ISSN 0582-9879 Acta Biochimica et Biophysica Sinica 2004, **36**(6): 412–418 **CN** 31-1300/Q

Characterization of MR-1, a Novel Myofibrillogenesis Regulator in Human Muscle

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Abstract The actin-myosin contractile apparatus consists of several thick filament and thin filament proteins. Specific regulatory mechanisms are involved in this highly ordered process. In this paper, we reported the identification and characterization of a novel myofibrillogenesis regulator, MR-1. The *MR-1* gene was cloned from human skeletal muscle cDNA library by using a strategy that involves EST data base searching, PCR and RACE. The *MR-1* gene is located on human chromosome 2q35 and encodes a 142 aa protein. Northern blot revealed that the mRNA level of MR-1 was highest in the skeletal muscle and certain level of MR-1 expression was also observed in heart, liver and kidney. Immunohistochemical assay confirmed that the MR-1 protein existed in human myocardial myofibrils. It was found by yeast two-hybrid screening and confirmed by *in vitro* binding assay that MR-1 could interact with sarcomeric proteins, such as myosin regulatory light chain, myomesin 1 and β-enolase. These studies suggested that MR-1 might play a regulatory role in the muscle cell and it was worth investigating further.

Key words MR-1; gene cloning; heart; muscle; yeast two-hybrid

Myofibrils are components of both cardiac and skeletal muscle cells. Myofibrillogenesis is a highly complex process that involves the expression and assembly of muscle proteins into sarcomeres [1]. The key components of sarcomeres are two filamentary proteins, actin and myosin. Sarcomeric myosin is the molecular motor that transduces energy from the hydrolysis of ATP into directed movement and drives sarcomere shortening and muscle contraction. Each myosin hexamer is composed of two heavy chains, two essential light chains (ELC), and two regulatory light chains (RLC) [2]. The M-band is the transverse structure in the center of the sarcomeric A-band, which is responsible both for the regular packing of thick filaments and for the uniform distribution of the tension over the myosin filament lattice in the activated sarcomere [3]. Although some proteins from the Ig-superfamily, such as M-protein and myomesin, are the major candidates for the role of M-band bridges, the exact molecular organization of the M-band is not clear. Muscle-specific β-enolase is also present in the M-band of striated muscle and associated with the sarcomere by binding aldolase and troponin. The structural characteristics of β-enolase would permit their accumulation near the muscle contractile apparatus, so that the ATP produced via glycolysis could be more efficiently delivered and used for contraction [4].

We recently identified a novel gene, *MR-1*, from human skeletal muscle cDNA library (AF417001). MR-1 is highly expressed in skeletal muscle and heart. Yeast two-hybrid screen and *in vitro* GST pull-down assay indicated that MR-1 could interact with myosin regulatory light chain, myomesin and β-enolase. Sarcomere-associated localization of MR-1 was observed by immunohistochemical microscopy using isolated human cardiac myofibrils. This observation suggests that MR-1 is involved in the formation of multi-protein complexes near

Received: February 18, 2004 Accepted: April 18, 2004
This work was supported by a grant from the Beijing Major Project of Science and Technology (No. H020220020310)
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the muscle contractile apparatus. The gene cloning, chromosomal location, expression profile and possible function of MR-1 were reported here.

Materials and Methods

Materials

Human skeletal muscle Matchmaker cDNA library, multiple-tissue Northern blot and yeast two-hybrid kits were purchased from Clontech. Plasmids pGEX-5X-1 and pcDNA3 were purchased from Pharmacia and Invitrogen, respectively. *Escherichia coli* strains DH5α and BL21(DE3) were used for cDNA cloning and expression of GST-MR-1 fusion proteins, respectively. TNT® coupled transcription/translation system and Random primers DNA labeling system were purchased from Promega. All DNA restriction endonucleases, T4 ligase, and the LA-*Taq* PCR amplification kits were obtained from TaKaRa Inc.. All chemical reagents were purchased from Sigma unless otherwise noted. *L*-[^{35}S]methionine, [α-^{32}P]dCTP and glutathione-Sepharose 4B were products from Amersham Pharmacia. The rabbit anti-MR-1 polyclonal antibody was self-made. Biotinylated goat anti-rabbit secondary antibody, ECL immuno-detection kits and DAB kits were purchased from Santa Cruz.

