Complex fiber-type-specific expression of fast skeletal muscle troponin I gene constructs in transgenic mice

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SUMMARY

We analyzed, in transgenic mice, the cellular expression pattern of the quail fast skeletal muscle troponin I (TnIfast) gene and of a chimeric reporter construct in which quail TnIfast DNA sequences drive expression of E. coli beta-galactosidase (β-gal). Both constructs were actively expressed in skeletal muscle and specifically in fast, as opposed to slow, muscle fibers. Unexpectedly, both constructs showed a marked differential expression among the adult fast fiber subtypes according to the pattern IIb>IIx>IIa. This expression pattern was consistent in multiple lines and differed from the endogenous mouse TnIfast pattern, which shows approximately equal expression in all fast fibers. These observations indicate that distinct regulatory mechanisms contribute to high-level expression of TnIfast in the various fast fiber subtypes and suggest that the outwardly simple pattern of equal expression in all fast fiber types shown by the endogenous mouse TnIfast gene is based on an intricate system of counterbalancing mechanisms. The adult expression pattern of the TnIfast/β-gal construct emerged in a two-stage developmental process. Differential expression in fast versus slow fibers was evident in neonatal animals, although expression in fast fibers was relatively weak and homogeneous. During the first two weeks of postnatal life, expression in maturing IIb fibers was greatly increased whereas that in IIa/IIx fibers remained weak, giving rise to marked differential expression among fast fiber types. Thus at least two serially acting (pre- and post-natal) fiber-type-specific regulatory mechanisms contribute to high-level gene expression in adult fast muscle fibers. Unexpected similarities between TnIfast transgene expression and that of the myosin heavy chain gene family (which includes differentially expressed IIb-, IIx- and IIa-specific members) suggest that similar mechanisms may regulate adult fast muscle gene expression in a variety of unrelated muscle gene families.

Key words: muscle gene regulation, transgenic mice, skeletal muscle fiber type, troponin I, β-galactosidase

INTRODUCTION

Skeletal muscle fibers fall into several biochemically and physiologically specialized classes termed fiber types whose specific properties result, in part, from the differential expression of muscle protein isoforms encoded by multigene families (reviewed in Burke, 1986; Gauthier, 1986; Emerson and Bernstein, 1987; Buckingham, 1989; Wade and Kedes, 1989; Pette and Staron, 1990; Gunning and Hardeman, 1991; Bandman, 1992). Different fiber types may arise from distinct precommitted myoblast lineages that differ in their muscle gene expression potentials (Stockdale, 1992). However, fiber-type-specific gene expression in adult muscle is plastic and can be altered by changes in external factors, notably innervation/electrical stimulation and hormonal milieu (Jolesz and Sreter, 1981; Pette and Vrbova, 1985; Izumo et al., 1986; Ausoni et al., 1990). The major fiber types in mammalian skeletal muscle include one slow (type I) and three fast types (IIA, IIB and IIx, also called IID (Schiavino et al., 1989; Termin et al., 1989)). Each contains a different myosin heavy chain (MHC) isoform encoded by a distinct gene (Weydert et al., 1985; Mahdavi et al., 1986, 1987; Yoon et al., 1992; Soussi-Yanicostas et al., 1993). Other muscle protein gene families appear to be less complex than that encoding MHC. In the case of the troponin subunits, myosin light chains, α-tropomyosin and sarcoplasmic reticulum Ca2+-pump, two principal genes/isoforms are expressed in skeletal muscle; one in slow fibers and another that is known, or assumed, to be expressed similarly in all fast fibers (reviewed in Pette and Staron, 1990). An additional level of complexity peculiar to the MHC gene family is the existence of embryonic and perinatal isoforms that are absent in almost all adult muscle tissues (Whalen et al., 1981; Periasamy et al., 1984; Weydert et al., 1987).

