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RESEARCH

Mouse Galactokinase: Isolation, Characterization, and Location on Chromosome 11

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Elevated galactose levels can be caused by several enzyme defects, one of which is galactokinase. Galactokinase deficiency causes congenital cataracts during infancy and presenile cataracts in the adult population. We have isolated the mouse cDNA for galactokinase, which shares extensive amino acid sequence homology, 88% identity, with a recently cloned human galactokinase. It is expressed in all tissues examined. In an interspecific backcross analysis galactokinase maps to the distal region of mouse chromosome 11, a region that is homologous to human chromosome 17q22–25. The availability of the mouse gene provides an opportunity to make a knockout model for galactokinase deficiency.

Genetic defects of galactose metabolism constitute a class of genetic disorders termed galactosemia. One form of galactosemia in humans is caused by an enzyme deficiency of galactokinase (Segal 1989). Galactokinase acts on the sugar substrate galactose to convert it to galactose-1-PO_4. Individuals with homozygous galactokinase deficiency become symptomatic in the early infantile period showing galactosemia, galactosuria, increased galactitol levels, cataracts, and in a few cases, mental retardation (Segal et al. 1979). Heterozygotes for galactokinase deficiency are prone to presenile cataracts ~20–50 years of age (Stambolian et al. 1986).

Galactokinase activity has been found in a variety of mammalian tissues, including liver, kidney, brain, lens, placenta, erythrocytes, and leukocytes. The isolation and characterization of the galactokinase gene has been completed in *Escherichia coli*, *Saccharomyces*, and *Streptomyces* lividans (Citron et al. 1984; Debouck et al. 1985; Adams et al. 1988). A potential human galactokinase, denoted GK2, with 29% homology to the galactokinase gene of *Saccharomyces*, was cloned by complementation in yeast and mapped to chromosome 15 (Lee et al. 1992). However, the role of GK2 in galactose metabolism remains unclear because active enzyme activity has not been demonstrated (beyond yeast complementation experiments) and mutations in GK2 have not been identified in patients with galactokinase deficiency. Recently, the cDNA encoding human galactokinase, termed GALK1, was cloned, functionally characterized, and mapped to chromosome 17q24 (Stambolian et al. 1995). Additionally, two distinct mutations were identified within GALK1 gene-coding sequences of two unrelated families that exhibited galactokinase deficiency and cataracts.

As a first step toward the generation of a murine model for galactokinase deficiency, we have cloned and characterized the murine galactokinase cDNA. The cDNA obtained shows 88% and 22% protein sequence identity to GALK1 and GK2, respectively. The mouse galactokinase gene was mapped to the distal portion of mouse chromosome 11, a region that is homologous to human chromosome 17q.

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RESULTS

Isolation and Characterization of Mouse Galactokinase cDNA Clones

Three cDNA clones that hybridized strongly with human galactokinase GALK1 cDNA (Stambolian et al. 1995) were isolated from a cDNA library constructed from mouse (strain 129) liver RNA. These clones were all found to contain partial cDNA inserts that encoded a portion of mouse galactokinase but lacked the amino-terminal domain of the protein. Clone pGlk012 contained the largest cDNA insert of 662 bp in length, which terminated with a poly(A) tract of nucleotides (not shown). The missing 5′ end of the galactokinase cDNA was obtained from mouse liver RNA by RT–PCR techniques, using primers corresponding to DNA sequences present within the mouse coding region of clone pGlk012 and the 5′-untranslated region (UTR) of the human GALK1 cDNA (including the ATG initiation codon) (Stambolian et al. 1995). A 900-bp RT–PCR product was found to contain the missing 5′ end of the mouse galactokinase cDNA. Independent RT–PCR reactions were performed, and the DNA sequences of these multiple PCR products were determined to confirm the DNA sequence of the mouse galactokinase cDNA and exclude the possibility of PCR-generated mutations occurring within the sequence.

The 900-bp RT–PCR product was combined with the insert of pGlk012 to generate a mouse galactokinase (*Glk1*) cDNA of 1316 bp in length following removal of the overlapping nucleotide sequence. This cDNA is terminated with a poly(A) tract of nucleotides that is preceded 12 bp upstream by a canonical eukaryotic polyadenylation signal, AATAAA (Fig. 1) (Proudfoot and Brownlee 1976). The cDNA encodes a peptide of 392 amino acids with a calculated molecular mass of 42,271 daltons.

