A Binding Site for Nuclear Receptors Is Required for the Differential Expression of the Aldolase A Fast-twitch Muscle Promoter in Body and Head Muscles*

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In hind limb muscles, the aldolase A muscle-specific promoter is specifically expressed in glycolytic fast-twitch fibers. Here, we show that in addition, it is expressed at higher levels in trunk and limb muscles than in neck and head muscles independent of their fiber-type content. We have identified by analysis of transgenic mice a DNA element that is required for this differential expression and, to a lesser extent, for fiber-type specificity. We show that members of the nuclear receptor superfamilly bind this element in skeletal muscle nuclear extracts. Interestingly, in gel mobility shift assays, different complexes were formed with this sequence in tongue nuclear extracts compared with limb or trunk muscle nuclear extracts. Therefore, binding of distinct nuclear receptors to a single regulatory sequence appears to be associated with the location-dependent expression of the aldolase A muscle-specific promoter.

One of the main characteristics of skeletal muscles is their diversity. In the adult, this diversity appears with regard to shape, anatomical position, mechanical function, energy requirement, and nerve stimulation of each individual muscle. At the cellular level, it is revealed by the existence of different types of myofibers characterized by expression of specific isoforms of structural proteins and metabolic enzymes (1, 2). The usual classification divides muscle fibers in four major types based on MHC isoform expression: type I (slow, oxidative), type IIA (fast, oxidative and glycolytic), and type IIB (fast, glycolytic). Several genes show a fiber type-specific pattern of expression, and for some of them, their regulatory sequences have been characterized, including tropinin I fast (3) or slow (4–6), MLC1f (myosin light chain 1 fast) and MLC3f (7–9), aldolase A (10, 11), and MHCβ (12). Some other genes are active in a subset of muscles, with no obvious link with a particular fiber type (e.g. ryanodine receptor-3 in diaphragm (13) or engrailed-2 in jaw muscles (14)). This kind of muscle diversity has also been revealed by random integration of transgenes into “special” loci (15) or by deletion analysis of regulatory sequences have been characterized, including tropo-
fragment to be microinjected was isolated on 1% agarose gel after digestion by AgeI and SacI, followed by electrophoresis and purification on an Elutip column (Schleicher & Schuell).

Transgenic mice were generated, identified, and propagated as described previously (21). For chloramphenicol acetyltransferase assays, various tissues were dissected from adult (at least 7-week-old) F1 transgenic animals, except for the few founders analyzed, for which transgene presence in each tissue was verified by Southern blotting. Chloramphenicol acetyltransferase activity was measured as described previously (11), with amounts of proteins up to 200 μg and reaction times up to 4 h. For each line, at least two different mice were analyzed.

**Histological Determination of Muscle Fiber Types—Gastrocnemius, tongue, masseter superficial, and digastricus muscles were dissected from adult mice. Muscles were frozen in isopentane at liquid nitrogen temperature and cross-sectioned in a microtome-cryostat. The sected from adult mice. Muscles were frozen in isopentane at liquid nitrogen temperature and cross-sectioned in a microtome-cryostat. The sected from adult mice.

**Northern Blots—**Total RNAs were prepared from several tissues and skeletal muscles of adult B6/CBA mice by the guanidium thiocyanate single-step procedure (25). Northern blot analysis was done as described previously (10) using a mouse pM-specific probe (gift from M. Colbert) (26) or an R45 ribosomal probe for standardization.

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**RESULTS**

**pM Is Highly Active in Fast-twitch Trunk and Limb Muscles, but Far Less in Those Localized in Head and Neck—**We investigated pM310CAT expression in a large number of muscles: from limbs (vastus lateralis, gastrocnemius, psoas major, soleus, extensor digitorum longus, tibialis anterior, and triceps brachii), trunk (sternomastoideus, longissimus dorsi, intercostals muscles, and diaphragm), and neck and head muscles (digastricus, mylohyoideus, masseter, and tongue) (Fig. 1). For the two independent lines studied, a high chloramphenicol acetyltransferase activity was detected in all trunk and limb muscles, with the exception of muscles devoid of type IIB fibers, soleus (types I and IIA) and diaphragm (types IIA and IIX) (7, 28), in which activity was at least 100-fold lower. This result reflects the specific expression of pM in type IIB glycolytic fast-twitch fibers. Surprisingly, in all head and neck (suprahyoid) muscles tested, activity was on the average 100-fold lower (1000-fold for the tongue) than in trunk and limb muscles. Since this difference may arise from a lack of type IIB fibers in these muscles, we determined their fiber-type composition and compared the ratio between pM activity and percentage of type IIB fibers. As shown in Table I, this ratio was close to 100 (arbitrary units) in three muscles of the limb and trunk, whereas it dropped to 2.3 and 1.4 in the masseter and digastrics, respectively, and even to <0.1 in the tongue. Therefore, in head and neck muscles, the level of activity of pM is not determined by the proportion of type IIB fibers; the weak activity of pM reflects an intrinsic difference between these muscles and those from limb and trunk, and not a different composition of fiber type (as judged by MHC expression). Below, this difference is referred to as “muscle location-dependent expression.”