Considerable progress has recently been made in the identification of cis-regulatory elements and trans-acting factors that direct the transcriptional activation of muscle genes during the differentiation of cultured embryonic myoblasts and myogenic cell lines (reviewed in Sartorelli et al., 1993).
However, because muscle cell cultures do not generate adult-type fibers, the characterization of gene regulatory mechanisms operating in adult muscle, including fiber-type-specific gene expression, must be addressed by in vivo approaches such as the transgenic mouse.

We have previously reported that, in transgenic mice carrying a quail gene encoding the fast skeletal muscle isoform of troponin I (TnIfast), quail TnIfast mRNA was produced efficiently and specifically in skeletal muscle and was considerably more abundant in fast- than in slow-fiber-enriched muscles (Hallauer et al., 1988). We now report the production of transgenic mice carrying a chimeric construct in which the 5′-half of the quail TnIfast gene (coding only for 5′-untranslated mRNA sequence) drives expression of a beta-galactosidase (β-gal) reporter gene. Histochemical analysis at the cellular level showed that β-gal expression is higher in fast than in slow fibers. This result locates fast fiber-specific cis-elements to the 5′-half (or immediately upstream) of the quail TnIfast gene and suggests that the fast fiber specific regulatory mechanisms probably operate at the transcriptional level.

In addition, we report an unexpected feature of the β-gal transgene expression pattern, i.e., a marked quantitative differential expression among the various fast fiber types in the pattern IIB>IIX>IIA. Re-examination, by in situ hybridization, of the original transgenic lines carrying the intact quail TnIfast transgene revealed that quail TnIfast mRNA is also differentially expressed in the same pattern. In contrast, endogenous mouse TnIfast mRNA is similarly abundant in all fast fibers. These findings reveal the existence of unanticipated differences in the transcriptional biology of the various fast fiber types. We speculate that the quail TnIfast constructs may lack functional cis-elements required for high-level expression in IIA/IIX fibers, but not in IIB fibers. A further implication is that the outwardly simple expression pattern of the endogenous mouse TnIfast gene and, perhaps, the fast isoform genes in other simple muscle gene families may in fact rest on a complex set of transcriptional mechanisms that compensate for fundamental differences among the IIA, IIB and IIX fast fiber types.

The TnIfast transgene overall differential expression pattern emerged in two developmental stages strikingly similar in general timing and character to two phases in the developmental expression pattern of the MHC gene family. These results identify two serially acting fiber-type-specific mechanisms contributing to high-level gene expression in adult fast muscle fibers. They also suggest that common or related mechanisms may regulate the fiber-type-specific expression of muscle gene families as outwardly dissimilar as those encoding troponin I and MHC.

**MATERIALS AND METHODS**

**DNA constructs and transgenic mice**

gC143 contains the quail TnIfast gene (4.4 kb), along with 0.53 kb and 1.5 kb of 5′ and 3′-flanking DNA (Baldwin et al., 1985; Konieczny and Emerson, 1987). Production of transgenic mouse lines with a 7 kb EcoRI-SalI fragment of gC143 (containing 375 bp and 275 bp of pBR322 DNA at the up- and downstream ends; Fig. 1) has been described (Hallauer et al., 1988). The present analyses involved animals from line 354.

TnILacZ1 was produced from a derivative of gC143, gC1012 (prepared by Yutzey et al. (1989) as a precursor to TnICAT1). In gC1012, all quail DNA downstream of the TnIfast ATG initiation codon in exon 2 has been discarded and the ATG codon has been mutated to ATC, creating a unique BglII site. (Also, the BamHI cloning sites at the ends of the quail DNA insert were destroyed by blunting/religation and the insert orientation is opposite to that in gC143.) gc1012 was BglII-linearized, Klenow-blunted and cut at the unique EcoRI site in pBR322, producing a 6.1 kb fragment containing most of pBR322 and including the quail TnIfast DNA insert up to the blunt BglII site in exon 2. TnILacZ1 was produced by ligating the 6.1 kb fragment with a 5.2 kb EcoRI-SmaI fragment from the β-gal expression plasmid pRSVZ (MacGregor et al., 1987). The 5.2 kb pRSVZ fragment contained the entire lacZ gene including the SV40 splice and poly(A) addition sequences, but no vector or RSV promoter DNA (see Fig. 1; the pRSVZ SmaI site is immediately upstream of the β-gal ATG initiation codon). Transgenic lines were produced by microinjection of the 7.7 kb EcoRI-SalI fragment of TnILacZ1 plasmid (Fig. 1) into CD1 zygotes, identification of founder animals by tail DNA Southern blot and matings with CD1 mice. β-gal expression was studied in hemizygous offspring. Except where indicated, the data reported here refer to line 29.

