Murine Mucopolysaccharidosis Type VII

Characterization of a Mouse with β-Glucuronidase Deficiency

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Abstract

We have characterized a new mutant mouse that has virtually no β-glucuronidase activity. This biochemical defect causes a murine lysosomal storage disease that has many interesting similarities to human mucopolysaccharidosis type VII (MPS VII; Sly syndrome; β-glucuronidase deficiency). Genetic analysis showed that the mutation is inherited as an autosomal recessive that maps to the β-glucuronidase gene complex, [*Gus*], on the distal end of chromosome 5. Although there is a > 200-fold reduction in the β-glucuronidase mRNA concentration in mutant tissues, Southern blot analysis failed to detect any abnormalities in the structural gene, *Gus-s*^{b}, or in 17 kb of 5′ flanking and 4 kb of 3′ flanking sequences. Surprisingly, a sensitive S1 nuclease assay indicated that the relative level of kidney *gus*^{mps} mRNA responded normally to androgen induction by increasing ~ 11-fold. Analysis of this mutant mouse may offer valuable information on the pathogenesis of human MPS VII and provide a useful system in which to study bone marrow transplantation and gene transfer methods of therapy.

Introduction

β-Glucuronidase (β-D-glucuronide glucuronohydrolase EC 3.2.1.31) is a lysosomal enzyme expressed in most, if not all, mammalian tissues (1). In mouse kidney and liver the enzyme is also found in the microsomes in association with the accessory binding protein, egasyn (2). The active enzyme is a tetrameric glycoprotein that degrades glycosaminoglycans by removing β-glucuronosyl residues at the nonreducing end of oligosaccharides (3, 4). Severe deficiency of this enzyme results in the accumulation of undegraded glycosaminoglycans in the lysosomes and produces the disease mucopolysaccharidosis type VII (5). This disease was first described in humans (6, 7) but there is also a canine model of β-glucuronidase deficiency (8).

Murine β-glucuronidase has been studied extensively because it provides a useful system for understanding mammalian gene regulation (1). The structural gene, *Gus-s,* is located on the distal half of chromosome 5 of the mouse. There are three common alleles designated *Gus-s*^{a}, *Gus-s*^{b}, and *Gus-s*^{h}, which are differentiated by electrophoretic mobility and heat stability (9–11). Three regulatory elements, designated *Gus-r, Gus-t,* and *Gus-u,* are closely linked to *Gus-s. Gus-r* is *cis*-acting and determines the level of *Gus-s* mRNA in the proximal tubule cells of the kidney in response to androgen induction (12). *Gus-t* is a *trans*-acting temporal regulator that determines the rate of β-glucuronidase synthesis in several mouse tissues during postnatal development (13, 14). *Gus-u* is a systemic *cis*-acting regulator that determines the relative levels of enzyme activity in all tissues (14, 15). These three regulatory elements in conjunction with the structural gene define the β-glucuronidase gene complex, [*Gus*].

In C3H strains of mice which are of the [*Gus*]^H haplotype (16), there are relatively low levels of β-glucuronidase activity in all tissues but the mice do not have any clinical symptoms. In lieu of a better mouse model, C3H mice have been utilized to study the correction of lysosomal enzyme deficiency by allogeneic bone marrow transplantation (17). Several years ago, three mice with dwarfing characterized by shortness of nose, limbs, tail, and body length were identified in a colony of B6.C-*H-2*^{bm1} mice at The Jackson Laboratory. The mutant gene was given the provisional name of adipose storage deficiency (*asd*) because, in addition to the obvious skeletal deformities, the animals were devoid of visually identifiable white adipose tissue (18). While studying this mutation at the genetic and biochemical levels, we found that the mutation is closely linked to the *Gus-s*^{b} allele and causes a severe, if not complete, deficiency of β-glucuronidase activity. Because this mutant mouse has a disease resembling human mucopolysaccharidosis type VII (MPS VII),[1] we have renamed the recessive mutation *gus*^{mps}. This paper describes our initial characterization of this mutation at the genetic, cellular, biochemical, and molecular levels. The results show that the mutation causes a severe lysosomal storage disease that we have named murine MPS VII. In addition, we discuss the importance of this mouse in developing methods of therapy for lysosomal storage diseases as well as providing information about the regulation of mammalian gene expression.

Methods

Animals. All animals used in these studies were from The Jackson Laboratory. They were obtained from the B6.C-*H-2*^{bm1}/ByBir-*gus*^{mps}/+ mutant strain maintained in the research colony of Dr. Birkenmeier, from the MOR/Rk strain maintained by T. H. Roderick, and from the DBA/2J production colony of The Jackson Laboratory. The mutant strain was maintained by brother–sister matings of *gus*^{mps}/+ animals and each animal was assigned a pedigree number that was recorded in the breeding records. The mice were fed Wayne Sterilizable Rodent

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1. *Abbreviations used in this paper:* MPS VII, mucopolysaccharidosis type VII; 4-MU, 4-methylumbelliferone; RFLP, restriction fragment length polymorphism.

Blox (Continental Grain Company, Chicago, IL), which is essential for the optimal health and reproductive performance of the mutant strain.

Transmission electron microscopy. Tissues were fixed in 2% glutaraldehyde, 1% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4. The tissues were then postfixed in 1% osmium tetroxide, stained *en bloc* with 0.5% uranyl acetate, dehydrated, and embedded in Epon-araldite. Ultrathin sections were stained again with uranylacetate followed by lead citrate.

