Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 299 (2002) 7–13
BBRC
www.academicpress.com

A fast fiber enhancer exists in the muscle regulatory factor 4 gene promoter

Christopher L. Pin[a] and Stephen F. Konieczny[b,*]

[a] *Departments of Paediatrics and Physiology and Pharmacology, Child Health Research Institute, University of Western Ontario, London, Ont., Canada N6C 2V5*
[b] *Department of Biological Sciences, Purdue University, West Lafayette, IN 47907-1392, USA*

Received 20 September 2002

Abstract

The development of skeletal muscle is a highly regulated process governed by the four myogenic regulatory factors (MRFs) MyoD, myf-5, myogenin, and MRF4. While these factors exhibit some unique functions, part of their individual activity can be attributed to different temporal and spatial expression patterns. To delineate the factors that control expression of the MRFs, we have begun a molecular dissection of the *MRF4* gene promoter. Through the generation of promoter/reporter gene constructs, we show that an 853 bp fragment, residing 4 kb upstream of the *MRF4* transcriptional start site (853AV), is able to enhance expression of the basal *MRF4* promoter 3–4-fold in myogenic cell cultures. Analysis of the 853AV enhancer in transgenic mice indicates that this region drives *MRF4* gene expression primarily in fast muscle fibers, suggesting that the normal adult *MRF4* expression pattern is regulated by a variety of control elements that may dictate fiber-type specificity.

Keywords: MyoD; Myf-5; Myogenin; MRF4; Transcription; Myogenesis

Skeletal muscle development is a well-coordinated event that requires the precise orchestration of the basic helix-loop-helix myogenic regulatory factor (MRF) gene family, which includes the four members *MyoD*, *myf-5*, *myogenin*, and *MRF4* [1]. Each MRF gene exhibits a specific temporal and spatial expression pattern that suggests that these proteins perform unique functions [2]. Studies in which individual MRF genes have been ablated in mice have supported the hypothesis that each MRF protein is important in development since individual MRF null animals produce subtle to severe skeletal muscle defects. For instance, loss of *myogenin* expression results in an almost complete absence of myoblast fusion [3], while the effects of losing *MyoD* are only observed when the regenerative ability of skeletal muscle is challenged [4]. Similarly, animals lacking both *MyoD* and *myf-5* fail to generate myogenic stem cells and die shortly after birth [5]. Even though these studies support a unique biological role for each of these proteins, recent evidence now suggests that the different expression patterns associated with the MRF genes underlie part of the differences observed in the individual MRF null animals. For instance, targeted insertion of the *MRF4* coding region into the *myogenin* locus results in restoration of early myoblast fusion, confirming that the *MRF4* protein can compensate for loss of the *myogenin* protein if expressed at the correct time and embryonic location [6]. This suggests that a higher level of control, upstream of MRF protein activity, is necessary for appropriate muscle development. Identifying the factors that regulate the individual MRF genes is crucial to fully understanding the complex processes associated with skeletal muscle differentiation.

One experimental design for establishing the genetic hierarchy of cell differentiation involves identifying regulatory elements in the promoters and enhancers of genes known to be involved in individual developmental processes. Such analyses have led to the precise identification of the regulatory elements necessary for both *myogenin* [7] and *MyoD* [8] gene expression. Transgenic mice in which various promoter elements have been used

* Corresponding author. Fax: 1-765-496-2536.
E-mail address: sfk@bilbo.bio.purdue.edu (S.F. Konieczny).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S0006-291X(02)02571-8

to drive expression of a bacterial *LacZ* reporter gene have allowed investigators to identify both temporally and spatially specific regulatory elements in the promoters of these genes. Similarly, analysis of the *troponin I* gene has suggested the existence of fiber-type specific elements and allowed for the potential elucidation of factors that lead to specific muscle gene expression [9,10]. These results highlight the importance for further delineating gene regulatory regions in order to advance our understanding of the hierarchy of key transcription factors, such as the MRFs, that lead to complete muscle differentiation.

While both *myogenin* and *MyoD* gene promoters have been well characterized, very little is known about the *MRF4* promoter. This is due, in part, to the close association of *MRF4* with the *myf-5* locus. Approximately 11 kb of DNA sequence separates these two genes and promoter elements for *myf-5* have been found within the *MRF4* exonic regions [11]. Recent evidence from Rigby and colleagues suggests that the expression of the *myf-5* and *MRF4* loci is controlled by unique regulatory elements, and that at least four separate elements are necessary for complete *MRF4* expression [12]. Previous work from our laboratory has also alluded to at least four specific promoter regions controlling thoracic and non-thoracic somite expression, adult expression and basal levels of expression, for the *MRF4* gene [13].