**In situ hybridization and northern blot analysis**

In situ hybridization and northern blot analyses were as described previously (Koppe et al., 1989). In situ hybridization used 35S-labeled DNA probes: for quail TnIfast mRNA, a 150 bp HindIII-PstI fragment from exon 8 of the cloned gene (Baldwin et al., 1985), and for mouse TnIfast mRNA, a 170 bp BglII-SphI fragment of the cDNA clone cM113 (Koppe et al., 1989). For quantitation, see below (image analysis). Northern blot hybridization used 32P-labeled DNA probes: for quail TnIfast mRNA, the insert of cDNA clone c112 (Hastings and Emerson, 1982) and for mouse TnIfast mRNA, a 470 bp SphI fragment of cM113 (Koppe et al., 1989). Northern blot sample loadings were adjusted to 20 µg total RNA by addition of quail liver poly(A)-lacking RNA. Hybridization was quantitated by densitometric analysis (LKB Ultrascan XL) of appropriately exposed X-ray films. Skeletal muscle α-actin mRNA, quantitated by reprobing blots with the insert of cDNA clone pHMtoA-1 (Gunning et al., 1983), served as an internal loading/transfer standard.

**Histochemical analyses**

**Fiber typing**

Isoform-specific anti-MHC antibodies (kindly provided by Stefano Schiaffino) were employed on frozen sections using immunoperoxidase detection, as in Schiaffino et al. (1989). The antibodies were SC-71 (anti-type IIA), BF-F3 (anti-type IIB) and BA-D5 (anti-type I). Type IIX fibers were identified by their lack of reaction. Alternatively, myosin ATPase histochemistry after preincubation at pH 4.3 (Brooke and Kaiser, 1970) was used to distinguish type I (slow) from type II (fast) fibers and analysis of serial sections by NADH-tetrazolium reductase histochemistry (Dubowitz et al., 1973) identified the type II fiber subtypes: type IIB fibers showed the least reaction, type IIA the most, and type IIX (and type I) were intermediate (Hämäläinen and Pette, 1993).

**β-gal**

Cryostat sections (20 µm) cut from frozen tissue blocks were fixed for 3 minutes at room temperature with 0.25% glutaraldehyde in buffer containing 43 mM K2HPO4, 9.5 mM NaH2PO4, 125 mM NaCl. β-gal histochemistry, with X-Gal substrate, was as described by Sanes et al. (1986) with reaction for several hours or overnight at room temperature or 37°C.
**RESULTS**

**TnILαcZ1 expression in adult muscle**

We prepared a TnIfast reporter gene construct by ligating the coding sequence for *E. coli* β-gal to a fragment of quail DNA containing the 5′-half (approximately) of the TnIfast gene. The TnIfast gene fragment included 530 bp of 5′-flanking DNA, exon 1 (a noncoding exon), intron 1 and the initial, noncoding, segment of exon 2. Fig. 1 shows maps of the construct, termed TnILαcZ1, and of gC143, the parent TnIfast gene construct. TnILαcZ1 contains all TnIfast regulatory cis-elements presently known from cell culture transfection studies (Konieczny and Emerson, 1987; Yutzey et al., 1989; Nikovits et al., 1990; Lin et al., 1991).

We produced transgenic mouse lines carrying TnILαcZ1 and examined β-gal expression in hindlimb skeletal muscle histochemically using X-Gal as substrate. Eleven lines were produced of which seven showed detectable β-gal staining. Six lines showed similar fiber-type-staining patterns, described in detail below. In one line, only a few fibers were stained and this line was not investigated further.

