Muscle-specific Regulation of a Transfected Rabbit Myosin Heavy Chain β Gene Promoter*

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We have examined the transcriptional regulation of the rabbit myosin heavy chain (HC) gene by using DNA-mediated transfection experiments. To analyze the regulatory activity of this HCB promoter in a myogenic background, cultured myoblasts from 12-day-old chick embryonic breast muscle were transfected with a chimeric gene containing 781 base pairs of the promoter region fused to the gene for chloramphenicol acetyltransferase (CAT). As indicated by the transient expression of chloramphenicol acetyltransferase, the activity of the promoter in myoblast cultures increased at least 32-fold following differentiation and was selectively inhibited when myogenesis was blocked with β-bromodeoxyuridine. Furthermore, RNAse protection experiments showed that the in vivo myosin HCB transcriptional initiation (or cap) site was utilized in the transfected skeletal muscle cells and also that the regulation of the exogenous promoter was similar to the induction of the endogenous skeletal α-actin gene. The results indicated that the exogenous promoter is regulated in a tissue- and stage-specific manner. By creating progressive 5′ deletions of the promoter, we showed that only the region extending ~294 base pairs upstream from the cap site is necessary for the muscle-specific expression. Linker-scanner mutagenesis of this region indicated that the positive regulation in differentiated skeletal muscle is mediated by at least two distinct elements within the 5′-flanking region of the myosin HCB gene.

Skeletal muscle is a convenient system in which to investigate the molecular mechanisms that regulate gene expression during terminal differentiation and development. The process of differentiation is well demarcated by the fusion of single cells into multinucleated myotubes and by the concomitant induction of genes for contractile, regulatory, and membrane proteins. Moreover, during subsequent development, many of the muscle-specific proteins that were initially expressed are replaced by other isoforms characteristic of adult muscle fibers.

One marker of myogenesis is the major subunit of myosin, the myosin heavy chain (HC), which is an important determinant of the enzymatic ATPase activity of myosin (1) and, hence, of the shortening velocity of muscle (2). Multiple molecular forms of myosin HC, each encoded by a separate gene, have been identified in fast, slow, and cardiac muscle (3). Unique forms of the protein may be associated with different fiber types. The expression of these isoforms follows a defined developmental pattern and is influenced by the neural input and the hormonal state of the muscle (4–9).

The myosin HCB gene is of particular interest because, in mammals, this gene is expressed early during the ontogeny of skeletal muscle and becomes restricted to slow twitch fibers during subsequent development. In addition, the myosin HCB is a major HC isoform expressed in cardiac muscle. S1 nuclease mapping (5, 10) and primer extension analysis of mRNAs indicate that the gene transcribed in slow skeletal muscle and cardiac muscle is the same. The modulation of myosin HCB gene expression in both tissues, however, is not identical (11). These differences probably reflect the distinct functional and metabolic requirements of the two tissues. Therefore, the analysis of myosin HCB gene regulation provides an opportunity to determine the relationship between the mechanisms that direct and modulate gene expression during muscle differentiation and during the subsequent development of adult skeletal muscle fibers, as well as to analyze the mechanisms that specify differences between cardiac and skeletal muscles.

We have previously isolated rabbit genomic clones specifying the α and β myosin HC and characterized their 5′-coding and -flanking sequences (12, 13). In this study, we examined the transcriptional regulation of the rabbit myosin HCB gene by using DNA transfection experiments. We showed that a 781-bp region containing the promoter and 5′-flanking sequences of the HCB gene is sufficient for both tissue- and developmental stage-specific transcription following introduction into primary cultures of skeletal muscle cells. By examining the effects of 5′ deletions and linker-scanner mutations, we demonstrated that transcription of the myosin HCB promoter is regulated by at least two discrete cis-acting regions located 184 and 275 nucleotides upstream from the transcriptional initiation (cap) site.

EXPERIMENTAL PROCEDURES

Cell Cultures—Primary cultures were prepared from the breast muscles of 12-day-old embryonic chickens essentially as described (14). Briefly, the muscles were dissected and minced, and the cells

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were dissociated with 0.125% trypsin and 0.0125% collagenase. To reduce the number of fibroblasts, the myoblasts were preplated twice on nongelatinized culture plates. The cells were filtered through sterile lens paper for removal of aggregates; they were then plated on gelatin-coated Petri dishes (Falcon brand, Becton Dickinson Labware) at a density of 8X10^4 cells/80-mm culture dish and grown at 37°C in Eagle's Minimum Essential Medium (MEM) containing 10% horse serum and 2% chicken embryo extract, with 10 units/ml penicillin and 10 μg/ml streptomycin. For the experiments involving 5-bromodeoxyuridine (BrdUrd), the cultures were maintained continuously in medium containing 10 μM BrdUrd.