Alignment of the predicted mouse galactokinase protein with human GALK1, GK2, and *E. coli* galactokinase shows a low to high degree of overall sequence similarity, ranging from ~22% to 88% amino acid sequence identity (Fig. 2). The mouse galactokinase sequence shares the greatest level of sequence conservation, 88% identity, with the human GK17 galactokinase protein. The mouse *Glk1* protein also shares 42% amino acid sequence identity with *E. coli* galactokinase. In contrast, the human GK2 and mouse *Glk1* proteins are only 22% identical. Importantly, three regions containing a galactokinase signature sequence (Debouck et al. 1985) and two ATP-binding motifs (Tsay et al. 1991) are identically conserved in the human and mouse galactokinases.

To examine the size and pattern of expression of mouse galactokinase mRNA in different mouse tissues, Northern blot analysis was performed using the 5′ end of the galactokinase cDNA as a hybridization probe. As shown in Figure 3, a single mRNA transcript of ~1.35 kb was detectable in all mRNA samples. This size is comparable to that of the *Glk1* cDNA, indicating that the isolated cDNA likely represents the entire mRNA transcript of the gene (with the exception of some 5′ UTR sequence).

Expression of Galactokinase Activity

The full-length *Glk1* cDNA was subcloned into the mammalian cell expression vector pCDN, forming vector pCDNGlk1, and transformed into COS cells. After 3 days of incubation, cell extracts were prepared and analyzed for galactokinase activity. COS cells transfected with the pCDN vector alone exhibited very low levels of galactokinase activity, amounting to a mean activity of 0.02 U/μg of protein. In contrast, galactokinase activity increased >10-fold to a mean activity of 0.80 U/μg of protein when COS cells were transfected with the pCDNGlk1 vector. This result functionally confirms that *Glk1* cDNA encodes mouse galactokinase.

Interspecific Backcross Mapping of Glkl

The murine chromosomal location of *Glk1* was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × *Mus spretus*) F_1 × C57BL/6J] mice. This interspecific backcross mapping panel has been typed for >1800 loci that are well distributed among all mouse autosomes and the X chromosome (Copeland and Jenkins 1991, and unpubl.). C57BL/6J and *M. spretus* DNAs were digested with several restriction enzymes and analyzed by Southern blot hybridization for informative restriction fragment-length polymorphisms (RFLPs) using a mouse *Glk1* cDNA probe (see Methods). A 6.1-kb *M. spretus*-specific *Bgl*II RFLP was used to follow the segregation of the *Glk1* locus in backcross DNAs. The mapping results indicated that *Glk1* is located in the distal region of mouse chromosome 11 (Fig. 4). Although 146 mice were analyzed for every marker shown in the haplotype

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                                                                          CAGAGCTGCAGGCGCGCGTC

ATG GCT GCT TGG AGA CCG CCC CGG GTC GAG GAG CTG CTG GCC GAG GCC CGC CGG GCC TTC    60
 M   A   A   W   R   P   P   R   V   E   E   L   L   A   E   A   R   R   A   F

ATG GAG GAG TTT GGA GCC GAG CCG GAG CTG GCA GTG TCG GCG CCG GGC CGC GTC AAC CTC   120
 M   E   E   F   G   A   E   P   E   L   A   V   S   A   P   G   R   V   N   L

ATC GGG GAG CAC ACG GAC TAC AAC CAG GGC CTG GTG CTG CCC ATG GCA CTG GAG CTC GTG   180
 I   G   E   H   T   D   Y   N   Q   G   L   V   L   P   M   A   L   E   L   V

ACC GTG ATG GTT GGC AGC CCC CGG ACA GAT GGG CTT GTT TCT CTT CTC ACC ACT TCC AAA   240
 T   V   M   V   G   S   P   R   T   D   G   L   V   S   L   L   T   T   S   K

GAT GCA GAC GAG CCC CAA AGA CTG CAG TTC CCA CTG CCC TCA GCC CAG TGG TCC TTG GAG   300
 D   A   D   E   P   Q   R   L   Q   F   P   L   P   S   A   Q   W   S   L   E

CCT GGA ATC CCA CAG TGG GCC AAT TAT GTC AAG GGA GTG ATT CAA CAT TAC CCA GCT TCC   360
 P   G   I   P   Q   W   A   N   Y   V   K   G   V   I   Q   H   Y   P   A   S