The most extensively characterized TnILαcZ1 transgenic line was line 29, which showed the strongest β-gal staining. Other lines showed either slightly (lines 49, 36, 39) or considerably (lines 15, 54) weaker β-gal staining; however, the β-gal expression pattern matched that of line 29 (longer X-Gal incubation times on weakly expressing lines gave results similar to shorter incubation times on strongly expressing lines).

Low-magnification microscopic analysis of the posterior crural muscles, the gastrocnemius, plantaris and soleus, showed muscle-specific and region-specific expression of the TnILαcZ1 construct (Fig. 2). β-gal staining was strong and rather uniform in the superficial region of the gastrocnemius. There was little staining in the soleus muscle and in nearby parts of the gastrocnemius, especially the zone laterally adjacent to the plantaris muscle (PALG in Fig. 2). Staining in the plantaris muscle and in the deeper regions of the gastrocnemius muscle was intermediate. Individual muscle fibers were stained to different degrees in a definite pattern of fiber-type-specific expression. This is shown in high-magnification analysis of a portion of the lateral gastrocnemius that we refer to as the complex zone, because all four fiber-types are well-represented (see Fig. 2 for location). In gastrocnemius complex zone, expression was according to the following pattern, IIB>IX>IIA>I (Fig. 3). Microdensitometry (Fig. 4) confirmed that each of the four fiber types showed a distinct mean staining intensity (optical density); all differences were highly significant.

The same pattern of fiber-type-specificity also applied to all other muscle regions examined, although not all regions contain all four fiber types. For example the soleus muscle contains predominantly type I and type IIA fibers with some IX (but no IIB) fibers (Donoghue et al., 1991). In soleus muscle, the β-gal expression pattern was IX>IIA>I (data not shown).

The fiber-type-specificity largely accounts for the overall appearance of the crural muscles following β-gal staining, as seen in Fig. 2. The superficial gastrocnemius consists almost exclusively of IIB fibers and shows the strongest, most uniform overall staining. In contrast, type IIB fibers are absent from the soleus muscle and the plantaris-adjacent region of the lateral gastrocnemius muscle. In addition, these muscle regions are particularly rich in type I fibers (which show the least β-gal staining). Because of these two factors, the soleus and plantaris-adjacent lateral gastrocnemius show very little β-gal staining. Other regions, such as the deep medial gastrocnemius, the plantaris and the gastrocnemius complex zone, have more complex mixtures of fiber types, including types I, IIA, and IIB and/or IX. These regions have intermediate overall staining intensities.

Although fiber type was a key determinant of TnILαcZ1 expression levels, additional regional factors also affected expression. For example, type IIB fibers in the superficial gastrocnemius were stained more intensely than type IIB...
fibers in the gastrocnemius complex zone and type IIA fibers in the gastrocnemius complex zone were stained more intensely than type IIA fibers in the soleus (data not shown). These secondary effects correlate roughly with position in the limb, expression being higher in muscle regions radially more distant from the limb axis.

Intact quail TnIfast transgene expression pattern

In our previous analysis (Hallauer et al., 1988) of transgenic mouse lines carrying the intact quail TnIfast gene construct gC143, we found that quail TnIfast mRNA was much more abundant in a predominantly fast muscle (gastrocnemius) than in a mixed fast + slow muscle (soleus), but we did not characterize expression at the level of individual fibers. We have since carried out in situ hybridization analysis of gC143 transgenic mouse gastrocnemius muscle and have found that quail TnIfast mRNA is expressed with the same pattern of fiber-type-specificity seen for β-gal in TnILacZ1 mouse muscle, i.e. IIB>IIX>IIA>I (Figs 5, 6).

In gC143 transgenic mouse muscle, we also compared the expression of transgenic quail TnIfast mRNA with endogenous mouse TnIfast mRNA by in situ hybridization with species-specific TnIfast cDNA probes in serial sections (Figs 5, 6). Mouse TnIfast mRNA was found to be similarly abundant in all the fast fiber types, i.e. in the pattern IIB–IIX–IIA–I (Figs 5, 6), consistent with our previous findings in rat muscle (Koppe et al., 1989). Therefore, although the transgenic quail TnIfast constructs and the endogenous mouse gene are similar in that they are expressed at high levels in fast (type II), but not slow (type I) fibers, they differ in their relative expression among the fast fiber types.

