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Mouse *Mef2b* Gene: Unique Member of MEF2 Gene Family[1]

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The myocyte enhancer factor 2 (MEF2) gene family, which belongs to the MADS [MCM1, agamous, deficiens, serum response factor (SRF)] superfamily, is thought to play an important role in differentiation of myocytes, including cardiomyocytes. To better understand the mouse *Mef2* gene family, the mouse *Mef2b* gene, which was found to be expressed in undifferentiated embryonal cells, was characterized. The *Mef2b* gene was found to be more than 30 kb in length, consisting of 11 exons. Eight exons correspond to coding regions and the remaining 3 exons for the 5′ part are alternatively used. Two internal exons are subject to alternative splicing, resulting in production of four subtypes of mouse MEF2B peptides. Fluorescence *in situ* hybridization (FISH) and inter-specific backcross analysis identified the *Mef2b* gene locus. *Mef2b* gene was expressed in heart or skeletal muscle of early mouse embryo, but not in those of adult mouse. Functionally, mouse MEF2B did not exhibit DNA binding with the MEF2 consensus element *in vitro*, but did cause transcriptional activation of the MEF2 element, although it was less effective than human MEF2B. Based on these results, mouse MEF2B seems to have a unique character, distinct from other MEF2 family members.

Key words: development, differentiation, gene, MEF2, transcription.

The myocyte enhancer factor 2 (MEF2), which belongs to the MADS [minichromosome maintenance 1 (MCM1), agamous, deficiens, serum response factor (SRF)] superfamily, is thought to play an important role in myocyte differentiation (*1-7*). MEF2s are encoded in multiple genes in higher eukaryotes, and there are four different *MEF2* genes encoding *MEF2A, MEF2B, MEF2C*, and *MEF2D* in human, of which all are highly expressed in skeletal muscle (*3, 5, 6*). Those *MEF2* genes are also thought to play an important role in cardiac muscle differentiation (*8*), though multiple factors including MEF2s, cardiac homeobox proteins (tinman, Nkxs, or Csx) and GATA factors are thought to interact together in that process (*8-11*).

Recently, we have found that a mouse MEF2B homologue is expressed in undifferentiated embryonal cells (*12*). Although mouse MEF2B has the MADS/MEF2 domain that is conserved in the MEF2 family and is homologous to human MEF2B, the tissue distribution of mouse MEF2B transcripts proved to be quite different from that reported for all other MEF2 family members, including human MEF2B (*12*). Mouse MEF2B was found to be little expressed in adult heart or skeletal muscle (*12*), while human MEF2B was highly expressed there (*3*). Although expression of human MEF2B is similar to that of other human MEF2s, human MEF2B peptide was reported to be small in size and to have different binding ability to DNA *in vitro* (*3*). Therefore, the function of mouse MEF2B might be distinct from that of other MEF2 factors, but the relationship between mouse MEF2B and human MEF2B or between MEF2B and other MEF2s is largely unknown. However, another group recently reported different results, showing that mouse MEF2B is expressed in adult muscle, but not in embryonal cells (*13*). Therefore, we have studied mouse *Mef2b* through structural analysis of the gene and functional analysis of the gene product, in order to confirm that mouse *Mef2b* gene has distinct properties among the *Mef2* gene family.

MATERIALS AND METHODS

All reagents were of analytical grade. Restriction enzymes and modifying enzymes are from Takara or Toyobo, unless otherwise specified. DNA and RNA were prepared from cells or tissues as described elsewhere (*14*). All cell lines were obtained from American Type Culture Collection (ATCC).

cDNA and Genomic DNA Library Screening—cDNA libraries from F9 mouse teratocarcinoma cells in lambda uni-ZAP, kindly provided by Drs. Shimada and Takihara of Osaka University, were screened as described before (*14*), by using an *Eco*RV/*Sma*I fragment (nt 249-nt 752) of mouse MEF2B cDNA reported previously (*12*) as the probe. Genomic DNA libraries from mouse in lambda FIX II (Stratagene) were also screened similarly. Cloned

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cDNAs and genomic DNAs were then subcloned into pBluescript II (Stratagene) and sequenced with automated instrumentation (Applied Biosystems). Sequences were verified by multiple runs for both strands.

Southern Blot Analysis—Genomic DNA was isolated from mouse kidney, restricted with endonucleases, electrophoresed in 0.7% agarose gels, and transferred to nylon filters as described before (*14*). Filters were hybridized with the respective MEF2B probe as described in the text.

Assignment of Chromosome Locus for the Mef2b Gene—The direct R-banding fluorescence *in situ* hybridization (FISH) method was used for chromosomal assignment of the mouse *Mef2b* gene. Preparation of R-banded chromosomes and FISH were performed as described before (*15*). The 1.2 kb fragment (nt +20-nt 1251) of mouse MEF2B (mMEF2B) cDNA inserted in the *Bam*HI-*Sal*I site of pUC13 was labeled by nick translation with biotin 16-dUTP (Boehringer Mannheim) following the manufacturer's protocol.

Recombinant animals for this study were generated by mating males of the feral-derived mouse stock, *Mus spretus*, with C57BL/6J females and backcrossing of the F1 females with *M. spretus* at the National Institute of Radiological Sciences, Chiba. Whole genomic DNAs prepared from kidneys of the backcross mice were digested with restriction endonuclease. The resulting fragments were separated by electrophoresis on 0.8% agarose gels and transferred onto nylon membrane. Radiolabeled 503 bp DNA fragment (*Eco*RV/*Sma*I) of the mMEF2B cDNA was used to determine the genotype of individuals. Microsatellite DNA marker loci for linkage analysis were chosen following the result of cytogenetic mapping by FISH, and purchased from Research Genetics (Huntsville, AL, USA). Genotyping of these markers was performed by polymerase chain reaction.