CCG CTC GTT GGC TTC AGT GCA GTG GTG GTC AGC TCA GTG CCC CTG GGG GGT GGG CTT TCC   420
 P   L   V   G   F   S   A   V   V   V   S   S   V   P   L   G   G   G   L   S

AGC TCA GCG TCT CTG GAA GTG GCC ACG TAC ACC TTC ATC CAG CAG CTC TGC CCA GAC TCG   480
 S   S   A   S   L   E   V   A   T   Y   T   F   I   Q   Q   L   C   P   D   S

GGG GCA ATA GCT GCC CGG GCC CAG GTG TGT CAA CGG GCT GAG CAC AGC TTC GCA GGG GTG   540
 G   A   I   A   A   R   A   Q   V   C   Q   R   A   E   H   S   F   A   G   V

CCC TGT GGC ATC ATG GAC CAA CTC ATC GCG CTG CTG GGG CAG AAA GGC TAT GCA CTA CTC   600
 P   C   G   I   M   D   Q   L   I   A   L   L   G   Q   K   G   Y   A   L   L

ATT GAC TGC AGG TCC CTG GAA ACA AGC CTG GTG CCA CTG TCA GAC CCC AAG CTG GCC GTG   660
 I   D   C   R   S   L   E   T   S   L   V   P   L   S   D   P   K   L   A   V

CTC ATC ACC AAC TCC AAT GTC CGC CAT TCC CTG GGC TCC AGC GAG TAC CCG GTT CGT CGG   720
 L   I   T   N   S   N   V   R   H   S   L   G   S   S   E   Y   P   V   R   R

CGA CAG TGT GAA GAA GTG GCC CAG GCC CTG GGC AAG GAG AGC CTT CGA GAG GTG CGG ATG   780
 R   Q   C   E   E   V   A   Q   A   L   G   K   E   S   L   R   E   V   R   M

GAG GAG CTT GAG GCG GGC AGG GAG CTA ATG AGC AAG GAG GGC TTC CGG CGG GCC CGG CAT   840
 E   E   L   E   A   G   R   E   L   M   S   K   E   G   F   R   R   A   R   H

GTG GTA AGC GAG ATC CGG AGA ACA GCC CAG GCA GCA GCT GCT ATG AGC CGA GGA GAC TAC   900
 V   V   S   E   I   R   R   T   A   Q   A   A   A   A   M   S   R   G   D   Y

AAG GCC TTT GGG CGT CTC ATG GTG GAG AGT CAC TAC TCA CTC AGG GAT GAC TAT GAG GTC   960
 K   A   F   G   R   L   M   V   E   S   H   Y   S   L   R   D   D   Y   E   V

AGC TGC CCT GAG CTG GAT CAA TTG GTT GAG GCC GCG CTC TCT GTG CCT GGG GTT TAT GGC  1020
 S   C   P   E   L   D   Q   L   V   E   A   A   L   S   V   P   G   V   Y   G

AGT CGC ATG ACA GGT GGT GGC TTT GGT GGC TGC ACC GTC ACA TTG CTG GAG GCC TCT GTT  1080
 S   R   M   T   G   G   G   F   G   G   C   T   V   T   L   L   E   A   S   V

GCC CCT CTT GTC ATA GAT CAT ATA CAG GAG CAG TAC AGT GGG ACA GCC ACC TTC TAC CTC  1140
 A   P   L   V   I   D   H   I   Q   E   Q   Y   S   G   T   A   T   F   Y   L

TCT CAA GCT GCC GAT GGA GCC CAG GTG CTG AGC TTG TGA GGTGTCCCTGAGACCGATGGACGCACA  1206
 S   Q   A   A   D   G   A   Q   V   L   S   L   *