Differential expression of quail and mouse TnIfast genes among fast fibers was also evident during low-magnification inspection of in situ-hybridized muscle sections (Fig. 7). In the deep regions of the gastrocnemius muscle (on the left of each muscle in the orientation shown in Fig. 7), the scattered slow fibers (revealed histochemically in Fig. 7A) stood out sharply as unlabeled spots following in situ hybridization with the mouse TnIfast cDNA probe (Fig. 7B). The intermingled fast fibers in these regions, which include a large fraction of IIA (and IIX) fibers, appeared to be as effectively labeled as the IIB-fiber-dominated superficial gastrocnemius (on the right of each muscle). In contrast, following in situ hybridization with the quail TnIfast probe, the deep regions were weakly labeled compared with the superficial gastrocnemius (Fig. 7C) and there was not a sharp contrast between uniformly labeled fast fibers and unlabeled slow fibers. This is consistent with equal expression of mouse TnIfast mRNA in all type II fibers, but unequal expression of quail TnIfast mRNA, i.e., lower expression in type IIA/IIX fibers (deep gastrocnemius) than in type IIB fibers (superficial gastrocnemius).

Differential expression of quail and mouse TnIfast genes was also evident in quantitative northern blot analysis (Fig.
Mouse TnIfast mRNA (Fig. 8A) was found to be 40% as abundant in soleus RNA (lane 7) as in gastrocnemius RNA (lane 2). Because gastrocnemius muscle consists predominately (~95%) of fast fibers, whereas the soleus muscle contains approximately equal numbers of fast and slow fibers, this result indicates approximately equal expression of mouse TnIfast mRNA, on a per fiber basis, in the fast fiber populations of both muscles. By contrast, quail TnIfast mRNA (Fig. 8B) abundance in soleus RNA was only 14% of gastrocnemius levels. Thus, on a per-fiber basis, the fast fibers of the soleus muscle (IIA/IIX) accumulate only one-third as much quail TnIfast mRNA as do gastrocnemius fast fibers (largely IIB), despite their equal expression of endogenous mouse TnIfast mRNA.

