Genetic Variation for Enzyme Structure and Systemic Regulation in Two New Haplotypes of the \( \beta \)-Glucuronidase Gene of \textit{Mus musculus castaneus}*

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Two new haplotypes of the [\textit{Gus}] gene complex have been characterized following their transfer from \textit{Mus musculus castaneus}, where they were found, to a C57BL/6J genetic background. The [\textit{GUS}] haplotype carries a new structural allele, Gus-\( s^a \), coding for enzyme with decreased thermolability and lacking an antigenic site present in other \( \beta \)-glucuronidase allozymes. The [\textit{GUS}] haplotype carries another new structural allele, Gus-\( s^d \), that codes for enzyme with increased thermolability and possessing the antigenic site. Both CS and CL \( \beta \)-glucuronidase have the same catalytic activity/molecule as the standard B allozyme from C57BL/6J mice.

Mice carrying either the [\textit{Gus}] or [\textit{Gus}] haplotype have reduced enzyme activity in all tissues examined at all stages of development. The reduced enzyme activity is partially accounted for by reduced rates of enzyme synthesis, and the remainder probably results from increased rates of enzyme turnover. \( \beta \)-Glucuronidase mRNA levels in these mice were not reduced suggesting that the observed reduction in enzyme synthesis is due to a decreased efficiency of translation for CS and CL mRNA.

Studies of gene regulation in mammalian systems have often relied on the naturally occurring variation in both structural and regulatory DNA sequences that is found among laboratory strains of animals. In mice, the availability of highly inbred strains provides a pool of genetically identical homozygous animals that can be used as the starting point for genetic analyses and biochemical studies of the mechanisms involved. Such studies have tended to focus on a limited number of experimental systems. Among these, \( \beta \)-glucuronidase has been extensively examined for genetic variation in its structure, intracellular location, intercellular transport, induction by androgenic steroids, and developmental regulation. Earlier studies (summarized in Ref. 1) have established the existence of a gene complex, [\textit{Gus}], located near the distal end of chromosome 5 in the mouse, that contains the enzyme structural gene, Gus-\( s \), together with several closely associated regulatory loci. These regulatory loci include a temporal gene, Gus-\( t \), determining the developmental program for enzyme synthesis, and Gus-\( r \), a regulator determining susceptibility of \( \beta \)-glucuronidase to induction by androgens.

Our current understanding of the \( \beta \)-glucuronidase system derives from such studies among the common inbred strains of laboratory mice. Altogether, over 300 inbred laboratory strains have been established from two subspecies of mice. \textit{Mus musculus musculus} and \textit{Mus musculus domesticus}. The taxonomic relationship between inbred mice and existing feral populations is somewhat uncertain particularly for the C57BL subfamily. However, several sources of information suggest that most laboratory strains are genetically similar to the house mice of Western Europe \textit{M. m. domesticus} and that they represent only a limited sampling of the total mouse gene pool (2,3). Surveys of 30-40 of the most common inbred strains originally identified the genetic variants that have been most intensively studied (4-6). A later survey of over 100 additional strains chosen for the diversity of their genetic origins (7) did identify additional strains carrying the same mutations but failed to uncover any new mutants. Since most of the remaining inbred mouse strains are closely related genetically to those already surveyed, it is unlikely that an appreciable reservoir of new mutants is available from this source.

In order to expand the source of genetic variants, we have surveyed house mice from diverse geographical locations. These mice are typically interfertile with laboratory strains and produce viable fertile \textit{F1} hybrids. A number of unusual genetic variants have been identified in \textit{M. m. castaneus} from Southeast Asia suggesting that this subspecies might be a likely source of [\textit{Gus}] variation. Our studies indicate that it carries at least two new forms or haplotypes of the [\textit{Gus}] complex designated [\textit{Gus}] and [\textit{Gus}]. These contain novel alleles of the [\textit{Gus}] structural gene and [\textit{Gus}] androgen regulator, as well as systemic regulation of enzyme expression. The regulatory phenotypes determined by the new haplotypes, as well as the structural properties of the \( \beta \)-glucuronidase allozymes coded by new structural gene alleles present in these haplotypes, have been examined after transfer of the gene complexes from [\textit{M. m. castaneus}] onto the C57BL/6 inbred strain background by repeated backcrossing. The congenic strains constructed in this manner provide a constant genetic background for comparing each of the new haplotypes with the standard [\textit{Gus}] haplotype normally found in C57BL/6, thus restricting our analysis to the phenotypic effects actually determined by the complex itself (7). This

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approach avoids ambiguities that might be introduced if \textit{M. m. castanews} and C57BL/6J possess other nonlinked genetic differences affecting various aspects of \(\beta\)-glucuronidase expression. The genetic variation in the androgenic induction of \(\beta\)-glucuronidase in kidney epithelial cells present in \textit{M. m. castanews} haplotypes was the subject of a separate report (8). Here we describe the enzyme structural changes and systemic regulatory phenotypes determined by the [Gus]^{CS} and [Gus]^{CL} haplotypes.