Cloning of *MR-1*

Two primers (Olig1, 5′-AGCGCGGTGAAGCGGGGG-TGGGATCTG-3′, and Olig2, 5′-ATAACTTTATTTGCC-TTTGGCTGG-3′) were generated according to the DNA sequence information from three EST clones (W60939, W60939 and AA0774597). 0.65 kb PCR fragment was amplified by using the human skeletal muscle cDNA library as the template. The PCR reaction was carried out in a volume of 50 μl containing 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM dNTP, 10 mM Tris-HCl, pH 8.3, 100 ng of each primer, and 25 u LA-*Taq* polymerase on a PTC-200 programmable thermal cycler (MJ Research Inc.). The PCR was conducted at 94 °C for 4 min followed by 30 cycles at 94 °C for 30 s, 55 °C for 50 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. To clone the full-length cDNA of human MR-1, 5′-RACE was employed using Marathon-Ready skeletal muscle cell cDNA according to the manufacturer's instructions (Clontech). Two sense primers Olig3 (5′-TACCACTACAATGGATG-3′) and Olig4 (5′-CTATTCGATGATGAAGATAC-3′) designed from the cDNA library vector pACT2, and two antisense primers Olig5 (5′-CCTTGTCCACTTCCCGCTTG-3′) and Olig6 (5′-GGGTTCTTGCCCCTCTTTCT-3′) designed from 0.65 kb PCR product were used in the primary (Olig3/Olig5) and secondary (Olig4/Olig6) PCR. 0.1 μl of the primary PCR product as template was used in the secondary PCR. The Olig7 (5′-GAGTAGTTCTCCTGG-GTCCG-3′) was designed based on the nest PCR product sequence. Using Olig7 and Olig2 as primers, the whole cDNA fragment of MR-1 was amplified from the human skeletal muscle cDNA library.

Plasmid construction

pGBKT7-MR1 was generated by subcloning the coding sequence of the entire *MR-1* (GenBank™ accession No. AF417001) in-frame with the GAL4 DNA-binding domain into the *Bam*HI/*Sal*I sites of the yeast two-hybrid vector pGBKT7 (Clontech). PCR primers were: Olig8 (5′-ATTAGGATCCAAATGGCGGCGGTGGTAGCT-GCT-3′, *Bam*HI site was underlined) and Olig9 (5′-AAT-TGTCGACTCAGGTCTGCACCCCAGACCC-3′, *Sal*I site was underlined). The *Bam*HI/*Sal*I fragment of *MR-1* was inserted into the same sites of pGEX-5X-1, and pGEX-MR1 plasmid was constructed and used to express GST-MR-1 fusion protein in *E. coli* BL21(DE3). All plasmids constructs were made using the standard techniques [5]. Each constructed plasmid was sequenced to verify the correct frame as well as the proper sequence of any linker introduced during the cloning procedure. Sequence analysis and oligonucleotides synthesis were performed by Sangon Co., Shanghai.

Yeast two-hybrid screening and interaction analysis

The Matchmaker GAL4 two-hybrid system 3 (Clontech) was used to identify new interaction partners for MR-1. First, the *Saccharomyces cerevisiae* strain AH109 was transformed with pGBKT7-MR1 using a small-scale lithium acetate/single-stranded carrier DNA/polyethylene glycol (LiAc/ss-DNA/PEG) transformation protocol [6]. Selected transformants were transformed with a human skeletal muscle Matchmaker cDNA library (in AD-vector pACT2; Clontech) using a large scale LiAc/ss-DNA/PEG transformation protocol. Positive transformants were selected on adenine, histidine, leucine and tryptophan lacking dextrose-containing synthetic (SD/Ade$^-$/His$^-$/Leu$^-$/Trp$^-$) selection media. All yeast clones were re-streaked onto SD/Trp$^-$/Leu$^-$ media and assayed for activity of β-galactosidase by a β-galactosidase plate assay [7,8]. AD-library plasmids were isolated from all *lacZ* positive clones and rescued in *E. coli* strain DH5α. For further analysis, every isolated library plasmid was re-transformed into the *S. cerevisiae* strain AH109 and additionally co-transformed

with the following DNA-BD plasmids: pGBKT7-MR1, empty pGBKT7, and pGBKT7-LAM (Clontech; encodes human lamin C and provides a control for a fortuitous interaction). Transformation reactions were plated onto appropriate minimal synthetic dropout media, followed by a β-galacto-sidase plate assay. Furthermore, the library insert was transferred from the pACT2-AD vector into the *Bam*HI site of the pGBKT7-BD vector, and *MR-1* (bait) was subcloned from the pGBKT7-BD vector into the *Bam*HI/*Xho*I sites of the pGADT7-AD vector, followed by a two-hybrid assay in yeast strain AH109.