**Linker-Scanner Mutagenesis**—A plasmid, pMHC-β, which contained the promoter region of the myosin HCB gene linked to the bacterial chloramphenicol acetyltransferase (CAT) gene, was constructed as follows. The 781-bp HindIII fragment of the genomic clone WMHC6β/1 (13) was isolated, the 5' protruding ends were filled in using the Klenow fragment of DNA polymerase I (Pharmacia LKB Biotechnology Inc.), and the fragment was inserted into the BgII site (also made blunt-ended) of the transfection vector pAIOCAT_M (15). Plasmid DNA was prepared according to the sodium dodecyl sulfate sulfate precipitation method, essentially as described by Wigler et al. (17). Plasmid DNA, in 125 mM CaCl₂, was added dropwise to an equal volume of 2X HBS buffer (50 mM HEPES, 280 mM NaCl, 115 mM Na₂HPO₄, pH 7.1). The precipitates were allowed to form for 10 min at room temperature and were then added directly to the cultures. After 4 h later the medium was replaced, and the growth of the cultures continued for an additional 24–72 h.

**Enzyme Assays**—The transfected cells were rinsed twice with cold phosphate-buffered saline and then scraped and collected in 0.5 ml of 40 mM Tris HCl, pH 7.8, 1 mM EDTA, and 140 mM NaCl. The cells were then pelleted in a microcentrifuge, resuspended in 0.25 M Tris HCl, pH 7.8, and disrupted by homogenization with a small pestle. The cell debris was removed from the lysates by centrifugation, and the supernatants were assayed for chloramphenicol acetyltransferase activity according to the procedure of Gorman et al. (18). The acetylation of [³⁵C]chloramphenicol (50–60 Ci/mmol, Du Pont–New England Nuclear) was monitored by thin-layer chromatography on silica gel plates (J. T. Baker Chemical Co.) using chlorormform:methanol, 9:1, as the solvent and visualized by autoradiography. For measurements of the conversion of chloramphenicol to its acetylated forms, the spots were scraped and collected and the radioactivity was measured by liquid scintillation counting. In most instances, the activity of β-galactosidase in the lysate was also assayed following the method of Miller (19), with 2-nitrophenyl-β-d-galactopyranoside used as the substrate (Boehringer Mannheim). Typically, 20 μl of lysate were used in a 20-min assay.

**RNase Protection Experiments**—Total RNA was isolated from transfected cultures as described by Chirgwin et al. (20). For the RNase protection experiments, labeled RNA probes were synthesized from plasmids containing either a PvuII fragment derived from the plasmid pMHC-β, which was subcloned into the Smal site of Bluescribe M13 (Stratagene Cloning Systems), or the S1 DNA-AhAII fragment from the 3' end of the chicken skeletal a-actin gene (kindly provided by Dr. B. Paterson, National Cancer Institute), which was cloned into the Smal site of the same vector. Complementary ³²P-labeled probes were transcribed using T7 RNA polymerase as described by the manufacturers. The probes were hybridized with 20 μg of total RNA in 80% formamide, 40 mM PIPES, pH 6.4, 0.4 mM NaCl, 1 mM EDTA at 45°C for 16 h. The hybridization mixture was then diluted in 10 mM Tris HCl, pH 7.5, 5 mM EDTA, 50 mM NaCl, incubated with 75–150 units of RNase T1 (Worthington) at 37°C for 60 min, treated with 40 μg/ml Proteinase K (Pharmacia LKB Biotechnology Inc.), and extracted with phenol. The RNA hybrids were precipitated with ethanol, and the protected fragments were fractionated by electrophoresis in 8% polyacrylamide–urea sequencing gels and visualized by autoradiography with a Lightning Plus intensifying screen (Du Pont, Cronex Division).

**Deletion Mutagenesis**—We created nested 5' deletions of the 781-bp HindIII fragment of the myosin HCB gene essentially as described by Kure (21) by using the exonuclease Bal31 (New England Biolabs). Plasmid subclones containing the deletions were digested with SmaI for release of the altered promoter region, which was then subcloned so that the XbaI-AvaI fragment of pMH-β was replaced for transfection. The locations of the deletion end points were confirmed by sequencing across the pmacl-plasmid junctions by the dideoxy chain termination method of Sanger et al. (22).

