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Molecular cloning of a mouse myosin I expressed in brain

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We have isolated two cDNAs that encode putative myosin I heavy chains by polymerase chain reaction amplification of brain cDNA with degenerate oligodeoxynucleotide primers representing myosin I-specific conserved amino acid sequences. We report the complete deduced amino acid sequence of one of these cDNAs. The sequence is most similar to those of the avian and bovine brush border myosin Is, with five putative calmodulin-binding repeats at the head–tail junction. Northern analysis demonstrates that this myosin heavy chain, unlike the brush border myosins, is expressed in many tissues.

Myosin heavy chain; Cytoskeleton; Cell motility

1. INTRODUCTION

The two major classes of myosins are conventional two-headed (myosin II) and one-headed (myosin I). Myosins are oligomeric proteins consisting of one or two heavy chains (MHCs) and varying numbers of light chains. The heavy chains of both classes have highly conserved N-terminal, or head, domains that generate mechanical force in the presence of ATP and actin. The C-terminal, or tail, domains differ between the two classes.

The myosin IIs are hexamers composed of a pair of heavy chains and two pairs of light chains. The heavy chains dimerize through their tail regions, forming an α-helical coiled-coil structure.

The single-headed myosin Is do not have α-helical coiled-coil tails, and thus the heavy chains do not dimerize [1,2]. The tails of some myosin Is have been shown to have actin- and phospholipid-binding activities. The lower eukaryote *Dictyostelium discoideum* has provided an excellent model for the study of amoeboid movement [3]. In *Dictyostelium*, myosin I has been localized to leading edges of pseudopodia of amoebae [4], consistent with myosin I isoforms playing an important role in pseudopod extension or stabilization. The subtle effects of ablation of one of the myosin I isoforms [5] suggest that the *Dictyostelium* myosin I isoforms have overlapping functions.

Extension of processes is also exhibited by many cell types during vertebrate development. Many elegant descriptive studies have analyzed mechanisms that are likely to be important in force generation and pathfinding, including actin treadmilling and forward transport of membrane proteins to the leading edge [6–8]. However, direct identification of the structural proteins involved, except actin, has been lacking. Nonconventional myosins have been proposed to mediate some of these mechanisms [9–11,8], and myosin I immunoreactivity has been observed [12] at the leading edge of the cell, but no vertebrate myosin Is (other than those of the brush border) have been identified until recently [13].

The *Dictyostelium* results suggest that mammalian cells also will have multiple myosin I isoforms. We have begun this approach by isolating cDNAs for two myosin I isoforms expressed in the mouse brain by PCR amplification, using degenerate primers based on conserved amino acid sequences.

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2. EXPERIMENTAL

RNA was prepared with RNAzol (Cinna Biotecx). Polyadenylated RNA was purified by oligo d(T)-cellulose spin columns (Pharmacia). Random-primed first- strand cDNA was prepared from this RNA using AMV reverse transcriptase (Invitrogen Copy Kit). Polymerase chain reaction (PCR) amplification of myosin I sequences was performed with the GeneAmp kit (Perkin Elmer Cetus) under standard conditions, using a 40°C annealing step, for 30 cycles. Approximately 1 ng of first-strand cDNA was amplified with primers 5′-ACI(T/C)TIAA(A/G)IIIGA(A/G)CA(A/G)GA(A/G)GA-3′ and 5′ -GG(T / C)TT(A / G)AT(A / G)CAIC(T / G)(A / G)AT(A / G)TA - 3′ (TLKXEQEE> and <YIRCIKP in Fig. 1, respectively; I = inosine). The PCR products were cloned using the TA cloning kit (Invitrogen).

The original PCR clone was used to screen an oligo d(T)-primed C57BL/6 brain library prepared in lZAPII (Stratagene). The largest (2.7 kbp) clone from that screen contained the 3′ terminus but lacked the 5′ terminus. To obtain the 5′ portion of the cDNA, an antisense oligonucleotide from the 5′ end of the original cDNA clone was used to prime brain cDNA synthesis. The cDNA was cloned using the Librarian II kit (Invitrogen) and approximately 10^5 colonies of the resulting library were screened with a DNA fragment from the 5′ end