In vitro transcription/translation and GST pull-down experiments

pcDNA3-MLC2, pcDNA3-Enolase and pcDNA3-myomesin (511–786) were constructed for the translation. *In vitro* transcription and translation was carried out in the presence of *L*-[^{35}S]methionine (Amersham Pharmacia Biotech) using the TNT phage T7-coupled reticulocyte lysate system according to the manufacturer's description (Promega Corp.). Reaction mixtures containing 25 μl of lysate, 40 μCi of [^{35}S]methionine (1000 Ci/mmol), 15 u of RNasin, 50 μM amino acid mixture (minus methionine) and 1 μg of DNA in a final reaction volume of 50 μl were incubated at 30 °C for 60 min. The constructed pGEX-MR1 plasmid was transformed into BL21(DE3) cells (Stratagene Corp.). GST-MR-1 fusion protein expression was induced by l mM IPTG for 5 h at 37 °C. Whole cell lysates in coating buffer [2×PBS, 1% Triton X-100 (*W*/*V*)] were prepared as described. The GST fusion protein was immobilized on glutathione-Sepharose 4B beads (Amersham Pharmacia Biotech) applying ap-proximately 100 μl lysate onto 50 μl of beads (50% slurry) in 1 ml coating buffer for one hour at 4 °C. The beads were washed three times with coating buffer and resuspended in binding buffer (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM DTT, 150 mM NaCl, 0.5% Triton X-100, 1 mg/ml leupeptin). 5 μl of *in vitro* translated protein were added to 50 μl of beads coated with 20 μg of GST-MR-1 fusion protein in 500 μl binding buffer. The mixture was incubated for 1.5 h at 4 °C and washed five times with binding buffer and resuspended in SDS sample buffer. The protein complexes were separated by SDS-PAGE using 15% (*W*/*V*) polyacrylamide gels. The gels were fixed, treated with Amplify (Amersham Pharmacia Biotech), dried and exposed using BioMax MR-1 film (Eastman Kodak, Rochester). The results of the CBB staining were used to confirm that equal amounts of each GST-protein in the different reactions were bound to the glutathione beads (data not shown). GST alone was used as negative control peptide.

Northern blot analysis

To study the expression pattern of the *MR-1* gene, a commercial Human 12-lane multiple tissue Northern (MTN™) blot was used. The full-length coding human MR-1 cDNA was labeled with [α-^{32}P]dCTP using the Random primers DNA labeling system and used as probe. Hybridization and washing were carried out according to the manufacturer's manual. The blot was dried and autoradiographed with an X-ray film and intensifying screen at –70 °C for 3 d.

Immunohistochemical assay

Human heart tissues were collected from patient corpse, and fixed in methacarn (60% methanol, 30% chloroform, and 10% glacial acetic acid) for 24 h. Tissues were subsequently dehydrated and embedded in 100% paraffin. Serial sections (6 μm) were made and subsequently rehydrated through a graded ethanol series to a final incubation in PBS. Endogenous peroxidase activity was quenched by incubating slides in methanol containing 0.3% hydrogen peroxide for 30 min, then subsequently rehydrated in PBS and blocked in a 1:50 solution of normal goat serum solution made up in PBS. Immunodetection was performed using rabbits anti-MR-1 polyclonal primary antibody (1:200 dilution) by incubation at 4 °C for 3 h and then using biotinylated goat anti-rabbit secondary antibody (1:5000 dilution) by incubation at 37 °C for 1 h. DAB methods were applied for detection and Image-Pro software was used for results analysis. Negative controls were incubated in the absence of primary antibody.

