

Cloning and sequencing of myosin heavy chain isoform cDNAs in golden-mantled ground squirrels: effects of hibernation on mRNA expression

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Rourke, Bryan C., Anqi Qin, Fadia Haddad, Kenneth M. Baldwin, and Vincent J. Caiozzo. Cloning and sequencing of myosin heavy chain isoform cDNAs in golden-mantled ground squirrels: effects of hibernation on mRNA expression. *J Appl Physiol* 97: 1985–1991, 2004. First published August 6, 2004; doi:10.1152/japplphysiol.00695.2004.—The golden-mantled ground squirrel is a small rodent hibernator that demonstrates unusual myosin heavy chain (MHC) isoform plasticity during several months of torpor, punctuated by bouts of rewarming and shivering thermogenesis. We measured MHC mRNA levels to determine whether pretranslational control mechanisms were responsible for differences in MHC_{2x} protein expression, as we previously observed between active and hibernating ground squirrels. We first cloned cDNA using the 3' rapid amplification of cDNA ends (3' RACE) technique and identified three sequences corresponding to MHC₁, MHC_{2x}, and MHC_{2b}. A DNA control fragment was developed to be used in conjunction with a coupled RT-PCR reaction to simultaneously measure MHC mRNA levels for each isoform in the skeletal muscle of ground squirrels. MHC mRNA and protein expression were strongly correlated, and type IIx and IIb mRNA levels were significantly different between active and hibernating ground squirrels. Pretranslational control of MHC protein is apparently an important process during hibernation, although the exact stimulus is not known. The techniques presented can be used to obtain MHC cDNA sequences and to measure mRNA expression in many vertebrate groups.

sequencing; *Spermophilus lateralis*; muscle atrophy; reverse transcriptase-polymerase chain reaction

A NUMBER OF STUDIES HAVE EXAMINED the plasticity of skeletal muscle, but these analyses are largely restricted to a small number of mammalian species. The response of skeletal muscle to mechanical unloading can typically be characterized by a loss of muscle mass and oxidative characteristics and by a conversion of myosin heavy chain (MHC) proteins from slow to faster isoforms (1, 2, 4, 5, 14). These changes in muscle composition generally would compromise both force production and fatigue resistance (4). Some rodent species naturally undergo long periods of torpor during hibernation, in which both the mechanical loading and the muscle activity are markedly reduced (7, 10, 11, 19); however, the effects of torpor on muscle physiology, particularly at the molecular level, are not well known.

In previous studies, we and others (12, 19) found that hibernating golden-mantled ground squirrels (*Spermophilus lateralis*) demonstrate only mild atrophy in the soleus and

gastrocnemius muscles despite 4–6 mo of relative inactivity, whereas diaphragm and plantaris muscles do not appear to atrophy. The MHC isoform composition of the atrophied muscles does not show typical slow-to-fast isoform transitions, as found in other mammalian models of disuse atrophy (1, 2). The gastrocnemius and plantaris muscles of hibernating ground squirrels have greater proportions of MHC_{2x} than found in active animals and have an unchanged relative abundance of MHC₁; the soleus and diaphragm muscles show no changes in MHC isoform profiles (12). These results are a marked departure from those commonly observed in rat and mouse models of muscle inactivity.

Because so little is known about the cellular and molecular responses of skeletal muscle to hibernation, the underlying mechanisms responsible for this departure remain undefined. The most obvious explanation may be that it is simply an effect of temperature (Q_{10}) that reduces the rate of protein degradation at low body temperatures during torpor (3, 15–17), thereby minimizing MHC isoform transitions and the loss of muscle mass. Another more subtle mechanism may be related to the interbout arousals that occur during hibernation. Ground squirrels need to rewarm periodically from torpor, and initial thermogenesis is provided by violent shivering of the skeletal muscles. The changes to MHC protein seen in hibernating muscle thus may be required to support this activity but also may be caused simply by the shivering itself, akin to a response to energetic exercise or chronic electrical stimulation. This brief but vigorous activity of the skeletal muscle during the bouts of rewarming may influence MHC protein via activation of exercise-sensitive transcriptional pathways, causing the observed changes in isoforms and the maintenance of muscle mass.

Given this background, we present a method of rapidly obtaining partial sequences of MHC genes for use in a coupled reverse transcriptase and polymerase chain reaction (RT-PCR) technique for quantifying relative MHC mRNA percentages from individual muscles. Using this approach, we tested the hypothesis that changes in MHC isoform expression during hibernation are controlled at the pretranslational level by changes in the relative abundance of MHC mRNA. The findings of this study demonstrate that hibernation preserves the slow MHC mRNA phenotype of the soleus muscle and induces a slower MHC mRNA phenotype (i.e., from fast MHC_{2b} to fast MHC_{2x}) in fast-twitch muscles like the plantaris and gastroc-

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nemius. This latter observation suggests that control pathways within muscle, rather than reduced temperature alone, play a role in mediating muscle phenotype during hibernation.

