A Novel Site in the Muscle Creatine Kinase Enhancer Is Required for Expression in Skeletal but Not Cardiac Muscle*

Expression of the muscle creatine kinase (MCK) gene in skeletal and heart muscle is controlled in part by a 5′ tissue-specific enhancer. In order to identify new regulatory elements, we designed mutations in a previously untested conserved portion of this enhancer. Transfection analysis of these mutations delineated a new control element, named Trex (Transcriptional regulatory element χ), which is required for full transcriptional activity of the MCK enhancer in skeletal but not cardiac muscle cells. Gel mobility shift assays demonstrate that myocyte, myoblast, and fibroblast nuclear extracts but not primary cardiomyocyte nuclear extracts contain a trans-acting factor that binds specifically to Trex. The Trex sequence is similar (7/8 bases) to the TEF-1 consensus DNA-binding site involved in regulating other muscle genes. To determine if TEF-1 interacts with Trex, selected TEF-1 binding sites such as GTT1c and M-CAT and two anti-TEF-1 antisera were used in gel shift assays. These experiments strongly suggest that a factor distinct from TEF-1 binds specifically to Trex. Thus it appears that MCK transcription is regulated in skeletal muscles through a Trex-dependent pathway while Trex is not required for MCK expression in heart. This distinction could account partially for the difference in levels of muscle creatine kinase in these tissues.

The differentiation of embryonic mesodermal cells into skeletal or cardiac muscle cells requires the controlled expression of numerous muscle-specific genes (1–5). Because the muscle creatine kinase (MCK)1 gene is expressed at different levels in mature skeletal and cardiac muscle cells (6), it is an instructive model for comparing the mechanisms that regulate expression of the same muscle-specific gene in both striated muscle tissues. The current study identifies a new regulatory element within the MCK enhancer, which exhibits such differential activity in skeletal and cardiac muscle cells.

Expression of the MCK gene in differentiated muscle cells is controlled by an array of cis-elements and trans-acting factors. Particular attention has been given to the analysis of a 206-bp upstream enhancer, which confers muscle-specific expression both in cultured cells and in transgenic mice (7–9). Mutational analysis, gel mobility shift studies, and footprinting assays have identified six transcriptional regulatory elements in the MCK enhancer (from 5′ to 3′): the CArG, AP2, A/T-rich, left and right E boxes, and MEF-2 sites (6, 10, 11). Each of these MCK gene control elements has been well conserved during mammalian evolution (7, 12–14), and similar nucleotide motifs have been implicated in the transcriptional control of numerous other muscle-specific genes (15).

Mutating these six MCK enhancer control elements causes different relative effects in cultured cardiomyocytes and skeletal muscle cells (6). Mutation of the MCK right E box site decreases enhancer activity more dramatically in skeletal than in cardiac muscle cells, while mutations of the CArG site or the A/T-rich site seem more deleterious in cardiac than in skeletal muscle cells. In contrast to these differential effects, mutations of the MEF-2 site or the left E box site decrease enhancer activity to about the same extent in both cell types. Unlike the other MCK enhancer control elements, the AP2 site seems to repress transcription in cultured skeletal and cardiac muscle cells as its mutation leads to increased expression in both cell types.

These six enhancer regulatory elements are the target sites for an array of muscle and non-muscle-specific DNA binding factors. The left and right E boxes contain the consensus core sequence CANNTG, which is the target for skeletal muscle-specific determination factors of the MyoD family of transcription factors (16). These two E boxes, which differ in their flanking regions, exhibit noticeably different transcriptional activities and protein binding properties (17, 18). Although E boxes are involved in the cardiac regulation of MCK as well as a number of other cardiac muscle genes (19–23),2 MyoD family members are not detected in the heart at any time during development (4). The MEF-2 site and the A/T-rich site are both rich in adenine and thymidine (25, 26). The MEF-2 site is the target of MEF2 proteins, which are also known as the Related to Serum Response Factor (RSRF) family (27, 28), and BBF-1, a serum-inducible factor identified in cardiac but not in skeletal muscle (29). MEF2 proteins have been shown to cooperate in transactivation with members of the MyoD family of transcription factors (30–33) and bind with lower affinity to the MCK A/T-rich site (34). The mouse MCK A/T-rich element is also a DNA-binding site for both the homeoprotein M-Hox and the ubiquitous Oct-1 factor (26, 34). The CArG motifs have been shown to bind serum response factors (28).