Genetic mapping. To determine the chromosomal location of the gus^{mps} mutation, heterozygous $gus^{mps}/+$ mice were outcrossed to either DBA/2J or MOR/Rk mice. The F1 progeny were then intercrossed and the F2 homozygous mutants were screened for the isozymes phosphoglucomutase-1 (*Pgm-1*), mitochondrial malate dehydrogenase (*Mor-1*), and β-glucuronidase (*Gus*) using standard cellulose acetate electrophoretic methods and staining procedures (11, 19, 20). DNA was isolated from the spleens of the F2 mutant animals. The DNA was digested with *Eco* RI or *Hind* III, Southern blotted, and then hybridized to cDNA probes of α-fetoprotein (*Afp*) or β-glucuronidase (*Gus*), respectively. The DNAs were scored using restriction fragment length polymorphisms (RFLPs) that were known to exist at these two loci.

Biochemical analysis. β-Glucuronidase activity was measured using a sensitive fluorometric assay (21, 22). Tissues were homogenized in 50 mM Tris-HCl pH 8.0 (2:1 volume to weight ratio) using a motor-driven pestle designed to fit into a conical 1.5-ml minicentrifuge tube. Enzyme assays were in 0.1 M sodium acetate, pH 4.6, in a final volume of 0.1 ml. The substrate was 4 mM 4-methylumbelliferyl-β-D-glucuronide and the reaction mixture was incubated at 37°C for 1 h. The reaction was stopped by the addition of 1.0 ml of 0.1 M sodium carbonate. Protein concentration was determined by the method of Lowry et al. (23). Activity was expressed as nanomoles of 4-methylumbelliferone (4-MU) released per milligram protein per hour.

RNA isolation and characterization. Total cellular RNA was isolated from mouse tissues by extraction with guanidine thiocyanate followed by centrifugation through CsCl (24). Poly A^+ RNA was isolated using oligo-dT cellulose chromatography. Northern blots were prepared after formaldehyde-agarose gel electrophoresis of the RNA (25). The Zetabind (AMF, Meriden, CT) nylon filters were hybridized to cDNA probes radiolabeled by the random hexamer method as described previously (26, 27).

For the S1 nuclease protection assays, part of the pGUS-1 cDNA clone representing exons 2 through 12 was subcloned into a Blue Scribe vector (Stratagene, LaJolla, CA). T7 RNA polymerase was used with [^{32}P]UTP to make labeled anti-sense RNA (cRNA) that was separated from the template by agarose gel electrophoresis. For each assay 100 μg of total RNA was suspended in a formamide hybridization buffer containing $1–5 \times 10^5$ cpm of the cRNA probe. The sample was denatured at 85°C and then allowed to hybridize at 65°C from 3 h to overnight. After hybridization, the samples were treated with 400 U S1 nuclease. To assay for the S1-resistant hybrid molecules, the samples were electrophoresed in nondenaturing polyacrylamide gels. The gels were dried and autoradiography performed at −70°C with an intensifying screen. To confirm that equal amounts of RNA were present in each assay, parallel assays were performed using a γ-actin cRNA probe. An additional control assay utilized *Escherichia coli* RNA rather than mouse tissue RNA in the hybridization reaction in order to verify the specificity of the bands detected.

Southern blot analysis. Genomic DNAs were digested with restriction enzymes, electrophoresed in 0.8% agarose gels, and transferred to Zetabind nylon filters by the Southern method (28). Probes were made from the various cloned DNAs using the random hexamer labeling method and were hybridized to the nylon filters as described previously (26).

Testosterone induction. In our initial experiments and for the Northern blots shown in this paper, female gus^{mps}/gus^{mps} mice were treated with testosterone by implanting capsules made from Silastic tubing (Dow Corning Corp., Midland, MI) in the nape of the neck as described previously (29). Each capsule contained either 0 or 5 mg of testosterone. Normal female mice of unknown genotype (+/+ or $gus^{mps}/+$) were included as controls to determine whether β-glucuronidase enzyme activity and mRNA concentration had been induced in the kidney in response to this treatment. For the S1 nuclease protection data that are presented in this paper, the mice received a 30-mg pellet of testosterone in the nape of the neck. The mutant animals responded in a similar fashion to each of these two methods of testosterone delivery.

Molecular clones. The cloned DNAs used to make probes for the Northern and Southern blots were obtained from several sources. The pGUS-1 cDNA clone of murine β-glucuronidase (12) was obtained from Dr. Ganschow. The subclones from the cosmid clone D23 (or PGY-1) of the $Gus\text{-}s^b$ allele from the YBR mouse strain (30) were provided by K. Paigen (University of California, Berkeley, CA). The α-fetoprotein cDNA clone, pHcII 440, was obtained from S. M. Tilghman (Princeton University, Princeton, NJ).

Results

History and clinical description of the gus^{mps}/gus^{mps} mouse. The mutation occurred on the congenic mouse strain B6.C-$H\text{-}2^{bm1}$/By, which was originally called H(z1). This strain carries a mutant $H\text{-}2K^b$ allele named $H\text{-}2K^{bm1}$. The strain originated from a C57BL/6By female mated to an irradiated BALB/cBy male (31). The $H\text{-}2K^{bm1}$ mutation was backcrossed for 10 generations to C57BL/6By mice and then inbred in order to establish the congenic strain mentioned above. Approximately 10 yr later in 1976, the first gus^{mps}/gus^{mps} animals were identified in the B6.C-$H\text{-}2^{bm1}$/By colony at generation F34. The parents and their offspring were used to develop a separate inbred colony of mice maintained by strict brother–sister matings of pedigreed animals. Mutant males are reproductively sterile for reasons independent of gonadal and reproductive tract morphology or of sperm number, morphology, and motility. Mutant females are capable of producing litters but, because of insufficient lactation, the offspring must be raised by foster mothers. Therefore, the strain, named B6.C-$H\text{-}2^{bm1}$/ByBir-gus^{mps}/+, is maintained by mating heterozygous $gus^{mps}/+$ siblings identified by their ability to produce mutant offspring.