RNA Analysis—Multiple tissue Northern blots (Clontech) were hybridized with mouse MEF2B specific probe from the *Eco*RV/*Sma*I fragment (nt 249-nt 752) of mMEF2B cDNA. A filter containing approximately 2 μg of purified poly(A)$^+$ RNA per lane was hybridized overnight at 65°C in 5×SSPE, 10×Denhardt's solution, 2% SDS, and 100 μg/ml ssDNA. The filter was then washed twice at room temperature for 15 min in 2×SSC and 0.05% SDS and twice at 50°C for 30 min in 0.1×SSC and 0.1% SDS, and finally detected by autoradiography.

Transcripts for mMEF2B were also analyzed by RNase protection assay using cRNA probes corresponding to a 224-base fragment of mMEF2B cDNA (nt 587-nt 810) as described before (*12*). cRNA probes corresponding to mouse alpha myosin heavy chain (αMHC) and beta actin (β-actin) were also used as references.

In addition, reverse transcription-polymerase chain reaction (RT-PCR) (*16*) was employed to analyze the transcripts of mMEF2B in heart or skeletal muscle in various stages of embryonal development. The first-strand cDNA synthesis was done in 20 μl containing 1 μg of total RNA, random hexamer primer (GIBCO-BRL), RNasin (Promega), all four dNTPs, and SuperScript II reverse transcriptase (GIBCO-BRL) according to the manufacturer's protocol. The cDNA reaction mixture was used for PCR with the primer pair (primer 2B28+: 5′-CCACCTTGCGAGCAAGACGC-3′, primer 2B3-: 5′-GGTGGTACAGGGAACATGAGAC-3′). The PCR products were electrophoresed on a 2% agarose gel.

DNA Binding Assay—DNA binding activity of mMEF2B peptide was studied by electrophoretic mobility shift assay (EMSA) as described before (*17*). The isolated mMEF2B cDNAs were subcloned into pBluescript II, and the resulting mMEF2B-pSKII constructs were linearized to obtain full-length cDNAs that encoded proteins (aa1-349, aa1-339, aa1-242, aa1-232) and a truncated translation product (aa1-107). RNA was synthesized with T7 RNA polymerase, and the resulting RNA was translated *in vitro* with rabbit reticulocyte lysate according to the manufacturer's suggested conditions (Promega). For EMSA with the *in vitro*-translated MEF2 proteins, the incubation reaction mixture contained 3 μl of the translated reticulocyte lysate, 0.2 ng of probe, and 0.4 μg of poly(dI-dC). The bound fraction and the free probe were electrophoresed on a 4% polyacrylamide gel (acrylamide:bis, 29:1) at 4°C. All probes and competitor DNAs were double-stranded synthetic oligonucleotides. Radio-labeled oligonucleotides corresponding to the MEF2 element of the MCK gene (5′-GATCCTCGCTCTAAAATACCCTGTC-3′) were used as described before (*3*).

Transcriptional Activation Analysis—Transcriptional activation was assessed by means of transient transfection assays as described before. C3H10T1/2 cells were maintained and transfections were performed by a liposome-mediated method as described before (*18*). MEF2 cDNAs were subcloned into a pMT2 vector as described before (*3*). We used 10 μg of the pE102MEF2×2CAT reporter construct (*3*) along with 5 μg of the MEF2 test construct as described in the text, as well as 2 μg of pCH110 as a control for transfection efficiency. Plasmid pE102MEF2×2CAT contains two MEF2 sites from the MCK enhancer located upstream of the minimal promoter from the embryonic myosin heavy chain (emb-MHC) gene, which drives the expression of the CAT reporter gene (*3*). Cell extracts were prepared and CAT activity was determined as reported previously (*17*).

RESULTS

Mouse Mef2b Gene Structure—cDNA libraries from F9 mouse teratocarcinoma cells were screened with a DNA probe corresponding to the specific region of mouse MEF2B cDNA stated above. Several cDNA clones were isolated and each contained sequences overlapping those given in our previous report for mMEF2B (Figs. 1 and 2). However, the region upstream of the predicted translation start site was different from the previously reported mMEF2B sequences for this region. This new 5′ sequence was also found in the mMEF2B transcript in P19 cells, while the two sequences for the 5′ region reported previously were found to be present there less abundantly (data not shown). In addition to the 5′ region, there were two regions (30 and 85 bases) in the midst of the translated region alternatively excluded, resulting in 4 patterns of transcripts in the coding region for mMEF2B. As a result, four different peptides (B-1, B-2, B-3, B-4) of 349, 339, 242, or 232 amino acids were encoded in the *Mef2b* gene. Except for the alternative use of these regions, all of the nucleotide sequences for the coding region were found to be identical to those of our previous report (*12*).

