Elements Regulating Cardiomyocyte Expression of the Human Sarcomeric Mitochondrial Creatine Kinase Gene in Transgenic Mice*

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Sarcomeric mitochondrial creatine kinase (sMtCK) is one component of a multiprotein, high energy channeling complex consisting of porin, mitochondrial creatine kinase, and adenine nucleotide translocase. To study the transcriptional mechanisms specifying sMtCK gene expression to the heart, transgenic mice were created carrying the 5′-flanking sequences of the human sMtCK gene ligated upstream of the human growth hormone (hGH) reporter gene. RNA blot hybridization demonstrated that the human sMtCK sequence, −485 to +6 base pair (bp), did not activate reporter gene expression to a detectable level. However, the human sMtCK sequence, −921 to +6 bp, expressed the hGH reporter gene at a high level in heart and skeletal muscle and at a very low level in esophagus and kidney, and it did not express the hGH gene in other organs tested (brain, lung, liver, spleen, bladder, uterus, and stomach). In situ hybridization revealed that reporter gene transcription was specified to cardiac and skeletal myocytes, recapitulating precisely the expression pattern of the endogenous gene. Sequence analysis identified several consensus binding sites between −921 and −757 bp, including four GATT motifs, one E box, and one MEF2 site. Further analysis of a third transgenic mouse strain demonstrated that the human sMtCK sequence, −757 to +6 bp, did not direct detectable expression of the hGH reporter gene. We conclude that this 160-bp genomic sequence, −921 to −757 bp, is necessary in specifying expression of the human sMtCK gene to the oxidative and highly metabolically active heart tissue.

ATP is the universal carrier of metabolic energy. In nonphotosynthetic eukaryotic cells, ATP is predominantly synthesized in mitochondria, where most of the energy-yielding oxidative reactions are localized. Emerging evidence supports the hypothesis that the dynamic multiprotein complex of porin, mitochondrial creatine kinase, and the adenine nucleotide translocase traverses the outer and inner mitochondrial membranes and forms a preferential pathway transporting energy out of the mitochondrion (1–3). Sarcomeric mitochondrial creatine kinase (sMtCK),1 one component of this complex, is expressed exclusively in the highly metabolically active and oxidative organs, heart and skeletal muscle, and may offer these organs metabolic advantages essential for fulfilling their function (4).

Transcriptional mechanisms regulating gene expression to skeletal muscle are well understood and controlled by the MyoD family of basic helix-loop-helix transcription factors (5). In the last few years, cardiac myogenesis and morphogenesis have begun to be understood at the molecular level with the cloning and characterization of essential cardiac transcription factors. These include Nkx2.5 (6, 7), eHAND and dHAND (8–11), GATA4 (12–14), and MEF2 (15, 16). Studies in Drosophila demonstrated that dorsal tube formation (Drosophila heart equivalent) requires the signaling pathway of the segment polarity gene, wingless (17, 18), and a homeodomain-containing gene, tinman (19, 20).

The spacial and temporal pattern of expression of these genes in vertebrates and flies suggests their crucial role in cardiac myogenesis and morphogenesis, which is further demonstrated in gene knock-out (D-MEF2 of Drosophila, Ref. 21; Nkx2.5 in mice, Ref. 22) and mRNA ablation studies (dHAND and eHAND, Ref. 11). However, numerous questions remain as to how these transcription factors are ordered in the pathway culminating in formation of the heart, how they interact with each other, and how they specify gene expression to the heart.

Myocytes possess unique energy metabolism pathways and, thus, express genes associated with these pathways (4, 23–26). To study the transcriptional mechanisms specifying energy metabolism-related gene expression to the heart, we isolated and characterized the human sMtCK gene and 4.6 kb of its 5′-flanking sequence (4, 24). This gene is located on chromosome 5, spans 37 kb of the genome, and encodes a single polypeptide of 419 amino acids. The 5′-flanking region of the human sMtCK gene possesses features of a highly regulated gene: a TATAA box at nucleotide −36, three CCAAT sequence homologues at positions −134, −155, and −175, and a non-GC-rich promoter region prior to the transcription start point.