The MCK enhancer does not contain all of the regulatory elements identified in other muscle genes. For example it does not contain functional Sp1 and CCAC sites found in regulatory regions of the α-actin (22) and myoglobin genes (35). The MCK enhancer also appears to contain no perfect match to the M-CAT sites, key elements in regulating the cardiac troponin T

1 The abbreviations used are: MCK, muscle creatine kinase; bp, base pair(s); MCBF, M-CAT binding factors; CAT, chloramphenicol acetyltransferase; TEF-1, transcriptional enhancer factor 1.

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(36), α- and β-myosin heavy chain (21, 37–40), and skeletal α-actin genes (41), and targets for MCBF (M-CAT binding factors) and the ubiquitous TEF-1 factor (42).

M-CAT sites were of particular interest to the present study because a newly identified MCK enhancer control element called Trex (Transcriptional regulatory element x) bears close similarity to the consensus MCBF/TEF-1-binding sites in other genes. We show here that the MCK enhancer Trex site is required for full transcriptional activity of the enhancer in MM14 skeletal muscle cell cultures. However, mutations in the Trex site that greatly reduce MCK reporter gene expression in these cultures exhibit virtually no effect in heart cell cultures. Furthermore, despite its similarities to the consensus TEF-1-binding site, we show that the Trex site is not a target for TEF-1, nor do previously identified TEF-1-binding sites (such as M-CAT or the SV40 enhancer GTIIc site) compete with the Trex sequence for specific Trex binding. A Trex-specific binding complex has been identified in MM14 skeletal myocyte, MM14 myoblast, NIH-3T3 fibroblast, and hybridoma cell nuclear protein extracts. However, no such complex was identified in nuclear protein extracts from neonatal cardiomyocytes. Trex may thus be partially responsible for the differential expression of MCK and potentially other genes in skeletal versus cardiac muscle cells.

MATERIALS AND METHODS

Plasmids—Plasmids (+ enh)80MCKCAT, (+ enh)80MCKCAT, 1256-MCKCAT, encoding chloramphenical acetyltransferase, and pUCSV2-pap, encoding the human placental alkaline phosphatase, have been described previously (6). Mutagenesis was performed by oligomer-mediated site-directed mutagenesis (43) with oligonucleotides synthesized as described previously (6). Mutagenic sites were introduced in 10-μl volumes. 1–2 μg of nuclear protein extracts were mixed with 10 μg of bovine serum albumin in 10 μl Tris-C1, pH 7.4, 0.1 mM NaCl, 0.1 mM EDTA, 0.5 μM dithiothreitol, 0.3 mM MgCl2, 0.5 mM phenylmethylsulfoxyl fluoride, and 4% glycerol and incubated with or without unlabeled oligonucleotide competitor at room temperature for 15 min prior to addition of 0.15 ng of oligonucleotide probe plus 360 ng of poly(d-I-d-C) as nonspecific competitor. The incubation was then continued for 10 min at room temperature prior to loading the mixture onto the gel. For antibody supershift experiments, 1 μl of TEF-1-antiserum replaced the competitors. Two TEF-1-specific antisera (50), denoted as N and C, were kindly provided by Drs. Irvin Davidson and Pierre Chambon (Institut de Chimie Biologique, INSERM-U.184, Strasbourg, France). N and C were prepared against an amino-terminal polypeptide (amino acids 16–25) and the carboxyl-terminal portion of TEF-1 (amino acids 167–426), respectively. Protein-DNA complexes were separated on a 4% (240:8) polyacrylamide gel in 0.5 X TBE buffer (51) at 20 V for 2–3 h. Gels were dried and exposed to Hyperfilm (Amersham Corp.). The sequences of the oligomers used for gel mobility shift assays were as follows: Trex, GGAGCGGTAGCTGCGTGTT; M-CAT, CGTGTTGCATTCCTCTCTGGATC. The underlined sites in M1 and TrexG-C indicate mutated bases, while in GTIIc and M-CAT they highlight consensus binding sites. M-CAT and GTIIc oligonucleotides (42) were generously provided by Dr. J anet Mar (Department of Internal Medicine, University of Texas, Houston, TX).

