Myosin Light Chain 3F Regulatory Sequences Confer Regionalized Cardiac and Skeletal Muscle Expression in Transgenic Mice

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Abstract. The myosin light chain 1F/3F locus contains two independent promoters, MLC1F and MLC3F, which are differentially activated during skeletal muscle development. Transcription at this locus is regulated by a 3' skeletal muscle enhancer element, which directs correct temporal and tissue-specific expression from the MLC1F promoter in transgenic mice. To investigate the role of this enhancer in regulation of the MLC3F promoter in vivo, we have analyzed reporter gene expression in transgenic mice containing lacZ under transcriptional control of the mouse MLC3F promoter and 3' enhancer element. Our results show that these regulatory elements direct strong expression of lacZ in skeletal muscle; the transgene, however, is activated 4–5 d before the endogenous MLC3F promoter, at the time of initiation of MLC1F transcription. In adult mice, transgene activity is downregulated in muscles that have reduced contributions of type IIB fibers (soleus and diaphragm). The rostrocaudal positional gradient of transgene expression documented for MLC1F transgenic mice (Donoghue, M., J. P. Merlie, N. Rosenthal, and J. R. Sanes. 1991. Proc. Natl. Acad. Sci. USA. 88:5847–5851) is not seen in MLC3F transgenic mice. Although MLC3F was previously thought to be restricted to skeletal striated muscle, the MLC3F-lacZ transgene is expressed in cardiac muscle from 7.5 d of development in a spatially restricted manner in the atria and left ventricular compartments, suggesting that transcriptional differences exist between cardiomyocytes in left and right compartments of the heart. We show here that transgene-directed expression of the MLC3F promoter reflects low level expression of endogenous MLC3F transcripts in the mouse heart.

During striated muscle development, a dynamic and complex pattern of structural gene expression generates the diversity of muscle types found in the adult vertebrate. Regulation of the majority of sarcomeric genes is under transcriptional control mediated by several families of regulatory proteins, including those of the MyoD and MEF2 transcription factors (Weintraub, 1993; Yu et al., 1992). Through interactions with cis-acting regulatory elements, these factors ensure the expression of specific genes whose products are required in particular subsets of cardiac and skeletal musculature. A large number of muscle-specific promoter and enhancer elements have been identified as important in tissue culture (see Rosenthal, 1989); dissection, however, of the complex spatial and temporal control of muscle-specific gene expression in vivo, which is not recapitulated in vitro, requires direct analysis of putative regulatory regions in transgenic mice. Transgenic studies have demonstrated the complexity of cis-acting elements controlling the expression of particular muscle-specific genes in different striated muscle types, for example, the separation of cardiac and skeletal muscle regulatory elements in mice containing upstream sequences from the desmin (Li et al., 1993) and MLC2V (Lee et al., 1992) genes. Similarly, transgenic studies have shown that adult skeletal muscle fiber-type diversity is mediated by distinct cis-acting elements that are required for slow (Banerjee-Basu and Buonanno, 1993) or fast (Donoghue et al., 1991b; Hallauer et al., 1993) fiber-type expression.

The diversity of muscle subtypes is illustrated by the alkali myosin light chain (MLC) family composed of three loci encoding four isoforms expressed in a developmentally regulated pattern in different striated muscle types (Barton and Buckingham, 1985). Alkali MLCs comprise two subunits of hexameric muscle myosin, and they are implicated in the velocity of muscle shortening (Lowey et al., 1993). The MLC 1F/3F locus encodes the two alkali MLC isoforms found in fast skeletal muscle: MLC1F and 3F differ at the amino terminal because of the use of two promoters and a differential splicing event such that exons 1 and 4 are MLC1F specific, and exons 2 and 3 are MLC3F specific (Fig. 1 a in this manu-

1. Abbreviations used in this paper: β-gal, β-galactosidase; CAT, chloramphenicol acetyltransferase; MHC, myosin heavy chain; MLC, myosin light chain; p.c., postcoitum; RT-PCR, reverse transcription PCR.
caudal-rostral gradient lie in the MLC1F promoter or en-
the mouse 3F promoter upstream of a
gradient appear to lie within the MLC regulatory elements
ghue et al., 1992a, b). The sequences responsible for the
et al., 1991a). This graded expression is cell autonomous,
and (b) whether the

An enhancer sequence located 3' to the MLCIF/3F locus
confers strong muscle-specific expression on the MLCIF
promoter in muscle cells in culture and in transgenic mice,
where a chloramphenicol acetyltransferase (CAT) reporter
gene is activated up to 1,000-fold over background levels ex-
clusively in skeletal muscle (Donoghue et al., 1988; Rosen-
thal et al., 1989). MLCIF-CAT transgenic mice containing
the 3' enhancer (IF-CAT-E) initiate transgene expression at
the time of myotome formation, when the endogenous
MLCIF gene is first expressed (Grieshammer et al., 1992),
and CAT expression in adult muscle is specific to fast fibers
(Donoghue et al., 1991b). Functional binding site motifs for
transcription factors of the MyoD and MEF2 families, in ad-
dition to a homeobox protein target site, are conserved in
both rat and human 3' enhancer sequences (Wentworth et al.,
1991; Rosenthal et al., 1990). Unexpectedly, IF-CAT-E
transgenic mice show a striking rostrocaudal positional
gradient of CAT expression, such that caudal muscles (e.g.,
extensor digitorum longus) express the transgene at levels 100-
fold higher than rostral muscles (e.g., masseter, Donoghue
et al., 1991). This graded expression is cell autonomous,
stable, and correlates with transgene methylation in partic-
ular muscles along the anterior-posterior axis (Dono-
ghue et al., 1992a, b). The sequences responsible for the
gradient appear to lie within the MLC regulatory elements
included in the transgene, although there is no gradient of
endogenous MLCIF expression. It remains unclear (a) how
the differential regulation of the MLCIF and 3F promoters
is controlled in the presence of a single enhancer element,
and (b) whether the cis-acting sequences responsible for the
caudal-rostral gradient lie in the MLCIF promoter or en-
hancer element. The experiments described in this paper
were designed to address these questions.