We also screened mouse genomic libraries and obtained

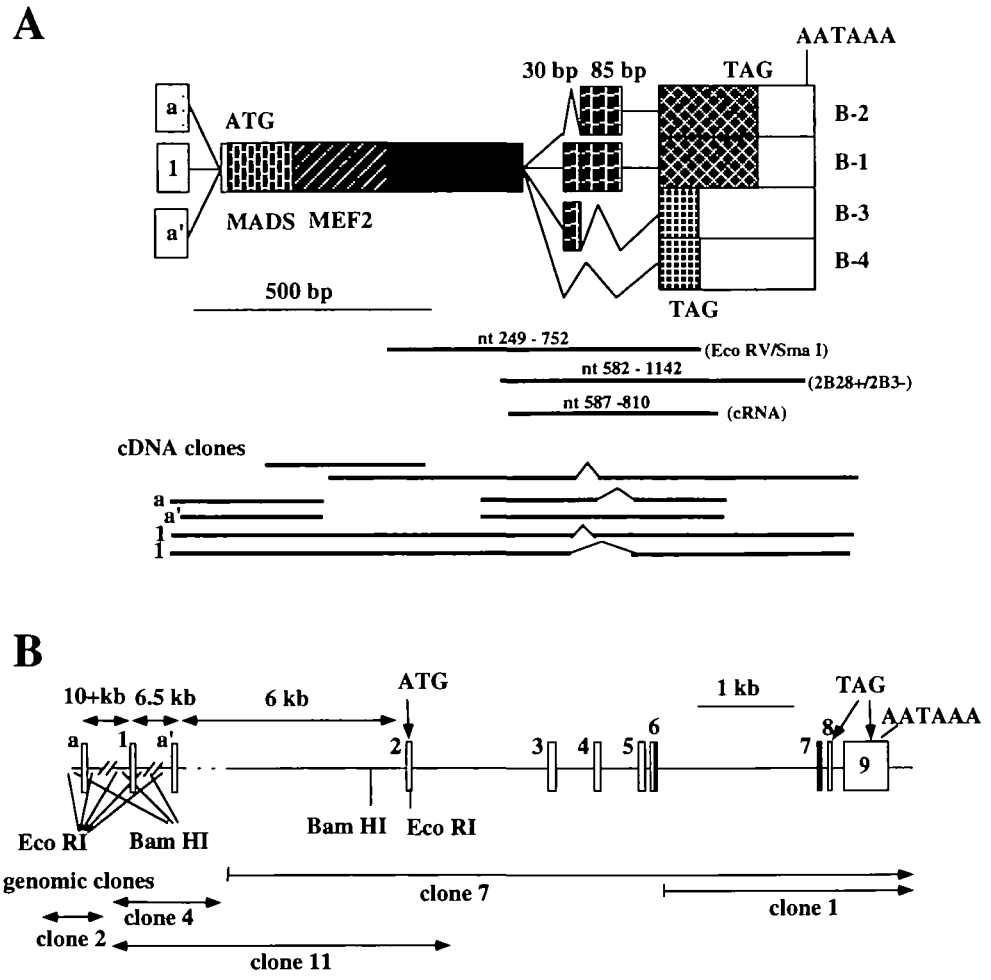

Fig. 1. **Structure of mouse MEF2B (mMEF2B) cDNA and gene.** A: mMEF2B cDNA has several forms generated by alternative splicing. Resulting *Mef2b* transcripts encode 4 different peptides (B-1, B-2, B-3, and B-4). The regions corresponding to the probes for Southern or Northern hybridization, as well as the positions of oligonucleotide primers for RT-PCR are indicated. B: Mouse *Mef2b* gene is more than 30 kb in length and consists of 11 exons. Three exons in the 5′ region are alternatively used as the first exon (exon a, 1, and a′) and 8 other exons include coding regions. Parts of exon 6 and exon 7 are subject to alternative splicing. The sequence data have been deposited in the EMBL/Genbank/DDBJ databases (D87828-D87836).

several clones spanning more than 30 kb that included the mouse *Mef2b* gene (Figs. 1 and 2). Sequence analysis revealed all of the exon-intron boundaries, as shown in Fig. 2. There were 11 exons in total, and three exons (exon a, 1, and a′) for the 5′ untranslated region were located dispersely and were alternatively used. Exon 1 corresponded to the cDNA reported here, used dominantly as the first exon, and exon a and exon a′ corresponded to the cDNAs reported previously as the most upstream sequences of mMEF2B cDNA. Eight exons (exon 2 through exon 9) from the 3′ end were located at about 7 kb, and included all of the coding regions. Two of those coding exons (exons 6 and 7) were subject to alternative splicing, producing four different peptides for mMEF2B. The two 3′ exons of *Mef2b* (exons 8 and 9) were less homologous to human *MEF2B* than the exons for the 5′ and mid coding regions (exons 2 to 7).

Southern Blot Analysis—Southern blot analysis was employed to see whether the isolated mouse *Mef2b* gene is indeed the mouse gene corresponding to human *MEF2B* gene. A Southern blot filter containing restricted mouse genomic DNA was first hybridized with a *Eco*RV/*Sma*I fragment (nt 249-nt 752) of mMEF2B cDNA, washed, and thereafter rehybridized with a *Xho*I/*Sma*I fragment (nt 253-nt 867) of human MEF2B cDNA in a less stringent condition. As shown in Fig. 3, both probes of mouse MEF2B and human MEF2B hybridized identical bands for the corresponding restriction digestion.

Assignment of Chromosomal Localization for Mouse Mef2b—The chromosomal assignment of the mouse *Mef2b* gene was made by direct R-banding FISH. As shown in Fig. 4A, the signals were localized on the R-positive C1 band of mouse chromosome 8 (*15*).