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                    TLKXEQEE> 5'
MHC I   NEKLQQΔFI-lTLK.EQEEyv.EgI.W.pΔ.yfdNkΔ.cDLIEek+p.Gi..ΔLD-.C.....p.g..D.
MHC II  NEKLQQFFNHHMFvLEQeEY.kEgI.W.fidfG.DlacIeLI.EK.Pmgi.sΔl-Eec.f....PkatDt
MIHC-K
MIHC-L                          YIREDIEWTHIDYFNNAIICDLIENNTN-GILAMLDEECLRPGTVTDE

MHC I   kfΔeK.......h.h........n............F.I+HYAG-VtYnΔ-GF.-KN+D.LF.DLΔ..Δ..
MHC II  sf..KL..qhlgk.nnf.....kpk.....kgkEahFsl.hYAGtV.y....wleKnkDPLn.tv..l...
MIHC-K                                                                      LK
MIHC-L  TFLEKLNQVCATHQHFESRMSKCSRFLNDTTLPHSCFRIQHYAGKVLYQVEGFVDKNNDLLYRDLSQAMWK

MHC I   s...ll.slFpe..........kK+P.TaGf..K.SΔ..Lmk.L..c.PHYIRCIKP
MHC II  .s......lf.....g..k...kkgsfTvsal.re.lnkLm..LrsThPHFvRCiIP
MIHC-K  ETMCSSMNPIMAQCFDKSELSDKKRPETVATQFKMSLLQLVEILRSKEPA
MIHC-L  AGHSLIKSLFPEG---NPAKVNLKRPPTRSSQFKASVATLMRNLQTKNPN
                                                        <YIRCIKP 3'
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Fig. 1. Partial amino acid sequences of myosin heavy chain head regions. MHC I and MHC II are consensus sequences as described by Pollard et al. [1]. MIHC-K and -L are sequences from the cloned PCR products. TLKXEQEE> and <YIRCIKP represent the MHC I consensus sequences used to synthesize the oligonucleotide primers used for PCR amplification.

of the original 3′ clone. Sequencing was performed by dideoxy chain termination. Northern blots were prepared by standard methods and hybridized with the first cDNA clone or a human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probe [14], labeled with ^{32}P using the Prime-It kit (Stratagene).

3. RESULTS AND DISCUSSION

Fig. 1 illustrates consensus sequences from myosin I and II heavy chains [1] in the region of the myosin I sequence targeted for amplification. We amplified approximately 1 ng of cDNA (GeneAmp kit, Perkin Elmer Cetus) with the primers TLKXEQEE> and <YIRCIKP (Fig. 1). We chose the primers to amplify myosin I sequences specifically. Although this region has highly conserved amino acid sequences at the 5′ and 3′ ends, the central region is not well conserved, so that clones obtained by this method should serve as very specific probes under stringent hybridization conditions.

PCR amplification of brain cDNA yielded a fragment of approximately 510 bp (data not shown), the expected size for a brush border myosin I heavy chain. We cloned the PCR products and sequenced 12 independent clones. Of the 12, we observed only two types and designated them MIHC-K and -L. In GenBank searches, both were very similar to the chicken and bovine brush border myosin Is (BBMIs). Their partial deduced amino acid sequences, compared with the avian and bovine BBMI sequences, are shown in Fig. 1. The amino acid sequence of MIHC-K is very similar to that of a cDNA clone for a bovine adrenal myosin I [13] (O. Reizes et al., personal communication).

MIHC-L was selected for further cloning. A 2.7 kbp cDNA was obtained by screening a brain cDNA library with the PCR clone. Sequencing of this clone demonstrated that it contained the PCR clone's sequence plus an additional 5′ 210 bp of sequence. The clone extended to the 3′ poly(A) tract (data not shown). We cloned the 5′ 1.3 kbp of the cDNA from a specifically primed library and its sequence was identical with the 5′ sequence of the first phage clone. The putative protein product of the 3629 bp assembled cDNA shows a high degree of identity (59% and 60%) and similarity (75% and 75%) with the bovine [15] and chicken [16] BBMI sequences, respectively. The murine BBMI sequence would be expected to be significantly more similar to the bovine than to the avian sequence due to its phylogenetic proximity. The lack of a significant difference suggests that MIHC-L does not represent the murine BBMI. An alignment of the three amino acid sequences is shown in Fig. 2. None of the potential MIHC-L translation start sites have excellent similarity with the consensus [17]; however, the second methionine shown has G at the −3 and −6 positions (data not shown).