We report an analysis of expression from the late activated
MLC3F promoter in vivo using transgenic mice containing
the mouse 3F promoter upstream of a lacZ gene (with a nu-
clear localization signal), and the mouse 3' enhancer down-
stream of the reporter gene (construct 3F-nlacZ-E). Our
results demonstrate that skeletal muscle-specific expression of
lacZ is precocious with respect to the endogenous
MLC3F transcript; transgene expression, however, is down-
regulated postnatally in skeletal muscles with reduced num-
bers of type IIB fibers in the adult (soleus and diaphragm). Unlike
1F-CAT-E transgenic mice, no positional gradient of
rostrocaudal expression is observed at the level of n LacZ tran-
script or product. We report the unexpected finding that
lacZ is expressed in a restricted subset of cardiac myocytes
from 7.5 d of development, and we demonstrate that there
is low level transcriptional activation of the endogenous
MLC3F promoter in the heart. This last observation reveals
a further level of complexity in transcriptional regulation at
the MLCIF/3F locus, and it leads to a reassessment of the
overlap in expression patterns between skeletal and cardiac
striated muscle–specific genes.

Materials and Methods

Transgene Construction

Plasmid pH4 contains the MLC3F promoter and 3F-specific exons plus
MLCIF-specific exon 4 on a 1.9kb HindIII fragment derived from λ phage
C3L isolated from the mouse MLCIF/3F locus (Robert et al., 1984; see Fig.
1a in this manuscript). The HindIII insert of pH4 was subcloned into PBlS
(Stratagene, La Jolla, CA) and modified by extension of MLCIF/3F se-
quences at the 5' and 3' ends and the introduction of a BgIII site, designed
for the dual purposes of transgenics and as a homologous recombination
replacement vector. (a) The BgIII site was introduced at the eighth MLC3F
exon 2 by site-directed mutagenesis in pBS (Stratagene) to gener-
ate pLCM6 (using oligonucleotide 1, see list at end of Materials and
Methods). pLCM6 was extended by 620 bp at the 3' end by the addition of
a PCR product generated from BALB/c mouse DNA using oligonucleo-
tide primers 2 and 3; amplification conditions were 94°C 1 rain, 57°C 1
min, and 72°C 3 min for 25 cycles (Taq DNA polymerase; Perkin-Elmer
Cetus, Norwalk, CT). The purified PCR product was digested with
BamHI, subcloned into pBlS, verified by DNA sequencing at 5' and 3' ter-
mini, and subsequently isolated on a BamHI/BspMI fragment for ligation
with a 260-bp pLCM6 XhoI/BspMI fragment into pLCM6 digested with
XhoI/BamHI to generate pLCM7. (b) To extend promoter sequences to
~2.0 kb, a 1.8-kb PCR amplified fragment (using primers 4 and 3, condi-
tions as above) was ligated onto the 5' end of pLCM7. This product con-
tained the expected restriction sites and correct terminal DNA sequence,
and it was subcloned into pBluescript (Stratagene) on a PvuII/EcoRI frag-
ment, and was subsequently excised as a PstI/Sacl partial fragment for liga-
tion with the 2.2-kb SacI/BamHI insert of pH4 as a 3.1-kb
PCR product using primers 8 and 9, cloned into pBluescript as a BamHI
fragment, and verified by DNA sequencing. The enhancer was subcloned
on a BamHI/BglII fragment containing the core 173-bp sequence from the rat enhancer (Donoghue et al., 1988; Wentworth et al., 1991), and corresponding to nucleotides
361-620 of the rat enhancer (Donoghue et al., 1988), was amplified as
a PCR product using primers 8 and 9, cloned into pBluescript as a BamHI
fragment, and verified by DNA sequencing. The enhancer was subcloned
on a BamHI fragment into the unique 3' BamHI site of pLCM8 as a 3.1-kb
NcoI-BamHI fragment that was annealed to a double-stranded oligonucleo-
tide (annealed oligos 6 and 7) designed to recreate the NcoI site containing
the lacZ ATG in frame with MLC3F sequences and to leave a BgIII over-
hang, generating p3F-nlacZ. The resulting 3F-nlacZ junction was verified
by DNA sequencing. (d) A 260-bp mouse MLCIF/3F 3' enhancer fragment
containing the core 173-bp sequence from the rat enhancer (Donoghue et al.,
1988; Wentworth et al., 1991), and corresponding to nucleotides
361-620 of the rat enhancer (Donoghue et al., 1988), was amplified as
a PCR product using primers 8 and 9, cloned into pBluescript as a BamHI
fragment, and verified by DNA sequencing. The enhancer was subcloned
on a BamHI fragment into the unique 3' BamHI site of p3F-nlacZ, in the
orientation in which the enhancer is found in the MLCIF/3F locus, to generate
p3F-nlacZ-E.

Muscle Cell Transfections

C2/7 skeletal muscle cells were grown under standard culture conditions
in DMEM with 20% fetal calf serum for proliferation, or 2% fetal calf serum
to induce differentiation. Cells were transfected with 10 μg of reporter con-
struct and 1 μg of RSV-luciferase vector per 6-cm dish, using the calcium-
phosphate method (see Biben et al., 1994). Cell extracts and luciferase as-
says were performed as in Biben et al. (1994). β-galactosidase assays were
performed as described in Sambrook et al. (1989); values were normalized with respect to luciferase activities to account for variability in transfection efficiency.

**Generation of Transgenic Mice**

The insert of p3F-nlacZ-E was excised as a 7-kb SphI/KpnI fragment, and was purified by gel electrophoresis and passage through an Elutip column (Schleicher & Schuell, Dassel, Germany). Transgenic mice were generated by microinjection of purified 3F-nlacZ-E insert into fertilized (C57BL/6J x SJL) F1 eggs at a concentration of 700 copies per picoliter using standard techniques (Hogan et al., 1986). Injected eggs were reimplanted into pseudopregnant (C57BL/6J x CBA) F1 foster mothers.