**Results**

The structure of the 5' end of the rabbit cardiac myosin HCB gene is shown schematically at the top of Fig. 1. The 5'-flanking region contains two small exons (open boxes) which are separated by a relatively large intron. Primer extension analysis of the myosin HCB mRNA revealed a single transcriptional initiation (cap) site (13), with the consensus eukaryotic promoter sequences TATA and CCAATT located at nucleotides -33 and -172, respectively. To examine the regulation of the myosin HCB promoter, we constructed a HCB-CAT fusion gene by subcloning a HindIII fragment between nucleotides -682 and +99 upstream of the CAT gene in the transfection vector pAIOCAT M. As shown in Fig. 1, the resulting plasmid, pMHC-β, contained the HCB cap site, the putative CCAATT and TATA boxes, and also 49 bp of the first intron of the gene.

**Tissue- and Stage-specific Regulation of the Myosin HCB Promoter in Skeletal Muscle**—To examine the muscle- or tissue-specific regulation of the HCB promoter, we prepared primary cultures from pectoralis muscle of 12-day-old embryonic chicks, as described under “Experimental Procedures.” Under the plating conditions used, the myoblasts replicate. Then, between 48 and 60 h in culture, they fuse to form...
multinucleated myotubes and begin to synthesize myofibrillar proteins (24). Typically, more than 70% of all cell nuclei are incorporated into the myotubes.

We transfected the myoblasts 1 day after plating and evaluated the expression of the myosin HCP promoter prior to fusion at 48 h and following fusion at 72 and 96 h in culture. To control for the transfection efficiency and for experimental variations between cultures, we co-transfected the plasmid pCH110, which contains the Escherichia coli β-galactosidase gene under transcriptional control of the SV40 early promoter, and we assayed the activity of both β-galactosidase and chloramphenicol acetyltransferase in the same cell extracts.

As shown in Table I, chloramphenicol acetyltransferase expression prior to fusion at 48 h was negligible, being comparable to the levels found in cells transfected with the promoterless CAT plasmid pAβCAT5M. Similar results were obtained following transfection of pMHC-β into a number of nonmuscle cells, including HeLa cells, the liver cell line HepG2, and CV-1 monkey kidney cells (25). These results indicate that the HCP promoter is inactive in nonmuscle and undifferentiated muscle cell backgrounds. In contrast, following terminal differentiation at 72 and 96 h, the expression of chloramphenicol acetyltransferase was induced markedly. β-Galactosidase activity increased approximately 2-fold in preversus post-differentiated cells, and similar increases were observed when the plasmid pSV2-CAT (18) was transfected (data not shown). Thus, when chloramphenicol acetyltransferase activity at 96 h is normalized to β-galactosidase activity, the myosin HCP promoter was induced approximately 32-fold. These results indicate that the HCP promoter is transcribed selectively in differentiated muscle cells.

To demonstrate further the dependence of promoter function on muscle differentiation, we examined the effects of inhibiting muscle differentiation with the thymidine analogue BrdUrd. In the presence of BrdUrd, skeletal myoblasts remain in the cell cycle, and cell fusion and the induction of contractile protein synthesis are blocked (26, 27). As shown in Table I, treatment of the cells with BrdUrd blocked the induction of pMHC-β at 72 and 96 h. Even though the β-galactosidase activity was relatively low at 96 h (0.08 versus 0.28 in the untreated cultures), the normalized chloramphenicol acetyltransferase activity was not elevated significantly above that in undifferentiated myoblasts. Thus, the activity of the transfected promoter mimics the requirement of the endogenous contractile protein genes for terminal differentiation.

**Initiation of the Chimeric Gene from the HCP Cap Site**—To establish that transcriptional initiation occurred from the HCP cap site and to show that induction of chloramphenicol acetyltransferase expression reflected changes in the corresponding mRNAs, we performed RNase protection experiments. The probes used in these studies are diagrammed in Fig. 2. A probe for the HCP-CAT fusion gene was generated from a subclone containing 152 bp of the CAT gene and 634 bp of 5′ terminal sequences of pMHC-β. This subclone was linearized with AvaI, and a 440-nucleotide RNA probe complementary to the mRNA was transcribed from the T7 promoter adjacent to the insert. The probe was hybridized to total RNA from the transfected muscle cell cultures, and the hybrids were treated with RNase T1. Because the cap site of