The sequence identity with the bovine and chicken BBMIs in the N-terminal (head) region is very high (Fig. 2). The putative calmodulin-binding repeats (also called the IQ-motifs [2]) are found at the head-tail junction. MIHC-L has 3 well-conserved repeats and 2 less conserved repeats (Fig. 2, bottom). The chicken BBMI has 3 or 4 of these repeats, the fourth produced by alternative mRNA splicing [18]. We have not yet determined whether alternative splicing occurs in this region of MIHC-L. The C-terminal tail also is similar to those of the BBMIs, suggesting that the MIHC-L tail may have similar phospholipid binding properties [19].

Northern analysis of polyadenylated RNA from adult mouse tissues (Fig. 3) indicates that expression of MIHC-L is widespread, with high transcript levels observed in liver, skin, kidney, and brain. Lower levels of the transcript are detected in small intestine than in liver or brain (Fig. 3, right). We also have detected transcripts in NIH3T3 fibroblasts (data not shown).

Quantitative comparison of the intensity of GAPDH hybridization (Fig. 3, bottom) indicates that the liver lane has less than one-third of the amount of polyadenylated RNA loaded in the small intestine lane; there-

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Bovine BB  mtllegsvgv edLVLLEPLe qEsLIrNLql RYEkkEiYTY IGnVlVSVNP YqqLPIYdlE fVakYRdYtF YELKPHIYAL
Chick BB   .......... .......... .......... ..qVVISVNP YkpLPIYtPE KVEEYhNCNF FavKPHIYAi
MHCI-L     ......migv gdMVLLEPLn eEtFIdNLkk RFDhnErYTY IGsVVISVNP YrsLPIYsPE KVEDYRNrNF YELsPHIFAL 74

Bovine BB  AnmAYqSLRD RDRDQCILIT GESGAGKTEA SKLVMSYVAA VCGKGEqVNs VKEQLLQSNP VLEAFGNAKT IRNnNSSRFG
Chick BB   ADDAYRSLRD RDRDQCILIT GESGAGKTEA SKLVMSYVAA VssKGEEVdk VKEQLLQSNP VLEAFGNAKT IRNDNSSRFG
MHCI-L     sDEAYRSLRD qDkDQCILIT GESGAGKTEA SKLVMSYVAA VCGKGaEVNq VKEQLLQSNP VLEAFGNAKT VRNDNSSRFG 154

Bovine BB  KYMDIEFDFK GfPLGGVItN YLLEKSRVVK QleGERNFHI FYQLLAGAdA QLLkaLKLER DtggYaYLNp DtSrVdGMDD
Chick BB   KYMDVEFDFK GDPLGGVISN YLLEKSRIVr hvkGERNFHI FYQLLAGgSA QLLqqLKLrp DcShYgYLNh EkSvlpGMDD
MHCI-L     KYMDIEFDFK GDPLGGVISN YLLEKSRVVK QprGERNFHV FYQLLsGASe eLLykLKLER DfSrYnYLsl DsakVnGvDD 234

Bovine BB  dANFkvLQsA MtVIGFsDeE IrqVLEVaAl VLKLGNVELi nEFQANGvpA SgIrDgRgvQ EIgELVGLns VeLERALCSR
Chick BB   AANFRaMQdA MaIIGFapaE VtAlLEVtAv VLKLGNVkLs ssFQAsGMEA SsIaEpRELQ EIsqLIGLDp stLEqALCSR
MHCI-L     AANFRtvrnA MqyVGFlDhE aeAVLEVvAa VLKLGNIEFk pEsrvNGLDe SkIkDknELk EIcELtsiDq VvLERAFsfR 314

Bovine BB  TmEtakEKVv TTLNViQAqY ARDALAKNIY SRLFnWLVNR INESIKVgTG ekRKVMGVLD IYGFEILEDN SFEQFVINYC
Chick BB   TVkvrdEsVl TaLsVsQgYY gRDALAKNIY SRLFdWLVNR INtSIqVkpG kqRKVMGVLD IYGFEIFqDN gFEQFIINYC
MHCI-L     TVEakqEKVs TTLNVaQAYY ARDALAKNlY SRLFsWLVNR INESIKaqTk vrkKVMGVLD IYGFEIFEDN SFEQFIINYC 394