Because no permanent cell line faithfully represents mature, beating cardiomyocytes, we generated two transgenic mouse lines to study the transcriptional regulatory activity of human sMtCK gene 5′-flanking region. Our results demonstrated that the 5′-flanking region of the human sMtCK gene contains elements specifying gene expression to heart and skeletal muscle and that the sequence between −921 and −485 bp (relative to the transcription start point) is essential for a high level, myocyte-specific gene expression. Further studies in transgenic mice narrowed the region to between −921 and −757 bp and pointed to the potential importance of several consensus bind-
ing sites within this region, including one MEF2 consensus, one E box, and four GATTC binding sites.

EXPERIMENTAL PROCEDURES

Human sMtCK Gene 5'-Flanking Sequence-Reporter Constructs for in Vitro Transient Transfection—To make 5'-nesteled deletion constructs, DNA fragments with varied 5'-ends and a common 3'-end (+6 relative to the transcription start point) of the human sMtCK gene 5'-flanking sequence were generated using convenient restriction sites (Fig. 1). The insert for one construct (pMtCK50CAT) was synthesized by polymerase chain reaction. These genomic DNA fragments were subsequently placed at the HindIII/XbaI site in the vector pCAT-Basic (Promega, Madison, WI) (Fig. 1).

Cell Culture and in Vitro Transient Transfection—Rat neonatal cardiomyocytes were isolated based on the method of Chien et al. (27). The cardiaco myocytes were plated at 2 × 10^5 cells/60-mm dish and incubated in serum-free PC-1 media (Vestrex, Portland, ME) at 37 °C, 5% CO₂, and 95% humidity. Transfection was performed by a liposome-mediated method (DOTAP, Boehringer Mannheim) with 20 μg of test DNA and 5 μg of the control plasmid, pMSV β-gal, which contains the β-galactosidase gene under the control of the Maloney murine sarcoma virus long terminal repeat. The medium was changed to PC-1/Dulbecco's modified Eagle's medium supplemented with 10% FBS 12-12 the day after transfection, and cells were harvested 48 h later. C57BL/6 mouse skeletal myoblasts, myotubes, and NIH/3T3 mouse embryo fibroblasts were plated, maintained, transfected, and harvested as described previously (4). Cell extracts were prepared by three cycles of freezing and thawing in 150 μl of 0.25 M Tris-HCl, pH 7.8. β-Galactosidase assay was performed, and the amounts of cell extract required for CAT assays were calculated accordingly. CAT assays were performed with 24 μl of [14C]chloramphenicol (Amersham Corp.). Acetylated chloramphenicol was separated by three rounds of xylene extraction and quantitated by liquid scintillation counting. Results were expressed as -fold induction over that of the promoterless and enhancerless vector, pCAT-Basic.

Constructs for Creating Transgenic Mice—The 2.15-kb human growth hormone (hGH) gene beginning at nucleotide +3 (relative to the transcription start point) (28) was inserted into the EcoRV site of the promoterless and enhancerless vector, pCAT-Basic (Promega, Madison, WI) (Fig. 1). The linear transgene fragments were placed at the HindIII site of the vector pBShGH. A HindII/PvuII restriction fragment of the human sMtCK gene 5'-flanking sequence, −921/6+6 bp (relative to the transcription start point), was placed into the EcoRV site of pBShGH. This construct was designated as pMtCK921hGH. A second construct, pMtCK485hGH, was created by placing the EcoRI/PvuII restriction fragment of human sMtCK gene 5'-flanking sequence, −485/6+6 bp, into the EcoRV site of the plasmids, which were subsequently released from the cloning vector, pBSI SK +, by HindIII and SacI as 3.1- (sMtCK921hGH) and 2.68-kb fragments (sMtCK485hGH), respectively (Fig. 2). The transgene sequences were verified by the dideoxynucleotide method (29) at both ends and through the junction regions between the 5'-end of the sMtCK sequence and the 5'-end of the hGH gene.