Results

Molecular cloning and characterization of MR-1

To identify novel genes in human muscle, we employed a strategy for cDNA cloning which has been successfully applied to identify many novel genes from human cDNA libraries [9–12]. We performed a screening of the EST database using several key words, such as heart and muscle, and identified 132 positive EST clones. The cDNA sequence from each positive clone was aligned with the known sequences in the GenBank using the BLAST program. In these analyses, three EST clones (W60863, W60939, and AA074597) were identified from human heart and shared a 443 bp region, which is different from any known genes. The overlapped cDNA is covered by three

regions of human chromosome 2q35 (AC021016). According to this information, several Oligs were synthesized. Using Olig1 and Olig2 as primers, a 0.65 kb PCR product was initially obtained from the human skeletal muscle cDNA library. 5′-RACE was performed to extend the 5′-direction of the cloned cDNA. A 755 bp PCR product with expected sequence was cloned from the human skeletal muscle cDNA library. This 755 bp cDNA contains an open reading frame of 426 bp, which encodes a protein of 142 amino acids (Fig. 1).

The predicted coding region of this ORF is preceded by an in-frame stop codon at the 4 nt position. At the 3′-end of the cDNA, one potential polyadenylation signal (AATAAA) is present at the 726 nt position. The protein encoded by this open reading frame is estimated as 15.4 kD and the calculated pI volume is 10.5. Alignment analysis in the GenBank database shows that this cDNA has no homologous gene, which suggested that it might be a novel gene. This gene was named as *MR-1* (myofibrillogenesis regulator 1, NCBI accession No. AF417001). The *MR-1* gene is located in human chromosome 2q35 because it is carried by the Homo sapiens BAC clone RP11-378A13 (GenBank Accession number AC021016). It spanned about 2887 bp of contiguous DNA. The *MR-1* gene is composed of 3 distinct exons ranging between 169 bp and 713 bp. Both the 5′ and 3′ acceptor splice sites in each of the introns followed the GT-AG consensus sequence for eukaryotic genes (Fig. 2). The exon 1 (212 bp) encoded whole 5′-untranslated region plus the first 22 amino acids. The exon 2 (169 bp) encoded the next 56 amino acids whereas the exon 3 (713 bp) contains the final 64 codons and an extensive 3′-untranslated region of about 182 bp (Fig. 2). A computer search of EST database with the amino acid sequence of MR-1 also identified MR-1 orthologs in mouse, rat, cow, and pig, but no detectable homologs were present in *Saccharomyces cerevisiae*, *Drosophila melenogaster*, *Caenorhabditis elegans*, *Fugu rubripe* or *Danio rerio*.

Distribution of MR-1 in human tissues

The expression pattern of human MR-1 across different tissues was detected using Northern blot analysis. The results are shown in Fig. 3. A band of approximately 0.8 kb was identified. This made it an independent confirmation that the full-length MR-1 mRNA was transcribed as predicted. The expression level of MR-1 was highest in the skeletal muscle and heart, relatively lower in kidney, liver, while the expression levels of human MR-1 in brain, colon, lung, peripheral blood leukocytes, placenta, small intestine, spleen, and thymus were barely detected.

Identification of the MR1-interacting proteins

Yeast two-hybrid screen was performed to identify proteins able to interact with GAL4 binding domain (BD)-MR-1 fusion protein. About 2×10^6 transformants were screened from the human skeletal muscle cDNA library, which fused with GAL4 activation domain (AD). A total of 94 colonies appeared on SD/Ade$^-$/His$^-$/Leu$^-$/Trp$^-$ selective medium, 8 of which exhibited significantly elevated level of β-galactosidase activity. These 8 plasmids were