Since the *MRF4* gene is expressed to high levels in the adult animal, and appears early in the process of muscle regeneration [14], it is important to understand how this key MRF gene is regulated. In this study we have characterized a potential *MRF4* regulatory enhancer that exists 4 kb upstream of the transcriptional start site. Previous studies have shown that this 5′ enhancer is capable of driving muscle-specific gene expression when tested in C2C12 myotube cultures [15]. In this study, we have extended these results and now show that the 5′ *MRF4* enhancer is capable of conferring specific expression of a *LacZ* reporter gene in adult mice in a fiber-type specific manner. The identification of such a regulatory element strongly supports the existence of fiber-type specific transcription factors and further documents the overall complexity associated with muscle gene regulation.

Materials and methods

Generation of promoter/reporter gene constructs. The 853AV *MRF4* enhancer has been previously described [15]. Constructs were generated with the 853AV region placed upstream of a *LacZ* reporter gene (encoding a nuclear localized β-galactosidase protein) either on its own (853-*LacZ*) or in combination with the basal *MRF4* promoter 853-(M + D + E+)-*LacZ*. Mutated constructs were generated by placing the 853AV enhancer upstream of the basal M + D + E+ promoter with mutations in either the MEF2 site (M − D + E+) or with combination mutations in the MEF2 site, TATA-box, and E-box (M − D − E) sites. In addition, sub-regions of the 853AV enhancer were generated by PCR, followed by cloning of individual fragments upstream of the M + D + E+ promoter. Primers specific for the 5′ portion of 853AV (5′-TCTCAGTGTGTAAGTGC-3′ and 5′-GTGAGTGTAGGAAGAA GT-3′) were used to amplify a region containing a consensus MEF2 site (ΔA), while primers specific for the 3′ portion of 853AV (5′-ACTTCTTCCTACACTCAC-3′ and 5′-AAATAATATCAGCACAT G-3′) were used to generate a section of the enhancer lacking the MEF2 site (ΔB).

Transfection and in vitro reporter gene assays. Primary chick myoblast cultures and standard C3H10T1/2 fibroblast cultures were maintained as previously described [13,16]. Cells were transfected using standard calcium phosphate precipitation. Each transfection consisted of 5 μg of a single *LacZ* reporter gene construct plus 0.1 μg of a CMV-Luc plasmid that served as an internal transfection efficiency control. Following transfection, cells were harvested for protein extracts and quantified for luciferase and β-galactosidase (β-gal) activity as described in [16]. A minimum of three independent transfections were performed for each experimental group.

Generation of transgenic mice. The 853-(M + D + E+)-*LacZ* construct was introduced by pronuclear injection as described in [13]. Founder mice were identified through PCR amplification of the *LacZ* gene and gene copy number was determined by standard Southern blot hybridization as previously described [17]. Founder mice were mated to C57/B6 inbred mice to generate the F1 generation.

Whole mount staining, histochemistry, and immunohistochemistry. Embryonic mice were obtained from pregnant transgenic females at embryonic (E) day E12.5, E14.5, and E16.5. Adult mice were sacrificed at 2–3 months of age. Whole mount β-gal histochemistry was performed as described by Pin et al. [13]. Sections (6 μm) from adult hind limb muscles were prepared using a Zeiss cryotome and stained for β-gal expression as described in [18]. The specificity of the antibodies used and the characterization of individual muscle fiber-types are described in [19]. The monoclonal antibodies used in this study are specific for slow (10D10), fast IIA (4A.74), and fast IIB/IIX (212F) myosin heavy chain isoforms.

In vivo quantification of LacZ expression. Protein extracts were obtained from the soleus (predominantly slow) and tibialis anterior (predominantly fast) hind limb muscles and processed for protein extracts as described in [18]. Protein extracts were quantified using a Bradford protein assay kit and 25 μg protein extract was used to determine β-gal expression as described above.

Results

Our previous studies on the *MRF4* promoter suggested that several different regulatory regions exist to control the complete spatial and temporal expression pattern for this gene [13]. One key region is located approximately 4 kb upstream of the *MRF4* transcription start site. This region, designated as 853AV, has been shown to function as an enhancer for the *MRF4* basal promoter (M + D + E+) when tested in myogenic cell lines [15]. In order to further characterize this putative enhancer, transient DNA transfections of primary chick myoblast cultures were performed with a variety of different enhancer/promoter gene constructs. Initial experiments confirmed previously published reports that the full length −8500 bp *MRF4* promoter is only moderately more active in myogenic cells when compared to the basal −336 bp *MRF4* promoter (Fig. 1). Analysis of