We then examined whether the absence of expression in head/neck muscles for the pM310CAT transgenes reflects the expression of the endogenous mouse aldolase A muscle-specific promoter. A Northern blot with total RNAs from different types of muscles was hybridized with a probe specific for mouse aldolase A exon M (Fig. 2); hybridization with the ribosomal probe R45 was used as a control for mRNA quantity and qual-

**TABLE I**

| Muscle | Fiber type | pM activity | pM/% IIB |
|-------|------------|-------------|---------|
| G     | I IIA IIX IIB | 3.7 17.7 35.0 43.5 | 4250 98 |
| TA    | I IIA IIX IIB | 1.0 13.3 19.3 67.5 | 5920 88 |
| Dig   | I IIA IIX IIB | 5.1 33.4 20.1 42.5 | 3630 85 |
| MS    | I IIA IIX IIB | 0 6 33 61 | 143 2.3 |
| Tg    | I IIA IIX IIB | 0 4 42 54 | 3.3 0.06 |

* Fiber type was determined by immunohistochemical staining of serial sections of mouse muscles with antibodies to specific type of MHCs.

* Values are the means of pM310CAT transgenic line 20 and 98 CAT activities (expressed in cpm/μg of protein/min of reaction).

* G, gastrocnemius; TA, tibialis anterior; T8, intercostal muscle 8; Dig, digastricus; MS, masseter superficial; Tg, tongue.

* Data for fiber type are from Ref. 14.
The M1 Sequence Is Required for Selective Expression of Aldolase A Transgenes in Trunk and Limb Skeletal Muscles—To identify the sequence(s) involved in the preferential activity of pM in trunk/limb muscles, we studied the expression pattern of transgenes harboring distinct segments of the pM regulatory region (Fig. 3A). We compared the activity of several transgenes in tongue and longissimus dorsi muscles and in several lines for each transgene (Fig. 3). On average, a 140-fold ratio of the activity in tongue and LgD muscles was observed for the construct M-tkCAT, compared with the 1000-fold ratio in pM310CAT transgenes; pM location-dependent expression is conserved without the proximal sequences (TATA box and exon M). The ratio of the activity in tongue and LgD muscles was diminished and probably reflects the fact that, usually, M-tkCAT transgenes were less active in trunk/limb muscles than pM310CAT; the M enhancer is probably more potent when linked to its innate minimal promoter. For the ΔAT-tkCAT transgenes, it should be first noticed that there was no detectable activity in muscles tested in four out of the nine transgenic lines studied. Nevertheless, in all lines with the “active” transgenes, we still observed a 220-fold ratio on average between tongue and LgD muscles. Thus, the sequences sufficient for muscle location-dependent expression are localized between base pairs −160 and −35. Further 5′-deletions of the pM regulatory sequences resulted in total loss of transgene activity (16). All four transgenic MΔE-tkCAT lines (sequences from base pair −235 up to the M1 site) were active, and there was an average 70-fold differential expression between tongue and LgD muscles. This result indicates that the E box and the SP1-binding site are also dispensable for a correct pattern of expression. Thus, the sequences needed for a graded expression between head and more caudal muscles were mainly localized in the region shared by the ΔAT and MΔE fragments: namely, the MEF3-, MEF2/NF-1-, and M1-binding sites.