Genomic DNAs of C57BL/6 and *M. spretus* were studied by means of Southern blot analysis. A comparison of C57BL/6 and *M. spretus* identified restriction fragment length variants (RFLVs) between the two *Mus* species for *Bam*HI, *Apa*I, and *Hin*dIII in the *Mef2b* gene. Linkage mapping of the mouse *Mef2b* gene was performed with *Bam*HI-digested DNA samples from a total of 146 interspecific backcross mice between (C57BL/6 × *M. spretus*) F1 females and *spretus* males. We examined the concordance of the segregation RFLVs identified by Southern blot hybridization with the segregation of four microsatellite marker loci, *D8Mit6, D8Mit9*, and *D8Mit15*, and the segregation of mouse *Mef2b* in the backcross mice shown with the microsatellite DNA markers as anchors in Fig. 4B. Comparative pairwise loci analysis showed that the gene order and recombination frequency for the *Mef2b* gene on chromosome 8 is: centromere-*D8Mit6*-(10/146)-*D8Mit9*-(3/146)-*Mef2b*-(26/146)-*D8Mit15*-telomere (Fig. 4B). The corresponding map distances between loci in centi-Morgans ± standard error are: *D8Mit6*-6.8 ± 2.1-*D8Mit9*-2.1 ± 1.2-*Mef2b*-17.8 ± 3.2-*D8Mit15* (*19, 20*).

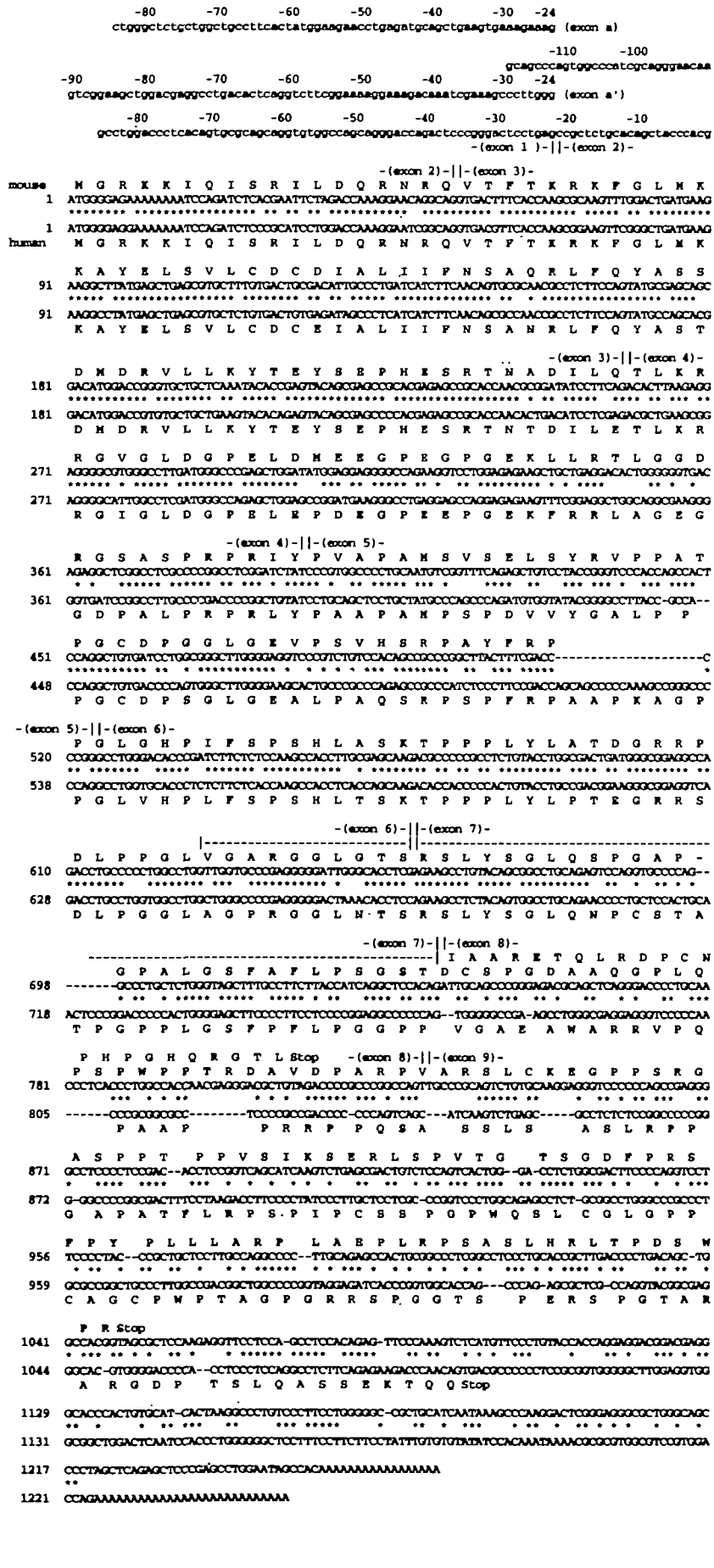

Fig. 2. **Sequence comparison of between cDNAs for mouse MEF2B and human MEF2B.** Nucleotide sequences of cDNA for mouse MEF2B (mouse) and human MEF2B (human) as well as deduced amino acid sequences are shown. Boundaries of each exon in the *Mef2b* gene are also indicated. Nucleotide number 1 denotes nucleotide A of the first ATG codon.