Transgenic Mouse Line Creation—The linear transgene fragments were suspended in 10 mM Tris-HCl, pH 7.5, 0.25 mM EDTA and injected into the pronuclei of the fertilized eggs of the C57BL/6 × SJL F2 hybrid mice in collaboration with DNX (Princeton, NJ). Mouse carrying the transgene were tested in three types of cultured cells: rat neonatal cardiomyocytes, C6C12 mouse skeletal myocytes, and 3T3 mouse embryo fibroblasts. The plasmid, pCAT-Basic, which does not contain a promoter or enhancer, and the plasmid, pCAT-Control, which possesses both the SV40 promoter and enhancer, were also tested in each experiment. High levels of CAT activities in the pCAT-Control transfected cell lines (C6C12 myotubes, 792-fold over that of pCAT-Basic; 3T3 fibroblasts, 181-fold; cardiomyocytes, 161-fold) demonstrated the presence of efficient general transcription/translation machinerys in these cells (Fig. 1A).

The construct, pMtCK1308CAT, expressed the CAT gene at a high level in myocytes as compared with 3T3 cells (C6C12 myotubes, 166-fold over pCAT-Basic; cardiomyocytes, 75-fold; 3T3 cells, 9-fold). As additional 5'-flanking sequence (2271 bp)

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is the hybridized signal in cpm. The calculated transgene copy numbers of three independent founder lines of the construct, sMtCK921hGH, were 112, 29, and 2, respectively (Fig. 2B, lanes 3, 4, and 5). Comparable loading between lanes was shown with the hybridized signal of the mouse α-actin gene (Fig. 2C).

Southern blot analysis also suggested that the transgene, sMtCK921hGH, was organized into a head-to-tail concatamer and integrated independently in host genome among these three founder lines (Fig. 2B, lanes 3–5). The common fragment has the same apparent molecular weight of the transgene itself (lane 1) and is released from the concatamer by EcoRV, which cuts once within the transgene (at −485 of the human sMtCK sequence). The two fragments with novel lengths correspond to the 5′- and 3′-junction fragments between the concatemer and the host genome. We conclude that the three lines result from independent integration events and have different transgene copy numbers. Because the pattern and level of expression of the reporter gene can be influenced by integration site environment and transgene copy number, independent founder lines are essential to control for such effects.

The Endogenous Mouse sMtCK Gene Is Exclusively Transcribed in Heart and Skeletal Muscles—Expression of the endogenous mouse sMtCK gene was examined extensively with RNA blot analysis (Fig. 3B). The endogenous sMtCK mRNA was detected exclusively in sarcomeric muscles, namely heart (both atria and ventricles), soleus, and diaphragm. The signal was more intense in diaphragm and ventricles and less in atria and soleus. No signal was detected from all other tissues tested, including brain, lung, liver, spleen, kidney, bladder, uterus, esophagus, and stomach. These results demonstrate that the sMtCK gene is highly and exclusively expressed in sarcomeric muscles and suggest that a transcriptional mechanism(s) specifying gene expression to muscle tissues must exist.
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**Fig. 2.** Transgenic mice carrying constructs of the human sMtCK 5′-flanking sequences placed at the 5′ end of the hGH reporter gene. A, constructs used for creating transgenic mice. The two constructs were created by ligating the human sMtCK gene 5′-flanking sequences, −485/+6 bp (sMtCK485hGH) or −921/+6 bp (sMtCK921hGH), to the entire hGH gene beginning at +3 (transcription start point is +1) in pBluescript II SK+ (Stratagene). The two constructs were subsequently released from the vector as the linear construct, sMtCK485hGH, and hybridized with the 32P-labeled probe of the construct, sequences illustrated. B, Southern blot analysis of the genomic DNA from the sMtCK921hGH transgenic mouse line digested with EcoRV and hybridized with the 32P-labeled, 451-bp DNA fragment, constructs used for creating transgenic mice. The same filter as in Fig. 1B, were chosen for detailed analysis. hGH radioimmunoassay detected hGH in their sera (line 1602, 12.3 μg/ml; line 1606, 5.8 μg/ml; line 1609, 0.5 μg/ml; the normal murine serum growth hormone level is 10–100 ng/ml). RNA blot hybridization results from one adult mouse (line 1606, 7 months old, copy number 29) are provided in Fig. 3A. Abundant hGH mRNA was detected in atria, ventricles, soleus muscle, and diaphragm. Similar to the endogenous sMtCK gene, the signal was more intense in ventricles and diaphragm and less so in atria and soleus. Low level signal was also noted in esophagus and kidney. The two bands of the transgene hGH mRNA detected on RNA blot (Fig. 3A) might be the result of differential polyadenylation or precursor hGH mRNA processing. hGH mRNA was not detected in any other tissues tested, including brain, lung, liver, spleen, bladder, uterus, and stomach.