![Figure 2](image_url)

**Figure 2.** RNase protection of mRNAs prepared from transfected cells. 32P-Labeled RNA probes were generated as described under “Experimental Procedures” and are depicted in the upper portion of the figure. Primary myoblasts cultured in the absence and presence of 10 μM BrdUrd (BrdR, as indicated) were co-transfected with 5 μg of pMHC-β along with 0.5 μg of pSV2-CAT as an internal control. Total RNA was extracted at either 48 or 72 h in culture. Either 20 μg (Bluescyrbe M13/β probe) or 5 μg (Bluescyrbe M13/α-actin probe) of RNA were hybridized with the appropriate probe, and the hybrids were digested with 75 and 150 units of RNase T1 (adjacent lanes) for 30 min at 37 °C. Probe lengths take into account nucleotides added during blunt-ended ligation. Fragments protected from RNase digestion were resolved on a denaturing 5% polyacrylamide gel and visualized by autoradiography. Molecular size markers (M) are 32P-labeled Sau3A digest of pBR322. BS, Bluescyre M13.
the HCβ promoter is located 99 nucleotides upstream from the CAT gene, utilization of the correct cap site would result in a protected fragment of 259 nucleotides. Similarly, we synthesized a 216-nucleotide labeled RNA probe that was complementary to 185 nucleotides of the 3’-nontranslated region of the chicken skeletal α-actin mRNA. As shown in Fig. 2, RNA from differentiated cultures transfected with pMHC-β protected a fragment of 259 nucleotide, verifying transcriptional initiation from the HCβ cap site. In agreement with the enzymatic analysis, the chimeric transcript was expressed only following differentiation at 72 h, and it was absent in cells grown in the presence of BrdUrd. By contrast, the expression of the co-transfected SV40-CAT fusion gene (152-nucleotide fragment) was comparable both before and after differentiation. Because fragments corresponding to the first exon of the HCβ gene were not observed, splicing at that donor site does not modulate the appearance of the chimeric gene transcript. Thus, the induction of both muscle- and stage-specific expression is regulated pretranslationally, most likely by transcription.

We also compared the function of the HCβ promoter to the expression of skeletal α-actin mRNA in the same RNA samples. As indicated by the protected 185-nucleotide fragment, a low level of skeletal α-actin mRNA was present in myoblasts, which was significantly up-regulated with differentiation at 72 h, and inhibited with BrdUrd treatment. Thus, the transcription of the transfected HCβ promoter paralleled the induction of an endogenous myofibrillar protein gene.

Analysis of 5’ Deletion Mutants—To determine which selected regions mediate stage-specific activation of the HCβ promoter, we constructed and evaluated progressive 5’ deletions for transient expression in the primary muscle cell cultures. The top of Fig. 3 depicts the end points of the various deletion mutants. The truncated promoter was placed adjacent to the same vector sequences in all constructs. The results (Fig. 3, bottom, and Table I) show that regulated expression of the promoter (induction with differentiation and suppression by BrdUrd) was lost when sequences between -294 and -226 were deleted. The loss of promoter function is not due to the alteration of essential promoter elements such as the CCAAT and TATA boxes and, as shown in Table II, does not result from variations in transfection efficiency. These results were reproduced in five separate experiments using different plasmid preparations. Thus, the region between -294 and -226 is necessary for stage-specific activation.

Although the relative chloramphenicol acetyltransferase expression among the active mutants varied somewhat, the relative expression of mutant -294 is notable. It is an order of magnitude above that of pMHC-β and of the other functional mutants, indicating that sequences upstream of -294 may suppress promoter function.

Analysis of Linker-Scanner Mutants—To localize more precisely the cis-acting elements involved in muscle-specific regulation of the HCβ promoter, we constructed eight different linker-scanner mutations within the region between -355 and -172. This region spanned the area that was necessary for promoter function in the 5’ deletion mutants. Some of the mutants also contain small deletions; nevertheless, we still refer to them as linker-scanner mutants. Fig. 4 shows the location of each linker-scanner mutation and the specific base changes in each. The bottom of Fig. 4 shows a representative transient expression of each mutant following differentiation of transfected primary muscle cultures. Mutations 1-1 and 5-5 had the most drastic effects, consistently abolishing the activity of the promoter. The remaining mutants were functional, although mutation 6-1 was less active than the rest. As shown in Fig. 4, mutation 1-1 alters the sequences corresponding to the cap site are given for each mutant, along with representative results of the corresponding chloramphenicol acetyltransferase assays (lower part of figure). 20 µl of cell extract were assayed for chloramphenicol acetyltransferase activity during a 30-min incubation. BUDR, 5-bromodeoxyuridine; CM, [3H]chloramphenicol; 1-AC and 3-AC, acetylated forms of [3H]chloramphenicol.