**Identification of Transgenic Mice**

DNA was prepared from mouse tails (Laird et al., 1991), and was analyzed by Southern blot or PCR. For Southern blot analysis 15 μg of DNA was digested with restriction endonucleases in the presence of 100 μg/ml BSA, subjected to electrophoresis, and transferred onto Hybond N+ membranes (Amersham Corp., Arlington Heights, IL) for hybridization in 0.5 M NaPO4 (pH 7.6), 7% SDS plus 100 μg/ml salmon sperm DNA at 65°C overnight; filters were washed in 0.1x SSC, 0.01% SDS at 65°C. Hybridization probes were either from the lacZ gene (kb NcoI/ClaI fragment) or, to determine copy number, 3F MLC3F fragments (either 1-kb XbaI/BglII or 450-bp EcoRI/BglII plasmid fragments). For PCR analysis, one primer in the proximal MLC3F promoter (10) and one within lacZ (11) were used, generating a 1,060-bp product. Amplification conditions were 95°C 1 min, 62°C 1 rain, and 72°C 3 rain, for 25 cycles. Since the 3F-nlacZ-E transgene is strongly expressed in adult skeletal muscle, transgenic mice were also identified by in toto X-gal staining (see below) of skinned sections of mouse tails.

**Analysis of Transgene Expression**

Heterozygous and homozygous transgenic males were crossed with non-transgenic females (C57BL/6J x SJL) F1, or CD1. Embryos were dated taking 0.5 d.p.c. as the day of the vaginal plug, and they were dissected in 1x PBS, fixed in 4% paraformaldehyde (for 30 min to overnight depending on the size of the embryos), rinsed in 1x PBS, and colored in X-gal solution (X-gal, U. S. Biochemical Corp., Cleveland, OH; Sanes et al., 1986) at 32°C for periods of 30 min to overnight. Transgenic embryos and individual adult muscles were analyzed by whole-mount microscopy or cryostat sectioning at -20°C. Sections were stained for β-gal activity, counterstained for 5 min in 1% eosin, dehydrated, and mounted in Cytoseal mounting medium (Stephens Scientific, Riverdale, NJ). Antibodies were applied to cryostat sections as described by Tajbakhsh et al. (1994). Slow myosin heavy chain antibody (DS) was supplied by S. Schiaffino (University of Padua, Italy).

**In Situ Hybridization**

RNA-RNA hybridization was performed as described by Sassoon and Rosenthal (1993); two 35S-labeled nucleotides (UTP and CTP) were incorporated into the riboprobes. Exposure times were from 5-14 d. The MLC3F probe was as described by Lyons et al. (1990a). A probe within the first intron of the mouse MLC3F/3' locus was synthesized from a plasmid clone containing a 5-kb BamHI/HindIII subfragment of the intron, derived from λ phage C (Robert et al., 1986). This plasmid was cut with Xbal and a 500-nt riboprobe complementary to a region ~2 kb upstream of the MLC3F transcriptional start site synthesized using T3 RNA polymerase, and it was hydrolyzed for 20 min before hybridization. A lacZ antisense probe was generated by T3 RNA polymerase from C1-digested lacZ-SV40 poly(A) in pSOK (Stratagene). The RNA product was 2 kb long, and it was hydrolyzed for 45 min before hybridization.

**Reverse Transcription Polymerase Chain Reaction**

Total RNA from adult tissue was extracted with guanidium thiocyanate followed by centrifugation on a caesium chloride cushion (Sambrook et al., 1989). 1 μg of total RNA was mixed with 50 ng pd(N)6 random primers (Pharmacia, Uppsala, Sweden). The mixture was heated for 10 min at 70°C, chilled on ice, and adjusted to 1x first-strand buffer (Gibco BRL, Gaithersburg, MD), 10 mM DTT, and 0.5 mM dNTPs. RNasin (1 μl [10 U]; Amersham) and reverse transcriptase (1 μl [200 U] SuperScript RNaseH-; Gibco BRL) were added (final volume = 20 μl). Tubes were incubated for 1 h at 42°C, 5 min at 95°C, spun, and chilled on ice. Four different polymerase chain reactions were performed per cDNA synthesis. 5 μl of cDNA solution were amplified in 1x Taq buffer (Amersham) with 50 pmol of each primer, 100 μM dNTPs, and 2 U Taq polymerase (Amersham). Different numbers (15, 20, and 25) of cycles of amplification were carried out to estimate the level of the RNA of interest. Cycle times were 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, followed by 60°C for 2 min and 72°C for 5 min. Oligonucleotide pairs used were: 12 and 13 for MLC3F (163-bp product), 14 and 15 for MLC3F (163-bp product), 16 and 17 for a-cardiac actin (115-bp product), 18 and 19 for MRF4 (mouse gene, 270-bp product), and 20 and 21 for S6f ribosomal protein (102-bp product). Samples were separated on 5% TBE nondenaturing polyacrylamide gels that were subsequently electroblotted onto Hybond N* (Amersham) for 1 h at 15 V using a trans-blot apparatus (Bio Rad Laboratories, Hercules, CA). The filter was washed for 10 min in 0.4 M NaOH, rinsed in 2× SSPE and heat at 1 h at 80°C. Hybridization was carried out at 65°C as above, followed by washes in 0.1x SSC, 0.1% SDS at 55°C. Hybridization probes were MLC3F/3F exon 1 for MLC3F products, MLC3F/3F common exon 5 for MLC3F products, and a PCR-amplified fragment from the mouse MRF4 locus (gift of M. Primig, Pasteur Institute, Paris, France) for MRF4 products.

**Oligonucleotides**

1. 5'CATTCCCTCAGGAGATCTGTCG3'.
2. 5'CATTCCCTCAGGAGATCTGTCG3'.
3. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
4. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
5. 5'TCCCTTGACTTGTGTTGACTG3'.
6. 5'TCCCTTGACTTGTGTTGACTG3'.
7. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
8. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
9. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
10. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
11. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
12. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
13. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
14. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
15. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
16. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
17. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
18. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
19. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
20. 5'ACAGTTCTTCAGGAGATCTGTCG3'.
21. 5'ACAGTTCTTCAGGAGATCTGTCG3'.