The M1 sequence has been shown to be important for pM expression since its mutation resulted in a strong diminution of pM activity in myotubes in primary cell culture and in hind limb muscles of transgenic mice (21, 22). We tested whether the muscle location-dependent expression is conserved in constructs with either a mutated M1 site (mM1) or a deletion of this site (MΔM1-tkCAT) (Fig. 3). Mutation of the M1-binding site resulted in the loss of the differential activity between tongue...
and trunk muscles (an average of 0.4-fold). With the thymidine kinase promoter, deletion of M1 from the MτE fragment resulted in complete absence of expression for three out of the four transgenic lines obtained (MΔB-tkCAT), indicating that in this context, M1 is critical for promoter activity. In addition, in the only line with an expressed transgene (MτCAT), the LgD/tongue activity ratio was only ~4. Comparison of the DNA constructs with an intact M1 sequence to those with a mutation or deletion of this site revealed that M1 is required for a 20-fold (at least) difference in transgene activity in tongue and LgD muscles (p < 0.001, with χ² test; similar results were obtained with masseter instead of tongue or vastus lateralis instead of LgD).

From the data presented in Table II, it appeared that mutation of M1 caused a drop in activity in all fast-twitch muscles studied down to the level of pM310CAT transgenes in the tongue. This loss of activity is particularly dramatic in limb and trunk muscles (from 1200 to 8000-fold), but is also observed (only 70-fold) in masseter, which was the highest expressing head muscle studied. Taken together, these results suggest that M1 behaves like a muscle location-dependent cis-activator; M1 is essential for a high level of expression of pM in fast-twitch trunk and limb muscles, but it is dispensable in the tongue. Interestingly, this sequence is conserved in the human, rat, and mouse promoters (see Fig. 5A) (29).

M1-binding Site and Fiber Specificity—The mutation of M1 also reduces the fiber specificity of pM transgenes. Expression in diaphragm (composed of type IIX and IIA fibers) was far less affected (only 50-fold) by M1 mutation than that in type IIB fiber-containing limb and trunk muscles (Table II). Thus, the mutation or deletion of M1 resulted in a loss of the differential activity between type IIB fiber-rich trunk and limb muscles and diaphragm (Fig. 4). However, fast-twitch fiber specificity is conserved since mM1 transgenes were still expressed at a higher level in gastrocnemius muscles than in slow-twitch soleus muscles (devoid of type IIB fibers and with rare IIX fibers) (Fig. 4 and Table II). It is unclear whether the ratio of the activity in fast gastrocnemius and slow soleus muscles was significantly reduced or not since there is a great variation among the different mM1 lines; nevertheless, in three lines out of five (four out of six if MΔB-tkCAT-67 was included), this ratio dropped to <10-fold, whereas for pM310CAT and M-tkCAT transgenes, chloramphenicol acetyltransferase activity was at least 130-fold higher (average of 175-fold) in gastrocnemius than in soleus muscles, suggesting a potential involvement of M1 in fast-twitch specificity.

The M1 Sequence Is Bound by Nuclear Receptors—Transgenic studies have shown that M1 is required for the muscle location-dependent activity of pM310CAT transgenes. A similar sequence is found in the same position (relative to the transcription start site) in the mouse pM promoter, which has the same pattern of expression (Fig. 2). Comparison of M1 sequences in human, mouse, and rat aldolase A genes revealed a conserved motif (a/gGGt/gCA) directly repeated with a 1-nucleotide-long spacer (DR1) (Fig. 5A); this sequence looks like a potential binding site for members of the nuclear receptor superfamily (30). When GMSA were performed with skeletal muscle nuclear extracts (from back muscles), two main complexes were formed specifically with M1 (Fig. 5B); this sequence looks like a potential binding site for members of the nuclear receptor superfamily (30). When GMSA were performed with skeletal muscle nuclear extracts (from back muscles), two main complexes were formed specifically with M1 (Fig. 5B); the formation of these two complexes (named A and B) was competed by the addition of an excess of an M1-binding site, but not by an oligonucleotide corresponding to the mutation of the M1 site introduced in transgenic mice. Complex A was more abundant in skeletal muscle nuclear extracts than in liver or spleen nuclear extracts (Fig. 5B). As an excess of a consensus DR1-binding site for nuclear receptors prevented the formation of both complexes A and B (Fig. 5C), we assumed that the main binding activity on M1 is due to nuclear receptor complexes.