Table II

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Chloramphenicol acetyltransferase activity % conversion</th>
<th>β-Galactosidase activity A400</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMHC-β</td>
<td>2.8</td>
<td>0.79</td>
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<tr>
<td>Mutant position</td>
<td></td>
<td></td>
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<tr>
<td>-627</td>
<td>5.0</td>
<td>0.77</td>
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<tr>
<td>-561</td>
<td>4.9</td>
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</tr>
<tr>
<td>-484</td>
<td>2.8</td>
<td>0.54</td>
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<td>-355</td>
<td>8.4</td>
<td>0.60</td>
</tr>
<tr>
<td>-294</td>
<td>21.7</td>
<td>0.64</td>
</tr>
<tr>
<td>-226</td>
<td>0.22</td>
<td>0.42</td>
</tr>
<tr>
<td>pA5CAT3M</td>
<td>0.23</td>
<td>0.83</td>
</tr>
</tbody>
</table>
3-AC
1-AC
CM

FIG. 4. Functional assay of linker-scanner mutants of the myosin HCβ promoter region. The BglII linker sequence CAGATCTCG was inserted into the myosin HCβ promoter region as described under "Experimental Procedures." The location of each control plasmid pCH110. The cells were harvested after 72 h in incubation.

Sequence Comparisons—We have compared the sequences altered by linker-scanner mutations 1-1 and 1-5 to the 5'-flanking regions of various contractile proteins genes, including chicken skeletal α-actin (28), human cardiac α-actin (29), mouse creatine kinase (30), rat (31) and chicken (32) embryonic skeletal myosin HC, and chick myosin light chains 1 (33) and 2 (34), to identify conserved sequences that may also serve a regulatory function. Because the functionally relevant sequences could overlap some of the boundaries of the distal and proximal regions (i.e. those not immediately flanked by a linker-scanner mutation), we extended those boundaries by 8 nucleotides to make the comparisons.

For the distal region corresponding to linker-scanner mutation 1-1, there were no extensive sequence homologies with the regulatory regions of other contractile protein genes. By contrast, in comparisons with the proximal region we identified one sequence within the myosin HCβ gene that showed significant identity with sequences from a number of contractile protein genes (Table III). The putative consensus sequence is found only in promoter regions necessary for muscle-specific expression and not in other flanking sequences of these genes. Interestingly, this sequence is very similar to the binding site of a cellular transcriptional activator, AP-2 (35). When combined with the mutational analyses that indicate the necessity for at least two functional regions, these results suggest the possibility that a general cellular activator of transcription may be involved in the muscle-specific activation of the myosin HCβ gene and possibly of other contractile protein genes as well.

TABLE III

Putative consensus elements within proximal regulatory regions

<table>
<thead>
<tr>
<th>Gene</th>
<th>5' regulatory boundary</th>
<th>Sequence</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit myosin HCβ</td>
<td>−294</td>
<td>ACTCATGCC</td>
<td>−201/−192</td>
</tr>
<tr>
<td>Chicken myosin LC1/3</td>
<td>−296</td>
<td>ACTCATGCC</td>
<td>−1905/−1914</td>
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<tr>
<td>Chicken myosin LC9</td>
<td>−50</td>
<td>ACTCATGCC</td>
<td>−60/−51</td>
</tr>
<tr>
<td>Mouse MCK</td>
<td>−350</td>
<td>ACTCATGCC</td>
<td>−315/−322</td>
</tr>
<tr>
<td></td>
<td>−1256</td>
<td>ACTCATGCC</td>
<td>−162/−170</td>
</tr>
<tr>
<td></td>
<td>−1211</td>
<td>ACTCATGCC</td>
<td>−1220</td>
</tr>
<tr>
<td>AP-2</td>
<td>TCAGCG</td>
<td>ACTCATGCC</td>
<td>−1211−1220</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, we have initiated the functional analysis of the rabbit myosin HCβ gene promoter by transient transfection in a myogenic system consisting of chick embryonic skeletal muscle cells. The induction of the transfected HCβ promoter and its suppression by BrdUrd demonstrate that the activity of the promoter is dependent on myogenesis. In addition, our mutational analyses show that the modulated expression is regulated transcriptionally by at least two cis-acting regions of the myosin HCβ promoter. Thus, this transfection system provides the opportunity to delineate the mechanisms that control selective gene expression during terminal differentiation.