METHODS

Animal Care and Muscle Collection

Frozen tissue samples from fall-active and winter-hibernating golden-mantled ground squirrels were kindly provided by W. K. Milsom. Animals were cared for in accordance with the guidelines of the University of British Columbia. Adult squirrels were captured in Summer 2001 by a commercial trapper and maintained in the laboratory before they were divided into two groups, active and hibernating. Active animals were killed in October, and the remaining animals were placed in a short-photoperiod (2 h light, 22 h dark) cold room at 5°C; they entered hibernation several weeks later and were killed after ~4 mo. All animals were euthanized by pentobarbital sodium injection. Gastrocnemius, soleus, plantaris, tibialis anterior, and diaphragm muscles and the heart were excised, wrapped in foil, and frozen immediately in liquid nitrogen. All tissue was stored at -80°C until further analysis.

Myosin Isoform Sequencing and RT-PCR Primer Selection

RNA isolation. Total RNA was isolated from ~25 mg of frozen skeletal and cardiac muscle from golden-mantled ground squirrels, subsequent to homogenization in Tri-Reagent and 1-bromo-3-chloropropane reagent (Molecular Research Center, Cincinnati, OH). Precipitation of RNA followed isopropanol addition and two washes of 75% ethanol; the final sample was spun dry in a vacuum centrifuge. The RNA was solubilized in 10 µl of water, heated to 50°C for 2 min, and stored at -80°C. The concentration of RNA in each sample was determined by optical density at 260 nm (Beckman DU 640B spectrophotometer). For samples used subsequently in the isolation of myosin sequences, verification of integrity of RNA was performed by electrophoresis of 1 µg RNA on a 1% agarose Tris-borate-EDTA gel, containing ethidium bromide. Undegraded samples had clear bands containing 28S and 18S ribosomal RNA. A 1-µg portion of total RNA from each of the plantaris, soleus, gastrocnemius, and diaphragm muscle samples was also reverse transcribed separately (SuperScript II, Invitrogen, Carlsbad, CA) for later measurement of MHC mRNA expression in individual muscles. Each reaction cocktail [100 µl of oligo(dT), 200 µg of random primers, 4 µl of 5× first-strand buffer, 2 µl of 0.1 mM DTT, 1 µl of dNTP, 1 µl of SuperScript II in 20-µl reaction] was incubated for 50 min at 42°C and then for 15 min at 72°C. Resulting cDNA from each sample was stored at -20°C until use in subsequent PCR reactions.

MHC sequence generation. The rapid amplification of cDNA ends (3' RACE; Invitrogen) technique was applied to 3–5 µg of total RNA from one sample each of tibialis anterior, diaphragm, and heart muscle types. Briefly, cDNA was created from sample RNA via SuperScript II and was appended with an adapter primer of known sequence at the 3' end. Amplification of target DNA was then performed by choosing an additional PCR primer specific to the gene or genes of interest; we utilized a 20-bp oligonucleotide of known identity to all skeletal muscle myosin genes in rats and humans (5'-AGAAGGAGCAGGACACCAGC), which lies ~500 bp upstream of the stop codon (9, 20). The PCR (Robocycler, Stratagene, La Jolla, CA) was carried out on 1 µl of cDNA with the following cycle conditions: 1 cycle at 96°C for 3 min; 25 cycles at 96°C for 1 min, at 56°C for 45 s, and at 72°C for 50 s; and 1 cycle at 72°C for 3 min. These cycles were carried out in 25 µl of PCR reaction buffer [18.85 µl of water, 1 µl of 50 mM MgCl₂, 0.5 µl of dNTP, 0.5 µl of 10 pmol/µl AUAP (adapter primer), 0.5 µl of 10 pmol/µl common MHC primer, 0.15 µl *Taq* DNA polymerase]. The resulting 600- to 700-bp fragments, containing a presumed combination of several MHC isoforms and other potentially

non-MHC-related sequences, were eluted from 1.5% agarose Tris-acetate-EDTA (TAE) gels (Qiagen, Valencia, CA) into 35 µl of water.

Because the sequences for each MHC gene were so similar in size and thus comigrated, direct sequencing after gel electrophoresis could not be performed at this stage, and eluted sequences were ligated (Rapid DNA ligation kit, Roche, Indianapolis, IN) to a pGEM-T vector (Easy Vector system, Promega, Madison, WI). For each sample, 1–2 µl of cDNA were added to a reaction cocktail (2 µl of pGEM-T vector, 2 µl of 5× DNA buffer, 4–5 µl of water, 10 µl of 2× buffer, 1 µl of ligase) for 30 min at room temperature, followed by termination at -20°C. Competent DH5α cells (Invitrogen) were transformed by adding 2 µl of the resulting ligation reaction to 50 µl of cells on ice for 30 min. The cells were briefly heat-shocked, then added to 950 µl of Luria-Bertani (LB) medium, and incubated for 1 h at 37°C. Cells were collected by centrifugation and were plated overnight on LB medium agarose plates containing 100 µg/ml ampicillin and X-gal. Colonies that contained the DNA insert were distinguished by blue/white screening resulting from β-galactosidase activity; ~20–30 colonies per plate were then selected for incubation overnight at 37°C, in 3 ml of LB medium and ampicillin. The DNA was extracted by MiniPrep (Qiagen), and 1 µl was electrophoresed on a 0.8% agarose-TAE gel to verify the presence of the insert in the vector, by size comparison to the vector alone. Confirmed inserts were then selected for sequencing reactions.