### Table II

Transgene activity in some head, trunk, and limb muscles

| Transgenic lines                      | Tg | MS | LgD | Tri | G | Sol | D |
|--------------------------------------|----|----|-----|-----|---|-----|---|
| pM310CAT-20                         | 1.8| 128| 6880|     |   |     |   |
| pM310CAT-98                         | 4.8| 157| 3190|     |   |     |   |
| mM1–4                                | 13.1| 6.1| 6.67| 0.54| 0.12| 0.03| 5.85|
| mM1–13                               | 6.9| 1.69| 3.29| 2.33| 1.38| 0.01| 0.54|
| mM1–16                               | 0.8| 1.3 | 0.1 | 0.85| 0.15| 0.02| 0.4 |
| mM1–20                               | 0.07| 0.77| 10 | 0.8 | 0.66| 0.01| 0.14|
| mM1–48                               | 0.25| 0.37| 0.56| 0.18| 0.3 | 0.08| 0.13|
| MΔB-tkCAT-67                        | 2.87| 2.74| 10.9| 2.19| 6.8 | 0.6 | 0.34|
| M-tkCAT-8                            | 0.4| 1.28| 112 |     | 131| 173 | 4.7 |
| M-tkCAT-86                           | 0.6| ND | 840 |     |   | 870| 6.3|

* Tg, tongue; MS, masseter superficial; Tri, triceps brachii; G, gastrocnemius; Sol, soleus; D, diaphragm; ND, not determined.

[FIG. 4. Fiber specificity is altered in the absence of M1. Values are ratios of transgene activity in the soleus to that in the gastrocnemius (Gastroc./Soleus) or in diaphragm to the mean of transgene activity in three glycolytic fast-twitch muscles: LgD, gastrocnemius, and triceps brachii (IIB/Diaphragm). Points show values for independent transgenic lines (+M1, pM310CAT (●)) and M-tkCAT (○); −M1, mM1 (○) and MΔB-tkCAT (●), and the mean ratio is represented by a bar. mM1 differs significantly from pM310CAT for type IIB fiber/diaphragm (p < 0.01 by Student’s t test). The difference for the gastrocnemius/soleus ratio in transgenes with or without M1 is significant only if M-tkCAT and MΔB-tkCAT lines are included (p < 0.05).]
Other complexes were formed with M1 (marked by asterisks), but they were poorly competed by an excess of unlabeled M1 probe and thus corresponded to nonspecific binding (see C for competition experiments). The upper band (marked by a diamond), whose formation was only moderately competed by an excess of the M1 oligonucleotide, probably corresponds to an SP1-like protein since it was very efficiently competed by an excess of the SP1 consensus site (data not shown).

Many nuclear receptors are able to bind to DR1 sequences, including the retinoic acid receptors RAR and RXR, peroxisome-activated receptors, and orphan nuclear receptors like COUP-TF/ARP1 and TR2/TR4 (30). As the M1 site mediated a muscle location-dependent activity, we investigated the nature of the complexes bound to this sequence with nuclear extracts from muscles of diverse origins: tongue (head muscle) and longissimus dorsi, diaphragm, and soleus. For normalization of the nuclear extracts, binding assays were performed with an upstream stimulatory factor (USF)-binding site; in each case, upstream stimulatory factor binding activity was detected in similar amounts (Fig. 6A). For the M1 probe, body muscle nuclear extracts (from trunk or limb, fast or slow twitch) displayed the same pattern as longissimus dorsi nuclear extracts (Fig. 6B and data not shown for gastrocnemius and vastus lateralis); complexes A and B were formed at least in equal amount, with usually more of complex A than complex B (to an extent that depended on the body muscle considered). With tongue nuclear extracts, the complexes formed were different. First, complex A was only weakly detected (Figs. 6B and 7A); in addition, no complex B was detected, whereas a third complex C appeared, migrating slightly slower than complex B observed with LgD nuclear extracts (Fig. 7A). Complexes B and C were better separated when GMSA was performed with a 6% acrylamide gel (Fig. 7A) compared with a 5% acrylamide gel (Fig. 6B); however, under this condition, complex A could not be easily distinguished from the additional SP1-like binding activity. In the following experiments, GMSA was performed with either 5 or 6% acrylamide gels, depending on which complex was considered.

We then asked if known nuclear receptors participated in

**Fig. 5.** Members of the nuclear receptor superfamily bind M1. A, the sequence of the M1 site was compared in human, mouse, and rat aldolase A genes. The three conserved sequences look like a DR1 site whose consensus sequence is given below. Arrows indicate the repeated sequence. B, gel shift assays were carried out on M1 with skeletal muscle (M), liver (L), and spleen (S) nuclear extracts. The specific complexes A and B are indicated, and asterisks mark nonspecific binding (see C for competition experiments). C, the M1 probe was incubated with muscle nuclear extracts (N.E.), and the specificity of complexes formed was tested by the addition of a 60-fold excess of unlabeled oligonucleotides. The oligonucleotides used were as follows: M1, GCCGGAGGCGAGGGTCATTAGA; mM1, GGCGGGAAgAtctCAGGGGTCATTAGA; and DR1, GGCGGGCCAGGTCAAGGCTTATTAGA.