RESULTS

MCK Enhancer Sequence Alignment—Nucleotide sequence comparisons of the MCK gene 5′ enhancers from mouse (7), rat (12), human (13), and rabbit (14) identify regions that appear to have been conserved during mammalian evolution and which may therefore be important for enhancer function. Fig. 1A shows the alignment of 65 bp between the mouse MCK enhancers—residues 1241, and 65 bp, 74% are conserved across the four species. While the mouse and rat sequences are the most similar, even the mouse and human sequences are 88% identical. In comparison only 49% of the entire 206-bp enhancer is identical between the same four species. Interestingly, nucleotide alignment of the four mammalian MCK enhancers identifies an additional highly conserved region located between the AP2 and A/T-rich sites (Fig. 1A). Due to its high degree of evolutionary conservation and to its sequence similarity to the M-CAT control elements of other muscle genes, this region (subsequently named “Trex” for Transcriptional regulatory element x) was analyzed for its transcripational activity.

Effects of Trex Region Mutations on MCK Reporter Gene Expression in Skeletal and Cardiac Muscle Cell Cultures—To investigate the possibility that an additional regulatory site exists between the AP2 and A/T-rich sites, a 10-nucleotide mutation (M1) was constructed to test this region (Fig. 1B). The mutation was carried in constructs shown in Fig. 1C and tested by transient and stable transfection assays of MM14 skeletal muscle cells and by transient transfections of rat primary cardiomyocytes (Fig. 2). In the context of the full-length promoter 1256MCKCAT, mutation M1 decreased enhancer activity 2–4-fold in both transiently and stably transfected MM14 myocyte cultures (Fig. 2A). In contrast, the 1256MCKCAT enhancer construct
Fig. 1. Muscle creatine kinase gene enhancer. A, partial nucleotide sequence comparison of the mouse (7), rat (12), human (13), and rabbit (14) MCK enhancers. Residues are compared with the mouse sequence, and identical nucleotides are indicated by a dot. Known regulatory sites are labeled in boldface. Non-conserved residues are indicated in lowercase letters. Gaps have been introduced to optimize the alignment. The Trex region is underlined. B, mutations introduced into the mouse MCK enhancer Trex region are indicated in boldface. The 22-bp oligonucleotide TrexG-C containing a single underlined base pair change is also indicated. C, constructs tested in mutational analysis. The enhancer-containing plasmids are shown schematically with known regulatory sites in stippled boxes and introduced mutations in black boxes. E indicates E box motifs. Each mutation (M1, M2, and M3) was tested separately. 1256MCKCAT is composed of the 206-bp enhancer with the −1050 through +74-nt promoter fused to a CAT gene. (+en)80MCKCAT and (−en)80MCKCAT are composed of the 206-bp enhancer in its native, (+), or reversed (−) orientation linked to the −80-bp basal MCK promoter and fused to CAT.

bearing the M1 mutation was expressed at a similar level to the wild-type enhancer in rat primary cardiomyocyte cultures (Fig. 2A). These results suggest that mutation M1 disrupts a positive regulatory site that is required for full transcriptional activity of the enhancer in skeletal myocytes but not in cardiomyocytes. A more dramatic effect of mutation M1 was observed in constructs containing the enhancer, in either its normal (+) or reversed (−) orientation, fused to a truncated promoter (+en)80MCKCAT and (−en)80MCKCAT. In these contexts the M1 mutation caused a near 10-fold decrease in enhancer activity in both transient (Fig. 2B) and stable transfection assays of MM14 myocytes (data not shown). The greater relative effects of Trex site mutations when tested in (+en)80MCKCAT versus 1256MCKCAT contexts may be due to compensatory effects of sequences in the −1050 to −81-bp region contained in the later construct; however, no Trex site has been identified in this region.