Bovine BB  NEKLQQVFIE MTLKEEQEEY kREgIpWvkV EYFDNgIICn LIEhNqrGIL AMLDEECLRP GvVsDsTFLa KLNQlFskHs
Chick BB   NEKLQQIFIl MTLKEEQEEY VREaIqWTpV EFFDNsIICD LIENskvCIL AMLDEECLRP GTVnEDTFit KLNQIFAsHk
MHCI-L     NEKLQQIFIE LTLKEEQEEY IREdIeWThI DYFnNaIICD LIENNtnGIL AMLDEECLRP GTVtDETFLe KLNQVcAtHq 474

Bovine BB  HYESKvTqNA qrqyDhSMgL SCFRIcHYAG KVTYNVnsFI DKNNDLLFRD LSQAMWKARH pLLRSLFPEG DPkqaSLKRP
Chick BB   rYESKeTlNA khvtDvSLPL rCFRIhHYAG KVTYNVtGFI EKNNDLLFRD LSQAMWaARH tLLRSLFPEG DPqrpSLKlP
MHCI-L     HFESrmskcs rflnDttLPh SCFRIqHYAG KVlYqVeGFV DKNNDLLYRD LSQAMWKAgH sLikSLFPEG nPakvnLKRP 554

Bovine BB  PTaGaQFKsS VtTLMKNLYS KNPNYIRCIK PNEhqqrghF sfELVsvQaq YLGLLENVRV RRAGYAYRQA YgsFLERYrL
Chick BB   PTtGSQFKAS VATLMKNLYS KNPNYIRCIK PNDtKtAmlF tpDLVlaQVR YLGLMENVRV RRAGYAFRQl YqPFLERYKM
MHCI-L     PTrsSQFKAS VATLMrNLqt KNPNYIRCIK PNDkKaAhiF nesLVchQIR YLGLLENVRV RRAGYAFRQA YePcLERYKM 634

Bovine BB  LSRsTWPRWn GGDqEGVEkv LgELsmssEE LAFGkTKIFI RSPkTLFyLE EqRrlRLqqL ATLIQKtYRG WrCRTHYQLM
Chick BB   LSRkTWPRWt GGDREGaEVL LaELkfPpEE LAYGhTKIFI RSPRTLFdLE krRqQRvaEL ATLIQKmFRG WcCRkrYQLM
MHCI-L     LckqTWPhWk GpaRsGVEVL FnELeiPvEE hsFGrsKIFI RnPRTLFqLE DlRkQRLeDL ATLIQKlYRG WkCRTHFlLM 714

Bovine BB  RKSQIVISsW FRGnMQkKhY rkMKaSALLI QAFVRGWKAR K..... ..... ..... .....
Chick BB   RKSQIlISAW FRGhMQrnRY kQMKrSvLLl QAYaRGWKtR rm..... ..... ..... .....
MHCI-L     krSQVVIaAW YRryeQqKRY qQiKsSALvI QsYIRGWKAR Kilrelkhqk rckeaattia aywhgtqarr elkrlkeear 794

Bovine BB  ..... .....n YRKYFRSgAa liLSNFIYks MVQKFLLGLK NdLPSpSILD KkWPSAPYKY FntANhELqR
Chick BB   ..... ..... YRrYFRSdAc trLSNFIYrR MVQKYLMGLq knLPpMaVLD rtWPpAPYKF LsdANqELKs
MHCI-L     rndavaviwa ywlglkvrre YRKFFRanAg kkiyeFtlqR iVQKYLLeMK NkMPSLSpiD KnWPSrPYlF LdsthkELKR 874

Bovine BB  lFHqWKCKKF RDQLsPkQve VLrEKLcASE LFKgKKAsYP QSVpiPFhGD YlGLqrNPKl QKLKggeEgp IlMAEtVvKV
Chick BB   IFyrWKCKKY REQLTPQQra mLqaKLwprq LFKDKKALYa QSlqQPFrGE YLGLtqNrKY QKLqavakDK lVMAEaVqKV
MHCI-L     IFHlWrCKKY RDQFTdQQkl IyeEKLeASE LFKDKKALYP sSVgQPFqGa YLeinkNPKY kKLKdaiEEK IIiAEvVnKI 954

Bovine BB  NRgNaKTsSR ILLLTkgHvI itDmKnpQaK tVIPLnslaG VSVtSFkDGL FsLHLsEiSs vGSKGEFLLV SEHvIELlTK
Chick BB   NRANGKTvpR lLLLTteHLV LADpKaaQpK mVlsLcDIqG aSVSrFsDGL LALHLKEtSt AGgKGDLLLV SpHLIELvTr
MHCI-L     NRANGKstSR IFLLTnnnLl LADqKsgQiK seVPLvDVtk VSmSSqnDGF FAvHLKEgSe AaSKGDFLFs SDHLIEMaTK 103