The Transgene sMtCK921hGH Is Not Expressed at a Detectable Level—Fourteen founder mice were created for the construct, sMtCK485hGH, and the transgene was successfully transmitted to their offspring (data not shown). We tested for hGH expression at the protein and mRNA levels by radioimmunoassay for hGH immunoreactivity in sera of progeny of the 14 founder mice (hGH detection limit, 0.5 ng/ml); RNA blot hybridization on total RNA isolated from ventricles and soleus muscles of 12 independent strains; and RNA blot analysis of total RNA from organs of two independent founder mice, including brain, lung, diaphragm, atria, ventricles, soleus, liver, spleen, kidney, bladder, uterus, esophagus, and stomach.

**Fig. 3.** Human sMtCK gene 5′-flanking sequence, −921/+6 bp, recapitulates the expression pattern of the endogenous mouse sMtCK gene in heart and skeletal muscle. A, expression of the reporter hGH gene. Total RNA was isolated from 13 tissues of an sMtCK921hGH transgenic mouse (line 1606, 7 months old), and 10 μg was loaded onto each lane. The blot was hybridized with a digoxigenin-labeled, antisense cRNA probe (240 bp) from hGH exon 5. The probe was detected with an alkaline phosphatase-conjugated antibody against digoxigenin and a standard color reaction for alkaline phosphatase. B, expression of the mouse endogenous sMtCK gene. The same RNA samples as in A were used. Hybridization was performed with a digoxigenin-labeled, 475-bp antisense cRNA probe transcribed from the mouse sMtCK cDNA exons 6–9.

**The Transgene sMtCK921hGH Is Expressed in Heart and Skeletal Muscle**—Five founder mice carrying the transgene, sMtCK921hGH, were created. They were all noticeably larger in size as compared with their nontransgenic littermates (data not shown). Three adult mice, each of an independent strain as shown in Fig. 2B, were chosen for detailed analysis. hGH radioimmunoassay detected hGH in their sera (line 1602, 12.3 μg/ml; line 1606, 5.8 μg/ml; line 1609, 0.5 μg/ml; the normal murine serum growth hormone level is 10–100 ng/ml). RNA blot hybridization results from one adult mouse (line 1606, 7 months old, copy number 29) are provided in Fig. 3A. Abundant hGH mRNA was detected in atria, ventricles, soleus muscle, and diaphragm. Similar to the endogenous sMtCK gene, the signal was more intense in ventricles and diaphragm and less so in atria and soleus. Low level signal was also noted in esophagus and kidney. The two bands of the transgene hGH mRNA detected on RNA blot (Fig. 3A) might be the result of differential polyadenylation or precursor hGH mRNA processing. hGH mRNA was not detected in any other tissues tested, including brain, lung, liver, spleen, bladder, uterus, and stomach. In another founder strain (1602), reporter gene expression was also detected in heart and skeletal muscle, but ectopic expression was observed in brain (data not shown). Ectopic expression may have resulted from the influence of integration site environment, since this differs between founder lines. These results demonstrate that the human sMtCK genomic sequence, −921/+6 bp, can recapitulate precisely the pattern of tissue expression of the endogenous sMtCK gene in sarcomeric muscles.