To determine if Trex is involved in silencing MCK gene expression in cells that do not express MCK, NIH-3T3 fibroblasts and MM14 myoblasts were transfected with 1256MCKCAT constructs. As expected, the wild-type MCK enhancer construct was expressed at a low background level (data not shown), consistent with the lack of endogenous MCK expression in these cell types. Mutation M1 did not result in elevated levels of reporter gene expression, indicating that Trex is not responsible for silencing the MCK gene in fibroblasts and myoblasts.

To further delineate the Trex site the sequence between the AP2 and A/T-rich sites was examined via two smaller mutations, M2 and M3 (Fig. 1B). Mutation of bases in the 5'-region of the M1 mutation (mut M3) had no effect on expression of the (enh)80MCKCAT reporter gene when tested in either the positive or negative enhancer orientation (Fig. 2B). These results indicate that none of the residues modified by M3 are critical parts of Trex. In contrast, disruption of bases within the more 3'-sequence by mutation M2 impaired expression severely, reducing the enhancer function about 8-fold (Fig. 2B). Since the nucleotide substitutions made in mutation M2 differ markedly from those made in mutation M1, it seems very unlikely that both mutations would create negative regulatory sites with similar repressor activity. The most straightforward interpretation is that the M1 and M2 mutations both disrupt the same positive control element.

Transfections of MM14 myocytes or 3T3 fibroblasts with either −80MCKCAT or a multimerized Trex sequence ligated to the basal MCK promoter, (Trex)3 ATCAT, result in background levels of CAT gene expression (data not shown), indicating that four copies of the Trex site are not sufficient to promote transcription. Therefore, the Trex site appears to require additional regulatory elements in order to confer transcriptional activity to skeletal muscle genes.

The Trex Site Exhibits Specific Nuclear Protein Binding—DNA-protein binding experiments were used to characterize trans-acting factors that interact with the MCK Trex site. A 22-bp Trex oligonucleotide was bound in gel mobility shift assays by nuclear proteins from MM14 skeletal myocytes, myoblasts, and NIH-3T3 fibroblasts (Fig. 3, lanes 2, 5, and 8) as well as by nuclear extracts from cardiac fibroblasts and F5D hybridoma cells (data not shown). The binding of nuclear proteins was sequence-specific as assessed by competition with excess unlabeled oligonucleotides bearing the wild-type Trex sequence (Fig. 3, lanes 3, 6, and 9) and the lack of competition by excess oligonucleotides bearing the functionally inactive M1 sequence (Fig. 3, lanes 4, 7, and 10). A similar competition
CATGCTT) located in the SV40 enhancer A domain (52). The lined mismatches, with the high degree of sequence similarity, except for the two underlined base pair. Several complementary approaches have proposed a consensus TEF-1-binding site (A/G)CATNC(C/T)(T/A) that differs from the Trex site by the single underlined base pair. Several complementary approaches were thus used to determine whether TEF-1 is indeed the Trex binding factor.

Gel mobility shift assays showed that the specific complex formed by Trex and nuclear proteins from MM14 myocytes was not abolished by competition with unlabeled GTIIc site or M-CAT site oligonucleotides (Fig. 4A, lanes 2-4 and lanes 5-7). Similarly, this complex persisted upon addition of a 22-mer oligonucleotide (Fig. 1B, Trex G-C) containing a 1-base pair mutation that converts the Trex site into a sequence equivalent to the TEF-1 consensus sequence (Fig. 4B, lanes 17-19). Cross-completion studies showed that binding to GTIIc, Trex G-C, or to M-CAT probes was not altered by addition of unlabeled Trex oligonucleotide (data not shown). Since binding to Trex is not altered by increasing amounts of known TEF-1-binding sites, it appears that TrexBF is not TEF-1.