Fig. 1 shows a 269-d-old mutant animal compared with a normal littermate. The mutant phenotype is easily discernible since the affected animals are smaller and have shorter, stubby limbs as well as a shorter, thicker tail. The most unusual feature is the peculiar facial dysmorphism shown in Fig. 1 *B*. The nasal bones are reduced markedly in size, resulting in a pug-nosed appearance. Although the mutant animals look grossly normal at birth, the abnormal appearance of the body and face described above is usually evident by the time the animals are weaned at 21 d of age. The syndrome is characterized further by early sudden death from unknown causes. Fig. 2 shows the ages at which adult male and female mice died in the colony during the previous 2 yr. Although normal C57BL/6J mice live to 850 or more d of age, the mutant male animals lived an average of 170±62 d (n = 38) while the females had a slightly shorter mean lifespan ($P < 0.025$, Student t test) of 141±67 d (n = 53). Of the 91 animals included in these statistics, only 10 lived longer than 241 d, the oldest a female who lived 340 d.

The mutant animals have several other unusual features. In addition to the peculiar facies, they are dwarfs with severe skeletal deformities. Smears of the peripheral blood showed abundant granulocytic inclusions in nucleated cells. Electron and light microscopy revealed evidence of vacuolar storage in many tissues. Fig. 3 shows the pronounced cytoplasmic vacu-

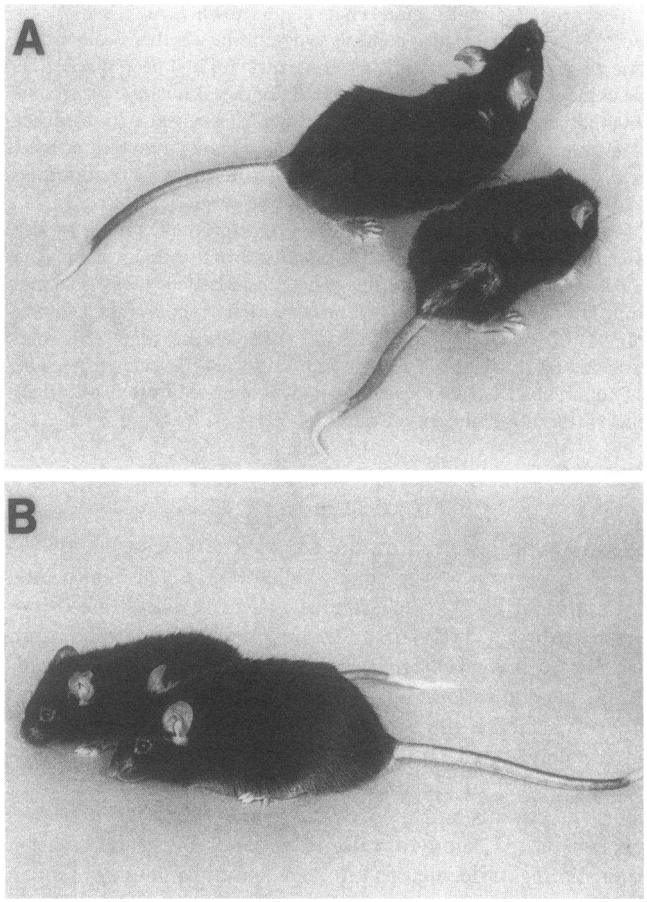

Figure 1. Appearance of gus^{mps}/gus^{mps} mice. Two photographs of a male gus^{mps}/gus^{mps} mouse standing next to a normal littermate male +/? mouse for comparison. The mice are 269 d of age. (*A*) The mutant is the smaller of the two animals. (*B*) The mutant is standing behind the normal mouse and has the shortened nose characteristic of the face in murine MPS VII.

olization present in endothelial cells of spleen and liver. These findings are remarkably similar to human patients with β-glucuronidase deficiency (6, 22). However, the specific diagnosis of murine MPS VII required the genetic and biochemical data presented below.

Genetic analysis of the gus^{mps} mutation. Initial genetic studies confirmed that the mutation was autosomal recessive. Of 1,179 animals that were born to heterozygous parents over a 2 yr period and survived to weaning, 233 (19.8%) were gus^{mps}/gus^{mps} and 946 (80.2%) were +/?. The female to male ratios for normal and mutant animals were 0.86 and 0.74, respectively.

Genetic analysis to determine the chromosomal location of the mutant gene led to the identification of the biochemical defect as β-glucuronidase deficiency. In the first 36 mutant animals obtained from a DBA/2J intercross, the recombination frequency with phosphoglucomutase-1 (*Pgm-1*) was 31% (23/72 chromosomes). Because this was less than the 50% level expected for random segregation, these results suggested that the mutation was on chromosome 5. Linkage to chromosome 5 was confirmed using an α-fetoprotein (*Afp*) cDNA clone, pHcII 440, that detects an *Eco* RI RFLP between C57BL/6J and DBA/2J. Of the 27 F2 mutant animals (54 chromosomes) typed for both *Pgm-1* and *Afp*, we found 37.03±6.75% and 20.37±5.48% recombination, respectively, between these loci and gus^{mps}.