GGCATGAGGCGGAGGCAGCAGGTGTGTGCCGCAGCTCTGCCTCGGGCATCCTCCTTCTCTATCTGGGTGCTTAATAAAC  1285

GTGTGCCTCTT
```

Figure 1 Nucleotide and deduced amino acid sequence of the *Glk1* cDNA. Amino acids (represented by the one-lettered code) are indicated below their respective codons beginning with the initiation methionine codon (M). Nucleotide positions are numbered at *right*. The underlined nucleotide sequence indicates canonical eukaryotic polyadenylation signals. The poly(A) tract of the cDNA is not shown. Nucleotides preceding the first ATG are from the 5′ UTR.

analysis (Fig. 4), up to 176 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are centromere–*Pkca*–13/149–*Glk1*–6/176–*Thbp*, where *Pkca* is protein kinase C-α and *Thbp* is prolyl 4-hydroxylase-β polypeptide. The recombination frequencies (expressed as genetic distances in cM ± the S.E.) are centromere–*Pkca*–8.7 ± 2.3–*Glk1*–3.4 ± 1.4–*Thbp*.

DISCUSSION

We have described the isolation of a mouse cDNA that encodes a protein that shares 88% sequence identity with human galactokinase GALK1. Evidence that the isolated cDNA encodes mouse galactokinase follows: (1) The encoded protein shares significant sequence homology with all known galactokinases and, more notably, contains several sequence motifs, including a galactokinase signature sequence and a putative ATP-binding domain that is highly conserved among all galactokinases; (2) the cDNA produced a 10-fold increase of galactokinase enzyme activity when expressed in COS cells; (3) the cognate gene of the cDNA was mapped to a distal location on chromosome 11, a region consistent with the location of the human homolog on chromosome 17q. Collectively, these results establish that we have cloned the mouse galactokinase gene, which we designate *Glk1*.

The mouse galactokinase gene had been mapped previously by enzyme activity on starch gel electrophoresis to chromosome 11 in a panel of Chinese hamster/mouse somatic cell hybrids (Mishkin et al. 1976; Kozak and Ruddle 1977; McBreen et al. 1977). Subsequently, its location was positioned on the distal region of mouse chro-

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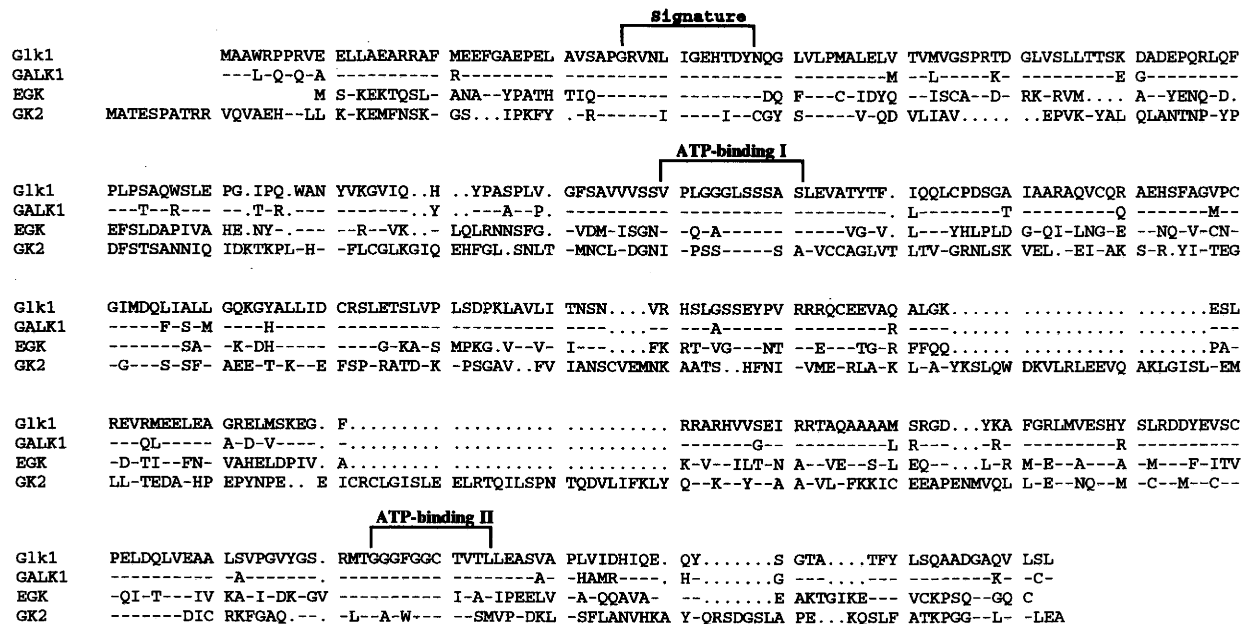

Figure 2 Amino acid sequence comparison of galactokinases for *E. coli* (EGK), human GK2, human GALK1, and mouse *Glk1*. Dashes indicate amino acid identity, whereas amino acid differences are shown in their corresponding position. Dots represent gaps which are introduced to maximize identity. The galactokinase signature sequence and two putative ATP-binding domains are depicted by brackets above the sequence.

mosome 11 based on (1) its syntenic relationship with *Umph-2* (Wilson et al. 1987), and (2) the homology between distal chromosome 11 and human chromosome 17q21–q25, where the human galactokinase gene had been mapped by somatic cell hybrid analysis. Our placement of *Glk1* on the distal region of mouse chromosome 11 (Fig. 4) agrees with this previous mapping data. Recently, the human galactokinase gene was mapped by in situ hybridization to chromosome 17q24 (Stambolian et al. 1995), which is consistent with our location of the mouse gene on chromosome 11.