Further evidence that the TrexBF differs from TEF-1 was obtained by immunological studies using anti-TEF-1 sera that recognize both human and mouse TEF-1 (40). Addition of either N-terminal or C-terminal anti-TEF-1 polyclonal antisera to MM14 nuclear extracts produced no change in migration of the Trex binding complex (Fig. 5, lanes 6 and 7). However, both antisera caused a supershift when the same skeletal muscle extracts were combined with GTIIc oligonucleotides (Fig. 5, lanes 2 and 3). As a control, no supershift was observed upon addition of nonimmune serum (Fig. 5, lanes 1 and 5). This result indicates that MM14 nuclear extracts contain TEF-1 or a TEF-1-related protein that can participate as part of a GTIIc binding complex. However, the DNA binding activity defined as TrexBF is not TEF-1.

Taken together these results indicate that both TEF-1 and a previously unrecognized factor, TrexBF, are present in MM14 nuclear extracts. However, despite the close sequence similarity between the MCK Trex site and the TEF-1-binding sites, TrexBF is not TEF-1.

The Trex Binding Factor Is Not Present in Cardiomyocyte Nuclear Extracts—Results from transfection experiments showed that the Trex region is not necessary for cultured cardiomyocytes to express 1256MCKCAT; yet, TrexBF could be
present in cardiomyocytes. To test this hypothesis, cardiomyo-
cytenuclearextractswereusedingelmobilityshiftassays.No
shifted bands were observed when cardiomyocyte nuclear pro-
teinextractswereaddedtothelabeledTrexprobe(Fig.6,lanes
1-3), suggesting that no cardiomyocyte nuclear-protein recog-
nizes and binds to the Trex sequence. To ascertain the quality
of the cardiac nuclear extracts, the presence of other specific
binding factors in these extracts was investigated using a
TrexG-Cprobe(Fig.6,lanes4–7)andthepreviouslydescribed
MCK CArG site (data not shown) (6). Two DNA-protein com-
plexes were formed between cardiomyocyte nuclear proteins
andtheTrexG-C-labeledprobe(Fig.6,lane4).Bothcomplexes
were competed by the addition of a 100-fold molar excess un-
labeled Trex G-C sequence (Fig. 6, lane 5) or GTIIc sequence
(Fig. 6, lane 6). Conversely, neither complex was abolished by
addition of unlabeled wild-type Trex sequence (Fig. 6, lane 7).
These results suggest that cardiomyocyte nuclear extracts do
not contain any TrexBF and confirm that they contain intact
binding proteins for the TEF-1 consensus site (40, 42).

To examine the possibility that cardiomyocyte extracts con-
tain an inhibitor that prevents binding to Trex, up to a 3-fold
excess of cardiomyocyte nuclear extract was added to a skeletal
extract and tested in gel mobility shift assays. In no case was
there a specific reduction of skeletal muscle extract binding to
Trex (data not shown), indicating that no inhibitor of Trex
binding exists in these extracts.

**DISCUSSION**

A Novel Regulatory Element, Trex, Is Required for Full Trans-
scriptional Activity of the MCK Enhancer in Skeletal Myo-
cytes—This study has identified a novel cis-acting regulatory
element, Trex, within the MCK enhancer. Transfection anal-
yses in MM14 myocytes indicate that the Trex site functions as
a positive control element. The relative loss in skeletal muscle
transcriptional activity due to Trex mutation M1 in 1256MCK-
CAT is comparable with the loss of activity resulting from
mutations in the MEF2 site, the left E box, or the CArG MCK
enhancer elements (6). The same Trex mutation neither de-
creased nor increased the low level of reporter gene expression
in proliferating myoblasts or NIH-3T3 fibroblasts. This sug-
MCK Trex Site

| PROBE    | Trex | TrexG-C |
|----------|------|---------|
| EXTRACT  | CARDIOMYOCYTES |
| COMPETITOR | - Trex M1 - Trex G-C GTIC Trex |