These results suggested that gus^{mps} was located on the distal half of chromosome 5. This was confirmed using an intercross with MOR/Rk mice. MOR mice carry the $Mor\text{-}1^b$ and $Gus\text{-}s^a$ alleles, while the mutant strain carries the $Mor\text{-}1^a$ and $Gus\text{-}s^b$ alleles (20). The gene order on chromosome 5 is *Pgm-1, Afp,* β-glucuronidase (*Gus*), and mitochondrial malate dehydrogenase (*Mor-1*). Among 74 mutant animals typed for *Mor-1,* there was 6.08±1.96% recombination between gus^{mps} and *Mor-1.* To complete the genetic studies, the mutation was mapped to the [*Gus*] complex using a β-glucuronidase cDNA clone, pGUS-1, that detects a *Hind* III RFLP between the structural genes $Gus\text{-}s^a$ and $Gus\text{-}s^b$ (16). Out of 40 DNAs tested (80 chromosomes), no recombination occurred between gus^{mps} and $Gus\text{-}s^b$. Thus, at the 95% upper confidence limit, these two loci are located within 3.7 centimorgans of one another. In addition, these results showed that the congenic strain carrying the gus^{mps} mutation had the C57BL/6By $Gus\text{-}s^b$ allele rather than the BALB/cBy $Gus\text{-}s^a$ allele. Therefore, the radiation treatment given the original BALB/cBy father of the H(z1) mouse could not have been the direct cause of the gus^{mps} mutation. In all probability, gus^{mps} is a spontaneous mutation of the $[Gus]^B$ haplotype that occurred in the B6.C-$H\text{-}2^{bm1}$/By colony.

Biochemical characterization of gus^{mps}/gus^{mps} mice. While the chromosome mapping studies were in progress, we attempted to biochemically type the mutant mice for the two β-glucuronidase alleles present in the second intercross. However, when the cellulose acetate plates were stained for β-glucuronidase activity by a simultaneous dye coupling method using naphthol-AS-BI-β-D-glucuronide as substrate (11), no activity was observed. This finding indicated that the biochemical defect was probably β-glucuronidase deficiency. To measure more accurately the specific activity of β-glucuronidase in various tissues, we used a sensitive fluorometric assay with 4-methylumbelliferyl-β-D-glucuronide as the substrate (21, 22). This method detected extremely low levels of what was presumably β-glucuronidase activity. Assays of liver, kidney,

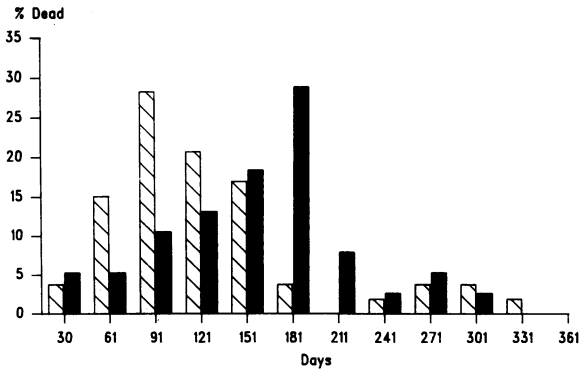

Figure 2. Lifespan of adult male and female gus^{mps}/gus^{mps} mice. This histogram shows the age in days at which 38 male (*solid bars*) and 53 female (*striped bars*) mutant mice died of natural causes in the colony over a 2-yr period. The histogram is a plot of the percentage of the animals for each sex that died within each consecutive 30-d period versus the minimum age in days represented in each time period. These data were obtained from animals that lived long enough to be weaned and had reached a minimum age of 30 d.