**Fig. 6.** Binding activity of different muscle nuclear extracts. GMSA were performed with nuclear extracts from back muscle (LgD), diaphragm (D), soleus (Sol), and tongue (Tg). An upstream stimulatory factor (USF)-binding site (GAAGATCGGGGACACATGTGGGGCGAAG) was used to control the amount and quality of nuclear extracts (A). A 5% acrylamide gel was used to separate slow migrating complexes A (B).

**Fig. 7.** Presence of RAR, RXR, and ARP1 nuclear receptors in the complexes formed with M1. M1-bound complexes formed with nuclear extracts from tongue (Tg), LgD, diaphragm (D), and soleus (Sol) were separated on 6% (A) and 5% (B) acrylamide gels. M1-labeled oligonucleotide was incubated with nuclear extracts of different muscles in the presence of anti-RAR antibodies (A), anti-RXR antibodies (B, lanes 1–4), or anti-COUP-TF antibodies (B, lanes 9–12). The same complexes were observed with two independent nuclear extracts, and quantification of the relative amount of supershifted complex B in LgD muscles was performed in several separate GMSA with a Shimadzu densitometer.

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complexes A, B, and C by using specific antibodies. The addition of an anti-RAR antibody prevented the formation of complex C with tongue nuclear extracts, but had no effect on complex B formation in body muscles (Fig. 7A) or on complex A. The addition of an antibody directed against RXR proteins entirely supershifted this complex C in the tongue, but only partly (≈40% according to quantification performed with a Shimadzu densitometer) supershifted complex B in LgD muscles (Fig. 7B). In both nuclear extracts, complex A was not affected by this anti-RXR antibody. When an antibody directed against COUP-TF/ARP1 was added to the nuclear extracts, we observed a nearly complete supershift of both complexes A and B in body muscles, whereas this antibody supershifted only poorly (if at all) complex C formed with M1 by tongue nuclear extracts (Fig. 7B). Nuclear extracts from soleus, diaphragm, gastrocnemius, and triceps muscles gave the same results as LgD nuclear extracts (data not shown).

In skeletal muscles, ARP1 transcripts were far more abundant than COUP-TF1 transcripts (data not shown). Therefore, the COUP-TF-immunoreactive complexes formed with M1 should correspond to homo- or heterodimers containing the ARP1 orphan nuclear receptor. We performed GMSA with in vitro translated ARP1 proteins; the retarded complex (corresponding to ARP1 homodimers) migrated slightly faster than complex B (Fig. 8). This observation suggests that while complex B could perhaps include ARP1 homodimers in addition to RXR-containing complexes, complex A is probably a heterodimer between ARP1 and another unknown protein.

In summary, these experiments show that nuclear receptors are able to bind M1 in muscle nuclear extracts. With tongue nuclear extracts, RAR/RXR heterodimers formed the main binding activity (complex C). In body muscle nuclear extracts, M1 is mainly bound by complexes containing ARP1 orphan nuclear receptors; complex B corresponds to ARP1- and RXR-containing complexes (including ARP1/RXR heterodimers and perhaps some ARP1 homodimers), whereas complex A probably corresponds to a heterodimer between ARP1 and another unidentified protein.

**DISCUSSION**

**Position-dependent Regulation of Muscle-specific Genes**—In this paper, we show that the muscle-specific promoter of the human aldolase A gene is expressed both in a fiber type- and muscle location-dependent manner. Previous works from other groups have already shown that muscle-specific transgenes could be differentially expressed in diverse muscles depending on their position: MLC1f transgenes display a rostro-caudal gradient of expression together with a preferential activity in fast-twitch fibers (7, 19), but this pattern was not observed with the endogenous gene; whereas in our case, the pM310CAT transgene mimics endogenous promoter expression. In addition, we have identified the sequence (M1) that is responsible for the high level of pM activity in trunk and limb muscles. Recently, Hauschka and co-workers (17) have shown that a muscle creatine kinase transgene with all its E boxes mutated was expressed with a pattern very reminiscent of the pM pattern: higher expression in fast-twitch muscles than in soleus muscles, except for the tongue. Thus, activation of the muscle creatine kinase promoter depends on E boxes in tongue, heart, and, to a lesser extent, soleus muscles, whereas distinct elements control its activity in fast-twitch body muscles. It may be worth noting that the aldolase A gene is regulated in a similar but slightly different way: the gene is transcribed from two distinct promoters, one active in fast-twitch limb and trunk muscles (pM; this study) and another (pII) that functions predominantly in heart, soleus, and tongue muscles.