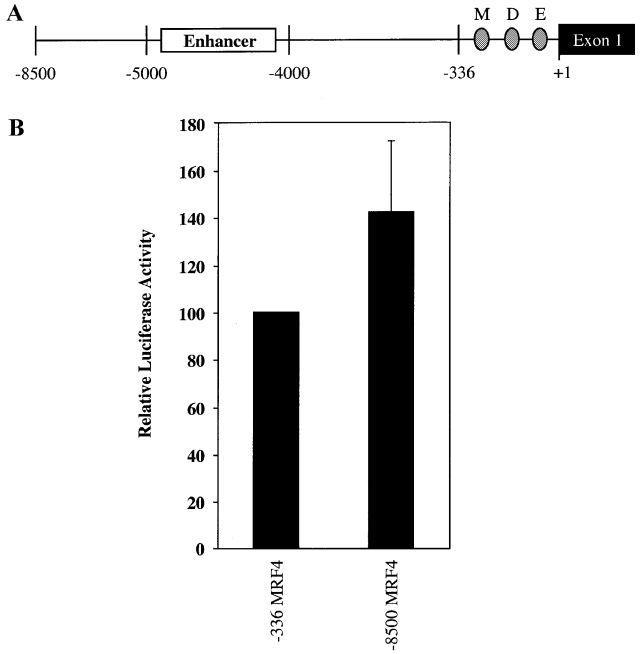

Fig. 1. Structure of the *MRF4* promoter region. (A) Schematic of the full-length *MRF4* promoter showing the basal promoter (−336) containing the MEF2 (M), TATA (D), and E-box (E) sites. The 853AV enhancer lies between −5000 and −4000 from the transcription start site. (B) The entire promoter fragment (−8500 *MRF4*) produces moderately higher gene expression in muscle cells when compared to the M + D + E+ basal promoter.

the *MRF4* basal promoter has revealed three essential elements necessary for correct expression [16]. These include a MEF2 site (M), a TATA-box (D), and an E-box (E) site. Each of these elements is essential to drive normal *MRF4* gene expression. For example, mutation of the MEF2 site (M − D + E+), or mutation of the MEF2, TATA, and E-box sites (M − D − E−), results in a 75–90% reduction in transcriptional activity associated with the basal *MRF4* promoter (Figs. 2A and B).

In order to further test the 853AV enhancer region, we generated additional gene constructs in which the enhancer was placed upstream of the wild type *MRF4* basal promoter [853-(M + D + E+)-*LacZ*] or upstream of mutated *MRF4* basal promoters (Fig. 2A). As shown in Fig. 2B, the 853-(M + D + E+)-*LacZ* gene exhibits a 3.5-fold enhancement over the (M + D + E+)-*LacZ* gene, supporting the role of this region to enhance *MRF4* promoter activity. Interestingly, removal of the MEF2 site (M) from the basal promoter [853-(M − D + E+)-*LacZ*] lowers the enhancer-driven activity of this reporter gene by 40% (Fig. 2B). However, the activity of this enhancer construct remains approximately 10-fold higher when compared to a mutated MEF2 basal promoter construct (M − D + E+-*LacZ*), suggesting that the 853AV enhancer overcomes the loss of the basal promoter MEF2 site. As predicted, mutation of the E-box and TATA-box elements (M − D − E−) completely abolishes the ability of the 853AV region to enhance *MRF4* promoter activity. Further characterization of the enhancer demonstrated that it is active only when positioned near a functional promoter. The enhancer alone (853-*LacZ*) does not support transcriptional activation of a *LacZ* reporter gene (Fig. 3A).

Although it is clear that the 853AV region is capable of enhancing the transcriptional activity of the *MRF4*