Developmental emergence of the TnILacZ1 expression pattern

In rodents, most or all skeletal muscle fibers are generated during prenatal life (see, e.g., Ontell and Kozeka, 1984; Ontell et al., 1988). However, the fibers undergo considerable postnatal growth and maturation, including MHC isoform transitions (see below). We analyzed the emergence of the adult TnILacZ1 expression pattern during early postnatal life. Upon macroscopic inspection, muscle sections from neonatal or early postnatal TnILacZ1 transgenic pups showed positive staining for β-gal (nontransgenic control muscle sections showed no staining); however, the staining was weak compared with that of parallel-processed adult transgenic muscle sections. The overall β-gal staining intensity increased up to and beyond two weeks of age, but there was little or no increase after 6 weeks (data not shown). At the cellular level, the staining intensities of superficial gastrocnemius fibers (entirely type IIB in the adult) were much greater in adult than in parallel-processed postnatal day 2 muscle (Fig. 9). In contrast, the type II fibers of the soleus muscle (entirely types IIA and IIX in the adult) did not undergo a similar increase in staining intensity during postnatal life. Therefore, the differential expression of the TnILacZ1 transgene among the various fast fiber types emerges largely during postnatal life and involves a pronounced increase in the β-gal concentration in type IIB fibers. However, the differential expression in fast versus slow fibers is clearly evident in neonatal, or postnatal day 2 (Figs 9, 10) animals.
The postnatal divergence of β-gal expression levels among the fast fiber types was also evident during direct microscopic inspection of the gastrocnemius complex zone. In the adult, this region is a striking mosaic of differential expression in all four of the adult muscle fiber types (see Fig. 3). However, as shown in Fig. 10A, in postnatal day 2 animals, there appeared to be fewer levels of β-gal expression. At this stage, fast and slow fibers could be distinguished by the specific reaction of the latter with anti-type I MHC antibody (Fig. 10C), although neither the IIA nor IIB adult fast MHC isoforms were detectably expressed (data not shown; the fast fibers at this stage presumably express the perinatal MHC isoform (Whalen et al., 1981; Periasamy et al., 1984; Weydert et al., 1987)). Fast fibers showed more intense β-gal staining than did slow fibers, but there was not a wide range of staining intensities among the fast fibers (Fig. 10A). The coefficient of variation (s.d./mean) of staining intensities of complex zone fast fibers was found to be considerably less in postnatal day 2 than in adult muscle (0.17 versus 0.60). In addition, the staining heterogeneity of complex zone fast fibers in postnatal day 2 muscle was little or no greater than that observed in the fast fibers of the superficial gastrocnemius or soleus muscles at the same stage (coefficients of variation=0.17, 0.16 and 0.20, respectively). This, despite the greater heterogeneity of prospective adult fast fiber type of complex zone fast fibers (IIB, IIA, IIX in similar numbers), than those of the superficial gastrocnemius (exclusively IIB precursors) or soleus (mostly IIA, with some IIX). These quantitative data confirm the visual impression in Fig. 10A that postnatal day 2 complex zone fast fibers are relatively homogeneous in terms of β-gal expression and indicate that there is, among the fast fiber population at this stage, little or no differential expression of the TnILacZ1 transgene according to prospective adult IIA/IIB/IIX fiber type.
In postnatal day 2 muscle, the β-gal staining intensities of superficial gastrocnemius fibers were slightly, but significantly, higher than those of the soleus fast fibers (Fig. 9). It is unlikely that this difference reflects the different prospective fiber type fates of these fast fiber populations, unless there is regional asynchrony of fast fiber type differentiation. Alternatively, the difference may reflect other regional factors not directly related to fiber type, such as the radial position effect in adult muscle noted above. In any case, this differential expression is far less pronounced than in adult muscle (Fig. 9), hence any differential expression of TnILacZ1 that may exist at early postnatal times among the various prospective fast fiber types is relatively weak compared with what emerges during later postnatal maturation.

By postnatal day 15, the complex zone β-gal staining pattern appeared similar to the adult condition, with a wide range of staining intensities among fast fibers (Fig. 10B). In agreement with previous MHC studies (Mahdavi et al., 1987; Weydert et al., 1987; Russell et al., 1988), we found that IIA and IIB MHC were first both detectable and differentially expressed (defining IIA and IIB fibers) at the beginning of the second postnatal week. Therefore, the differential expression of the TnILacZ1 construct among the fast fiber types becomes evident near the time that these distinct fast fiber subpopulations begin to express distinct adult fast MHC isoforms.
DISCUSSION

We have found that two quail TnIfast gene constructs show a pattern of fiber-type specific expression that resembles that of the corresponding endogenous mouse TnIfast gene, but with an unexpected difference. Like the endogenous mouse TnIfast gene, the quail transgene constructs are expressed at high levels in fast but not in slow fibers. However, unlike the endogenous mouse TnIfast gene, which is expressed at similar levels in all fast fiber types, the quail transgene constructs are differentially expressed in the various fast fiber types in the pattern IIB>IIX>IIA. The differential expression in fast versus slow fibers is apparent around the time of birth, but the differential expression among the fast fiber types emerges largely or entirely during postnatal growth and maturation. These findings have several implications for muscle gene regulation.