**Results**

To examine the in vivo activity of the MLC3F promoter and 3' enhancer, we generated transgenic mice containing a nlacZ reporter gene under the control of a ~2kb MLC3F promoter and 3' enhancer sequence (3F-nlacZ-E, Fig. 1). The construct contains 2 kb 5' and 3' of the MLC3F transcriptional start site, thus encompassing both MLC3F-specific exons (Fig. 1b). An Escherichia coli lacZ gene containing a nuclear localization signal was cloned in frame within the second MLC3F-specific exon such that a fusion protein including the first eight MLC3F amino acids is produced from a transcript containing the entire MLC3F 5' UTR. The correct MLC3F splicing pattern is maintained (as determined by reverse transcription PCR [RT-PCR], data not shown). nlacZ was selected as a reporter gene for sensitivity of both whole-mount and histological assays, while the inclusion of a nuclear localization signal distinguishes specific from nonspecific signals. The 3' enhancer element is a 260-bp mouse sequence, containing the conserved E-box and MEF2 motifs demonstrated to be necessary for function of the 173-bp minimal rat enhancer fragment (Wentworth et al., 1991); the mouse and rat enhancer DNA sequences are
with the enhancer is 60-fold higher than that containing no enhancer (average of five experiments). The MLC3F promoter in tissue culture. Two independent lines of transgenic mice containing the 3F-nlacZ-E construct were obtained, each containing 5–10 copies of the transgene. Both lines display strong β-galactosidase (β-gal) activity in skeletal muscle such that in adult mice, nlacZ expression is apparent in the majority of skeletal muscles. Adjacent nonmuscle tissues (including blood vessels, connective tissue, and nerve) are negative for transgene expression (Figs. 2 and 3).

**MLC3F-nlacZ-E Expression in Developing Skeletal Muscle**

In the developing mouse embryo, the first skeletal muscle cells appear in the myotomal compartment of the somites, which differentiates in a rostral to caudal gradient from ~8.5 d p.c. (see Buckingham, 1992). 3F-nlacZ-E transgenic mice express nlacZ in the myotome from 9 d p.c., positive cells first appearing in rostral somites at about the 18-somite stage, initially scattered throughout the myotome. The last 7–11 formed somites do not express the transgene. Fig. 2 a shows a 24-somite mouse embryo (9.5 d p.c.) with transgene expression in the myotome of the 13 most rostral somites (see also Fig. 2 d). Note that at this stage, the more rostral somites express the transgene at a higher level than more caudal somites, consistent with a rostrocaudal gradient of somite maturation. Endogenous MLC3F transcripts are not detected at this stage, and they first appear from 13.5 d; MLC3F transcripts, in contrast, are detectable from 9.5 d by in situ hybridization (Lyons et al., 1999a). The 3F-nlacZ-E transgene is, therefore, activated 4 d earlier than the endogenous MLC3F promoter in the myotome.

Transgene expression in the developing limb musculature of 3F-nlacZ-E mice is also early with respect to endogenous MLC3F transcripts; at 10.5 d p.c., β-gal–positive cells are observed in the forelimb bud, and separation into distinct premuscle masses is seen by day 11.5 p.c., when three dorsal and two ventral groups of positive cells are observed (Fig. 2 b). In the hindlimb, significant numbers of β-gal–positive cells accumulate from 11.5 d (Fig. 2 b), and distinct premuscle masses are detectable from 12.5 d p.c. β-gal–positive cells are detected in developing muscle masses surrounding the eye from 11.5 d p.c. In addition to expression in skeletal muscle, β-gal–positive cells are also observed in the heart throughout development, and transiently in the brain and developing ear (from 9 to ~15 d p.c.). Expression at these sites is observed in both 3F-nlacZ-E lines. Unexpectedly, endogenous MLC3F transcripts are expressed in the heart (see later); very low levels of endogenous transcript were detected by RT-PCR in otic vesicle (after 35 cycles, data not shown), but not in brain RNA, at 9.5 d p.c. Transgene expression in the developing ear becomes restricted to the dorsal extremity of the endolymphatic duct at 12.5 d. At later stages of development, the transgene is clearly expressed in the majority of, if not all, skeletal muscle masses throughout the embryo (Fig. 2 c).

**Postnatal Downregulation of Transgene Expression in Diaphragm and Soleus Muscles**

After birth and during adult life, the 3F-nlacZ-E transgene is expressed at high levels in the majority of skeletal muscles. Expression of the transgene, however, is downregulated in two muscles during postnatal development, such that in adult mice, extremely low levels of nlacZ expression are observed in the soleus and diaphragm. These differences reflect the particular fiber-type distribution of these muscles; both muscles are adapted for fatigue resistance in adult mice, and they lack a significant number of myosin heavy chain (MHC) type IIB fast fibers.

Crural (lower leg) muscles of 3F-nlacZ-E transgenic mice express nlacZ equivalently at fetal stages (Fig. 3 a); however, in adult mice, significantly reduced transgene expression is observed in the deep crural muscles, especially in the soleus (Fig. 3 b). This decrease in β-gal activity reflects decreased transgene transcription in these muscles as determined by in situ hybridization using an antisense lacZ riboprobe (Fig. 3 c). This pattern of expression appears to reflect the abundance of fibers containing type I (Fig. 3, c and d) and type
Figure 2. Transgenic embryos at different stages of development illustrating expression of the 3F-nlacZ-E transgene in embryonic and fetal skeletal muscles. (a–c) Whole-mount embryos stained with X-gal; (a) A 24-somite 9.5-d p.c. transgenic embryo (line 2) where the transgene is active in the 13 most anterior somites. In addition to expression in the myotome (M), the nlacZ reporter gene is active at this stage in the heart (H), the otic vesicle (OV), and the brain. (b) An 11.5-d p.c. transgenic embryo (line 1), showing expression in developing myotomal muscles and the premuscle masses of the limb buds; FL, forelimb, HL, hindlimb. (c) A 14.5-d p.c. embryo (line 2) showing widespread expression in skeletal muscles. A transverse section through a 10.5-d transgenic embryo shows β-galactosidase activity in the myotomal compartment of the somite (d). Bar, 100 μm.
Figure 3. 3F-nlacZ-E expression in crural and diaphragm muscles. (a) At 17.5 d p.c., β-galactosidase activity is equivalent throughout the crural muscles of a 3F-nlacZ-E transgenic embryo, as seen in a cryostat section stained with X-gal. nlacZ activity is downregulated in the soleus muscle of an adult (4 mo old) transgenic mouse (b). Reduced time of X-gal coloration reveals that transgene expression is also low in the region of the lateral gastrocnemius adjacent to the plantaris muscle (c, asterisk); both this region and the soleus muscle are rich in type I immunopositive fibers, as seen in an adjacent section reacted with MHC I antibody (d). The section in c was photographed using an orange filter. The low expression in adult soleus muscle is at the level of transgene transcription; in situ hybridization with an antisense lacZ riboprobe shows reduced activity in soleus compared to gastrocnemius muscle (e). The punctate signal reflects the perinuclear subcellular localization of the nlacZ transcript, and it is comparable to in situ hybridization localization of the CAT transcript in 1F-CAT-E transgenic mice (Sassoon and Rosenthal, 1993). In the diaphragm of a 2-d postnatal mouse, transgene expression remains high (f); after 3 wk, transgene expression levels in the diaphragm decrease (g), remaining highest in nuclei at the center of the fibers (Sy, potential synaptic nuclei) and at the myotendinous junction (Te). G, gastrocnemius; S, soleus; E, extensor digitorum longus; TA, tibialis anterior; F, fibula; T, tibia. Bars, 100 μm.
IIA MHC in this muscle (Lewis et al., 1982; Donoghue et al., 1991b; Wigston and English, 1992). Levels of β-gal activity are also reduced in the plantaris-adjacent region of the lateral gastrocnemius muscle, which in the mouse is also particularly rich in type I fibers (Fig. 3c, asterisk; Hallauer et al., 1993). In the diaphragm, transgene expression is strong throughout the muscular component in utero, and it decreases between 2 and 3 wk after birth (Fig. 3f and g). At this time, it has been shown that the proportion of MHC I-expressing fibers is increasing in the rat diaphragm (Laframboise et al., 1991); the adult mouse diaphragm contains a mixture of fibers containing type I, IIA, and IIX(D) MHC, and it is devoid of fibers containing type IIB MHC (Zardini and Parry, 1994). Interestingly, nlacZ expression in the diaphragm remains highest in a specific subset of nuclei localized at synaptic and myotendinous junctions (Fig. 3g), comparable with endogenous acetylcholine receptor subunit expression (Hall and Sanes, 1993; Piette et al., 1993). These results support the finding that the nlacZ gene is predominantly active in type II fast fibers (with IIB fibers expressing the transgene at a higher level than IIA fibers), and they are comparable with the observations of Donoghue et al. (1991b) in 1F-CAT-E transgenic mice. The 3F-nlacZ-E construct, therefore, includes the cis-acting DNA sequences which restrict β-gal expression to fast muscle fibers in vivo.