**The Transgene sMtCK921hGH Is Expressed in Cardiac and Skeletal Myocytes at a Level Comparable with That of the Endogenous sMtCK Gene**—To determine the cell type in sarcomeric muscles that expresses the hGH reporter gene, in situ hybridization was performed. Paraffin-embedded tissue sections were prepared and hybridized with cRNA probes labeled

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**The Transgene sMtCK485hGH Is Not Expressed at a Detectable Level**—Fourteen founder mice were created for the construct, sMtCK485hGH, and the transgene was successfully transmitted to their offspring (data not shown). We tested for hGH expression at the protein and mRNA levels by radioimmunoassay for hGH immunoreactivity in sera of progeny of the 14 founder mice (hGH detection limit, 0.5 ng/ml); RNA blot hybridization on total RNA isolated from ventricles and soleus muscles of 12 independent strains; and RNA blot analysis of total RNA from organs of two independent founder mice, including brain, lung, diaphragm, atria, ventricles, soleus, liver, spleen, kidney, bladder, uterus, esophagus, and stomach. Expression of the hGH reporter gene was not detected by any of these tests in any of the 14 strains. These results show that the sMtCK sequence, −485/+6 bp, did not direct detectable expression of the hGH reporter gene to the organs examined, including heart and skeletal muscle. Thus, crucial cis-acting, activating elements are not located within this region.
Corresponding pattern of expression of the reporter hGH gene and the endogenous mouse sMtCK gene in heart and skeletal muscle demonstrated by in situ hybridization. Organs (heart, soleus, and gastrocnemius) of an sMtCK921hGH transgenic mouse (line 1606, 7 months old) were excised, fixed in Bouin’s solution, paraffin-embedded, and sectioned. In situ hybridization was performed with the same cRNA probes used in RNA blot analysis in Fig. 3, but they were labeled with [α-³²P]UTP. After hybridization, the tissue sections were exposed to x-ray film for 3 days. Panels A, B, C, and D, heart; panels E, F, G, and H, soleus (two small cross-sections) and gastrocnemius (two larger tissues). First column, antisense cRNA hybridization; second column, sense cRNA hybridization.

The human sMtCK gene 5’-flanking sequence, −921/+6 bp, directs myocyte-specific reporter gene expression in heart and skeletal muscle at a level comparable with that of the endogenous sMtCK gene. After in situ hybridization was performed, the sections were embedded in and exposed to an autoradiographic emulsion for 4 days. Counterstaining was performed with eosin and hematoxylin. Under dark field microscopy, the low level, background silver granules (white spots) in sense probe hybridization (first column) permitted the use of a higher illumination level to show the cell (green) and nucleus (yellow) contours (panels A, D, G, J, M, P, S, and V). For antisense hybridization (second column), the intense signal limited the illumination level under dark field microscopy (panels B, E, H, K, N, Q, T, and W). As such, the bright field images (third column) of the areas shown in the second column were provided to orient cells and nuclei (panels C, F, I, L, O, R, U, and X). A, right atrium; V, interventricular septum; G, gastrocnemius; and S, soleus. Magnification is ×400.

As is evident in panels I, L, U, and X. The respective dark field views are provided in the second column (panels B, E, H, K, N, Q, T, and W). These results demonstrate that cardiac and skeletal myocytes are the cells that express abundant hGH reporter gene mRNA. Because the probes and tissue sections were handled similarly, the comparable signal intensity between the hGH mRNA and the sMtCK mRNA also suggests a comparable level of expression between the two mRNAs. Altogether, these data demonstrate that the human sMtCK genomic sequence, −921/+6 bp, is sufficient to direct myocyte-specific expression both in heart and skeletal muscle and that the pattern of expression faithfully recapitulates that of the endogenous sMtCK gene.