Bovine BB  lCRaTLDATq mqLPVtVtEE FSVkFkeGsl tVKVIQGpgG GGtgklstKK KGSRcLEVtV q
Chick BB   LhqTlMDATa QaLPlsIaDq FStrFpkGdV aVtVVesakG GGdVPvC.KK rGShkMEIlV h
MHCI-L     LYRTTLsqTk QkLnIeIsDE FlVqFrqdkV cVKfIQGnqk nGsVPtC.Kr KnnRlLEVaV p 1095
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RVAELATLIQKMFRGWCCRKRYQ
LMRKSQILISAWFRGHMQRNRYK
QMKRSVLLLQAYARGWKTRRMYRRY      chicken (residues 654 - 724)

RLEDLATLIQKIYRGWKCRTHFL
LMKRSQVVIAAWYRRYEQQKRYQ
QIKSSALVIQSYIRGWKARKILRELKHQK
RCKEAATTIAAYWHGTQARRELKRLKEEA
RRNDAVAVIWAYWLGLKVRREYRKF      MIHC-L (residues 690 - 818)
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Fig. 2. (Top) Alignment of the deduced amino acid sequence of MIHC-L with those of the bovine [15] and chicken [16] brush border MHCs. Numbering refers to the MIHC-L sequence. Residues conserved in at least 2 of the 3 sequences are capitalized. The nucleotide sequence has been submitted to the EMBL database (accession number X69987). (Bottom) Alignment of the putative calmodulin-binding repeats of MIHC-L with those of the chicken brush border MHC. Conserved residues are shown in bold type.

fore, the steady-state level of MIHC-L transcripts in liver is more than three times greater than the level in small intestine, if the GAPDH transcript level is assumed to be constant. These data, and the lack of sequence similarity differences between MIHC-L and the bovine and avian BBMI sequences, argue that MIHC-L does not represent the murine BBMI, since both of the BBMI RNAs are expressed at much higher levels in intestine than in other organs [15,16].

Two major transcripts were observed with lengths of

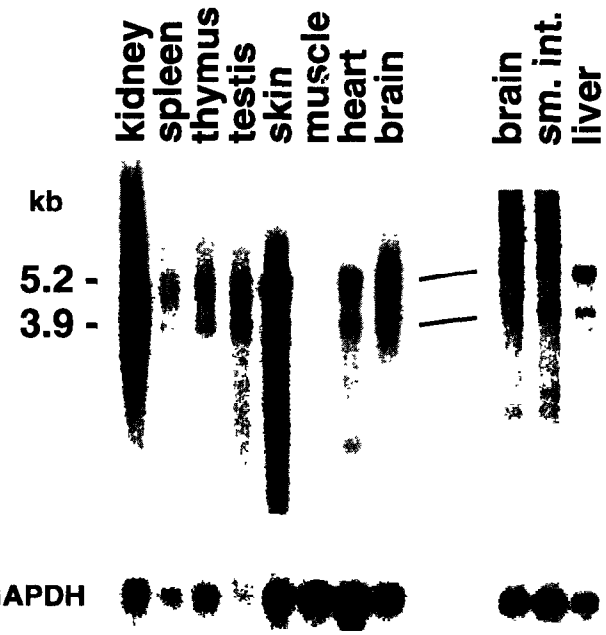

Fig. 3. Northern blot analysis of MIHC-L RNA expression in tissues from adult C57BL/6 mice. Transcript lengths were determined relative to the 9.5 kb RNA ladder (BRL). To control for loading variations, the blots were also hybridized with a human GAPDH probe, shown below each blot. Radioactivity was quantitated and printed using a Molecular Dynamics phosphor imager, on a linear grey scale.

3.9 and 5.2 kb. Although smears are shown in the kidney, skin and brain lanes (Fig. 3, left), both transcripts can be resolved with a higher scale (data not shown). The size of the smaller transcript correlates well with that of the cloned cDNA sequence (3629 bp). We do not yet have any data on the structure of the larger transcript.

We are currently localizing the MIHC-L protein using anti-peptide antibodies to the C-terminal sequence and will attempt to ablate MIHC-L function both in cultured cells by antisense oligonucleotide treatment and antibody microinjection, and in the entire animal by homologous recombination.

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