Transgene Expression Is Not Graded along the Rostrocaudal Axis

Transgenic mice containing 1.2 kb of rat MLC1F promoter sequences upstream of the CAT reporter gene, plus a 900-bp rat 3' enhancer fragment downstream of the CAT gene, display a rostrocaudal gradient of reporter gene expression which is distinct from the gradient of somite maturation (Donoghue et al., 1991a). To determine whether the regulatory sequences from the MLC1F/3F locus included in the 3F-nlacZ-E construct confer a gradient of reporter gene expression, β-gal activity was analyzed in skeletal muscles along the body axis of 3F-nlacZ-E mice.

Apart from the rostrocaudal gradient of somite maturation, no positional gradient of transgene expression was observed either during in utero or postnatal development; the absence of a gradient of β-gal activity is demonstrated in the intercostal muscles at 15.5 d p.c. by whole-mount X-gal staining (Fig. 4a), and at 13.5 d p.c. by X-gal staining of cryostat sections (Fig. 4b). At these time points, the MLC1F-CAT transgene clearly displays a positional gradient (Grieshammer et al., 1992). To avoid saturation, β-gal detection was carried out under limiting conditions (reduced time of coloration). Since β-gal activity may not directly reflect nlacZ transcription (Gundersen et al., 1993), we confirmed by in situ hybridization that the nlacZ transcript is expressed at a uniform level in intercostal muscles at 16.5 d p.c. (Fig. 4c). Furthermore, we confirmed that the endogenous MLC3F transcript, like the MLC1F transcript (Grieshammer et al., 1992), is expressed uniformly along the rostrocaudal axis (Fig. 4d). These results demonstrate that the mouse MLC3F promoter and core 3' enhancer sequence do not confer a positional gradient of reporter gene expression in transgenic mice.

Transgene-directed and Endogenous MLC3F Expression in Cardiac Muscle

The MLC3F protein is exclusive to skeletal striated muscle (Barton and Buckingham, 1985). It was, therefore,
Figure 5. The 3F-nlacZ-E transgene is expressed in restricted regions of the heart. Cardiac expression in the adult is confined to the atria and left ventricular myocardium, as shown in a whole-mount photograph of an adult transgenic heart (a), and in an X-gal–stained cryostat section, where expression is strong in the interventricular septum, but is excluded from the right ventricular wall (b). In situ hybridization using an antisense lacZ riboprobe shows that this pattern of β-galactosidase activity reflects differential accumulation of transgene RNA (c). The transgene is expressed early in the myocardium of transgenic embryos, showing regionalization as early as 8.5 d.p.c. when the outflow tract (O) and presumptive right ventricle (arrow) are β-gal negative (d). At 9.5 d, a cryostat section through the heart shows that β-gal activity is restricted to the atria and presumptive left ventricular myocardium (e). RA, right atria; LA, left atria; RV, right ventricle; LV, left ventricle; S, interventricular septum; A, atria; V, ventricle; P, pericardium; M, myocardium; E, endocardium. Bar, 100 μm.
unexpected to observe strong nlacZ expression in cardiac muscle in both lines of 3F-nlacZ-E transgenic mice. In adult mice, cardiac expression of the transgene is regionalized, being largely confined to the left ventricle, interventricular septum, and atria (Fig. 5, a and b). Transgene expression in the left ventricle and interventricular septum is nonuniform, and regions of nonexpressing myocardiocytes are observed (Fig. 5 a); however, no transmural differences in expression are evident. Expression is stronger in the right than left atrium, while only occasional positive myocardiocytes are found in the right ventricular wall. This pattern of 3F-nlacZ-E transgene expression was observed by whole-mount X-gal coloration of isolated transgenic hearts (Fig. 5 a), by histological detection of β-gal activity in cryostat sections of adult hearts (Fig. 5 b), and by in situ hybridization to nlacZ transcripts in paraffin sections of adult hearts (Fig. 5 c). This pattern of 3F-nlacZ-E expression is distinct from all other described sarcomeric, cytoplasmic, and nuclear cardiac markers in the adult myocardium, and, therefore, it defines a previously undescribed restriction of transcriptional activity in the mouse heart.