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   1 atgtagtttctggtctgtaggcaggacggaaggagcgggggaggccccttacgcaaacta
  61 caattcccggcggggagcgcggtgaagcgggggtgggatctgaacatggcggcggtggta
                                                M  A  A  V  V    5
 121 gctgctacggcgctgaagggccgggggcgagaaatgcccgcgtcctccgggggattctc
      A  A  T  A  L  K  G  R  G  A  R  N  A  R  V  L  R  G  I  L   25
 181 gcaggagccacagctaacaaggcttctcataacaggacccgggccctgcaaagccacagc
      A  G  A  T  A  N  K  A  S  H  N  R  T  R  A  L  Q  S  H  S   45
 241 tccccagagggcaaggaggaacctgaaccccctatccccggagctggaatacattcccaga
      S  P  E  G  K  E  E  P  E  P  L  S  P  E  L  E  Y  I  P  R   65
 301 aagaggggcaagaaccccatgaaagctgtgggactggcctgggccatcggcttcccttgt
      K  R  G  K  N  P  M  K  A  V  G  L  A  W  A  I  G  F  P  C   85
 361 ggtatcctcctcttcatcctcaccaagcgggaagtggacaaggaccgtgtgaagcagatg
      G  I  L  L  F  I  L  T  K  R  E  V  D  K  D  R  V  K  Q  M  105
 421 aaggctcggcagaacatgcggttgtccaacacgggcgagtatgagagccagaggttcagg
      K  A  R  Q  N  M  R  L  S  N  T  G  E  Y  E  S  Q  R  F  R  125
 481 gcttcctcccagagtgccccgtcccctgatgttgggtctggggtgcagacctgaggagcg
      A  S  S  Q  S  A  P  S  P  D  V  G  S  G  V  Q  T  *       142
 541 ctgcgacgcctcctaggctattgactgttaagtcctcaggtttggcccagattccagttcg
 601 tgcctctgaggtccaccagagggcgcatgaagcccaggctgttgccaaaccctaccctgc
 661 cccacaccaaggagccagccaaaggcaaataaagttattgagtgtttagtaga
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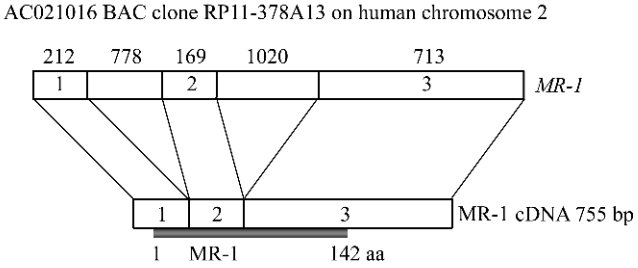
Fig. 1 Nucleotide sequence of the coding and flanking region of human MR-1

The deduced amino acid sequence is given below the nucleotide sequence in single-letter code. The asterisk indicates the stop codon, and potential polyadenylation signal is underlined. The nucleotide sequence was determined on both strands by automated sequencing.



Exon No.	Length (bp)	5′ Splice donor	Intron length (bp)	3′ Splice acceptor
1	212	**tccggg** gtaagg	778	ccacag **ggattc**
2	169	**ggcctg** gtgagt	1020	ccgtag **ggccat**
3	375	**agaaag**		

Fig. 2 Chromosomal localization of the human *MR-1* gene

The panel shows the exon and intron organization of the human *MR-1* gene. The 5′ acceptor GT and 3′ donor AG are underlined, the exonic sequences are printed in bold.

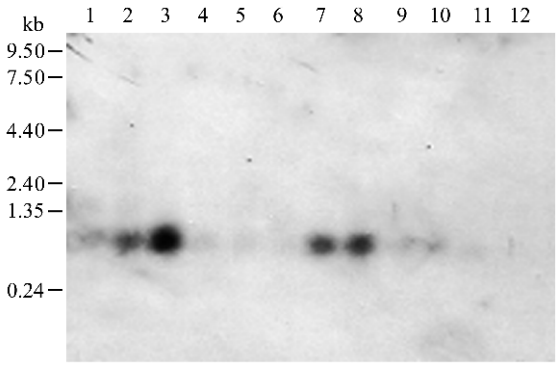


Fig. 3 Northern blot analysis of human MR-1 mRNA from various human tissues

Human tissue blot (CLONTECH) was probed with ^{32}P-labeled cDNA of MR-1. Hybridization and washing were carried out according to the manufacturer's manual. RNA in each line was from human tissues. 1, brain; 2, heart; 3, skeletal muscle; 4, colon (non mucosa); 5, thymus; 6, spleen; 7, kidney; 8, liver; 9, small intestine; 10, placenta; 11, lung; 12, peripheral blood leukocyte.