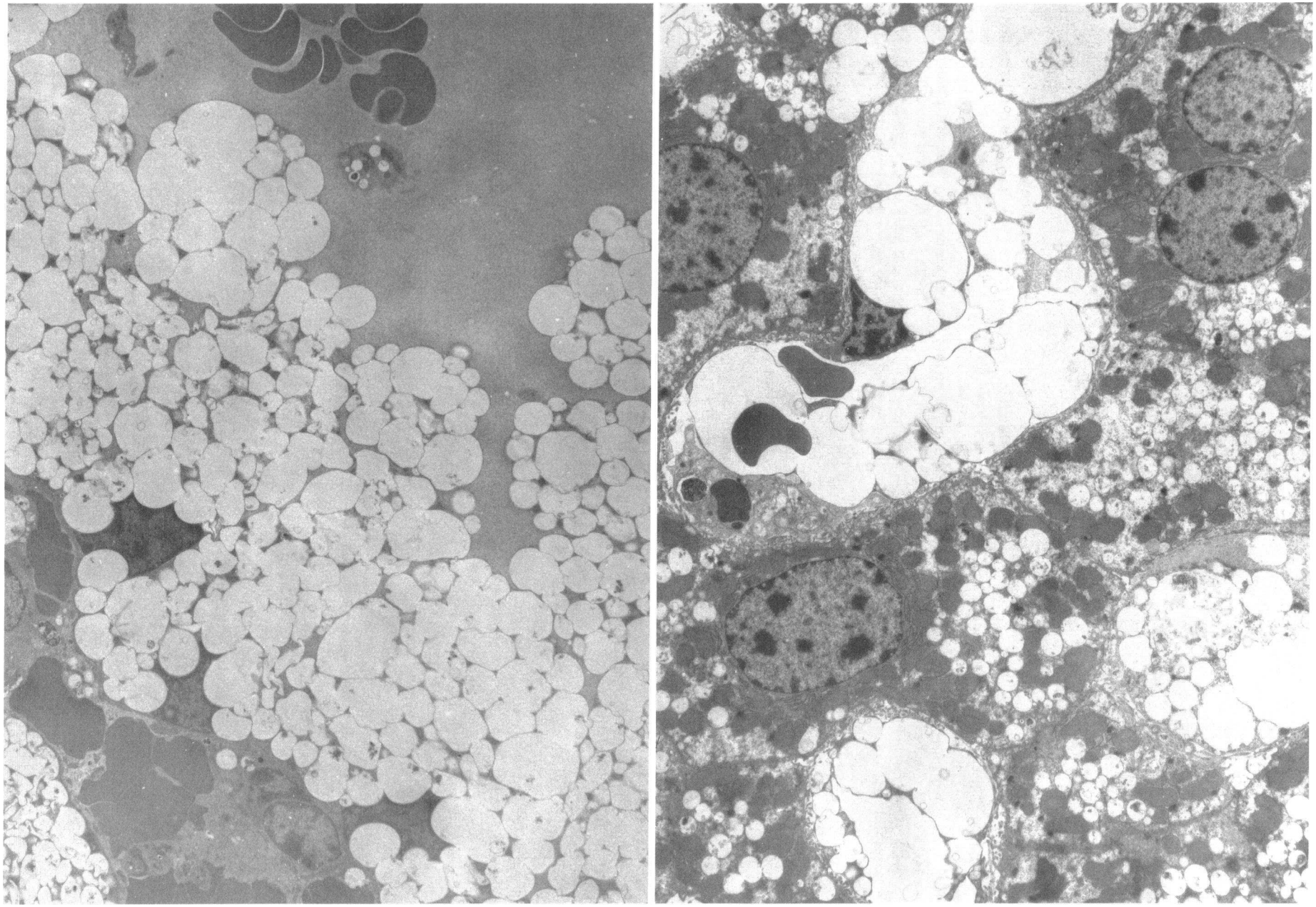

Figure 3. Electron micrographs of spleen and liver from a mutant mouse. Endothelial cells in both (*left*) spleen and (*right*) liver are severely distended by cytoplasmic vacuoles. ×2,800.

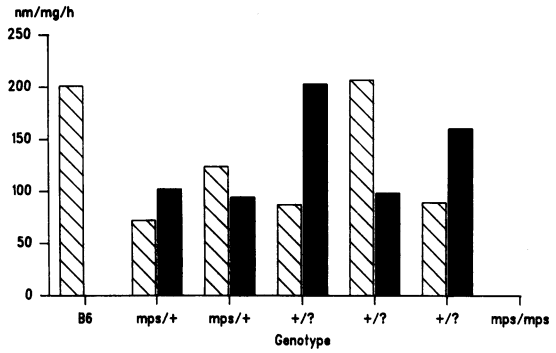

Figure 4. Liver β-glucuronidase activity in normal and mutant adult mice. This bar graph shows the specific activity of β-glucuronidase in livers from normal C57BL/6J mice (B6), phenotypically normal gus^{mps}/+ mice (mps/+), phenotypically normal mice of unknown genotype (+/?) that were either +/+ or gus^{mps}/+, and homozygous gus^{mps}/gus^{mps} mutant mice (mps/mps). Each pair of male (*stipled bars*) and female (*solid bars*) mice was a breeding pair obtained from the gus^{mps} mutant colony except for the C57BL/6J male mouse that was from the production stocks of The Jackson Laboratory.

brain, thymus, and spleen from mutant animals gave < 1% normal levels, and the specific activity was always < 1.0 nmol of 4-MU released/mg of protein per h. The levels of activity in kidneys and liver of mutant mice (male and female) at four months of age were 0.08±0.02 and 0.18±0.03 nmol of 4-MU/mg of protein per h, respectively. This is in striking contrast to the kidneys and liver of a control C57BL/6J male mouse which had β-glucuronidase levels of 79 and 201 nmol of 4-MU/mg of protein per h, respectively.

Next, we measured the levels of β-glucuronidase activity within the mutant strain. If the effects of the normal and gus^{mps} alleles on enzyme activity are additive in heterozygous animals, then the gus^{mps} allele is semidominant. Fig. 4 shows that the gus^{mps}/+ parents producing mutant offspring had levels of activity in liver that were approximately one-half of that observed in C57BL/6J mice. In contrast, parents that failed to produce mutant offspring after having at least 14 pups clearly showed that one parent appeared normal (+/+) while the other had reduced activity similar to the heterozygous parents. Since all of the obligate heterozygous animals had intermediate levels of β-glucuronidase activity, we concluded that the gus^{mps} allele is semidominant at the biochemical level even though gus^{mps}/+ mice otherwise appeared normal.

β-Glucuronidase mRNA levels. To determine the levels of β-glucuronidase mRNA in mutant mice, we isolated total cellular RNA from the kidneys of two sibling male mice that were 87 d of age. One was phenotypically normal (+/?) and the other was a mutant (gus^{mps}/gus^{mps}). A Northern blot containing 15 μg of each RNA was hybridized to a cDNA clone, pGUS-1, that represents ~ 1.7 kb of the 2.9-kb murine β-glucuronidase mRNA (12). Although the expected mRNA was detected in the normal animal, there was no hybridizing RNA of any size in the mutant RNA (data not shown). Since a mRNA at 10% of the normal level would have been detected, it appears that the deficiency of β-glucuronidase activity in the mutant animals is caused by a low concentration of β-glucuronidase mRNA.

