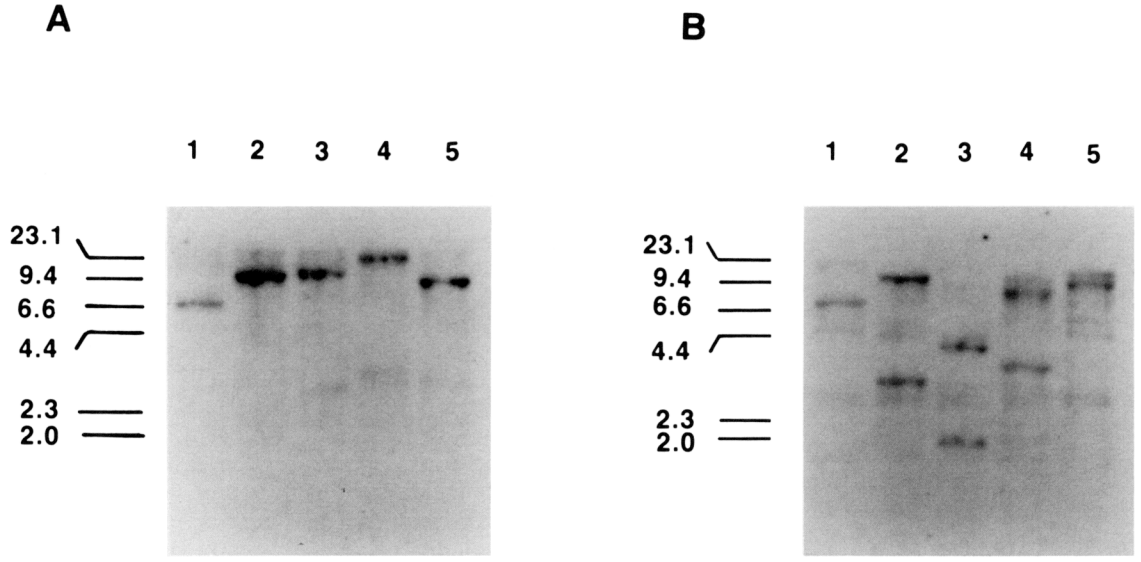

FIGURE 5. *Southern blots showing hybridization of cellular and smooth muscle myosin regulatory light chain complementary DNAs to genomic DNA. Total genomic DNA was isolated from chicken gizzard and digested with several restriction enzymes; then a Southern blot[22] was prepared. The blot was hybridized using a ^{32}P-labeled chicken smooth muscle regulatory light chain coding region probe* (panel A). *The probe was removed, and the blot was rehybridized with a ^{32}P-labeled chicken cellular regulatory light chain coding region probe* (panel B). *Blots were washed under low stringency conditions as described in "Materials and Methods." Sizes in kilobases of λDNA digested with* Hind*III are given to the left of each blot. Restriction enzymes used were* Bam*HI (lane 1),* Bgl *II (lane 2),* Eco*RI (lane 3),* Hind*III (lane 4), and* Xho *I (lane 5).*

to the smooth muscle–specific CsmRLC isolated from adult chicken gizzard (LC20-A) and nearly identical to an embryonic chicken gizzard myosin RLC isoform (LC20-B1).[16] The CceRLC cDNA isolated from adult chicken gizzard shares 81% nucleotide sequence homology with the CsmRLC coding region; the untranslated regions are divergent. Noteworthy is the conservation of amino acids threonine and serine found at positions 18 and 19 in all RLCs, because these amino acids in smooth muscle RLCs have been shown to be phosphorylated by myosin light chain kinase,[23–25] an important regulatory event in contraction. The differences in the nucleotide sequences of CsmRLC and CceRLC are predominantly at the third base position of the amino acid codons, which do not change the protein sequence, suggesting that there is a strong evolutionary pressure to conserve the myosin RLC protein structure. The cDNAs encoding the vertebrate myosin RLC isoforms investigated herein also differ in length: the smooth muscle (CsmRLC) isoform is 1,075 nt long, the novel cellular isoform (CceRLC) is 1,378 nt long, and the embryonic gizzard LC20-B1 cDNA is 899 nt long. This difference in length is due to additional nucleotides in the 3′utr of CceRLC cDNA. The 3′utr of the CceRLC cDNA contains two polyadenylation signals at nucleotide positions 828 and 1,357. The polyadenylation signal at position 828 of the CceRLC cDNA is in approximately the same position of the 3′utr as that of the LC20-B1 cDNA (position 827), although there is no evidence that the polyadenylation signal at position 828 of the CceRLC mRNA is utilized. Finally, the 3′utr of the CceRLC cDNA contains one T stretch (26 of 29 nucleotides) at positions 547–576 and a second T stretch (11 of 13 nucleotides) at positions 590–603. This feature is not shared with any of the known smooth muscle myosin RLC isoform cDNAs, and its significance is not known.