The developing heart is the earliest site of transgene expression in 3F-nlacZ-E mice. At 7.5 d of development, two groups of β-gal-positive cells are observed in the anterior mesoderm. Transgene expression is regionalized early: at 8.5 d p.c., the outflow tract myocardium is β-gal negative (Fig. 5 d). By 9.5 d p.c., β-gal expression is localized to the regions of presumptive left ventricle and atria (Fig. 5 e); significant expression is excluded from the region of the presumptive right ventricle, outflow tract, and sinus venosus. Right ventricular exclusion of transgene expression therefore precedes ventricular septation; the 3F-nlacZ-E transgene thus provides an early marker for specific subregions of the developing myocardium.

The observation that nlacZ is expressed in the heart in two lines of 3F-nlacZ-E transgenic mice led us to reexamine expression of the endogenous MLC3F gene in cardiac tissue. MLC3F protein and accumulated mRNA were not detected in cardiac muscle by two-dimensional protein gel or Northern analysis in previous studies (Barton et al., 1985a, b). We demonstrate here, however, using RT-PCR, that the endogenous MLC3F transcript is present in RNA isolated from whole adult mouse hearts, although at a significantly lower level than in RNA from adult skeletal muscle (Fig. 6, a and b). In contrast, α-cardiac actin is expressed at high levels in cardiac muscle and low levels in skeletal muscle. The myogenic sequence MRF4, which is specific for skeletal muscle, provides a negative control in these experiments (Fig. 6 b). The expression of endogenous MLC3F transcripts in the four compartments of the adult heart was analyzed (Fig. 6 c). While the endogenous gene is expressed at higher levels in atrial than in ventricular myocardium, no significant differences between left and right ventricles were observed. S16 ribosomal protein transcripts are uniformly expressed in the four cardiac chambers (Fig. 6 c).

These findings were confirmed by in situ hybridization; endogenous MLC3F transcripts were detected in the heart of nontransgenic mice at a time before their appearance in developing skeletal muscle (10.5 d p.c., Fig. 7 b). The MLC3F antisense in situ probe is derived from the 5' UTR of the MLC3F transcript (Fig. 1 a, probe B), and it does not cross-hybridize to other members of the alkali light chain gene family (Lyons et al., 1990a). In agreement with our RT-PCR observations, the endogenous MLC3F gene is expressed at higher levels in atrial than ventricular myocardium (Fig. 7 b); restriction of detectable levels of endogenous 3F transcript to the atria occurs between 12.5 and 14.5 d p.c. (Fig. 7, c and d). The low level of MLC3F ventricular expression at later stages precludes the use of in situ hybridization to analyze whether, like the 3F-nlacZ-E transgene, the endogenous 3F transcript shows right ventricular exclusion. Before 14.5 d, however, MLC3F expression is regionalized in the developing ventricles (Fig. 7 c). Since the sequence complementary to the MLC3F probe lies within the first intron of the primary MLCIF transcript, the probe can cross-hybridize to unprocessed MLCIF transcripts (see Fig. 1 a). Using a second probe complementary to unspliced MLCIF transcripts (within the first intron, 2 kb upstream of the MLC3F promoter; Fig. 1 a, probe A), we can differentiate between unprocessed MLCIF and MLC3F transcripts. At 14.5 d p.c., unprocessed MLCIF transcripts are readily detected in skeletal but not in cardiac muscle (Fig. 7, f and g), while the MLC3F probe hybridizes to transcripts in both muscle types, showing that the signal observed in the heart is specific for the 3F transcription unit (Fig. 7, d and e). Low levels of processed MLCIF transcripts were in fact detected in adult cardiac muscle by RT-PCR (Fig. 6 c); these transcripts, like MLC3F transcripts, were more abundant in skeletal muscle (Fig. 6 a).

Figure 6. Endogenous MLC3F transcript is expressed in the heart. By RT-PCR, MLC3F transcripts are detected in RNA isolated from adult hearts, although they are present at a lower level than in skeletal muscle RNA (a). In contrast, α-cardiac actin is represented at a higher level in cardiac than skeletal muscle RNA. PCR amplifications and Southern blot hybridization with an MRF4–specific probe (b). Expression of the MLCIF and 3F transcripts varies among the four compartments of the heart (c); both are present at higher levels in atrial than ventricular RNA. S16 ribosomal protein message, in contrast, is constant in the four compartments. After 15, 20, and 25 cycles of PCR reaction, products were Southern blot hybridized to either 3F or 1P probes, and filters were washed under conditions of high stringency (0.1x SSC). L, left; R, right; LV, left ventricle; RV, right ventricle, LA, left atria; RA, right atria; S, skeletal muscle; H, cardiac muscle.

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Figure 7 In situ hybridization to endogenous MLC3F transcripts. Bright- (a) and dark-field (b) micrographs of a section through a nontransgenic mouse heart at 10.5 d after in situ hybridization with an antisense MLC3F riboprobe (Fig. 1 a, probe B) showing 3F transcripts in both atrial and ventricular compartments. At 12.5 d p.c., ventricular MLC3F expression is both reduced and regionalized (c). By 14.5 d, MLC3F expression in the heart, as detected by in situ hybridization, becomes restricted to both right and left atria (d and e). An antisense probe complementary to sequences in the first intron of the MLC1F/3F gene (Fig. 1 a, probe A) detects abundant unprocessed MLC1F message at 14.5 d p.c. in developing skeletal muscle, but not in cardiac muscle (f and g). Note the punctate signal obtained with this probe, which hybridizes to unspliced nuclear transcripts. P, pericardium; V, ventricle; A, atria; RV, right ventricle; LV, left ventricle; RA, right atria; LA, left atria; D, diaphragm; T, tongue; IC, intercostal muscles. Bars, 100 μm.
atrial than ventricular RNA. By in situ hybridization, however, MLC1F transcripts could only be detected in the heart at early stages of development (before 10.5 d, data not shown).

**Discussion**

In this study, we demonstrate that the MLC3F promoter and 3' enhancer are sufficient to confer strong muscle-specific expression and fast fiber-type distribution on a nlacZ reporter gene in transgenic mice. Correct temporal activation of the MLC3F promoter, however, is not observed. We have revealed an additional level of regulation at this complex locus, showing that the MLC3F promoter is transcribed in the embryonic and adult mouse heart, describing a novel pattern of cardiac gene activity.