Sequencing reactions (~200 total, bidirectional) were performed with ABI Prism BigDye (Applied Biosystems, Foster City, CA) and HalfBD enhancer reagent (Genetix, Charlestown, MA), following the manufacturer's provided protocol, and were analyzed on an ABI Prism 3100 capillary sequencer (University of California, Irvine, DNA Core Facility). Primers for the reactions (SP6 and T7) were from sites on the pGEM vector flanking the insert sequence. Sequences of ~600 bp, representing the last ~550 bp of each gene, and an additional 40–50 bp of the 3' untranslated region, were compared by BLASTn analyses (NCBI) to other myosin sequences, and putative notations of fast and slow isoforms were made. Three sequences were identified as likely distinct isoforms, including one slow and two fast; however, sequences differed by as few as 40 bases in the coding region but were much more variable in the 3' untranslated region.

Bootstrapping analyses were performed to more conclusively identify the respective isoforms with their cloned sequences (Fig. 1), using the MEGA software package (8). A total of 46 sequences for MHC genes were retrieved from GenBank, encompassing nine mammalian, three fish, and two avian species; these sequences represent the majority of available sequences that have previously been identified as MHC genes. The sequence information used for the analysis was limited to the ~500-bp region beginning with the 20-bp region identical to all isoforms, used above for the 3' RACE, and ending with the stop codon. A neighbor-joining method was used to construct the tree, with distances estimated by the Tajima-Nei algorithm; 500 iterations were carried out for the bootstrap analysis. Trees generated through the minimum-evolution method and maximum parsimony analysis provided similar topologies, where the clones were consistently identified uniquely as slow MHC₁, fast MHC_{2x}, and fast MHC_{2b}.

Primers for the two fast isoforms were chosen by utilizing the higher variability in the 3' untranslated region, and modified in length until a common annealing temperature of 56°C was obtained. The sequence isolated from heart muscle was sufficiently different from the fast isoforms upstream of the stop codon, and the type I-specific primer was derived from the coding region. Three simultaneous PCR reactions were performed on an individual muscle sample, with each reaction (2 µl of cDNA diluted 1:2 H₂O, 14.85 µl of water, 2.5 µl of 10× PCR buffer, 1 µl of 50 mM MgCl₂, 0.5 µl of 10 mM dNTP, 2 µl of 10 pmol/µl specific primer, 2 µl of 10 pmol/µl common MHC primer, 0.15 µl of *Taq* DNA polymerase; 56°C annealing temperature and PCR cycle conditions as above) comprising the common primer and one of the specific primers for an isoform (MHC₁: 5'-CGC-