Finally, we have aligned our interspecific linkage map of distal chromosome 11 with a composite linkage map that reports the map location of many uncloned mouse mutations (compiled from the Mouse Genome Database, a computerized data base of mouse linkage information maintained at The Jackson Laboratory, Bar Harbor, ME). None of the mutations close to *Glk1*, that is, *cod*, *tn*, and *js* (Lyon and Searle 1989), have a phenotype that might be expected for a mutation (i.e., cataracts) at this locus. However, it is still possible that one of these mutations is caused by a defect in *Glk1*. Additional studies will be required to rule out this possibility.

The isolation of the mouse galactokinase cDNA now makes it possible to use it as a probe to clone its genomic counterpart. This gene will then be used to create mouse knockout strains, thus providing a mouse model that will be useful for evaluating the role of galactokinase in cataract formation.

METHODS

cDNA Cloning

A cDNA library was constructed from liver poly(A)$^+$ RNA extracted from a female mouse (129 strain), primed with

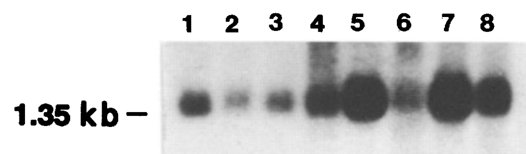

Figure 3 Expression analysis of the galactokinase gene. Northern blot (Mouse Multiple Tissue Northern Blot, Clontech) was probed with a 474-bp 5′ cDNA fragment of *Glk1*. The size is indicated at *left* in kb. (Lane *1*) RNA from heart; (lane *2*) brain; (lane *3*) spleen; (lane *4*) lung; (lane *5*) liver; (lane *6*) skeletal muscle; (lane *7*) kidney; and (lane *8*) testis. Tissues for RNA extraction were removed from 9- to 10-week-old mice. The blot probed with the *Glk1* probe was exposed to X-ray film overnight.

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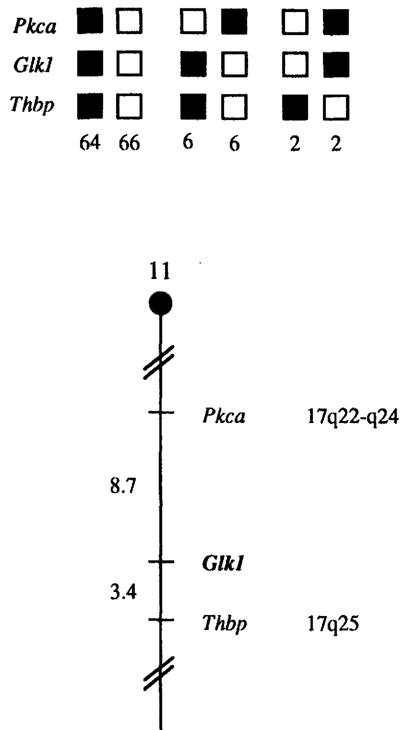

Figure 4 *Glk1* maps in the distal region of mouse chromosome 11. The segregation patterns of *Glk1* and flanking genes in 146 backcross animals are shown at the *top*. For some individual pairs of loci, >146 animals were typed. Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*) F_1 parent. (■) The presence of a C57BL/6J allele; (□) the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the *bottom* of each column. A partial chromosome 11 linkage map showing the location of *Glk1* in relation to linked genes is shown below the columns. Recombination distances between loci in cM are shown to the *left* of the chromosome, and the positions of loci in human chromosomes, where known, are shown to the *right*. References for human map positions can be obtained from GDB (Genome Data Base), a computerized data base of human linkage information maintained by The William H. Welch Medical Library of The Johns Hopkins University (Baltimore, MD).

oligo(dT) and packaged into the Lambda ZAP vector following the manufacturer's suggestions (Stratagene). The cDNA was size-fractionated before ligation on a Sephacryl S-400 gel filtration column. The fraction containing 0.5–6 kb was ligated and yielded a library complexity of 1.25 × 10^6 clones. Plaques were transferred to nylon filters (MSI), and hybridization was done in 50% formamide, 5× SSPE, 5× Denhardt's solution, 100 ng/ml of salmon sperm DNA, and 0.1% SDS using the full-length human galactokinase cDNA, GALK1, as a probe. The filters were finally washed with 0.2× SSPE, 0.1% SDS, at 50°C and exposed to X-ray film at –70°C overnight. Positive clones were plaque purified. Three partial cDNAs were isolated from the initial screening.

RT–PCR