TnIfast gene regulation

Our previous studies (Hallauer et al., 1988) showed that properly initiated and spliced quail TnIfast transcripts in gC143 transgenic mice were abundant only in skeletal muscle, suggesting that the transgene was expressed specifically in skeletal muscle fibers. The present in situ hybridization analysis of gC143 mouse muscle and histochemical studies of TnILacZ1 transgene expression, confirm this tissue-specificity. These TnIfast gene constructs are expressed in skeletal muscle fibers, but not non-muscle elements present adjacent to or within the muscles, including connective tissue (septae and cartilage), blood vessels and nerve. Neither construct is efficiently expressed in the myocardium (Hallauer et al., 1988; data not shown). These results indicate that the cis-regulatory elements required to drive tissue-specific expression in skeletal...
Fiber-type specificity of TnIfast transgenes

muscle in vivo are present in the cloned quail DNA segment. Moreover, these regulatory elements are functionally autonomous and appear to work in diverse chromosomal contexts, assuming that independent transgenic lines represent different genomic insertion sites.

The TnILacZ1 construct shows the full pattern of specificity of the intact quail TnIfast gene. This indicates that all levels of cell-type-specificity, i.e., muscle versus non-muscle, skeletal versus cardiac, fast versus slow and IIB>IIX>IIA, are directed by cis-regulatory elements present in the DNA common to both constructs, i.e. within the 5′-half of the TnIfast gene and not further than ~530 bp upstream of the RNA start site. Moreover the regulatory mechanisms must be based either on transcriptional control, or on post-transcriptional mechanisms operating through exon 1, intron 1 or the initial segment of exon 2.

Transfection studies have identified several TnIfast gene cis-regulatory elements that contribute to expression in cultured muscle cells, including elements within 500 bp of the promoter and an important muscle-specific enhancer in the first intron (Konieczny and Emerson, 1987; Yutzey et al., 1989; Nikovits et al., 1990; Lin et al., 1991). All of these known elements are contained within the segment of DNA shared by gC143 and TnILacZ1 and it is probable that they make some contribution to the observed expression of these constructs in muscle fibers in vivo. Our results indicate that any additional elements required for efficient expression in adult muscle fibers, as distinct from cultured muscle cells, also reside within the 5′-half of the gene, as do elements directing fast versus slow fiber specificity.

The IIB>IIX>IIA pattern

The IIB>IIX>IIA hierarchy of expression of quail TnIfast transgene constructs among fast fiber types in adult muscle was unexpected and contrasts with the similar levels of expression of the endogenous mouse TnIfast gene in all fast fiber types. The differential expression hierarchy of the transgenes presumably reflects differences in the transcriptional biology of the adult fast fiber types. Yet the endogenous mouse TnIfast gene does not seem to respond to these differences. It is unlikely that the transgene pattern results from an artificial and irrelevant incompatibility between bird genes and mammalian muscle fibers, because similar patterns have also been generated by transgene constructs based on mammalian muscle genes (see below). A more plausible explanation is that distinct cis-regulatory elements are required to support high-level expression in the different fast fiber types and the quail transgenes construct lack IIA/IIX-specific elements (or they are rendered non-functional by bird/mammal evolutionary divergence). This idea implies that the IIB>IIX>IIA expression hierarchy exhibited by the transgenes may be a simpler and more fundamental pattern (i.e. a ‘basal fast’ pattern) than the uniform fast fiber expression pattern of the endogenous mouse TnIfast gene.

A similar IIB>IIX>IIA differential expression among fast fiber types was seen in transgenic mice carrying a reporter gene construct driven by enhancer and promoter elements of the rat fast myosin light chain 1 (mlc1f) gene (Donoghue et al., 1991). Although the pattern of endogenous mlc1f expression has not been characterized, it is a striking fact that the two fast muscle-specific genes that have been tested in transgenic mice to date both show a similar IIB>IIX>IIA specificity.