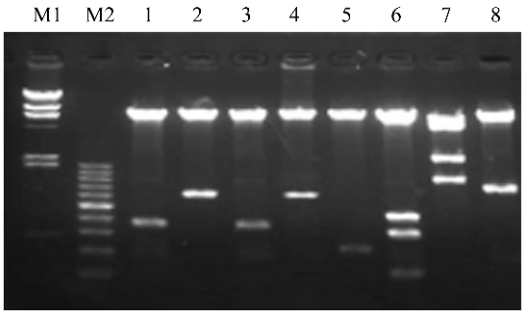


Fig. 4 Electrophoresis pattern of *Eco*RI/*Xho*I double digestion of 8 positive AD containing plasmids

M1, λ/*Hin*dIII, from top to down: 23, 9.4, 6.6, 4.4, 2.3, 2.0 kb; M2, 2.0–0.2 kb ladder marker; 1–8, No. 3, 10, 29, 38, 43, 62, 82, and 91 positive AD plasmids digested.

isolated and the digestion pattern was shown in Fig. 4. Comparison of the amino acid sequences indicated that AD10 and AD38 were overlapped with C-terminal 89 amino acids (269–357 aa) of homo eIF3 subunit 5 (NM003754), with identity 93% and 97%, respectively. AD62 showed high similarity with Myomesin1 (NM003803, identity 91%). AD43 and AD91 resemble homo sapiens β-enolase 3 (ENO3, NM053013) with overlapped region of 147 amino acids (25–171 aa, identity 95% and 97%, respectively). AD29 was similar to MRLC2 (AF363061, identity 91%); AD3 was very similar to homo copper metabolism gene MURR1 (NM152516, identity 97%); AD82 was similar to homo sapiens MRIP1 (AF359283, identity 90%).

Retransformation of these isolated library plasmids into the pGBK-MR1 containing yeast AH109 or alone was accomplished to confirm the interaction specificity. To eliminate a false positive, we moved the inserts from the pACT2-AD to the pGBKT7 vector and handled *MR-1* in the same way. Co-transformants containing the switched plasmids could also activate the reporter genes. This confirmed the interaction between MR-1 and other proteins.

Confirmation of the interaction between MR-1 and β-enolase, MRLC2, or myomesin by *in vitro* GST pull-down assay

As an independent approach to test whether the interaction detected in the two-hybrid analysis could be

detected by biochemical means, we performed GST pull-down assays. For this experiment, myosin light chain, myomesin (residues 511–786) and β-enolase were transcribed and translated *in vitro* by reticulocyte extracts in the presence of [^{35}S]methionine. Expression of GST-MR-1 fusion protein in *E. coli* (DE3) which contained the pGEX-MR1 recombinant plasmid was accomplished as described in Methods. The *in vitro* translated [^{35}S]methionine-labeled proteins were incubated with either GST or GST-MR-1 in the presence of glutathione-Sepharose beads. The beads were pelleted, washed extensively, and bound proteins were eluted by boiling the beads in SDS-loading buffer. The samples were then subjected to SDS-PAGE and ^{35}S-labeled species detected by autoradiography of the dried gel were selectively retained on the GST-MR-1 beads, in contrast no significant retention of GST was observed (Fig. 5). These results therefore verified the interaction detected in the two-hybrid analyses and indicated that the interaction between MR-1 and MRLC2, or myomesin, or β-enolase is likely direct.

Expression of MR-1 protein in isolated cardiac myofibrils

Striated muscles are characterized by a very precise organization of contractile proteins into repeating structural subunits, the sarcomeres. A sarcomere is defined as the region between two Z-discs that anchor the thin (actin) filaments, with the thick (myosin) filaments being anchored in the center via a structure called the M-band. Both myomesin and β-enolase have been found in M-band [4]. In order to study the biological role of MR-1, we did the immunohistochemical staining by using isolated cardiac myofibrils. The results indicated that MR-1 was expressed in human myocardial myofibrils (Fig. 6).

Discussion

Myofibrillogenesis is a complex process that depends on the coordinated assembly and integration of a number of cytoskeletal scaffolding and signaling proteins. There are three proposed stages during the assembly of myofibrils: premyofibrils to nascent myofibrils to mature myofibrils [13]. As premyofibrils become nascent myofibrils, myomesin also accumulate in the cytoplasm and gradually organize into a periodic pattern of primordial M-line structure prior to the incorporation of titin into M-line [14,15]. Myomesin binds directly to titin and is thought to promote the proper orientation and incorporation of its C-terminus into developing M-line [16]. Myomesin also binds directly to sarcomeric myosin, suggesting that it may link titin to myosin filaments [17].