It is evident that, in skeletal muscles (soleus and gastrocnemius), only some cells showed intense reporter mRNA signal.
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FIG. 6. Known consensus sequences present in the human sMtCK 5'-flanking region. −921/+6 bp. Consensus sequences absent from this region are M-CAT ((G/A)CATNC(T/C)(T/A)); Refs. 39–42) and CarG (CC(A/T)₆GG; Refs. 36 and 43–46).

(Fig. 5, panels N and Q), while others showed background signal level. This is also observed for the endogenous sMtCK mRNA signal (Fig. 5, panels T and W). It is likely that the highly oxidative, slow twitch myocytes are the cells with the mitochondrial and should express the transgene and the sMtCK gene to a high level.

Because the human sMtCK sequence, −921/+6 bp, expresses the reporter hGH gene in cardiac and skeletal myocytes and the sMtCK sequence, −485/+6 bp, did not direct a detectable level of reporter gene expression, we conclude that crucial cis-acting elements regulating sMtCK gene expression to sarcomeric tissues reside within the sequence −921/+485 bp.

DISCUSSION

Our study in transgenic mice has demonstrated that the 5'-flanking sequence of the human sMtCK gene, −921/+6 bp, is necessary and sufficient to direct the reporter gene expression to cardiac and skeletal myocytes at a level comparable with that of the endogenous mouse sMtCK gene, suggesting the presence and functioning of crucial cis-acting elements regulating gene expression to the cardiac and skeletal myocytes within this region. Comparison of the two transgenic mouse lines proves that the sequence between −921 and −485 bp is mainly responsible for this high level, myocyte-specific expression of the human sMtCK gene.

The nucleotide sequence between −921 and +6 bp is shown in Fig. 6. Some well known consensus transcription factor binding sites regulating gene expression in sarcomeric tissues are noted. 1) There are two clusters of MEF-1/MyoD consensus binding sequences (E boxes, CANNTG): a proximal cluster of five E boxes between −30 and −300 and a distal cluster of five E boxes between −570 and −830. 2) Two A/T-rich MEF2 (myocyte-specific enhancer-binding factor 2) consensus sequences ((C/T)T(A/T)(A/T)AAATA(A/G), found in the control regions of many sarcomeric genes (15, 16, 35), exist in the human sMtCK gene control region. One MEF2 element (ATATTTTAA) is located at −782 in reverse orientation. The second MEF2 element also exists in reverse orientation at −472 (CTATTTTAA). Moreover, this sequence lies in a context similar to that of the rat myosin light chain 2 gene (GGGGCTATTTTTAA) important for its cardiac specific expression (36). 3) Although perfectly matched GATA consensus sequences are absent from −921 to +6 bp ((A/T)GATA(A/G); Refs. 12–14 and 37), the closely matched GATA motif is present at −783, −488, and −486 (in reverse orientation). Another closely related GATT motif (38) is present at −910 and −895, and this same motif in antisense is found at −883, −863, −536, and −173. We noticed that, within a 50-bp region (−910 to −860 bp), there are four GATT consensus sequences (−910, −893, −883, and −863). The significance of this packed localization of the GATT motifs within a short genomic region will need to be examined. Other consensus sequences important for gene expression in muscles were absent from the human sMtCK 5'-flanking sequence, −921/+6 bp. These include the M-CAT motif ((G/A)CATNC(T/C)(T/A)) from chicken cardiac troponin T gene (39–42) and the CarG motif (CC(A/T)₆GG) from the human α-cardiac actin gene (43–46).

We previously reported (4) that the proximal three E boxes of the human sMtCK 5'-flanking sequence are not important for its tissue specificity, because transient transfection of constructs sequentially including these E boxes did not result in selective expression of the CAT reporter gene in C₆C₁₂ myotubes as compared with 3T3 and HepG2 cells. Our current study examining expression of the sMtCK485hGH construct in transgenic mice supported this previous observation from cell culture. In addition, our results proved that the proximal group of five E boxes and the MEF-2 motif at −472 bp are not sufficient to activate sarcomeric tissue-specific expression.