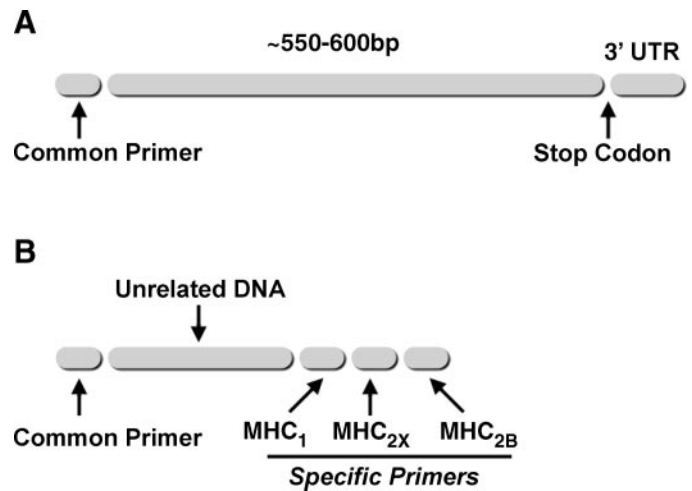
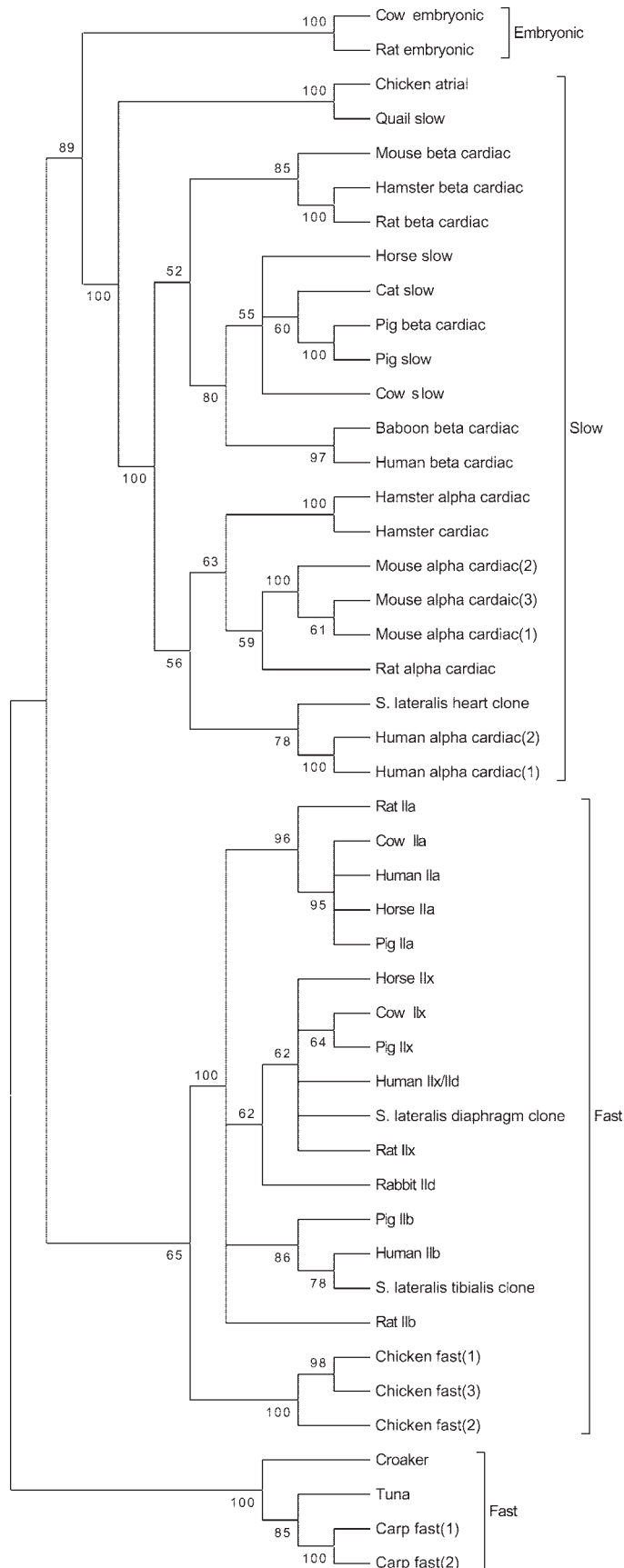


Fig. 2. A: representations of the amplified MHC sequences obtained by the 3' rapid amplification of cDNA ends (3' RACE) technique. Isolation of myosin-specific sequences was accomplished by utilizing a 20-bp oligonucleotide (common primer) with 100% identity to all known myosin genes in rats. The location of this region is ~500 bp upstream of the stop codon; the 3' untranslated region (3' UTR) is also obtained. B: construction of the synthetic control fragment begins with a 392-bp ion-channel gene sequence, unrelated to MHC genes. The MHC common primer is added to the 5' region of this fragment, and the specific primers are then added by successive oligonucleotide overlap extension at the 3' end. The final control fragment is 488 bp and can be used as a template in individual PCR reactions measuring MHC mRNA expression; the length of amplified PCR products depends on the specific primer used: MHC₁ (432 bp), MHC_{2x} (460 bp), and MHC_{2b} (488 bp).

CAATGTCACGGCTCTTG; MHC_{2x}: 5'-CCAAAAGTAATAAGTA-CAAAACAGAGTG; MHC_{2b}: 5'-CTCTGCAGATTTTATTTCACT-GATATAC). Primers were checked for sequence identity against other published sequences in GenBank and were unrelated to any other skeletal muscle genes.

Synthetic control design and quantitation of MHC mRNA expression. To allow for semi-quantitative comparison of relative percentage of mRNA expression for each isoform, a synthetic control fragment was constructed by oligonucleotide overlap extension and amplification by PCR (18). A fragment previously used for similar RT-PCR analyses of MHC isoforms in the rat (20) was modified; the fragment (see Fig. 2B) is composed primarily of a 392-bp sequence, unrelated to MHC genes and derived from a sequence for an ion-channel gene. The 5' end had been appended with the 20-bp MHC common primer, and the 3' end had previously been appended with the specific primers for rat MHC genes. The 5' region of type I-specific squirrel MHC primer was especially designed with 10 bp of overlap to the ion-channel gene sequence, and PCR was performed as above, utilizing 2 μ l of control fragment as a template and including the common MHC primer. This yielded a fragment composed of, in 5' to 3' orientation, the common primer + ion-channel + type I primer sequences. The newly synthesized sequence was eluted from a 2% agarose-ethidium bromide gel, and 2 μ l of this product were used as a template for the