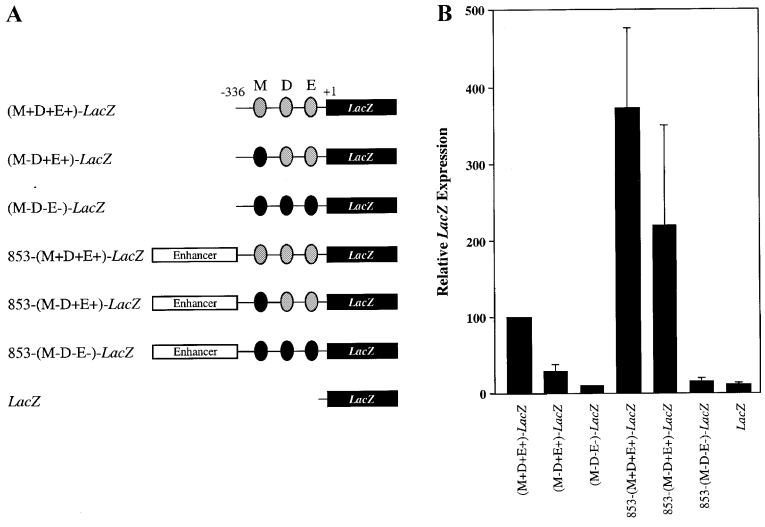

Fig. 2. The 853AV region enhances expression of the (M + D + E+) basal *MRF4* promoter. (A) Promoter/enhancer constructs used in transient DNA transfection assays. Light gray ovals indicate that the sites have been maintained, while the dark gray ovals indicate that the sites have undergone targeted point mutations. Each construct was tested in the presence or absence of the 853AV enhancer. The empty vector (*LacZ*) contains the *LacZ* gene without any promoter elements. (B) Transient DNA transfections of the constructs from (A) into chick primary myoblast cultures. All values are given as a percentage of the (M + D + E)-*LacZ* reporter gene. The 853AV region enhances expression in the presence or absence of a functional MEF2 site, but not in the combined absence of all three regulatory regions.

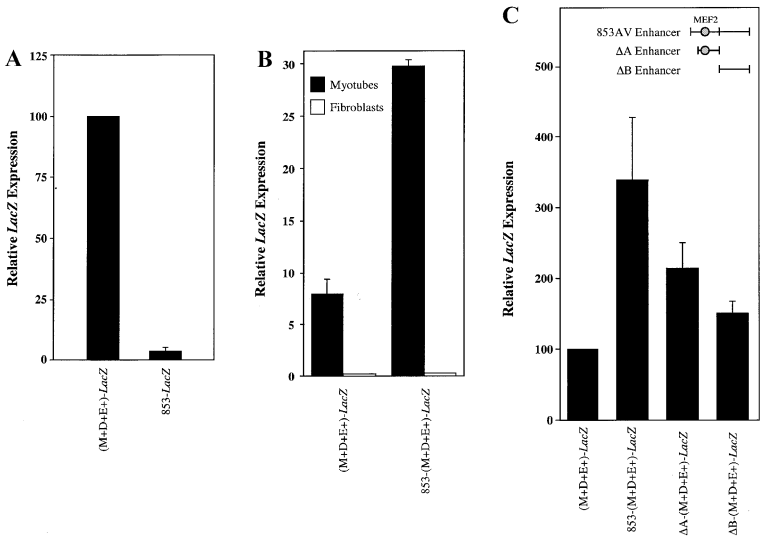

Fig. 3. Further characterization of the 853AV enhancer region. (A) The 853AV enhancer does not direct reporter gene expression in the absence of the (M + D + E+) *MRF4* basal promoter. Expression of *LacZ* from a construct containing only the 853AV enhancer remains at background levels, while the (M + D + E+)-*LacZ* gene shows significant expression in muscle cells. (B) The 853AV region functions as an enhancer only in myotubes (black bars). No expression is observed in non-muscle cells (white bars). (C) Deletions of different regions of the 853AV enhancer reveal the importance of regulatory elements within the 5′ portion of the enhancer region which contains a single MEF2 binding site. While not maintaining the same level of expression as the full-length 853AV region, the 5′ portion of the enhancer (ΔA) increases reporter gene expression when compared to the (M + D + E+)-*LacZ* reporter gene. The 3′ portion of the enhancer (ΔB) also appears to retain modest enhancer activity over the M + D + E+ promoter, but not to the same level as constructs containing the MEF2 site.

gene, we wished to determine if this enhancer activity is skeletal muscle specific. Therefore, we individually tested the basal promoter (M + D + E+)-*LacZ* gene construct and the basal promoter construct containing the enhancer [853-(M + D + E+)-*LacZ*] in myotube and fibroblast cultures. As shown in Fig. 3B, the 853AV enhancer produces a 3.5-fold increase in reporter gene expression in differentiated muscle cells, but this enhancer is completely inactive in fibroblasts, suggesting that transcriptional regulators that likely bind to DNA target sequences within the enhancer function, or are present, only in terminally differentiated muscle cells.

To further determine the elements responsible for the enhancement activity of 853AV, two individual truncations of the enhancer were produced and tested with the *MRF4* basal promoter. These constructs were designed to maintain (ΔA) or to delete (ΔB) a region of the enhancer containing a consensus MEF2 binding element (Fig. 3C). Transient DNA transfections into primary chick myotube cultures revealed that more than a single element within the enhancer is necessary to obtain full enhancer activity (Fig. 3C). Constructs containing the MEF2 site [ΔA-(M + D + E+)-*LacZ*] exhibit expression levels only slightly lower than the full-length enhancer. However, this MEF2 site is not solely responsible for enhancer activity since constructs lacking this key muscle transcription factor binding site [B-(M + D + E+)-*LacZ*] still maintain moderate enhancer activity over the (M + D + E+)-*LacZ* gene.