Because, in transgenic mice, the construct sMtCK921hGH expresses the transgene and the construct sMtCK485hGH does not express the reporter gene at a detectable level, we conclude that human sMtCK 5'-flanking sequence between −921 and −485 bp is crucial for specifying gene expression in both the cardiac and skeletal myocytes. These data from transgenic mice are supported by results from the in vitro transient transfection results (Fig. 1B), which showed that inclusion of the sequence between −485 and −921 bp dramatically increased the level of reporter CAT expression (52-fold versus 9-fold over pCAT-Basic).

We created an additional transgenic mouse strain carrying the 5'-flanking sequence of the human sMtCK gene, bp −757/+6, ligated in the 5' end of the hGH reporter gene. Analysis of several founder strains using RNA blot and radioimmunoassay did not detect hGH mRNA in tissues or circulating hGH immunoreactivities in sera, thus narrowing further the sequence crucial for gene expression to myocytes to between −921 to −757 bp. Within this 160-bp region, several known transcription factor consensus binding sites exist and include four GATT motifs (GATT at −910 and −893 bp; AATC at −883 and −863 bp), one E box (CACGGT at −827 bp), and one MEF2 consensus (ATATTTTAA at −782 bp). Preliminary results from gel electrophoresis mobility shift, site-specific mutagenesis, competition, and antibody supershift studies established that GATT at −893 and −863, E box at −827, and the MEF2 at −782 can bind, respectively, GATA-related proteins, upstream stimulatory factor 1 and MEF2 and that the GATT-binding protein is not a GATA4. The transcriptional regulatory activities of these consensus binding sites are now being examined with site-specifically mutated promoter constructs in cell lines and transgenic mice.²

However, it is not known whether, within −921 to −757 bp of the human sMtCK gene, elements specifying gene expression in the heart will segregate from those required for skeletal muscle expression, as has been documented for some contractile protein genes expressed in both the heart and slow twitch muscles, such as the mouse slow/cardiac troponin C gene (47),

³ W. Qin and A. W. Strauss, manuscript in preparation.
the rat cardiac myosin light chain-2 gene (48), and the mouse β-myosin light chain gene (49). Skeletal myogenesis is controlled, in part, by the MyoD family of basic helix-loop-helix transcription factors (5). These genes are exclusively expressed in skeletal myocytes and specify gene expression by binding to the E box consensus, CANNTG, found in the control region of numerous muscle-specific genes. These transcription factors are not expressed in the heart.

The recent cloning and characterization of several genes encoding transcription factors expressed in the heart indicates that divergent mechanisms have evolved regulating cardiac myogenesis and morphogenesis (6, 7, 9, 11). Gene or mRNA ablation demonstrates that MEF2, Nkx2.5, and the HAND genes regulate gene expression in the heart (11, 21, 22). There are many unknown aspects of these transcription factors.

MEF2 is a diverse group of transcription factors: there are four MEF2 genes in vertebrates (mef2A, -B, -C, and -D); Mef2A, -C, and -D transcripts have multiple alternatively spliced isoforms; and each transcript has a distinct pattern of tissue distribution (50). Further investigation is required to determine whether the overlapping pattern of expression of these MEF2 transcripts defines specificity of gene regulation. The Nkx2.5 gene is activated early in the embryo and expression maintained throughout adulthood (6). Because this gene was cloned by homeodomain homology screening, downstream target genes are mostly unknown and need to be identified. The HAND genes are expressed transiently during early embryo development, and target genes remain to be identified. In addition, these transcription factors are also expressed in other tissues as well as in the heart. It remains to be determined how cardiac specific gene expression is achieved.

Moreover, mechanisms must also exist to regulate expression of metabolism-related genes in response to energy requirements of the highly metabolically active and oxidative cells, such as the cardiac and skeletal myocytes. How cellular energetics and cardiac myogenesis are coordinated at the transcriptional level can now be investigated. Study of an energy metabolism-related gene, the mHCK, which is exclusively expressed in heart and skeletal muscle, will add to our understanding of transcriptional regulation of gene expression during cardiac myogenesis.

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