When examined in congenic strains each of the two new [Gus] haplotypes directs the synthesis of a novel structural form of \(\beta\)-glucuronidase. These differ from each other and from all previously known alleles of \(\beta\)-glucuronidase in thermostability, and in one case antigenicity, indicating the presence of two new alleles of the enzyme structural gene, Gus-s. Both of the \textit{M. m. castanews}-derived haplotypes also carry new alleles that alter the systemic regulation of \(\beta\)-glucuronidase expression. This regulation affects \(\beta\)-glucuronidase activity in all tissues and at all stages of development by a constant factor. These alleles alter rates of \(\beta\)-glucuronidase protein synthesis but not levels of \(\beta\)-glucuronidase mRNA as determined by hybridization. This suggests that the new haplotypes produce mRNA molecules with reduced translation efficiency. Because the altered rates of enzyme synthesis are not sufficient to account for all of the decreased enzyme activity observed, it appears that \textit{M. m. castanews}-derived \(\beta\)-glucuronidase also turns over faster than the enzyme from C57BL/6J mice. A systemic effect on \(\beta\)-glucuronidase activity has been reported for the [Gus]^{h} haplotype derived from the mouse strain C3H/HeJ, and the locus responsible has been termed the systemic regulator, Gus-u (9). For the time being we shall consider the systemic effects demonstrated by the \textit{M. m. castanews} haplotypes to be a new allele or alleles of Gus-u.

**EXPERIMENTAL PROCEDURES**

**Animals**—The inbred mouse strain C57BL/6J (B6) was originally obtained from the Jackson Laboratory (Bar Harbor, ME). The congenic strains B6.CL and B6.\textit{p} carry two new haplotypes of the glucuronidase gene complex [Gus] found in \textit{M. m. castanews} and transferred onto the genetic background of C57BL/6J mice by repeated backcrossing. After 7 generations of backcrossing an intercross was carried out to obtain homozygous animals, and these became the parents of the inbred congenic lines used for most of these experiments. B6.CL animals used for most recent experiments were backcrossed to B6 for 15 generations before the intercross. For structural studies enzyme was obtained from tissues of 2–4-month-old mice.

**Enzyme Activity**—Tissues were homogenized using a Polytron homogenizer (Kinematica, Switzerland) in 10 volumes of 0.02M imidazole, 0.25M sucrose, pH 7.4. \(\beta\)-Glucuronidase was assayed using a fluorometric procedure with 4-methylumbelliferyl-\(\beta\)-D-glucuronide as substrate (10). One enzyme unit is equivalent to the hydrolysis of 1 \(\mu\)mol of substrate/h at 37 °C. In general, samples containing 0.001–0.0010 unit of activity were used for assay. \(\beta\)-Glucuronidase activity in all tissues and at all stages of development was measured according to Laemmli (11) as modified by Swank and Paigen (12). Relative rate of synthesis was determined by conventional electrophoresis.

**Determinations of mRNA**—RNA was isolated from the guanidine HC1 medium in the presence of 1.15 M NaCl, 0.02M Tris-Cl, pH 7.4, were partially purified as for rate of synthesis determinations (8, 18), and different amounts of enzyme activity were precipitated quantitatively with anti-\(\beta\)-glucuronidase antibody. Precipitates were dissociated in loading buffer and subjected to SDS-gel electrophoresis (14). Gels were stained for proteins in 0.025% Coomassie Brilliant Blue, 50% isopropanol, 10% acetic acid, and destained in 7% acetic acid, 10% methanol. Stained protein was quantitated by densitometry, and the areas under the \(\beta\)-glucuronidase peaks were determined by cutting and weighing the paper.