Fig. 6. Trex-specific binding activity is undetectable in cardiac myocyte nuclear extracts. Nuclear extracts from primary rat cardiomyocytes were mixed with 5'-labeled Trex oligonucleotide (lanes 1-3) or TrexG-C, a single base pair mutant Trex oligonucleotide that conforms to the TEF-1 consensus binding sequence (lanes 4-7). No nuclear proteins from the cardiomyocyte extract form a shifted complex with the Trex probe (lanes 1-3). In contrast, two DNA-protein complexes formed between cardiac proteins and the TrexG-C labeled probe (lane 4). Both complexes are competed by addition of a 100-fold molar excess unlabeled Trex G-C sequence (lane 5), as well as by excess GTIIC sequence (lane 6). Neither complex is abolished by addition of excess unlabeled wild-type Trex sequence (lane 7).

suggests that the Trex site and associated factors do not act as an inhibitor of MCK gene expression in myoblasts or non-muscle cells. When examined in the (enh)80MCK CAT configurations, mutation M1 was even more deleterious than mutations in most of the other enhancer elements and nearly as deleterious as mutations of the right E box. The relative effects of the smaller overlapping mutations M2 and M3 suggest that the crucial Trex bases are located within the central and 3'-portion of the conserved Trex region.

Trex Is Inactive in Cardiomyocytes—Results from transient transfection assays of primary rat cardiomyocytes indicate that Trex does not function as a positive control element in cardiac muscle cells since the M1 mutation has no significant effect on reporter gene expression (Fig. 2A). This finding is consistent with the observation that TrexBF is undetectable in cardiomyocyte nuclear protein extracts (Fig. 6), most likely due to its absence in these cells. However, an alternative explanation is that TrexBF is present in cardiomyocyte extracts but post-transcriptionally modified and thereby unable to bind DNA. Behavior of this type has been reported for MyoD, which is present in skeletal muscle myoblasts (53) and yet unable to bind control elements such as the MCK enhancer right E box (MEF-1) site (10). This inhibition appears to be due to members of the Id family, which inhibit differentiation by sequestering E proteins (E12/E47), thereby preventing MyoD family members from forming DNA-binding heterodimers (54, 55). The possibility that cardiomyocyte extracts might contain an inhibitor that prevents TrexBF binding was tested by adding increasing amounts of cardiac muscle nuclear extracts to skeletal muscle extracts in gel mobility shift assays. No inhibition of skeletal muscle TrexBF binding was detected using this strategy. Taken together, our data are consistent with the hypothesis that TrexBF is not present in cardiomyocytes and with the corollary that the MCK Trex site is functionally silent in these cells.

TrexBF Is Present in Cultured Skeletal Muscle and in Non-muscle Cells—Trex binding complexes of similar mobility are observed in skeletal myocyte, myoblast, and fibroblast nuclear extracts (Fig. 3). Although the Trex site does not appear to be active on its own, the presence of TrexBF among proliferating and differentiated skeletal muscle cells as well as among several non-muscle cell types suggests that TrexBF may also be involved in regulating non-muscle genes. Why Trex participation in such control mechanisms should be precluded in cardiomyocytes is unclear. However, the requirement for Trex seems to correlate with the higher levels of MCK gene expression in skeletal versus cardiac muscle cells (6).

Although TrexBF is present in various cell extracts, its activity has not been defined. TrexBF's activity could be regulated in these cell types through several mechanisms, for example by post-translational modification (e.g. phosphorylation) or by interaction with ubiquitous or cell-type specific accessory factors. Considering the fact that TrexBF activity is found in both replicating and postmitotic MM14 cells, mitogen-regulated phosphorylation is probably not involved in TrexBF activity. The alternative mechanism, involving protein-protein interactions, has been proposed for regulation of the cardiac troponin T gene. In this gene the ubiquitous TEF-1 factor binds to M-CAT control elements (42), but its full transcriptional activity requires intermediary factors, termed TIFs (50). Understanding the mechanisms involved in regulating TrexBF function awaits its cloning and characterization.