It appears from studies on chick embryos (31, 32) that head and neck muscle precursor cells come from unsegmented mesoderm and from the most rostral somites, whereas more caudal somites give rise to limb and trunk muscles. Furthermore, recent work by Buckingham and co-workers (33) demonstrated that myogenesis proceeds differently in somites that will give rise to body muscles and in head mesoderm that will make head muscles; mice lacking functional Myf-5 and Pax3 genes do not make body muscles, whereas they still form apparently normal head muscles. It is therefore tempting to establish a link between somitic origin and pM activity. Besides, in addition to a different embryologic origin, head muscles and particularly the tongue do not have the same physiological role as limb muscles. Since muscle activity is known to influence muscle gene expression (34, 35), pM body muscle-selective expression may also result from the contribution of extrinsic factors that could be regionally expressed or modulated by the physiological and contractile status of each muscle. Current work is in progress to distinguish between these two nonexclusive models.

**Nuclear Receptors and Muscle Location-dependent Expression**—We have identified M1 as a DNA-binding site required for a high level of activity of pM in trunk and limb muscles. With nuclear extracts from these muscles, the M1 site is recognized by nuclear receptors. While complexes formed with M1 in GMSA are similar with trunk and limb muscle nuclear extracts, the results are very different with tongue nuclear extracts; in this case, M1 is predominantly bound by complexes containing RAR/RXR heterodimers instead of ARP1, RXR, and probably other nuclear receptors. Thus, the M1 binding activity in muscle nuclear extracts changes in parallel with the transcriptional activation mediated through this sequence. The GMSA data suggest that ARP1 may be important for pM regulation as an activator since an ARP1-immunoreactive activity formed the main complexes detected in muscles in which M1 is important for pM expression. This observation may be surprising since ARP1 is known to behave mainly as an inhibitor of transcription, either as an active inhibiting factor (36, 37) or more frequently by competition with other nuclear receptors for a common DNA-binding site (27, 38–40), when tested in cotransfection studies. However, in other reports, it was demonstrated that ARP1 could be an activating transcription factor, depending on promoter context (41, 42), on exogenous factors (like dopamine or cAMP) (43), or on interaction with cofactors (44). Since complex A containing ARP1 activity formed with M1 did not migrate in GMSA like ARP1 ho-

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3 F. Spitz, unpublished observations.
modimers, it suggests that this complex is a heterodimer between ARP1 and another protein. One can propose that this ARP1 dimerization partner is a key element for enhancing pM activity; the observation that complex A was far more abundant in body skeletal muscle than in tongue muscles or non-muscle tissues suggests that this protein may be muscle-enriched. In chick primary myotubes, overexpression of ARP1 alone has no effect on pM310CAT activity (data not shown), suggesting that something (this unknown dimerization partner, activating exogenous signals, or a putative ligand) is missing in this cell system compared with the in vivo situation.

Alternatively, the absence of complex B and the presence of complex C in tongue compared with body muscles may provide another potential mechanism of location-dependent regulation for pM. Identification of proteins involved together with ARP1 in the formation of complex A or characterization of the RAR/RXR isoforms forming complexes B and C will be crucial to test functionally the role of these complexes in pM regulation.

Fiber- or Muscle Subtype-specific Regulatory Mechanisms—Previous works had identified MEP3 and NF-1 DNA-binding sites as key elements for pM activity in hind limb muscles (16, 21). From the present work, the M1 site appears to be essential to stimulate transcription from pM in fast-twitch trunk or limb muscles. We also show that the mutation of M1 alters pM fiber specificity, which makes M1 one of the few identified cis-acting elements that may be involved in muscle fiber-specific expression. In previous in vivo footprinting experiments, dimethyl sulfate accessibility to M1 was shown to be different in soleus and gastrocnemius muscles (21). However, GMSA did not reveal any difference for M1-bound proteins in slow- and fast-twitch muscle nuclear extracts. In addition, it should be emphasized that fiber specificity is not suppressed by the mutation of M1, suggesting that additional fiber-specific elements are present elsewhere in the 310-base pair enhancer and that these regulatory mechanisms may enable a fine-tuning of the level of gene expression, muscle by muscle, as well as a differential adaptation of distinct muscles to modifications of humoral, neural, and contractile status.

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