In studies of human aldolase A gene constructs in transgenic mice, Concordet et al. (1993) found that removal of a previously identified enhancer resulted in a marked increase in the preferential expression from the pN promoter in a predominantly fast muscle (extensor digitorum longus) as opposed to soleus muscle. This finding led to the hypothesis of a second, previously unknown, enhancer having a high level of specificity for fast glycolytic (presumably IIB) fibers and this has two points of relevance to the present discussion. Firstly, although the IIB fiber-specificity of the postulated second aldolase A enhancer has not been directly established by single fiber or in situ hybridization analysis, its highly specific expression in a type IIB-fiber containing muscle, extensor digitorum longus, as compared with soleus muscle, corresponds to the pattern of expression both our quail TnIfast gene constructs and the mlc1f construct of Donoghue et al. (1991). Secondly, the increased presumed-IIB fiber-type specificity of the pN promoter following removal of an enhancer is consistent with the idea that the pattern of IIB fiber-enriched expression may be a fundamental one that can be achieved with a smaller number of regulatory elements than is required to direct a broader pattern of expression in multiple muscle fiber types.

Developmental regulation: similarities to MHC gene family

The TnILacZ1 transgene adult expression pattern emerges in two stages. In neonatal or two day-old pups, the TnILacZ1 gene is expressed in fast, but not in slow fibers and it is expressed at similar, low levels in all fast fibers. Differential expression among the various fast fiber types emerges during the first two weeks of postnatal life and involves a great increase in expression in IIB fibers. These results indicate that at least two serially acting mechanisms contribute to high-level expression in adult muscle fibers; one associated with early myogenesis (perhaps myoblast differentiation) that activates expression of the gene to a relatively low level and a second that quantitatively increases that expression during the postnatal maturation of IIB fibers.

The adult pattern of MHC isoform expression is also known to emerge in two developmental stages. In the first stage, fast and slow fibers arise but the various adult fast fiber types are not yet differentiated: the slow fibers express type I MHC (in addition to embryonic MHC) and the fast fibers express embryonic and perinatal MHC isoforms, not adult IIA, IIB, IIX fast MHC isoforms (Narusawa et al., 1987; Condon et al., 1990). In the second stage, the adult fast fiber types arise; IIA, IIB, IIX MHC gene expression is activated and expression of embryonic/perinatal MHC isoforms is eclipsed (Whalen et al., 1981; Periasamy et al., 1984; Mahdavi et al., 1987; Weydert et al., 1987; Russell et al., 1988). In rodents, the first stage occurs before birth and the second occurs during the first week(s) of postnatal life. (In birds a more complex but generally similar picture applies, in which adult repression of embryonic/perinatal fast MHC isoforms is less comprehensive than is the case in mammals (Crow and Stockdale, 1986; Gauthier and Orfano, 1993).)

There are striking parallels between TnILacZ1 transgene expression and the developmental regulation of the mouse
MHC gene family. In both cases, there is a prenatal stage in which the fast-versus-slow fiber type distinction is made, but in which the fast fibers form a rather homogeneous population. This is followed by a phase during the first two weeks of postnatal life during which the adult fast fiber types, with their characteristic gene expression profiles, emerge. These similarities may reflect fundamental connections between the developmental systems regulating troponin I and MHC genes. In particular, the TnIfast transgene expression pattern that we document here resembles that of the MHC IIB isoform. Both are expressed predominantly in type IIB fibers in the adult and high-level expression in IIB fibers arises during the first weeks of postnatal life. Related mechanisms may regulate these genes. Moreover, the element(s) that we postulate for the endogenous mouse TnIfast gene that permit equal expression in IIA and IIB fiber types presumably operate by specifically enhancing expression in the IIA fiber type. Such a mechanism could be related to those that direct the IIA-fiber-specific expression of the MHC IIA isoform.

Our findings, and related observations of others (Donoghue et al., 1991; Concordet et al., 1993), imply that the gene regulatory systems of type IIA, IIX and IIB fibers are significantly different, not only for the MHC gene family, but also for simpler muscle gene families. The apparent simplicity of the expression pattern of the endogenous TnIfast gene, and perhaps other muscle protein fast-isoform genes, i.e., expression at similar levels in all fast fiber types, may belie a complexity of transcriptional control mechanisms that compensate for fundamental differences among the fast fiber types. This aspect of our findings increases the known complexity of muscle gene regulation. However, the similarities between the TnIfast transgene expression pattern and those of the MHC gene family suggest that unifying principles based on shared mechanisms may simplify our understanding of the regulation of diverse muscle gene families.

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