Fig. 1. A cladogram of nucleotide sequences from known myosin heavy chain (MHC) isoforms and 3 ground squirrel (*Spermophilus lateralis*) clones (from the heart, diaphragm, and tibialis anterior muscles). Each sequence begins with the region of sequence identity, ~500 bp upstream from the stop codon, and does not include the 3' untranslated region. The tree represents a condensed topology of slow and fast isoforms from available taxa and was constructed by neighbor-joining analysis, with distances estimated through the Tajima-Nei method. Bootstrap analysis was performed through 500 iterations, and bootstrap values of >50 only are shown. The squirrel heart clone was identified as slow MHC₁, the diaphragm clone as fast MHC_{2x}, and the tibialis clone as fast MHC_{2b}.

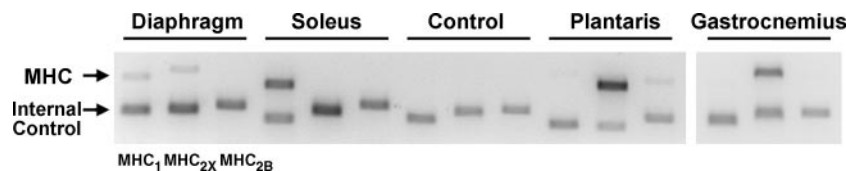


Fig. 3. A Sybr green-stained agarose gel showing MHC mRNA expression in 4 squirrel muscle types. The *top* band, when present, is amplified cDNA representing MHC mRNA expression for each isoform. The *bottom* band represents the amplified control fragment and is always present, provided that the PCR reaction proceeded normally. Three individual, simultaneous reactions are performed on each muscle sample, using the same starting template of a mixture of muscle cDNA and diluted control fragment but utilizing different specific MHC isoform primers. The third sample from *left* (Control) was not loaded with muscle cDNA to demonstrate the amplification of the internal control fragment in the absence of other cDNA. Sybr green stain may modestly affect the migration distance when used in loading buffer, and fragment size was verified independently with ethidium bromide-stained gels.

subsequent PCR reactions, wherein the MHC_{2x} and MHC_{2b} primers were added to the fragment in similar fashion. The final control fragment is depicted in Fig. 2B.

Individual determination of MHC mRNA expression (Fig. 3) was performed in 2 μ l of template [1 μ l of cDNA and 5.5 μ l of control fragment diluted to approximately attomole (10^{-18} mol) concentration] in three separate PCR reactions (conditions as above). PCR products were separated on a 2% agarose gel and visualized with Sybr green stain (Molecular Probes, Eugene, OR). Bands were analyzed with ImageQuant software (Molecular Dynamics, Sunnyvale, CA), and relative MHC mRNA percent was calculated from the band intensities as

(Specific MHC mRNA/corresponding control)/

$$\frac{(\text{MHC}_1 \text{ mRNA/control} + \text{MHC}_{2x} \text{ mRNA/control} + \text{MHC}_{2b} \text{ mRNA/control})}{\text{Internal Control}} \times 100\%$$