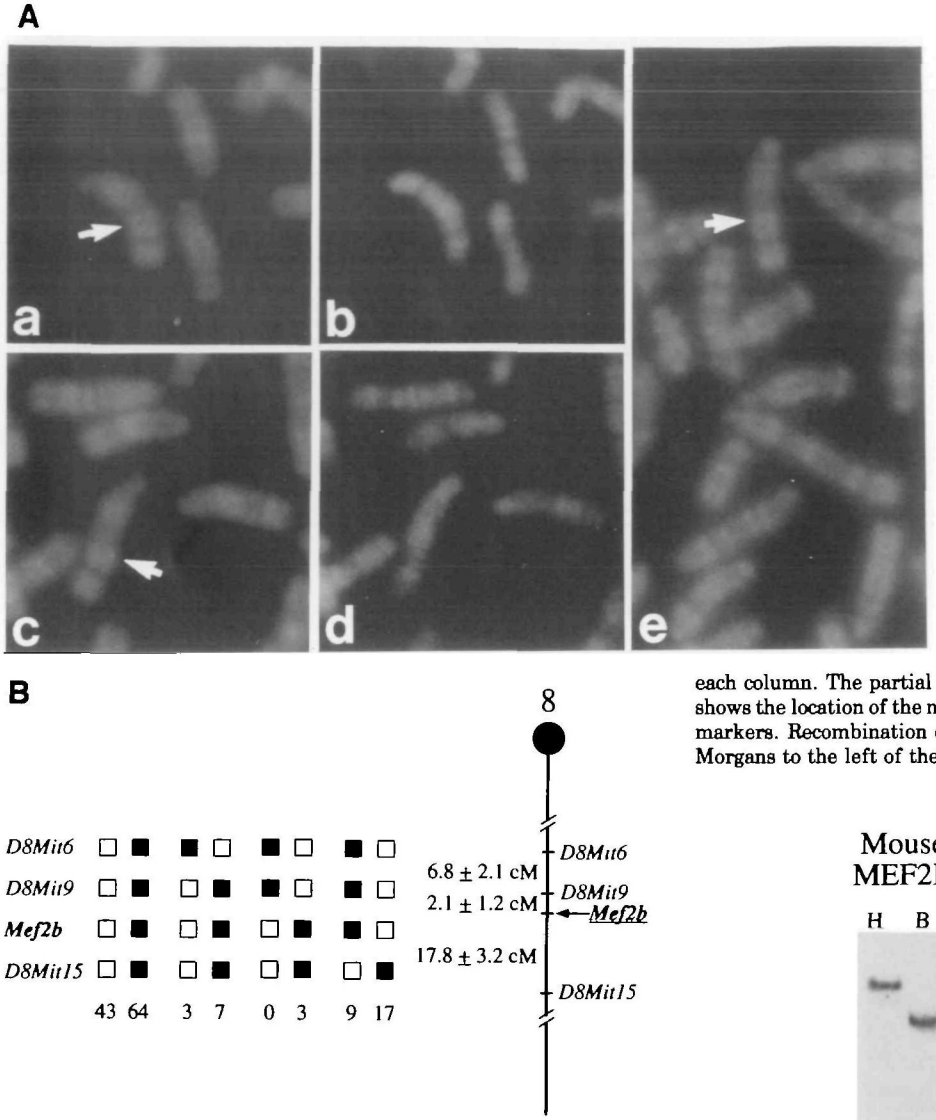

Fig. 4. **A: Fluorescence *in situ* hybridization.** Chromosomal localization of the *Mef2b* gene on mouse R-banded chromosomes using a 1.2 kb cDNA fragment as a biotinylated probe. The hybridization signals are indicated by arrows. The signals are localized at R-positive C1 of mouse chromosome 8. The metaphase spreads were photographed with Nikon B-2A (a, c, e) and UV-2A (b, d) filters. R-band and G-band patterns are demonstrated in (a, c, e) and (b, d), respectively. **B: Location of mouse *Mef2b* on chromosome 8.** The segregation patterns of the mouse *Mef2b* with flanking microsatellite DNA markers, *D8Mit6*, *D8Mit9*, and *D8Mit15*, in the backcross mice are shown at the top. Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6×*M. spretus*)F1 parent. The open squares represent the presence of the *M. spretus* allele, and the closed squares represent the presence of the C57BL/6 allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. The partial chromosome 8 linkage map at the bottom shows the location of the mouse *Mef2b* in relation to the flanking DNA markers. Recombination distances between loci are shown in centi-Morgans to the left of the chromosome.

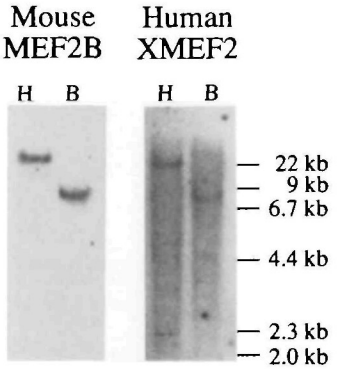

Fig. 3. **Southern blot analysis.** Ten micrograms of mouse genomic DNA was restricted with *Hin*dIII (H) or *Bam*HI (B). The blotted filter was first hybridized with mouse MEF2B probe at 65°C, washed and subject to autoradiography (Mouse MEF2B). The same filter was rehybridized with human MEF2B probe at 50°C, washed and subject to autoradiography (Human XMEF2).