Fig. 5 shows the results of another attempt to detect mRNA in mutant mice by Northern blot analysis. We isolated poly A^+ RNA from the kidneys and liver of a testosterone-treated mutant female mouse and prepared a Northern blot containing 20 μg of each mRNA. Control lanes contained 1.0 and 5.0 μg of kidney and liver poly A^+ RNAs, respectively, that were obtained from a testosterone-treated +/? normal female mouse. After hybridization to the pGUS-1 probe, several bands were detected in the mutant kidney. A dominant small band represented a mRNA that is about 1.0 kb in size. The origin and nature of this mRNA is unknown. Another larger band comigrated with the β-glucuronidase mRNA that was present in the lanes containing mRNA from the normal mouse. The intensity of these bands on the film indicated that if the comigrating band present in the mutant kidney was β-glucuronidase mRNA, then its concentration was at least 200-fold less than that found in normal kidney. Interestingly, the mutant liver poly A^+ RNA lane had only a very faint band at this position and it was apparent only on the original autoradiogram after an 88-h exposure. This large relative difference between the β-glucuronidase mRNA levels in mutant kidney and liver from testosterone treated mice is similar to that seen in normal mice where testosterone causes a > 10-fold induction of renal β-glucuronidase mRNA levels (32).

To measure the testosterone induction of β-glucuronidase mRNA in gus^{mps}/gus^{mps} mice, we used a S1 nuclease protection assay with anti-sense RNA transcribed from the pGUS-1 cDNA clone. The 1.4-kb subclone of pGUS-1 used to make the cRNA probe contains exons 2 through 12 of the β-glucuronidase gene and thus represents most of the coding sequences. The results of the S1 protection analysis are shown in

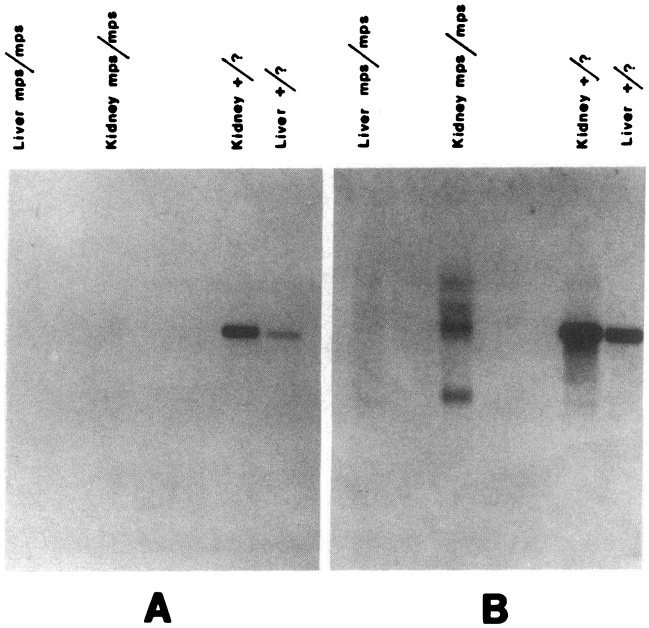

Figure 5. Northern blot of kidney and liver mRNA. Polyadenylated mRNA was isolated from the kidneys and livers of testosterone-treated female mice. The Northern blot contains 20 μg of liver mRNA from a mutant mouse (*Liver mps/mps*), 20 μg of kidney mRNA from the same mutant mouse (*Kidney mps/mps*), 1.0 μg of kidney mRNA from a normal mouse (*Kidney +/?*), and 5.0 μg of liver mRNA from the same normal animal (*Liver +/?*). The blot was hybridized to the pGUS-1 probe and the resulting autoradiograms were obtained after either (*A*) an 18-h exposure or (*B*) an 88-h exposure.