The regulated transcription of the myosin HCβ promoter in avian skeletal muscle cells probably reflects developmental processes that normally induce closely related chicken myosin HC isoforms. In mammals, myosin HCβ is expressed in adult cardiac muscle and slow twitch fibers, and it is co-expressed along with an embryonic myosin HC in developing skeletal muscles (5, 7, 10). The pattern of expression is similar in avian muscles, except that the cardiac and slow myosin HC co-expressed in embryonic skeletal muscle have different primary structures. The expression of the transfected mammalian gene in avian embryonic muscle cells could reflect a program in which cardiac contractile protein isoforms are induced following differentiation (36), it could be related to the early commitment of some cells (or nuclei) toward a slow twitch fiber phenotype (37), or both.

Using linker-scanner mutagenesis, we showed that muscle- and stage-specific regulation is dependent on at least two distinct regions of the gene. Neither region can direct the regulated expression of the promoter following the disruption of the other. Moreover, the distal region alone does not function as an activator (or enhancer) when placed upstream of a heterologous thymidine kinase (38) gene. Thus, both regions are necessary for stage-specific regulation. The distal
region appears to mediate a positive effect on transcription because, following its deletion, the promoter remains inactive in undifferentiated muscle. The distance of this region from consensus promoter elements such as the CCAAT and TATA sequences and the existence of intervening linker-scanner mutations that do not alter promoter function strongly suggest that this region encompasses a discrete regulatory element. By contrast, the downstream or proximal region that is just 10 bp upstream from the putative CCAATT box contains the sequence ATGCCATAACAAT, which also resembles the CCAAT consensus sequence. Therefore, it is possible that the proximal sequences interact with general transcription factors, such as CCAAT box binding proteins (39, 40). Recent results suggest that the boundaries of the proximal region extend beyond those of the initial linker-scanner mutation 5-3. Studies on 5′ deletions of the skeletal α-actin gene (41), in combination with the analysis of protein-binding factors within the 5′-flanking region (42, 43), suggest that distinct sequence elements near to and overlapping the CCAAT box are involved in muscle-specific regulation. Therefore, it is possible that this area contains multiple functional elements involved in either general or muscle-specific expression of the myosin HCβ gene.

The sequence comparisons of the myosin HCβ distal and proximal elements with other myofibrillar protein genes show that the proximal region encompasses another sequence that is intriguing. AP-2 is an enhancer-binding protein that is involved in the regulation of the SV40 promoter and a number of cellular genes (35, 44). Its role could be a secondary one of mediating the effects of other activators because by itself the protein is not a major stimulus of transcription (45). The functional properties of AP-2 are consistent with a model of muscle-specific regulation whereby the binding of AP-2 or an AP-2-like protein to the proximal region mediates the effect of other elements. Thus, muscle-specific expression of contractile protein genes may involve general cellular activators of transcription.

Transfection experiments involving other myofibrillar protein genes implicate multiple regulatory domains that are distributed over a wide distance of the promoter and gene. At least three domains having either positive or negative effects have been identified in the rat embryonic skeletal myosin HC gene (31). In the quail troponin I (46) and the mouse creatine kinase (30) genes, muscle-specific expression appears to be controlled by sequences relatively close to the core promoter, but it is also modulated by sequences either upstream of the promoter or within the first intron (42). In addition, a muscle-specific enhancer has been identified downstream of the rat LC1/LC3 gene (47). Our transfection results suggest that sequences located between −335 and −294 suppress the activity of the promoter. Although we cannot rule out possible variations in activity arising from the juxtaposition of vector and gene sequences, the data suggest the presence of an additional negative modulator in this region.

The functional definition of two elements within the myosin HCβ gene presented here enables the characterization of the putative trans-acting factors necessary for transcription of the gene in differentiated muscle. Moreover, the myosin HCβ gene is also expressed in cardiac muscle. Thus, delineating the regulatory elements that function in skeletal muscle will allow us to determine whether the same mechanisms define the potential for transcription in cardiac muscle and to elucidate some of the processes involved in specifying different muscle types.

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Regulation of a Transfected Myosin Heavy Chain β Promoter