Evidence is now presented, given this report and previous studies,[16,18] for the existence of at least three myosin RLC isoform RNAs expressed in chicken gizzard. Expression of at least one isoform (LC20-B1) may be translationally controlled[16]; it remains to be established whether the CceRLC gene expression is similarly controlled. The CceRLC, CsmRLC, and LC20-B1 myosin RLC isoform cDNAs are all distinct based on nucleotide and encoded protein sequences, although they are highly homologous. The coding region homology between the CceRLC and LC20-B1 cDNAs is particularly striking (>99% at the nucleotide and amino acid levels). The untranslated regions of these cDNAs also share identity for 33 nucleotides past their termination codons, although the remainder of the 3′utr are divergent and of different lengths. The high degree of homology at the nucleotide level in the coding region of the CceRLC and LC20-B1 cDNA clones, the identity in a 33 nt stretch of the 3′utr, and the strong overall homology among all three cDNA clones provide evidence for the possibility that the myosin RLC isoforms comprise a gene family, arising from duplication of a single ancestral gene. The extremely high homology of the coding regions between the CceRLC and LC20-B1 cDNAs (as well as the Southern blot data) suggests that these myosin RLC mRNAs may arise from an alternative splicing

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mechanism. Confirmation of these possibilities will await chromosomal assignment of the myosin RLC isoforms as well as isolation of genomic clones of the chicken myosin RLC locus.

We have used untranslated region probes from the CsmRLC and CceRLC cDNAs to clearly demonstrate both qualitative and quantitative differences in expression of the myosin RLC isoform mRNAs in various chicken tissues. High level expression of the previously cloned CsmRLC[18] mRNA is found predominantly in smooth muscle tissue. In contrast, the novel cellular isoform mRNA encoding CceRLC is expressed in all tissues examined, including gizzard, brain, aorta, and kidney. This expression pattern was also observed for a recently cloned cellular myosin heavy chain.[17] Based on the signal intensities and the exposure times of Figure 4, the expression of the cellular isoform mRNA of myosin RLC is not more than 5% of the expression level of smooth muscle myosin RLC mRNA in gizzard and aorta tissues. It is interesting to note that published Northern blot analysis of RNA expression in chicken gizzard,[16] using the LC20-B1 coding region cDNA probe, revealed two hybridizing bands at approximately 1.1 and 1.4 kb, whereas only the 1.1-kb RNA species was detected with a 3′utr probe. Since only the 1.1-kb RNA hybridized to the 3′utr LC20-B1 probe, the authors had speculated that the 1.4-kb RNA may represent a related message. It is possible that the 1,378 nt CceRLC cDNA that we have isolated may correspond to the 1.4-kb RNA detected by the LC20-B1 cDNA probe,[16] because the coding regions are nearly identical (as mentioned above). More importantly, the 3′utr probe of the CceRLC cDNA only detects a 1.4-kb RNA, as shown in the Northern blot of Figure 4B, since the 3′utr of the myosin RLC isoform cDNAs are divergent.

Previous studies by Taubman et al,[10] using rat aortic myosin RLC cDNA isolated from aortic smooth muscle cells in culture, suggested that smooth muscle and nonmuscle (cellular) myosin RLC isoforms are identical and encoded by a single gene. In contrast, we have recently characterized a human smooth muscle–specific myosin RLC cDNA from human umbilical artery and have shown that this myosin RLC isoform is expressed only in smooth muscle tissues, but not in other muscle types.[11] This highly differentiated cell-type–specific myosin RLC isoform is also expressed in some, but not all, nonmuscle cells. Based on these studies, we suggested that smooth muscle and nonmuscle (cellular) myosin RLC isoforms are distinct and are encoded by different genes. The results presented in this report confirm our previous observations and also provide direct evidence that smooth muscle and cellular myosin RLC isoforms are encoded by different genes. Although hybridization of the myosin RLC isoforms described in this report to chicken genomic DNA revealed a simple pattern consistent with single copy number, several faint bands also appeared. Additional faint bands were also observed in the genomic Southern blot of a cellular myosin heavy chain cDNA.[17] These results, as well as our own, may represent cross hybridization to additional myosin heavy chain or myosin RLC isoforms, respectively, of cellular, smooth, or sarcomeric type.

In view of all of the above described findings including mRNA expression patterns, it is not very surprising to note that the cDNA reported here, which we propose corresponds to the cellular myosin RLC isoform, shows greater homology (95%) in amino acid sequence to the rat aortic myosin RLC than to CsmRLC (92% homology). It is interesting to speculate whether the difference in amino acid sequence between myosin RLC isoforms at positions 4–7, 69–73, and 114–121 have any relevance to the function of these isoforms in different cell types.

Smooth muscle contractile activity is important for many physiological activities, including blood movement, food propulsion, and uterine contraction. Cellular contractile activity appears to be necessary for several phenomena including cytokinesis, cellular movement (motility), and adhesion to solid support. In addition, as part of the cytoskeletal network, cellular contractile proteins play an important structural role in maintaining cell morphology and membrane fluidity. Our finding of two distinct myosin RLC isoforms encoded by two different genes, as well as previous reports on cellular myosin heavy chain[17] and LC20-B1,[16] reinforces the importance of the myosin RLC isoforms for the respective cellular structural/contractile activities. Moreover, our preliminary studies, using human smooth muscle–specific myosin RLC cDNA, indicate that the smooth muscle–specific isoform is selectively repressed when fibroblast cells undergo transformation (manuscript in preparation). The availability of smooth and cellular (nonmuscle) myosin RLC cDNA probes will, therefore, facilitate studies on genomic organization of the myosin RLC locus as well as regulation of differential expression of myosin RLC isoforms in the normal and transformed states.

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KEY WORDS • cloning • myosin regulatory light chain

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