Poly(A)$^+$ RNA from strain 129 liver was used. The first-strand cDNA was synthesized using primer 1949 (5′-CCTTGTAGTCTCCTCGGCTCATA-3′) which was designed to the 3′ end of the cloned mouse galactokinase cDNA. To generate the specific 5′ cDNA product, the PCR reaction was performed using a nested primer, 1950 (5′-CTCCGGATCTCGCTTACCACAT-3′), located 5′ to primer 1949, and another primer, DJ4 (5′-CAGAGCTGCAG-GCGCGCGTCA-3′), designed to the human galactokinase 5′ UTR. The thermal cycler (MJ) was programmed for 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min. The DJ4/1950 primer pair gave a specific band of ~900 bp. The RT–PCR product was directly subcloned into the TA vector (Invitrogen) for sequencing.

Sequence Analysis

Sequencing of both strands was performed from isolated plasmid. Five separate subclones of the RT–PCR product were sequenced to rule out the possibility of any *Taq* polymerase errors. Sequencing was done on an automated ABI 373A sequencer. Alignments were done using MacVector software (Kodak).

Expression in COS Cells

An *Eco*RI–*Kpn*I cDNA fragment encompassing the entire coding region of the mouse galactokinase gene was subcloned into the COS cell (Gluzman 1981) expression vector pCDN (Aijar et al. 1994) generating the clone pCDNGlk1. COS cells were grown to 60%–80% confluency and then transfected with 50 µg of DNA/per flask of pCDNGlk1 or pCDN control as described previously (Caltabiano et al. 1989). After incubation at 37°C for 3 days, the cells were washed with phosphate-buffered saline, scraped from the plate with a rubber policeman, pelleted by low-speed centrifugation, and frozen on dry ice. Cell lysates were prepared for the galactokinase assay (Stambolian et al. 1985) by dissolving in 10 mM phosphate buffer (pH 7.2).

Northern Blot Analysis

A Northern blot containing 2 µg of poly(A)$^+$ RNA per lane from different mouse tissues was purchased from Clontech Laboratories (Palo Alto, California) and probed with a ^{32}P-labeled 474-bp cDNA fragment from the 5′ end of the mouse galactokinase cDNA according to the recommendations of the manufacturer. Following hybridization, the filter blot was washed in 0.1× SSPE, 0.1% SDS, at 65°C and exposed to Kodak X-ray film at –70°C with an intensifying screen overnight.

Interspecific Mouse Backcross Mapping

Interspecific backcross progeny were generated by mating

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(C57BL/6J × *M. spretus*) F_1 females and C57BL/6J males as described (Copeland and Jenkins 1991). A total of 205 backcross mice were used to map the galactokinase locus (*Glk1*). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described (Jenkins et al. 1982). All blots were prepared with Hybond N^+ nylon membrane (Amersham). The probe, a 600-bp *Eco*RI–*Xho*I mouse cDNA fragment, was labeled with [α-^{32}P]dCTP by nick translation (Boehringer Mannheim); washing was done to a final stringency of 1.0× SSCP, 0.1% SDS, at 65°C. A 7.5-kb fragment was detected in *Bgl*II-digested C57BL/6J DNA, and a 6.1-kb fragment was detected in *Bgl*II-digested *M. spretus* DNA. The presence or absence of the 6.1-kb *M. spretus*-specific *Bgl*II fragment was followed in backcross mice.

A description of the probes and RFLPs for the loci linked to *Glk1* including *Pkca* and *Thbp* have been reported previously (Buchberg et al. 1989; Morishige et al. 1993). Recombination distances were calculated as described by Green (1981), using the computer program SPRETUS MADNESS. Gene order was determined by minimizing the number of double and multiple recombination events across the chromosome.

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