Distribution of Mouse Mef2b Gene Product—Northern blots and RNase protection assay were employed to study the level of RNA transcripts for *Mef2b* in various mouse tissues and cell lines. Northern blot analysis using total RNA from any of the adult mouse tissues could not identify transcripts for *Mef2b* (data not shown). Then, Northern blot analysis with poly(A)$^+$ RNA identified transcripts for *Mef2b* in spleen, lung, and testis of adult mouse after longer exposure (3 days), while there was almost no transcript in adult heart, brain, or skeletal muscle (Fig. 5A). The size of *Mef2b* transcripts was 1.6 kb in spleen or lung, and 1.9 kb in testis. RNase protection assay showed that the undifferentiated P19 cells expressed transcripts for *Mef2b* most abundantly, but all other cultured cells tested, including myocytic cells (Soleus8 myoblasts, myotubes) or lymphocytic cells (L1210, J774) did not (Fig. 5B). RT-PCR showed that the undifferentiated P19 cells expressed more *Mef2b* transcripts of subtype B-1 or B-3 than the differentiated P19 cells (Fig. 5C). Although transcripts for other *Mef2b* subtypes were also detected predominantly in undifferentiated P19 cells, transcripts for the subtype B-2 were relatively dominant in differentiated P19 cells. RT-PCR also showed that mouse embryonal heart or skeletal muscle expressed transcripts for *Mef2b* in the early stages (before 14 d.p.c.), but adult heart or skeletal muscle did not express it (Fig. 5C). In adult mouse, some *Mef2b* transcripts were identified in testis or spleen. Southern hybridization revealed that the transcripts for *Mef2b* expressed in these embryonal muscles were of subtype B-2 only (data not shown).

Properties of Mouse MEF2B—We undertook experiments to compare the DNA-binding activity of cloned mMEF2B peptides with that of human MEF2B (hMEF2B) or human MEF2A (hMEF2A). Expression vectors were constructed with identical sequences of 5′ untranslated

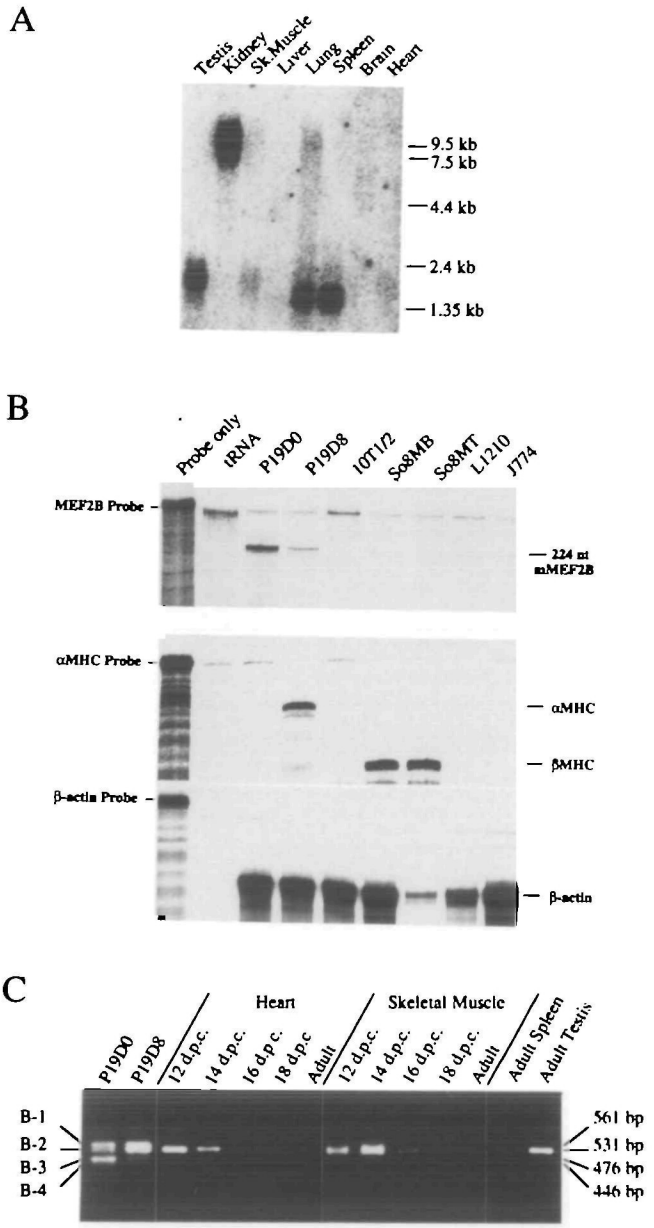

Fig. 5. **Expression of *Mef2b* gene transcripts.** A: Northern blot analysis of mouse RNA from multiple tissues. B: RNase protection assay. cRNA probes corresponding to mMEF2B cDNA (nt 587-nt 810) as well as mouse alpha myosin heavy chain (αMHC) and beta actin (β-actin) were also used. Ten micrograms of total RNA was extracted from cultured cells. P19D0: P19 mouse embryonal cells in the undifferentiated condition; P19D8: P19 mouse embryonal cells in the differentiated condition (*12*); 10T1/2: 10T1/2 fibroblasts; So8MB: Soleus 8 mouse myoblasts in the undifferentiated condition; So8MT: Soleus 8 mouse myotubes in the differentiated condition; L1210: L1210 mouse lymphocytic cells; J774: J774 mouse lymphocytic cells. Protected bands for mMEF2B, αMHC, βMHC, or β-actin are also indicated. C: Reverse transcription-polymerase chain reaction. Expression of *Mef2b* gene transcripts in mouse embryonal heart and muscle as well as in P19 cells, adult heart and skeletal muscle was analyzed. One microgram of total RNA extracted from the tissues or cultured cells [P19D0: P19 mouse embryonal cells in the undifferentiated condition; P19D8: P19 mouse embryonal cells in the differentiated condition (*12*)] were reverse-transcribed and subjected to PCR amplification. mMEF2 subtypes and the corresponding size of the amplified DNA were indicated. d.p.c.: days post coital.