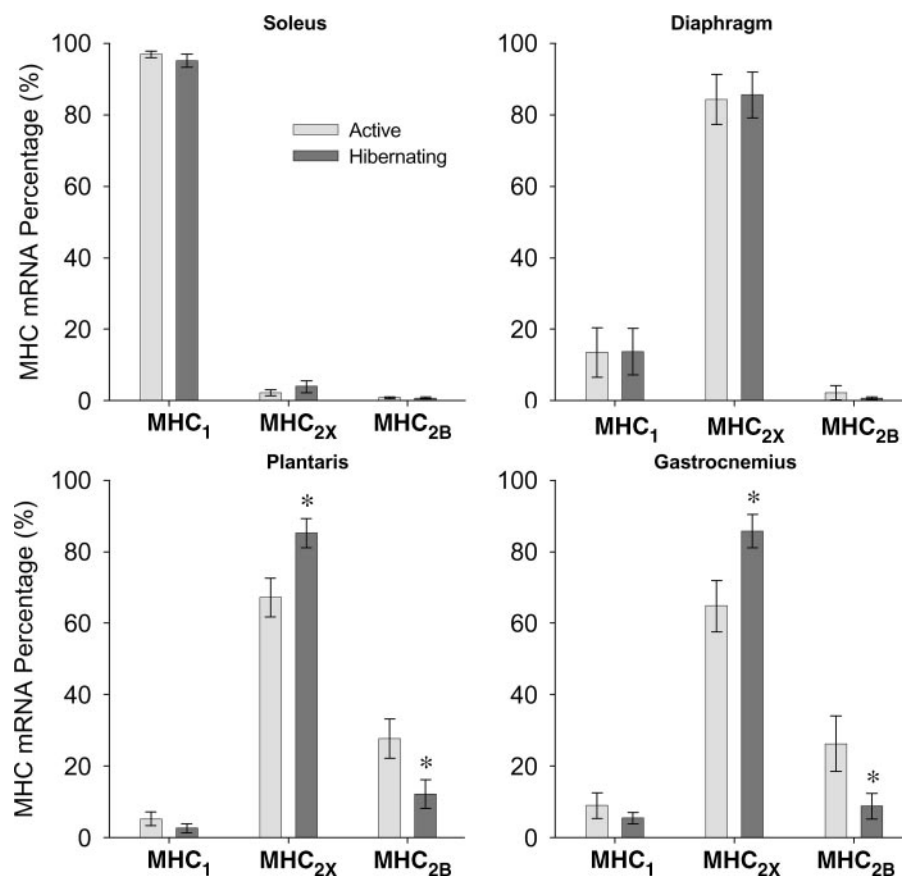
MHC Protein Isoform Expression

A 15- to 20-mg sample of each whole muscle was homogenized and used for SDS-PAGE analysis of relative MHC protein isoform percentages, as described previously (12, 13). Gels were silver stained (Bio-Rad) and documented with a digital camera, and the relative percentage of MHC isoforms was determined through densitometry (ImageQuant).

RESULTS

We isolated three putative MHC cDNA sequences (accession nos. AY551935, AY551936, and AY551937) in over 100 clones from ground squirrel muscle; these represent nucleotide sequences of the last ~ 600 bp of the MHC mRNA and include the 3' untranslated region. Approximately 97% of the sequences were identified as MHC genes, but non-MHC genes

Fig. 4. Relative mRNA expression for each MHC isoform, as determined by RT-PCR. Soleus ($P > 0.05$, $n = 25$) and diaphragm ($P > 0.05$, $n = 14$) muscles did not demonstrate differences in relative mRNA expression between active and hibernating animals. MHC_{2x} mRNA expression was relatively greater in the hibernating plantaris ($P = 0.0199$, $n = 24$) and gastrocnemius ($P = 0.0166$, $n = 26$), whereas MHC_{2b} mRNA expression was relatively less ($P = 0.044$, plantaris; $P = 0.026$, gastrocnemius). Data are means \pm SE. * $P < 0.05$.



were occasionally encountered. BLASTn analyses were helpful in tentatively identifying two sequences as corresponding to fast MHC₂ isoforms. Squirrel clones were identified further as slow MHC₁ and fast MHC_{2x} and MHC_{2b} by bootstrap analysis (Fig. 1). Subsequent RT-PCR reactions comparing mRNA expression in muscles of known MHC protein isoform composition provided additional evidence that each sequence corresponded to slow MHC₁, fast MHC_{2x}, or fast MHC_{2b}. Western blots have previously been used to identify the MHC protein expression of the four muscles used in the current study (12), and the MHC_{2a} protein was notably absent as well. The sequence categorized as the slow isoform was isolated uniquely from cardiac muscle and is therefore unlikely to be misidentified. Although both α - and β -cardiac myosin are expressed, α would not be expressed in skeletal muscle, whereas the β sequences and slow type I skeletal myosin are identical. The sequence labeled as MHC_{2x} was found as the predominant sequence in diaphragm muscle, which expresses only MHC₁ and MHC_{2x} protein isoforms. The MHC_{2b} labeled sequence was isolated uniquely from tibialis anterior muscle, which expresses both fast MHC_{2x} and MHC_{2b}.

Relative mRNA expression for each MHC isoform in individual skeletal muscles reveal differences between active and hibernating ground squirrels (Fig. 4). Plantaris and gastrocnemius muscles from hibernating animals showed significant differences in MHC_{2x} ($n = 24$, $P = 0.0199$ and $n = 26$, $P = 0.0116$, respectively) and MHC_{2b} ($P = 0.0116$ and $P = 0.026$) mRNA expression, which very closely mirrored changes in MHC protein isoforms measured previously in the same animals (12).

Correlation analyses were performed to support our categorization of each isoform as MHC₁, MHC_{2x}, or MHC_{2b} and to validate our RT-PCR technique. Relative percent mRNA expression for each MHC isoform was compared with the corresponding relative percent MHC protein isoform expression from the same muscle (Fig. 5), including data from both hibernating and active animals. No differences were found in the results of correlation analyses performed on active animals alone ($n = 43$) or on pooled data from both active and hibernating animals ($n = 79$). For the MHC₁ ($P < 0.000$, $r^2 = 0.8745$) and MHC_{2x} ($P < 0.000$, $r^2 = 0.7508$) isoforms, correlations were robust. The MHC_{2b} ($P < 0.000$, $r^2 = 0.5157$) analyses were similarly significant; however, there were a number of instances in which MHC_{2b} mRNA expression was detected in the absence of MHC_{2b} protein. We do not believe this to be a failure of the primer or a matter of cross-reactivity with the MHC_{2x} sequence, as MHC_{2b} mRNA expression was not correlated with MHC_{2x} mRNA expression in any way.