**Relative Rate of Enzyme Synthesis**—The relative rate of synthesis of \(\beta\)-glucuronidase was measured using a modification of the procedure of Cleveland and Swank (18) as described elsewhere (6). Relative rates of synthesis represent the amount of label incorporated into \(\beta\)-glucuronidase protein divided by the amount incorporated into total protein.

**Determination of mRNA**—RNA was isolated as modified by Laskey and Parson (20). At least 4 animals of each strain were pooled for each RNA preparation. \(\beta\)-Glucuronidase mRNA was measured by hybridization with labeled complementary RNA transcribed in vitro. The template for this RNA synthesis was a fragment of cloned \(\beta\)-glucuronidase cDNA (21) that was subcloned into a vector containing a bacteriophage SP6 RNA polymerase promoter (22, 23) (vector was a gift from M. Green, Harvard University, Cambridge, MA). The details of the construction and transcription of this cDNA subclone are reported elsewhere (21). Labeled RNA was synthesized in vitro using SP6 RNA polymerase (24) (a gift from M. Chamberlin, University of California, Berkeley, CA). cRNA was obtained by hybridization conditions employed, the RNA probe was speciated by dot blot of total RNA on nitrocellulose (25). Hybridization conditions were: 50% formamide, 0.75M NaCl, 75mM Na citrate, 10% dextran sulfate, 10 ° Denhardt's solution (26), 0.5% SDS, 0.2 mg/ml denatured lambda sperm DNA, and 2–10 \(\times\) \(10^{8}\) cpm/ml [\(\text{\textsuperscript{32}}\text{P}\)]RNA for 48–60 h at 60 °C. Hybridized filters were washed 4 times at 70 °C in 15 mM NaCl, 1.5 mM Na citrate, 5 mM NaHPO\(_4\), pH 7, 0.15% Na, pyrophosphate, 0.2% SDS for 30 min each time. This was followed by 2 washes for 30 min each in 3 mM Tris-Cl, pH 8.9, at 20 °C. Washing continued with 0.5% SDS, 0.1% Na citrate, and the filter was exposed to X-ray film, after which they were cut up and the amount of labeled RNA hybridized to each kidney RNA spot was determined by scintillation counting. A dilution series of a standard preparation of total RNA (from the kidneys of male A/J mice treated for 6 weeks with testosterone) was included in each experiment and indicated that the

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2 The abbreviations used are: TES, N-tris(hydroxymethyl)methyl-2-amino-ethanesulfonic acid, SDS, sodium dodecyl sulfate.
radioactivity hybridized was directly proportional to the amount of
β-glucuronidase RNA bound to the nitrocellulose. One µg of this
standard RNA contained $1.8 \times 10^{-6}$ µg of β-glucuronidase mRNA
(21), and this standard was used to determine the mass fraction of
each total RNA sample that was β-glucuronidase mRNA.

RESULTS

Enzyme Structure

Heat Inactivation—The A and B allozymes of β-glucuronidase have similar thermolabilities, while the H allozyme is markedly more thermolabile (7, 27, 28). Among the M. m. castaneus mice tested two new allozymes of β-glucuronidase were found that differed from each other and from previously characterized β-glucuronidase allozymes in their thermolability. These differences were also seen after transfer of the [Gus] complex from M. m. castaneus to B6, and the new allozymes were characterized in extracts of B6 congenic strains (Fig. 1). One of the new allozymes exhibits a thermolability intermediate between the B and the H allozymes at 73 °C. We refer to this labile form as CL β-glucuronidase and Gus-s" as the allele of the corresponding structural gene. The other M. m. castaneus enzyme form is less thermolabile than any mouse allozyme that has been characterized previously; we refer to it as the CS allozyme and the corresponding allele of the structural gene as Gus-s".

In addition to the rate of inactivation at 73 °C, the kinetics of thermal denaturation at four additional temperatures in the range from 69.9–74.0 °C were tested. The log of these initial rates of inactivation, expressed as pseudo first order rate constants (min⁻¹), were linear functions of the reciprocal of the absolute temperature (Fig. 2). This allowed us to estimate the Arrhenius activation energy of the denaturation reaction from the slopes of the curves. Under the experimental conditions the activation energies in cal/mol for denaturation

![Fig. 1. Heat stability of β-glucuronidase. Homogenates were prepared as described, incubated for the indicated times at 73 °C, and the remaining enzyme activity determined. Liver homogenates were made from B6 (■), B6.CS (▲), B6.CL (●), and C57/Hef (□) mice.](image1.png)

![Fig. 2. Thermal inactivation of β-glucuronidase: Arrhenius plot. The natural logarithm of the rate of inactivation at 4 different temperatures is plotted against the reciprocal of the absolute temperature. Each point represents the average of 4 determinations. Liver homogenates were prepared as described in the text from B6 (■), B6.CS (▲), and B6.CL (●) mice.](image2.png)
New Haplotypes of the $\beta$-Glucuronidase Gene

**Fig. 3.** Electrophoretic mobility (a) and isoelectric focusing (b) of $\beta$-glucuronidase. Gels contained liver homogenate from the [Gus] haplotypes indicated and were stained for $\beta$-glucuronidase activity.