The mouse MLC1F/3F 3' enhancer drives strong differentiation-specific expression from the mouse MLC3F promoter in cultured muscle cells, confirming in vitro experiments carried out with the rat gene (Garfinkel and Davidson, 1987), and with the human enhancer on the rat promoter (Rosenthal et al., 1990). We subsequently analyzed the expression of the MLC3F promoter/3' enhancer construct in transgenic mice. During embryogenesis, the 3F-nlacZ-E transgene is activated in muscle cells at 9 d p.c., 4–5 d before the endogenous MLC3F transcript first appears (Lyons et al., 1990a). In IF-CAT-E transgenic mice, CAT expression is first detected in the myotome at 9–9.5 d p.c. (Grieshammer et al., 1992), as is the endogenous MLC1F gene. Reporter gene expression also initiates at the same time in the developing premuscle masses of the limb in 1F and 3F transgenic mice (11.5 d, this study and Grieshammer et al., 1992). Thus, while the 3' enhancer confers correct temporal regulation on the MLC1F promoter in vivo, and is clearly an important element for strong muscle-specific expression in vitro and in transgenic mice, it is not sufficient to activate MLC3F expression in vivo at the correct time. Furthermore, late activation of MLC3F transcription does not appear to be the result of developmental specificity residing in the proximal 3F promoter. Our observations contrast with findings at the β-globin locus, where a major part of the developmental specificity of the β-globin genes resides in the proximal promoters; for example, fetal isoforms are repressed in the adult by factors binding in their promoter regions, the common locus control region subsequently activating distally located adult isoforms (reviewed in Dillon and Grosfeld, 1993).

How the late activation of MLC3F transcription is controlled remains unclear, although we envisage two possible mechanisms: (a) that the 3' enhancer does not normally interact with the 3F promoter, and in our transgenic mice may override MLC3F regulatory signals, perhaps in the absence of a favored interaction with the MLC1F promoter, which is not included in the construct. Note, however, that in the endogenous locus, the MLC1F and 3F promoters are, respectively, 25 and 15 kb from the 3' enhancer. (b) Alternatively, or in combination with (a), there may be additional regulatory elements in the MLC1F/3F locus that are required for late MLC3F activation and are not included in the construct. We have identified a region of 800 nucleotides in the first intron of the MLC1F/3F gene that has myotubeline-specific enhancer activity and interacts preferentially with the MLC3F promoter in vitro. This intronic region is, therefore, a candidate sequence that may be required for correct MLC3F expression in vivo (Kelly, R., S. Alonso, A. Schneider, S. Tajbakhsh, and M. Buckingham. 1994. J. Cell. Biochem. 18D:499 [Abstr.]). The analysis of additional transgenic lines to address this question is underway.

Early developmental misregulation of the 3F-nlacZ-E transgene is contrasted by later downregulation in muscle types known to contain low levels of MLC3F protein. In adult transgenic mice, low β-gal levels are seen in the soleus and diaphragm, although both muscles express the transgene at high levels in utero. Other muscles, such as the tibialis anterior, continue to express high levels of β-gal in the adult. These changes correlate with the development of adult fiber-type diversity. Biochemical, physiological, and histological properties vary between muscle fibers, which can be subdivided on the basis of which MHC isoform they express (see Pette and Staron, 1990; Schiaffino and Reggiani, 1994). Fiber type content varies among different muscles, such that mouse soleus, for example, contains roughly equal proportions of slow (type I) and fast (type II, A×X) fibers (Lewis et al., 1982; Donoghue et al., 1991b), while the tibialis anterior contains >90% fast fibers (Donoghue et al., 1991b). The region of the gastrocnemius laterally adjacent to the plantaris muscle, where the transgene is also downregulated, is, like the soleus, rich in fibers containing type I and IIA MHC, and lacks glycolytic fibers containing type IIB MHC. Similarly, the diaphragm is rich in types I, IIA, and IIX(D) fibers, and devoid of type IIB fibers (Zardinl and Parry, 1994). These data suggest that (a) the 3F-nlacZ-E transgene is expressed preferentially in type II (fast) fibers, and (b) nlacZ expression is higher in a subset of type II fibers (most likely IIB). The 3' enhancer and MLC3F proximal promoter sequences included in the transgene are therefore sufficient to confer fast fiber-type specificity. Similar results were obtained by Donoghue et al. (1991b) in IF-CAT-E transgenic mice, which express CAT at a higher level in fast than slow fibers; furthermore, CAT levels vary among type II fiber subtypes in the order IIB>IIX(D)>IIA. The core enhancer element common to 1F-CAT-E and 3F-nlacZ-E transgenic mice may therefore be involved in selective fiber-type expression. Hallauer et al. (1993) also reported a IIB>IIA>I pattern of expression of a fast quail troponin I-lacZ construct in transgenic mice; in this case, the endogenous mouse gene was expressed evenly in all fast (type II) fibers. In contrast, a lacZ reporter gene under the control of the Pgk-1 promoter is expressed in fast fibers containing IIA or IIX(D) MHC, but not in type IIB fibers or slow type I MHC fibers (McBurney et al., 1994). Distinct cis-regulatory elements are thus likely to direct gene expression in different fast fiber types (see Schiaffino and Reggiani, 1994). We are currently comparing the pattern of transcription of the endogenous MLC3F gene with that of the 3F-nlacZ-E transgene in particular fiber types at a more detailed cellular level.