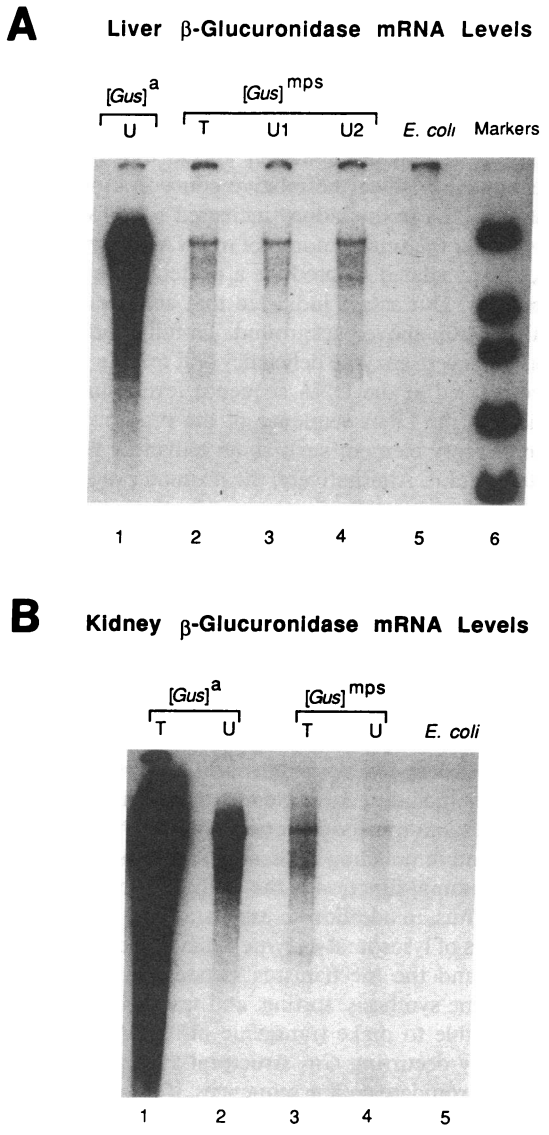

Figure 6. Testosterone induction of β-glucuronidase mRNA in mutant mice. (*A*) This autoradiogram shows the relative levels of β-glucuronidase mRNA in the livers of female mutant [*Gus*]^{mps} mice and normal [*Gus*]^{a} mice that either were or were not treated with testosterone. For each lane, 100 μg of total cellular RNA was hybridized with an antisense β-glucuronidase riboprobe followed by S1 nuclease treatment and gel electrophoresis. The source of RNA in each lane is as follows: lane *1*, three untreated [*Gus*]^{a} mice (*U*); lane 2, one [*Gus*]^{mps} mouse treated with testosterone (*T*); lanes *3* and *4*, each lane has one untreated [*Gus*]^{mps} mouse (U); lane *5*, *E. coli;* and lane *6*, size markers of 1448, 517, 396, 239, and 147 base pairs. (*B*) This autoradiogram is similar to *A* but shows the relative levels of β-glucuronidase mRNA in the kidneys of mutant and normal mice. The source of RNA in each lane is as follows: lane *1*, three [*Gus*]^{a} mice treated with testosterone (*T*); lane *2*, three untreated [*Gus*]^{a} mice (U); lane *3*, one [*Gus*]^{mps} mouse treated with testosterone (*T*); lane *4*, one untreated [*Gus*]^{mps} mouse (*U*); and lane *5*, *E. coli.*

Fig. 6. The liver RNA from mutant animals generated a 1.4-kb dsRNA fragment identical in size to that found in a normal liver. However, in the mutant mice, the β-glucuronidase mRNA was present at a much lower concentration. Testosterone treatment did not change the concentration of liver mRNA in either the mutant mouse or the normal mouse. A similar relatively faint 1.4-kb band was observed in the mutant kidney. However, the intensity of the bands indicated that the renal β-glucuronidase mRNA in mutant mice is present at an 11-fold higher concentration after testosterone treatment. Despite this response to testosterone, the β-glucuronidase mRNA concentration in the kidneys of mutant animals remains much lower than that seen in normal uninduced animals. Although it may not be possible to correlate the Northern blot results directly with these data, both experiments suggest that the β-glucuronidase gene is being transcribed in *gus*^{mps}/*gus*^{mps} mice and that a polyadenylated mRNA of normal or nearly normal size is produced. This mRNA is present at a concentration at least 200-fold less than that found in normal mice. The relative concentration of mutant kidney mRNA increases in response to testosterone treatment in a manner that appears to be similar to that seen in normal mice.

Structure of the Gus-s allele in mutant mice. To investigate the *gus*^{mps} mutation at the DNA level, DNA from +/+ and *gus*^{mps}/+ normal animals was compared with DNA from homozygous *gus*^{mps}/*gus*^{mps} mutant animals by Southern blot analysis. These DNAs were digested with *Bam* HI, *Eco* RI, and *Hind* III and then hybridized to a 3.2-kb *Bam* HI fragment obtained from the 5′ flanking region of a genomic clone of *Gus-s*^{b} from a YBR mouse (30). With all three enzymes, the mutant allele looked identical to the normal allele (data not shown). The pGUS-1 cDNA clone also failed to detect any differences between the normal and mutant DNAs. Therefore, Southern blot analysis did not reveal any large deletions, duplications, insertions, or rearrangements within a 35-kb segment of the mutant genome that contains the *Gus* structural gene. Either the mutation has not generated a RFLP that is detectable with the three restriction enzymes we tested or the mutation is located outside of the region covered by our probes.

Because methylation of specific DNA sequences sometimes correlates with levels of expression of certain genes, we used the methylation-sensitive enzyme, *Hpa* II, to determine the methylation status of some of the cytosine residues within the mutant *Gus* locus. Liver DNA from the mice used in the Southern blots described above, was digested with either *Hpa* II or the methylation-insensitive isoschizomer, *Msp* I. The DNAs were Southern blotted and hybridized to the pGUS-1 cDNA probe and the results are shown in Fig. 7. The mutant and normal DNAs looked identical when digested with *Msp* I and three major bands were resolved in the gel. The *Hpa* II digests were different from the *Msp* I digests, and at least six easily discernible bands were present in each lane. However, the *Hpa* II digests of all three DNAs were essentially identical to one another. These results indicate that methylation of cytosine residues has occurred at some of the *Msp* I sites in liver DNA, and that the sites which are methylated are identical in normal and mutant animals.

Discussion

This article describes a recently discovered mutant mouse at the genetic, cellular, biochemical, and molecular levels. The data show that the phenotype and clinical presentation of the disease in this mouse can be attributed to the lack of β-glucuronidase activity in the various tissues tested. We concluded

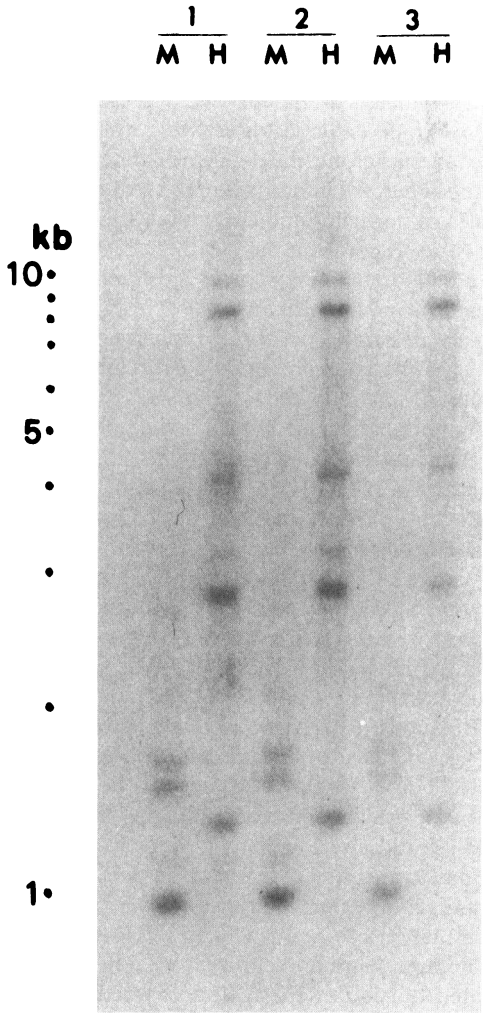

Figure 7. Methylation pattern of the β-glucuronidase gene in mutant mice. This is an autoradiogram of a Southern blot hybridized to the pGUS-1 cDNA probe. It contains liver DNA from (*1*) gus^{mps}/gus^{mps} mice, (*2*) gus^{mps}/+ mice, and (*3*) +/+ mice. Each DNA was digested with *Msp* I (*M*) and *Hpa* II (*H*).