DISCUSSION

There were several major findings of this study. First, although 3' RACE is a commonly employed technique, its application here is particularly useful in rapidly obtaining MHC sequence information, especially for the 3' untranslated region, which can be critical to developing primers. Second, we observed that bouts of torpor did not produce a slow-to-fast MHC mRNA isoform transition, as predicted from studies employing different models of inactivity in mice and rats. There may be several aspects of torpor that contribute to this unusual response, but changes in MHC protein could generally

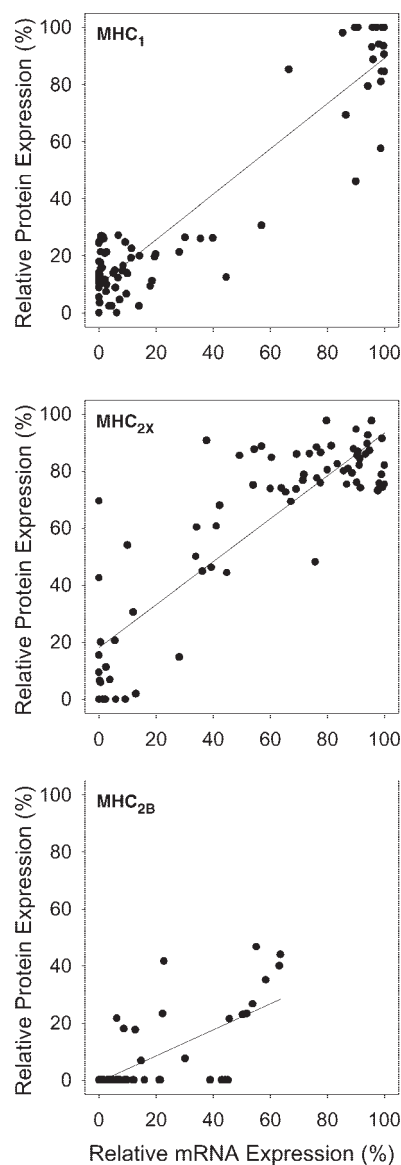


Fig. 5. Correlation analyses of relative MHC isoform protein expression and relative MHC mRNA expression. MHC protein data were obtained from SDS-PAGE analysis of whole muscle, and mRNA expression data were obtained by RT-PCR reactions, using primers derived from nucleotide sequences of ground squirrel MHC genes (see text). All correlations were highly significant ($P < 0.000$, $n = 79$; MHC₁ $r^2 = 0.87$, MHC_{2x} $r^2 = 0.75$, MHC_{2b} $r^2 = 0.52$).

be explained by similar changes in MHC mRNA (Fig. 6). Finally, we did not observe the presence of the fast MHC_{2a} mRNA isoform, a finding that is consistent with our previous protein analyses (12).

The technique described for obtaining sequences from MHC genes should be applicable across a wide range of vertebrate species and has been successfully used in our laboratory on several rodent species (*Spermophilus lateralis* and *Cynomys ludovicianus*, *leucurus*), a lizard (*Varanus exanthematicus*), and the black bear (*Ursus americanus*). Success of the 3' RACE technique is theoretically only dependent on the annealing of the gene-specific primer, the common MHC sequence utilized herein, and the sequencing of sufficient amplified products to ensure isolation of all isoforms. Although complete