In this work, through EST database searching and PCR combined with 5′ RACE, we cloned *MR-1* from human skeletal muscle cDNA library. MR-1 is highly expressed

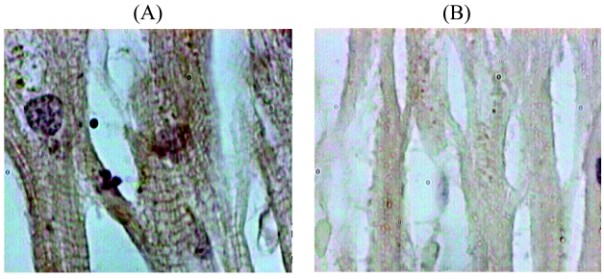


Fig. 6 Expression of MR-1 protein in isolated cardiac myofibrils (400×)

(A) Detected with human anti-MR-1 antibody. (B) Negative control.

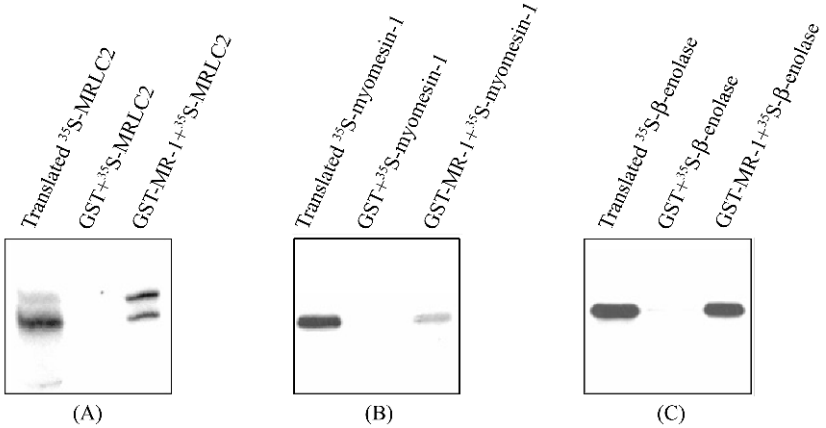


Fig. 5 *In vitro* binding of GST-MR-1 with ^{35}S-β-enolase (A), ^{35}S-MRLC2 (B) and ^{35}S-myomesin1 (C) proteins

in skeletal muscle and heart. Yeast two-hybrid screen and *in vitro* GST pull-down assay indicated that MR-1 could interact with myomesin, suggesting that MR-1 might regulate the function of myomesin in linking titin to myosin filament.

Myosin is found in almost every cell type in vertebrate, which is composed of one pair of heavy chain (MHC) and two pairs of light chain (MLC). MLC2 phosphorylation plays pivotal roles in smooth muscle contraction and actin-myosin interaction for stress fiber and contractile ring formation in non-muscle cells [18–20]. MLC mutations have been identified in the hypertrophic cariomyopathy (HCM) patients [21]. The striking structural regularity was required in the contractile apparatus by a series of complex events occurring in a highly coordinated manner, which guaranteed the correct protein-protein interactions to take place in right spatial and temporal order [22]. β-Enolase, a glycolytic enzyme, supplies energy for movement in muscle contraction process. It consisted of three subunits, α, β and γ, in homodimer and heterodimer, and distributed in tissue-specific manner. The β-enolase presented in the M-band of striated muscles, and was associated with binding aldolase and troponin.

The interaction of MR-1 with sarcomeric structural proteins and regulatory proteins involved in muscle contraction, its expression in various human muscle tissues and its presence in human myocardial myofibrils suggest that it may play an important role in the regulation of muscle contractile apparatus, which lays a basis for its further studies.