The *MRF4* gene exhibits a biphasic pattern of gene expression during skeletal muscle development. The initial expression of *MRF4* is found within the somites at E10.5 [2]. This expression is transient, however, and beginning at E14.5, a second wave of *MRF4* gene expression occurs in the body musculature. Previous studies from our laboratory have indicated that 8500 bp of the *MRF4* 5′ flanking sequence is responsible for at least part of the somitic and all of the normal adult *MRF4* gene expression. On its own, the M + D + E+ basal promoter does not produce detectable expression in transgenic mice, even though it exhibits muscle-specific activity in muscle cell cultures (16 and above). In order to determine if the 853AV enhancer supports transcriptional activity in vivo, we generated transgenic mice using the 853-(M + D + E+)-*LacZ* gene construct. Four lines of mice were generated containing the enhancer/promoter elements and characterized for β-gal expression. Analysis of the 853-(M + D + E+)-*LacZ* mice at E11.5 revealed no detectable *LacZ* expression (data not shown). However, beginning at E14.5, β-gal activity was observed in the limb and trunk musculature. By E16.5, animals exhibited extensive staining throughout all muscles examined (Figs. 4A and B). In comparison, transgenic animals containing only the (M + D + E+)-*LacZ* basal promoter construct showed no expression at any time point (Fig. 4C).

While all muscles examined in the 853-(M + D + E+)-*LacZ* mice were positive for *LacZ* expression in adult animals, a variable staining pattern was observed

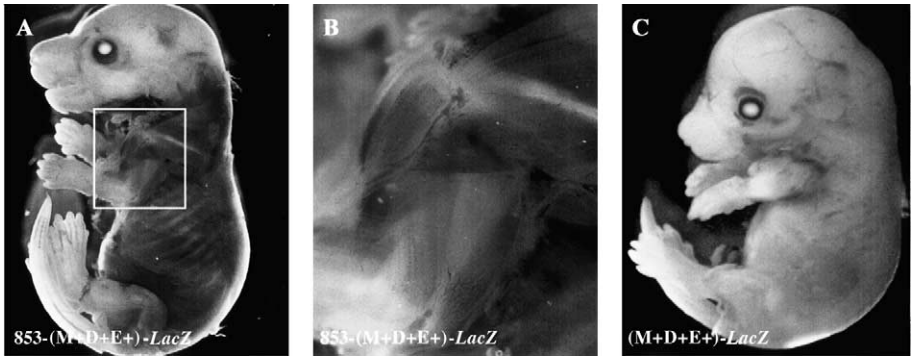

Fig. 4. Transgenic mice containing the 853-(M + D + E+)-*LacZ* gene generate late myogenic specific expression. (A) Addition of the 853AV region [853-(M + D + E+)-*LacZ*] to the *MRF4* basal promoter leads to enhanced in vivo expression of the reporter gene in E14.5 embryos. This expression is specific to skeletal muscle. (B) The boxed region in (A) has been magnified to reveal the specific expression pattern obtained from the 853-(M + D + E+)-*LacZ* gene. Analysis of earlier developmental time points failed to reveal any somitic staining (data not shown). (C) Mice containing only the (M + D + E+)-*LacZ* gene do not show any transgene expression.

throughout individual muscles (data not shown). To examine this phenomenon more closely, frozen sections from the tibialis anterior (TA) muscle were prepared and stained for β-gal activity using X-gal histochemistry. As shown in Fig. 5A, nuclear localized β-gal is restricted to individual nuclei within the fibers. Analysis of these sections revealed that the more superficial muscle fibers stained at a much higher rate than deeper muscle fibers. This staining pattern suggests that expression may be limited to fast fibers since for the TA muscle the deeper regions are typically slower in contraction speed [20]. To support this hypothesis, serial cross-sections were stained for fast IIA, fast IIB/IIX or slow (I) myosin heavy chain isoforms (Figs. 5B–D). As expected, the fastest fibers (IIB/IIX) are localized to the more superficial region of the TA muscle and correspond to the observed *LacZ* expression pattern (compare Figs. 5A and C). Individual β-gal positive fibers in the deeper regions of the TA correspond to individual fast fibers and not to slow muscle fibers (compare Figs. 5A and D). To support this argument, muscle protein extracts were isolated from the TA muscle (fast) and from soleus