that the mouse has a lysosomal storage disease that is the murine counterpart of a human disease, mucopolysaccharidosis type VII (MPS VII), described originally in a patient by William S. Sly and colleagues (6). Since this original article, there have been 19 patients reported in the literature (33). There is also a single report of β-glucuronidase deficiency in a dog (8). Although there are inbred strains of mice with high and low levels of β-glucuronidase activity, none have a severe enough deficiency to cause a disease with similarities to human MPS VII (15, 17). Therefore, as a null mutation, the gus^{mps}/gus^{mps} mouse is a unique resource that will have a broad application in answering many important scientific questions about (*a*) β-glucuronidase-deficient mucopolysaccharidosis and (*b*) regulation of *Gus* gene expression.

The [*Gus*] complex is one of the most thoroughly characterized genetic loci in the mouse (1). Some of its interesting genetic characteristics and various regulatory elements have been referenced previously in this paper. The gus^{mps} mutation has been mapped to the [*Gus*] complex on the distal half of chromosome 5. Based upon the physical appearance of the mice, it is a recessive mutation. However, at the biochemical level, it appears as a semidominant mutation because gus^{mps}/+ animals have one half the β-glucuronidase activity present in +/+ animals. Even though there is a large reduction in the β-glucuronidase mRNA concentration in the mutant tissues, the proximal tubule cells of the kidney appear to respond to testosterone induction since the relative concentration of β-glucuronidase mRNA in the kidney increased > 10-fold. It is not known whether the small amount of mRNA present in the mutant mice is translated to produce a protein with β-glucuronidase activity. Our assays indicated that activity, if present, was only slightly above background. To fully understand the cause of this severe enzyme deficiency will require that the mutation be defined at the DNA sequence level. One likely possibility is that the DNA sequence of the promoter or an associated regulatory element such as an enhancer has been altered in the mutant. Alternatively, the promoter may be capable of functioning normally as suggested by the testosterone induction experiment. The mRNA species observed on the Northern blots are consistent with the possibility that the defect is related to mRNA stability or processing. Whatever the cause of this mutation, further study of the genetic defect at the molecular level will lead to a better understanding of the regulation of β-glucuronidase gene expression.

It is also important to recognize the usefulness of this null mutation in providing a genetic background in which to study certain cellular processes. Lysosomes are involved in many cellular processes including killing infectious agents, degradation of hormones and transport proteins, turnover of intracellular proteins, and remodeling of tissues and bones. Crucial to appropriate lysosomal function is the trafficking of lysosomal enzymes (34). Thus, in addition to understanding the biological consequences of lysosomal enzyme deficiencies, it is necessary to understand the mechanisms responsible for normal lysosomal enzyme synthesis, sorting, and transport. It is now technically possible to make transgenic mice carrying either various naturally occurring *Gus* structural alleles or in vitro mutated β-glucuronidase coding sequences. If these transgenes are then placed on the gus^{mps}/gus^{mps} genetic background by appropriate genetic crosses, it should provide a valuable biological system in which to identify and characterize the molecular signals required for correct cellular processing and targeting of lysosomal enzymes.

In the area of clinical investigation, research on murine MPS VII may have important implications. Human MPS VII has been reported to have considerable phenotypic variation (33, 35). It is unclear which of the various symptoms and their severity relate directly to enzyme deficiency and which are also affected by genetic background and other disease processes. The murine gus^{mps} mutation is on a well-defined homogeneous genetic background. All of the mice are pedigreed and the colony has been maintained by strict brother–sister matings. Thus, the gus^{mps}/gus^{mps} mouse offers a model system in which to study the pathogenesis of a lysosomal storage disease in a large number of animals with a uniform genetic background. Although MPS VII is a rare disease, all of the human lysosomal storage diseases taken together have a significant clinical incidence. Studies using animal models of the human diseases may provide important information about the clinical course of the disorder and suggest ways to treat humans with similar lysosomal enzyme defects (17, 36). Two interesting possibilities for treatment of murine MPS VII are transplanta-

tion of normal syngeneic bone marrow cells and infection of mutant bone marrow stem cells with defective retroviruses that encode murine or human β-glucuronidase. Our preliminary results with bone marrow transplantation into sublethally irradiated (200–400 rads) mutant mice have shown remarkable reversal of storage disease pathology in many tissues. To date, 78% (14/18) of the animals that received transplants are still alive and are between 400 and 500 d of age (Birkenmeier et al., unpublished results). We predict that some may approach a normal lifespan and reach an age of at least 2 yr.

In summary, we have identified a murine lysosomal storage disease that has many similarities to human MPS VII. There is little if any β-glucuronidase activity present in these mice because of a very low level of β-glucuronidase mRNA. The exact nature of the defect at the DNA level has yet to be defined but the *gus*^{mps} mutation maps to the [*Gus*] complex on chromosome 5. The mice may be useful in further basic research involving regulation of gene expression and trafficking of lysosomal enzymes as well as clinical research involving lysosomal storage diseases and their treatment.

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