References

1 de Tombe PP. Cardiac myofilaments: Mechanics and regulation. J Biomech, 2003, 36(5): 721–730

2 Szczesna D. Regulatory light chains of striated muscle myosin: Structure, function and malfunction. Curr Drug Targets Cardiovasc Haematol Disord, 2003, 3(2): 187–197

3 Obermann WM, Gautel M, Weber K, Furst DO. Molecular structure of the sarcomeric M band: Mapping of titin and myosin binding domains in myomesin and the identification of a potential regulatory phosphorylation site in myomesin. EMBO J, 1997, 16(2): 211–220

4 Foucault G, Vacher M, Merkulova T, Keller A, Arrio-Dupont M. Presence of enolase in the M-band of skeletal muscle and possible indirect interaction with the cytosolic muscle isoform of creatine kinase. Biochem J, 1999, 338 (Pt1): 115–121

5 Sambrook J, Fritsch EF, Maniatis T eds. Molecular Cloning: A Laboratory Manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989

6 Gietz RD, Triggs-Raine B, Robbins A, Graham KC, Woods RA. Identification of proteins that interact with a protein of interest: Applications of the yeast two-hybrid system. Mol Cell Biochem, 1997, 172(1-2): 67–79

7 Duttweiler HM. A highly sensitive and non-lethal beta-galactosidase plate assay for yeast. Trends Genet, 1996, 12(9): 340–341

8 Fashena SJ, Serebriiskii I, Golemis EA. The continued evolution of two-hybrid screening approaches in yeast: How to outwit different preys with different baits. Gene, 2000, 250(1-2): 1–14

9 Gong L, Kraus N. Molecular cloning of cDNA encoding the alpha unit of CNGC gene from human fetal heart. Life Sci, 1998, 63(17): 1555–1562

10 Gong L, Yeh ET. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. J Biol Chem, 1999, 274(17): 12036–12042

11 Gong L, Millas S, Maul GG, Yeh ET. Differential regulation of sentrinized proteins by a novel sentrin-specific protease. J Biol Chem, 2000, 275(5): 3355–3359

12 Gong L, Kamitani T, Millas S, Yeh ET. Identification of a novel isopeptidase with dual specificity for ubiquitin- and NEDD8-conjugated proteins. J Biol Chem, 2000, 275(19): 14212–14216

13 Du A, Sanger JM, Linask KK, Sanger JW. Myofibrillogenesis in the first cardiomyocytes formed from isolated quail precardiac mesoderm. Dev Biol, 2003, 257(2): 382–394

14 Wang SM, Lo MC, Shang C, Kao SC, Tseng YZ. Role of M-line proteins in sarcomeric titin assembly during cardiac myofibrillogenesis. J Cell Biochem, 1998, 71(1): 82–95

15 Yang YG, Obinata T, Shimada Y. Developmental relationship of myosin binding proteins (myomesin, connectin and C-protein) to myosin in chicken somites as studied by immunofluorescence microscopy. Cell Struct Funct, 2000, 25(3): 177–185

16 Obermann WM, van der Ven PF, Steiner F, Weber K, Furst DO. Mapping of a myosin-binding domain and a regulatory phosphorylation site in M-protein, a structural protein of the sarcomeric M band. Mol Biol Cell, 1998, 9(4): 829–840

17 Obermann WM, Gautel M, Weber K, Furst DO. Molecular structure of the sarcomeric M band: Mapping of titin and myosin binding domains in myomesin and the identification of a potential regulatory phosphorylation site in myomesin. EMBO J, 1997, 16(2): 211–220

18 Geeves MA, Holmes KC. Structural mechanism of muscle contraction. Annu Rev Biochem, 1999, 68: 687–728

19 Wakabayashi T. Structural basis for the molecular switching in regulatory mechanism of muscle contraction. Tanpakushitsu Kakusan Koso, 2002, 47 (5): 553–567

20 Iwasaki T, Murata-Hori M, Ishitobi S, Hosoya H. Diphosphorylated MRLC is required for organization of stress fibers in interphase cells and the contractile ring in dividing cells. Cell Struct Funct, 2001, 26(6): 677–683

21 Poetter K, Jiang H, Hassanzadeh S, Master SR, Chang A, Dalakas MC, Rayment I *et al*. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. Nat Genet, 1996, 13(1): 63–69

22 Somlyo AP, Somlyo AV. Signal transduction and regulation in smooth muscle. Nature, 1994, 372(6503): 231–236

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