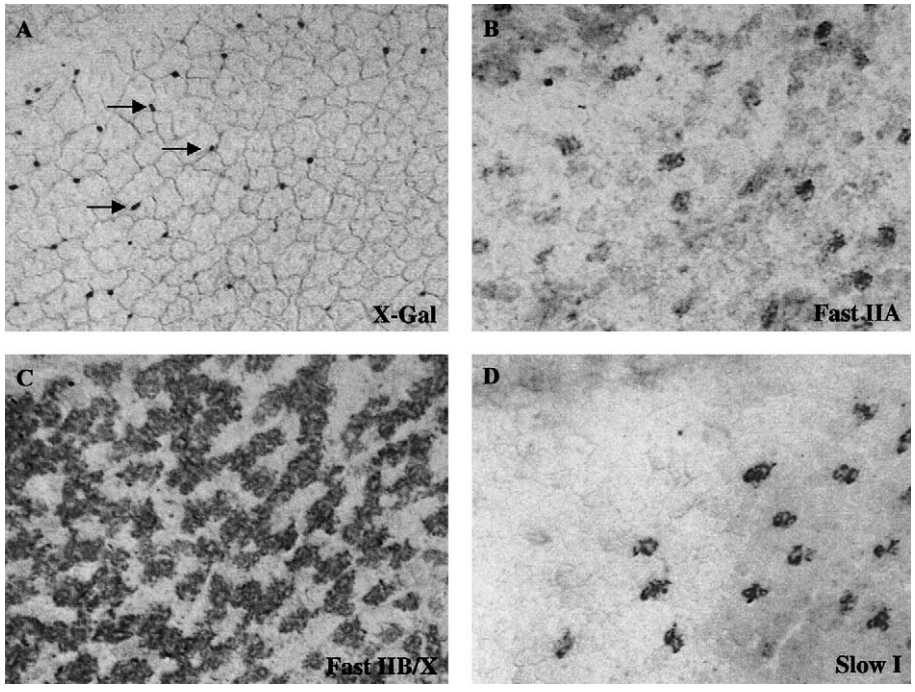

Fig. 5. Expression of the 853-(M + D + E+)-*LacZ* gene is limited to fast muscle fibers. (A) X-gal analysis of muscle sections reveals non-uniform expression of the 853-(M + D + E+)-*LacZ* reporter gene. Fibers to the superficial area of the tibialis anterior stain more readily than fibers to the superficial aspect of the muscle. Immunohistochemical analysis of adjacent serial sections for fast IIA (B), fast IIB/X (C), and slow I (D) myosin heavy chain isoforms indicates that the X-gal positive fibers are preferentially IIB/IIX. Arrows in (A) indicate X-gal positive nuclei.

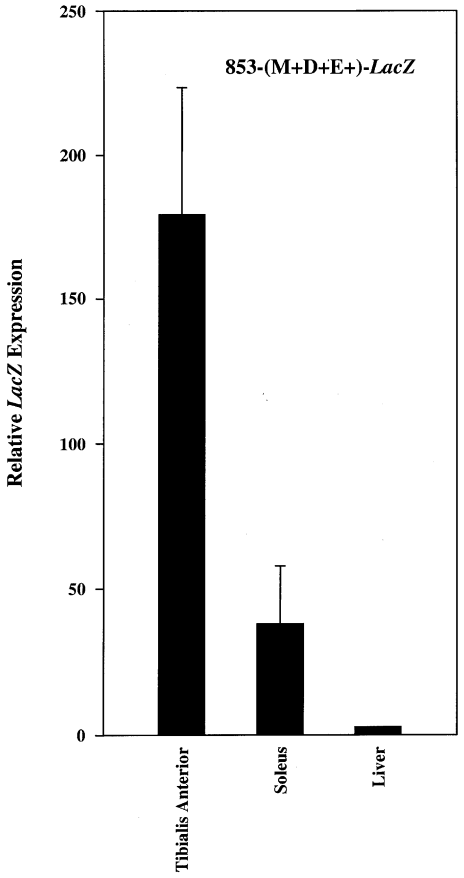

Fig. 6. The 853-(M + D + E+)-*LacZ* reporter gene exhibits a fast muscle restricted expression pattern. Quantitative X-gal analysis of protein extracts from the tibialis anterior (fast) and soleus (slow) muscles of transgenic animals reveals that β-gal expression is predominantly restricted to the fast tibialis anterior muscle. No expression is detected in non-muscle tissues such as liver.

muscle (slow) and were analyzed quantitatively for β-gal activity. As shown in Fig. 6, the TA muscle from transgenic animals expressed levels of β-gal that were 7-fold higher than observed in extracts from the soleus muscle. As expected, no β-gal activity was observed in non-muscle tissues such as liver. These results support the theory that the 853AV enhancer confers a predominantly fast fiber-type specific expression pattern on the *MRF4* gene.