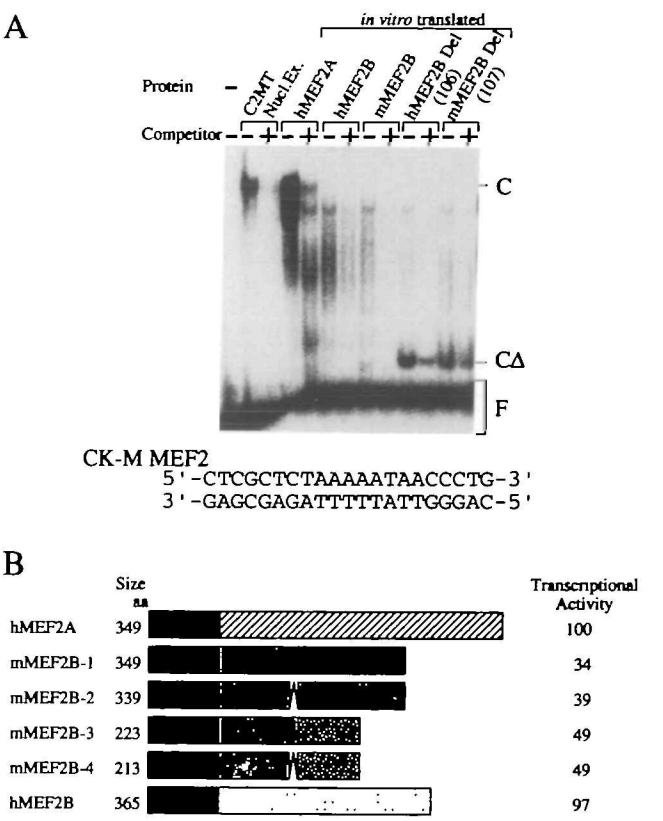

Fig. 6. **DNA binding and transcriptional activity of mouse MEF2B.** A: EMSAs were performed with *in vitro*-translated isoforms of mouse MEF2B (mMEF2B) as well as human MEF2A (hMEF2A) and MEF2B (hMEF2B) proteins. The MEF2 site from the mouse MCK enhancer was used as a probe. Deletion mutants of mouse [mMEF2B Del (107)] and human MEF2B [hMEF2B Del (106)] containing MADS/MEF2 domain without most of the carboxyl terminal (107 amino acids and 106 amino acids from the N-terminus for mMEF2B and hMEF2B, respectively) were also used for EMSA analysis. B: 10T1/2 cells were transiently transfected with 10 μg of the pE102MEF2×2CAT reporter gene and 5 μg of expression vectors encoding each isoform of mouse MEF2B (mMEF2B-1, -2, -3, -4), human MEF2B (hMEF2B), or human MEF2A (hMEF2A). CAT activity in cell extracts was determined as described before. Values are shown as the percentage of the activity observed for human MEF2A and are the average of at least three experiments.

region, and the same efficiency of translation were confirmed by ^{35}S-methionine labeling (data not shown). EMSA revealed that hMEF2A as well as C2 myotube nuclear extracts could indeed bind to the MCK MEF2 site specifically, but the full length of mMEF2B as well as hMEF2B did not show specific binding to the MEF2 site (Fig. 5A). However, truncated mMEF2B peptide, as well as truncated hMEF2B showed specific binding to the MCK MEF2 site when most of the carboxyl terminal regions were deleted and the MADS/MEF2 domains were retained (Fig. 6A).

To investigate MEF2 site-dependent transcriptional activation by the cloned mMEF2B, mMEF2B cDNAs, subcloned into the pMT2 eukaryotic expression vector, were cotransfected with the reporter gene, pE102MEF2× 2CAT, in C3H10T1/2 cells. The cloned mMEF2B factors showed less effective MEF2 site-dependent transcriptional activation, while the cloned hMEF2B or hMEF2A showed

much higher site-dependent transcriptional activation (Fig. 6B).

DISCUSSION

We isolated and characterized mouse *Mef2b* gene. While the entire gene structure of *Drosophila mef2* (*Dmef2*) gene has been reported (*21*), most of the MEF2 genes in higher eukaryotes have not been extensively studied, partly because of the large size of the genes. The structure of the mouse *Mef2b* gene that we identified resembles that of the *Dmef2* gene. Although the coding region of *Mef2b* gene is relatively compact, the 5′ untranslated region seems to be rather big. We also identified the chromosomal localization of *Mef2b* and characterized cloned peptides for the ability to bind to DNA *in vitro* and for site-dependent transcriptional activation ability. Although our results indicate that *Mef2b* is clearly the mouse homologue to human *MEF2B*, they nevertheless suggest that *Mef2b* has quite distinct characteristics from the other members of the MEF2 gene family.