TrexBF Is Not TEF-1—The MCK enhancer Trex site exhibits sequence similarity with the consensus MCBF/TEF-1-binding site defined by the alignment of muscle gene M-CAT sites with TEF-1 binding motifs (42). In addition to their role in cardiac troponin T gene regulation (56), M-CAT motifs are also involved in regulating the α- (21, 37) and β-myosin heavy chain genes (39, 40, 57, 58), as well as skeletal α-actin (41). The possibility that the MCK enhancer Trex site could be M-CAT or another TEF-1-binding site was examined. Gel mobility shift assays using M-CAT and other TEF-1-binding sites as competitors as well as supershift experiments using two anti-TEF-1 antisera confirmed that TrexBF is not TEF-1. Interestingly, the amino-terminal-specific antisera to TEF-1 slightly diminishes formation of the TrexBF complex (Fig. 5, lane 6). Because the DNA-binding domain of TEF-1 (TEA domain) is located in its N-terminal region, it is possible that antisera N recognizes a TEA-like domain in TrexBF.

Five TEF-1 isoforms have been characterized (59). Although we cannot rule out the possibility that TrexBF is a novel TEF-1 isoform, this seems unlikely since neither TEF-1 antisera supershifted the TrexBF complex (Fig. 5). In addition, a single base pair mutation that converts the MCK enhancer Trex site to a consensus TEF-1-binding site does not compete for Trex binding (Fig. 4B). This suggests that association of TrexBF with the Trex site is very sequence-specific. Since a single amino acid substitution in a helix-loop-helix protein can change the protein specificity for bases flanking the core E box motif (60), it is possible that TrexBF and TEF-1 may have very similar structures but that slight changes are sufficient to alter their binding specificity. An additional distinction between TEF-1 and TrexBF is that TEF-1 is enriched in skeletal mus-
and cardiac troponin C, cardiac troponin T, myosin light chain 2, instance, distinct enhancer fragments control expression of the late tissue-specific expression of the same gene (3, 62). For muscle (35, 38). However, in many cases heart and skeletal tissue-specific differences in a gene’s expression level in each type of muscle. Indeed, factors that are common to both striated muscle types. Indeed, since combinatorial interactions of multiple cis-acting elements underlie the regulation of muscle-specific genes, the relative ratios of common factors could be responsible for the quantitative differences in a gene’s expression level in each type of muscle (35, 38). However, in many cases heart and skeletal muscle cells use alternative transcriptional strategies to regulate tissue-specific expression of the same gene (3, 62). For instance, distinct enhancer fragments control expression of the cardiac troponin C, cardiac troponin T, myosin light chain 2, and β-myosin heavy chain genes in cardiac versus skeletal muscle cells (24, 56, 63, 64). Here we present evidence that a novel MCK enhancer regulatory element, Trex, is required for skeletal muscle but not for cardiac muscle expression of the MCK gene. Thus it appears that MCK is expressed in skeletal muscle through a Trex-dependent pathway and in heart through a Trex-independent pathway. It will be instructive to determine both how TrexBF is activated in skeletal muscles and how this factor is restricted in cardiacomyocytes. It will also be important to extend our observations to transgenic mice as well as to evaluate the role of Trex in other muscle genes.

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The activation and steady state expression of the same genes in both skeletal and cardiac muscles could imply transcription factors that are common to both striated muscle types. Indeed, since combinatorial interactions of multiple cis-acting elements underlie the regulation of muscle-specific genes, the relative ratios of common factors could be responsible for the quantitative differences in a gene’s expression level in each type of muscle (35, 38). However, in many cases heart and skeletal muscle cells use alternative transcriptional strategies to regulate tissue-specific expression of the same gene (3, 62). For instance, distinct enhancer fragments control expression of the cardiac troponin C, cardiac troponin T, myosin light chain 2, and β-myosin heavy chain genes in cardiac versus skeletal muscle cells (24, 56, 63, 64). Here we present evidence that a novel MCK enhancer regulatory element, Trex, is required for skeletal muscle but not for cardiac muscle expression of the MCK gene. Thus it appears that MCK is expressed in skeletal muscle through a Trex-dependent pathway and in heart through a Trex-independent pathway. It will be instructive to determine both how TrexBF is activated in skeletal muscles and how this factor is restricted in cardiacomyocytes. It will also be important to extend our observations to transgenic mice as well as to evaluate the role of Trex in other muscle genes.