Discussion

The unique spatial and temporal expression patterns attributed to the four myogenic regulatory factors play critical roles in the generation of myogenic cell lineages and in the maturation of adult skeletal muscle. Although each MRF protein has been shown to exhibit distinct activities, many of the differences attributed to each MRF are due to subtle differences in their expression patterns. For example, targeted ablation of the *myogenin* gene results in severe impairment of myoblast fusion [3]. However, this defect can be corrected by placing the *MRF4* coding region within the *myogenin* locus, thereby substituting one MRF protein for another [6]. While this observation suggests that the MRF proteins serve a redundant function, there remains additional evidence that supports subtle differences in their ability to promote muscle differentiation and regeneration [4]. Nonetheless, an important goal of muscle developmental biologists is to identify the molecular mechanisms that control the precise expression of each MRF gene since the timing and location of MRF gene expression is crucial to myogenesis.

Our previous work has characterized the *MRF4* gene promoter and has led to the identification of at least four regions that are necessary for full *MRF4* gene expression [13]. The first region is required for complete somitic expression and is located at least 8500 bp upstream of the *MRF4* transcriptional start site. A second region exists between −8500 and −6500 and confers thoracic somite specific expression. At least one other region located between −6500 and −336 is necessary for adult muscle expression while the region located within −336 and +72 is necessary for basal levels of gene expression. In this report, we have further characterized the promoter region and have identified at least one additional element that is necessary for adult *MRF4* gene expression. The characterization of the 853AV enhancer indicates that it confers fast fiber expression, suggesting that additional elements likely exist and are necessary for full adult *MRF4* transcriptional activity.

Sequence analysis of the 853AV enhancer reveals that it contains several E-box sites and one potential MEF2 site that may serve as a DNA binding site for muscle-specific MEF2 transcription factors [21]. While we have not directly tested the importance of the 853AV enhancer MEF2 site, addition of the enhancer to the *MRF4* basal promoter in which the proximal MEF2 site was destroyed restores most of the transcriptional activity. This preliminary result suggests that the MEF2 site within the 853AV enhancer has a critical role in controlling expression of the *MRF4* gene. Future studies will be aimed at determining the importance of individual MEF2 and E-box sites in regulating *MRF4* gene expression.

Fiber-type specificity of muscle gene expression patterns is a well-known characteristic of the myogenic program. For instance, studies on the *troponin I* (*TnI*) gene promoter have identified unique regulatory elements that drive fiber specific gene expression when tested in transgenic mice [22]. Analysis of the *MRF4* 853AV enhancer failed to reveal any sequence similarity with the SURE and FIRE elements previously described for *TnI* gene regulation [23]. The existence of a fiber-type specific regulatory element within the *MRF4* promoter is not surprising given that *MRF4* transcripts preferen-

tially accumulate to higher levels in slow muscle fibers [24]. What is surprising is our identification of a fast muscle-specific enhancer within the *MRF4* promoter. These results serve to illustrate the complexity of regulatory elements that control expression of the muscle regulatory factor genes in skeletal muscle tissue and underscore the importance for further characterization of these genes. Understanding the details on how each MRF gene is transcriptionally controlled will be essential to fully characterizing the molecular pathways that are involved in generating the myogenic lineage.

Acknowledgments

Transgenic mice described in this work were produced by the Purdue Cancer Center Transgenic Mouse Core Facility. This work was supported by a grant to S.F.K. from the National Institutes of Health (AR41115) and a postdoctoral fellowship to C.L.P. from the Muscular Dystrophy Association of Canada and Canadian Institutes of Health Research.