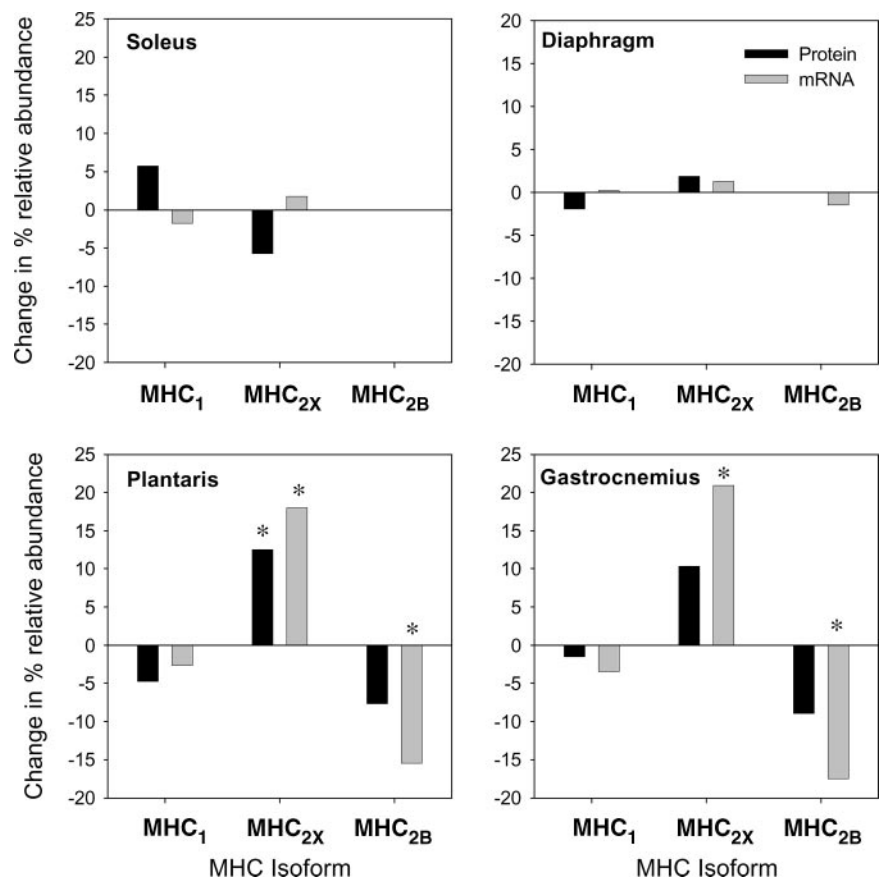


Fig. 6. Change in %relative abundance of MHC isoform protein and mRNA. Differences in MHC protein isoforms after hibernation are shown with the respective differences in MHC mRNA levels from the identical muscles. *Significant differences in isoform percentage between active and hibernating groups for either protein or mRNA data, $P < 0.05$.

sequences for MHC genes exist for several vertebrate species of interest, comparative physiologists studying muscle biology in nonclassical vertebrate models can measure MHC mRNA expression in which little or no sequence information is available. The method employed is rapid and sensitive and provides an internal control construct that more accurately quantitates MHC gene expression despite potential variation inherent in RT-PCR reactions (20).

Hibernating ground squirrels alter the MHC_{2x} protein isoform composition of plantaris and gastrocnemius muscles in unusual fashion during 4–6 mo of torpor (12), whereas other muscles are unchanged. We conclude that this likely is due to pretranslational control of the MHC genes and not as the result of differential protein degradation. However, it is not clear whether the pretranslational control mechanisms are influenced by the shivering activity of the animals during bouts of re-warming and at what time the mRNA expression changes, whether shortly upon entry to torpor or gradually throughout the hibernating period. The mRNA levels could be adjusted through a variety of signaling pathways in muscle and may be influenced by skeletal muscle activity itself; conversely, the mRNA levels may be regulated to accommodate the requirements of shivering thermogenesis in the muscles of hibernating individuals before the first bout of re-warming, continuing throughout the remaining hibernation period. There is a short time (2–3 wk) after the active animals were killed and before the hibernating animals entered the first bout of torpor during which mRNA levels could have been regulated by intrinsic muscle pathways independent of shivering activity. This could

be verified by measurement of mRNA levels during that interim phase, by obtaining of mRNA levels at additional time-points, and by close monitoring of muscle activity via electromyography throughout hibernation.

We also measured lower MHC_{2b} mRNA expression in hibernators than in active animals, and MHC_{2b} protein expression is completely lost in the gastrocnemius (12). Although the plantaris muscle does not show significant changes in IIB protein, other posttranscriptional mechanisms may be affecting the MHC_{2b} protein expression. In a number of muscles, we measured relatively high levels of MHC_{2b} mRNA expression, with no concomitant expression of MHC_{2b} protein (Fig. 5); in fact, the preponderance of MHC_{2b} mRNA apparently is not translated, particularly in hibernating muscles. This is not uncommon in skeletal muscle, as soleus muscles in the rat responding to hindlimb suspension or denervation evince significant expression of mRNA for the embryonic MHC isoform, without a concomitant expression of embryonic MHC protein (Ref. 6; Rourke, unpublished observation). The hibernating phase also is arguably not a steady-state condition for either mRNA or protein; nevertheless, correlations made with active and hibernating muscles independently are virtually indistinguishable. A temporal lag between protein translation may occur as a result of torpor, or suppression of MHC_{2b} protein translation may be occurring. Because MHC_{2x} protein expression is altered during torpor, it becomes unlikely that translation of MHC_{2b} protein would not proceed as well simply due to a lag.

The fast MHC_{2a} protein isoform is typically expressed at the lowest level of the four adult MHC isoforms found in mouse and rat hindlimb muscles, but MHC_{2a} protein was not detected in the muscle types used in this study for comparisons between active and hibernating animals (12). Trace quantities have been detected by SDS-PAGE analysis of tibialis anterior extracts, but these represented <5% of the total MHC isoforms when rarely seen in individual muscles. Some clones used in this study were derived from a tibialis anterior muscle, but we did not identify any cDNA sequences that might correspond to the MHC_{2a} gene and believe that the mRNA levels of MHC_{2a} in the examined muscles are not present in significant quantities to be detected.

Many interesting questions in the comparative physiology of muscle can now be extended to the pretranslational control of MHC gene products, utilizing the methods described herein. Measuring MHC mRNA expression may be most useful after steady-state conditions have changed, such as after exercise, fasting, migration, or hibernation phenomena and during other experimental manipulations.

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