The low level of transgene expression observed in the adult diaphragm in 3F-nlacZ-E mice (>90% IIA/X(D) fibers, Zardinl and Parry, 1994) is consistent with our observations in crural muscles. Interestingly, a subpopulation of nuclei in diaphragm fibers continues to express elevated transgene levels at focal synaptic and terminal myotendinous junctions, both regions being marked by elevated acetylcholinesterase activity (in the myotendinous junction at 20% of levels at the
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expression, yet the CAT gradient has been shown to be di-
of reporter gene expression, which suggests that the trans-
gene responds to intrinsic differences in positional informa-
tion along the body axis (Donoghue et al., 1991a). The
endogenous MLC1F gene, however, is not graded in ex-
pression, yet the CAT gradient has been shown to be di-
rected by sequences contained within the MLC-derived
fragments. It is not dependent on the reporter gene since a
gradient of expression is observed when CAT is subtituted
by either the acetylcholine receptor \( \gamma \)-subunit cDNA (Do-
 noghue et al., 1991a) or lacZ (Donoghue, M., J. Merlie, and
J. Sanes, personal communication). 3F-nlacZ-E mice
provide a second transgenic mouse strain containing regula-
atory sequences derived from the 1F/3F locus, and it was thus
of interest to determine whether the nlacZ gene is expressed
in a positionally graded manner in these mice. We have
demonstrated in this study that \( \beta \)-gal protein and mRNA lev-
els are not positionally graded in the intercostal muscles of
3F-nlacZ-E transgenic embryos, at a stage when the CAT
gradient is evident in IF-CAT-E mice. Our results show that
the MLC3F promoter and core 3' enhancer do not confer a
positional gradient of reporter gene expression in transgenic
mice, and they suggest that the cis-acting sequences respon-
sible for the gradient in IF-CAT-E mice lie outside the core
3' enhancer.

The MLC1F and 3F proteins are restricted to skeletal stri-
muscle (see Barton and Buckingham, 1985). Two other mouse alkali MLC isoforms are expressed in both skeletal
and cardiac muscle: MLCIV in slow skeletal fibers and adult
ventricle, and MLCIA in embryonic and fetal skeletal mus-
cle and adult atria (Barton et al., 1985a, b). We have now
demonstrated that both transgene directed and endogenous
MLC3F transcription occurs in cardiac muscle. Cardiac
MLC3F expression is likely to depend on regulatory se-
quencies in the MLC3F promoter region, since, in contrast
to the 3F-nlacZ-E transgene, the 3' enhancer does not drive
CAT expression in IF-CAT-E transgenic mouse hearts (Ro-
senthal et al., 1989). Furthermore, MLC3F transgenic mice
lacking the 3' enhancer express a high level of nlacZ in the
heart (Kelly, R., S. Alonso, and M. Buckingham, unpub-
lished observations).

3F-nlacZ-E transgene expression follows a previously un-
documented pattern of cardiac gene expression. As early as
8.5 d of development, at the tubular heart stage, transgene
expression is regionalized such that the outflow tract and a
region of the common ventricle (future right ventricle) are
nonexpressing. The 3F-nlacZ-E transgene thus provides an
early marker for positional specification in the developing
mouse heart, and it should help to address the question of
whether such spatial restriction arises from distinct lineages
of committed cardiac precursors, or by positional cues. The
two major cardiac alkali myosin light chain genes are, in con-
trast to the 3F-nlacZ-E transgene, widely expressed in the
myocardium at early stages, and only later in embryogenesis
do they become restricted to atrial or ventricular compart-
ments (Lyons et al., 1990b). MLC2V, however, the ventricu-
lar regulatory myosin light chain, shows earlier regionaliza-
tion to the ventricular compartment (by 11 d, O'Brien et al.,
1993). While many cardiac genes have been documented to
be expressed in atria or ventricles, few have been described
which demonstrate left/right compartmental differences.
Two examples of which we are aware are (a) atrial natriuretic
peptide, for which low level ventricular expression in the
adult (1% of atrial expression levels) is stronger in the left
than right ventricle (Gardner et al., 1986); (b) muscle crea-
tine kinase (MCK), which shows transient left/right asym-
metry with transcripts first appearing in the wall of the right
ventricle of the 12.5-d mouse heart; by 15.5 d, MCK tran-
scripts are expressed at a high level in all cardiac myocytes
(Lyons, 1994). Our results suggest that transcriptional differ-
ences exist between right and left ventricular compartments,
and that such differences precede septation (complete by 13
d) and associated secondary physiological constraints. Fur-
thermore, left/right transcriptional differences are mediated
by sequences included in the 3F-nlacZ-E construct.

By both RT-PCR and in situ hybridization we have shown
that our observations in transgenic mice reflect low level
transcription of the endogenous MLC3F gene in the mouse
heart. MLC3F expression is compartmentalized, with higher
transcript levels in atrial than ventricular RNA. Endogenous
MLC3F transcripts in embryonic hearts show some ventricu-
lar regionalization, but in adult hearts, in contrast to nlacZ
expression in transgenic mice, no left/right asymmetry is
evident; the cause of this difference remains unclear. The
low level of MLC3F transcription in the heart is likely to explain
why cardiac MLC3F expression was not previously observed
(Barton and Buckingham, 1985). Although the low level of
MLC1F protein has been reported in embryonic cardiac mus-
cle cells in the chick (Obinata et al., 1983), there is no evi-
dence for the presence of IF or 3F MLC isoforms in mam-
alian cardiac muscle (Whalen et al., 1982). Reexamination
of MLC protein isoforms present in the adult heart on silver-
stained two-dimensional gels revealed no trace of MLCIF
or MLC3F proteins either in whole mouse heart or in left
and right atrial and ventricular protein preparations (Butler-
Browne, G., V. Moully, F. Edom, R. Kelly, S. Alonso, and
M. Buckingham, unpublished data). Therefore, it seems un-
likely that MLC3F transcription in the heart is of functional
importance since MLC3F protein, if present, is at an ex-
tremely low level compared with that of the major cardiac
alkali MLC isoforms, MLCIA and MLCIV. It is possible that
cardiac MLC3F transcripts are subject to posttranscriptional
control. This would not be without precedent: Gorza et al.
(1993) have demonstrated that rat cardiac troponin I mRNA
accumulates in the ventricle of developing rat hearts from
day 11, whereas the protein is not detected in the ventricles
before day 18.

While a number of cardiac muscle isoforms are transiently
expressed during skeletal muscle development (including
MLCIA and \( \alpha \)-cardiac actin), there are also examples of
skeletal muscle protein isoforms that are transiently expressed
in the developing heart: these include \( \alpha \)-skeletal actin (Sas-
soon et al., 1988) and MLC2F (Faerman and Shani, 1993).
Our results extend the known overlap in genes activated at
early stages of both cardiac and skeletal myogenesis, and
they suggest that transcriptional activation requirements for both striated muscle types are shared by many muscle-specific genes.

Note added in proof. Similar results showing ML C3F expression in the heart have been obtained by Michael McGrew and Nadia Rosenthal (Cardiovascular Research Center, Massachusetts General Hospital, Charlestown, MA), according to a personal communication.

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