References

[1] L.A. Sabourin, M.A. Rudnicki, The molecular regulation of myogenesis, Clin. Genet. 57 (2000) 16–25.
[2] T.H. Smith, N.E. Block, S.J. Rhodes, S.F. Konieczny, J.B. Miller, A unique pattern of expression of the four muscle regulatory factor proteins distinguishes somitic from embryonic, fetal and newborn mouse myogenic cells, Development 117 (1993) 1125–1133.
[3] Y. Nabeshima, K. Hanaoka, M. Hayasaka, E. Esumi, S. Li, I. Nonaka, Y. Nabeshima, Myogenin gene disruption results in perinatal lethality because of severe muscle defect, Nature 364 (1993) 532–535.
[4] L.A. Megeney, B. Kablar, K. Garrett, J.E. Anderson, M.A. Rudnicki, MyoD is required for myogenic stem cell function in adult skeletal muscle, Genes Dev. 10 (1996) 1173–1183.
[5] M.A. Rudnicki, P.N. Schnegelsberg, R.H. Stead, T. Braun, H.H. Arnold, R. Jaenisch, MyoD or Myf-5 is required for the formation of skeletal muscle, Cell 75 (1993) 1351–1359.
[6] Z. Zhu, J.B. Miller, MRF4 can substitute for myogenin during early stages of myogenesis, Dev. Dyn. 209 (1997) 233–241.
[7] T.C. Cheng, M.C. Wallace, J.P. Merlie, E.N. Olson, Separable regulatory elements governing myogenin transcription in mouse embryogenesis, Science 261 (1993) 215–218.
[8] J.C. Chen, C.M. Love, D.J. Goldhamer, Two upstream enhancers collaborate to regulate the spatial patterning and timing of MyoD transcription during mouse development, Dev. Dyn. 221 (2001) 274–288.
[9] S. Calvo, P. Venepally, J. Cheng, A. Buonanno, Fiber-type-specific transcription of the troponin I slow gene is regulated by multiple elements, Mol. Cell. Biol. 19 (1999) 515–525.
[10] J.V. O'Mahoney, K.L. Guven, J. Lin, J.E. Joya, C.S. Robinson, R.P. Wade, E.C. Hardeman, Identification of a novel slow-muscle-fiber enhancer binding protein, MusTRD1, Mol. Cell. Biol. 18 (1998) 6641–6652.
[11] J.K. Yoon, E.N. Olson, H.H. Arnold, B.J. Wold, Different MRF4 knockout alleles differentially disrupt Myf-5 expression: *cis*-regulatory interactions at the MRF4/Myf-5 locus, Dev. Biol. 188 (1997) 349–362.
[12] J.J. Carvajal, D. Cox, D. Summerbell, P.W. Rigby, A BAC transgenic analysis of the Mrf4/Myf5 locus reveals interdigitated elements that control activation and maintenance of gene expression during muscle development, Development 128 (2001) 1857–1868.
[13] C.L. Pin, D.C. Ludolph, S.T. Cooper, B.J. Klocke, J.P. Merlie, S.F. Konieczny, Distal regulatory elements control MRF4 gene expression in early and late myogenic cell populations, Dev. Dyn. 208 (1997) 299–312.
[14] Z. Zhou, A. Bornemann, MRF4 protein expression in regenerating rat muscle, J. Muscle Res. Cell Motil. 22 (2001) 311–316.
[15] C.M. Kerkvliet, T.J. Hinterberger, Distal regulatory regions of the rat MRF4 gene, Biochem. Biophys. Res. Commun. 237 (1997) 170–176.
[16] P.S. Naidu, D.C. Ludolph, R.Q. To, T.J. Hinterberger, S.F. Konieczny, Myogenin and MEF2 function synergistically to activate the MRF4 promoter during myogenesis, Mol. Cell. Biol. 15 (1995) 2707–2718.
[17] C.L. Pin, J.M. Rukstalis, C.L. Johnson, S.F. Konieczny, The bHLH transcription factor Mist1 is required to maintain exocrine pancreas cell organization and acinar cell identity, J. Cell. Biol. 155 (2001) 519–530.
[18] C.L. Pin, A.C. Bonvissuto, S.F. Konieczny, Mist1 expression is a common link among serous exocrine cells exhibiting regulated exocytosis, Anat. Rec. 259 (2000) 157–167.
[19] C.L. Pin, P.A. Merrifield, Developmental potential of rat L6 myoblasts in vivo following injection into regenerating muscles, Dev. Biol. 188 (1997) 147–166.
[20] R.B. Armstrong, R.O. Phelps, Muscle fiber type composition of the rat hindlimb, Am. J. Anat. 171 (1984) 259–272.
[21] B.L. Black, E.N. Olson, Transcriptional control of muscle development by myocyte enhancer factor-2 (MEF2) proteins, Annu. Rev. Cell Dev. Biol. 14 (1998) 167–196.
[22] S. Banerjee-Basu, A. Buonanno, *Cis*-acting sequences of the rat troponin I slow gene confer tissue- and development-specific transcription in cultured muscle cells as well as fiber type specificity in transgenic mice, Mol. Cell. Biol. 13 (1993) 7019–7028.
[23] M. Nakayama, J. Stauffer, J. Cheng, S. Banerjee-Basu, E. Wawrousek, A. Buonanno, Common core sequences are found in skeletal muscle, Mol. Cell. Biol. 16 (1996) 2408–2417.
[24] E.H. Walters, N.C. Stickland, P.T. Loughna, MRF-4 exhibits a fiber type- and muscle-specific pattern of expression in postnatal rat muscle, Am. J. Physiol. Regul. Integr. Comp. Physiol. 278 (2000) R1381–R1384.