First, based on the cDNA clones isolated from F9 cells, there are several isoforms of mMEF2B due to alternative splicing of the mid coding regions as well as alternative use of the 5′ non-coding exons, resulting in four peptides consisting of 349 aa, 339 aa, 242 aa, and 232 aa. Therefore, all four members of the mouse MEF2 family encode isopeptides, while hMEF2B has been reported to encode only a single peptide so far. Although there is low homology in the 3′ region (exons 8 and 9) between mMEF2B and hMEF2B, the results of Southern hybridization suggested that *Mef2b* is a mouse homologue for human *MEF2B* (Fig. 3). Genomic DNA cloning revealed alternative splicing of exon 6 and exon 7, as well as all of the exon-intron boundaries. Furthermore, this is the first time that the 5′ upstream and 3′ downstream sequences of the MEF2 genes have been disclosed (sequence data have been deposited in GenBank/EMBL/DDBJ). Further studies on the identification of the gene structure of other MEF2 genes are needed.

FISH analysis and an interspecific backcross study revealed that the mouse *Mef2b* gene is located on mouse chromosome 8, and the assigned region for *Mef2b* on mouse chromosome 8 has a conserved linkage homology with the region reported for human *MEF2B* on chromosome 19p12 (*22*). This region is thought to be distal to the *D8Mit9/jund1* locus.

As we suggested in a previous report (*12*), there was almost no expression of mouse *Mef2b* gene in heart or skeletal muscle of adult mouse. The size of mouse *Mef2b* transcripts is relatively small (less than 2 kb) in comparison with those of other *Mef2* genes. These differences between *Mef2b* gene and other *Mef2* genes might account for the different properties. Although the *Mef2b* gene is subject to alternative splicing, resulting in multiple subtypes of the mMEF2B peptide in embryonal cells, the subtype B-2 of *Mef2b* transcript seems to be the major form of mMEF2B in mouse embryonal heart or skeletal muscle (Fig. 5).

DNA-binding activities of mMEF2B were similar to those of hMEF2B, in which *in vitro*-translated peptides of full length did not show specific binding to the MCK MEF2 sequence in EMSA. When truncated peptides translated *in vitro*, containing the MADS/MEF2 domain, were tested in EMSA, mMEF2B as well as hMEF2B showed specific binding to the MCK MEF2 sequence. Although these results might be due to our conditions of EMSA and might not reflect the specific binding ability of mMEF2B *in vivo*, we think that the specific binding ability of mMEF2B (and hMEF2B) is somewhat different from that of MEF2A (and also MEF2C or MEF2D). Regarding transcriptional activity, the cloned mMEF2B factors was less active for MEF2 site-dependent transcriptional activation than the cloned hMEF2B or hMEF2A. This is another difference between mMEF2B and hMEF2B, in addition to the different distribution of transcripts for both MEF2Bs, suggesting that the most carboxyl-terminal region of MEF2B peptide is important for transcriptional activation.

Our results are basically in agreement with the structure of the *Mef2b* gene reported recently by another group (*13*). However, that report did not include the most 5′ and 3′ regions of the gene, and there were several differences from our observations, including the number of exons for the coding region. We identified the coding region of the *Mef2b* gene as including 8 exons in about 7 kb while they found the same coding region containing 7 exons within 3.5 kb. They found only one exon (their exon 5) in the region where we identified two exons (exons 6 and 7), and we found this region to be subject to alternative splicing. They did not identify any alternative splicing in MEF2B transcripts. Although multiple MEF2B transcripts for isotypes produced by alternative splicing were found in mouse embryonal cells, differentiated cells or embryonal heart or muscle predominantly expressed one type of MEF2B transcripts. This might be the reason why the other study did not find alternative splicing in mouse *Mef2b*. However, the biological function of this alternative splicing remains to be found. Second, our findings on the exon-intron boundaries revealed that the differences of resulting amino-acid sequences between subtypes of mouse *Mef2b* or between mouse and human *MEF2B* start from exon 8. We speculate that alternative usage of the 3′ part of the gene might play a distinct role in mouse and human.

The most striking difference between the recent report (*13*) and ours is the pattern of *Mef2b* expression. Our results showed that mouse *Mef2b* is expressed in undifferentiated embryonal cells and that there is some expression in adult spleen, but not in adult heart or skeletal muscle (*12*). On the other hand, the recent report suggested that *Mef2b* is expressed more highly in differentiated skeletal muscles, based on a peptide study using anti-human MEF2B antibody instead of anti-mouse MEF2B antibody. Furthermore, our results showed that no other cells, including myoblasts as well as differentiated myotubes, contained many transcripts for *Mef2b* based on a study using mouse *Mef2b*-specific cRNA probe. Although some discrepancy between the RNA expression and MEF2 peptides has been suggested, we prefer to consider that mouse MEF2B has a distinct pattern of expression, since there are striking differences of the C-terminal amino-acid sequence between human MEF2B and mouse MEF2B, as shown in Fig. 2.

Other MEF2 factors, MEF2A, MEF2C, and MEF2D, have more similarity in their sequences, distribution pattern, and transcriptional activation between human and mouse than MEF2B. Therefore, another human MEF2 factor or derivative corresponding to mMEF2B might be present, although in Southern hybridization, each MEF2B

probe revealed only one gene. Taken together, we conclude that *Mef2b*, a unique member of the *Mef2* gene family, is expressed rather dominantly in embryonal cells and might have a function distinct from that of other known *Mef2* gene family members. The function of mMEF2B remains to be ascertained. More detailed studies on the *Mef2* genes, including *Mef2b* and others, are needed.

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