**Fig. 4.** Comparison of catalytic efficiencies for $\beta$-glucuronidase allozymes. Peak area of stained purified, $\beta$-glucuronidase protein is plotted against enzyme activity applied to SDS gels. $\beta$-Glucuronidase was purified from livers of B6 (●), B6.CS (▲), and B6.CL (●) mice.

approximately equal numbers of $\beta$-glucuronidase activity units were precipitated with antibody. The precipitated proteins were solubilized in SDS/urea/Tris buffer, separated by SDS-gel electrophoresis, and quantitated by scanning densitometry after staining for protein. There was no significant difference in the amount of $\beta$-glucuronidase protein corresponding to one activity unit of the CL, CS, and B allozymes indicating that the catalytic efficiencies of all three forms of the enzyme are essentially the same (Fig. 4).

CS enzyme lacks an antigenic determinant that is present in the B and CL allozyme. This is apparent using rocket immunoelectrophoresis of enzyme samples (Fig. 5). For a given amount of enzyme activity, which corresponds to an equal number of enzyme molecules, the area under the precipitation peak is larger for CS enzyme than for CL and B enzyme. That is, it requires a larger amount of antibody to neutralize an equivalent amount of the CS allozyme, indicating that there are fewer antibody molecules directed against the CS allozyme than against the other two allozymes. The decreased antigenicity of CS enzyme with the antibody used was confirmed by showing that a given volume of Sepharose-coupled anti-$\beta$-glucuronidase binds fewer units of CS enzyme than either CL or B6 enzyme (data not shown). Although the double immunodiffusion technique of Ouchterlony (16) produced no detectable spur at the junction of the CS and B6 precipitation lines, CS enzyme appears to lack at least one antigenic determinant present in B and CL enzymes.

**Enzyme Regulation**

**Tissue Enzyme Levels**—Congenic lines carrying the [Gus]$^{CS}$ and [Gus]$^{CL}$ haplotypes have half or less of the $\beta$-glucuronidase activity of B6 mice, and F$_1$ heterozygotes have intermediate levels (Table I). There does not appear to be any tissue specificity to the reduction in enzyme levels.

**Development Pattern**—Reduced enzyme activity is present throughout postnatal development of several tissues (Fig. 6).
There are no significant differences in the shape of the developmental profiles of β-glucuronidase between the two new haplotypes recovered from *M. m. castaneus* or between these and the standard *B* haplotype. In particular, the *CS* and *CL* haplotypes do not show any of the developmental changes associated with mutation at the *Gus-t* temporal gene (1, 30). Instead, the enzyme changes associated with the *CS* and *CL* haplotypes appear to reflect variation at a genetic locus similar to or identical with a previously defined systemic regulatory locus, *Gus-u*, present in the *GUS* complex (9).

Regulation of β-Glucuronidase Synthesis—To determine whether the reduced enzyme levels seen with the *CS* and *CL* haplotypes are the consequence of a reduced level of enzyme synthesis, the relative rate of β-glucuronidase synthesis was measured in livers of animals of the *B6* strain and the two congenic strains *B6.CS* and *B6.CL*. For this purpose animals were pulse labeled, β-glucuronidase was purified by a combination of physical and immunological methods and the radioactivity incorporated into β-glucuronidase compared to that incorporated into total protein. Both the *CS* and *CL* haplotypes showed reduced rates of β-glucuronidase synthesis (Table II). It is significant, however, that the reduced rate of synthesis was not sufficient to completely account for the difference in enzyme activity. This suggests that the *CS* and *CL* haplotypes are also characterized by an increased rate of β-glucuronidase turnover to account for the greater reduction in enzyme activity than enzyme synthesis. Ideally, we should like to have measured turnover differences directly; however, calculations of the amount of radioisotope required suggested that such experiments were not practical.

β-Glucuronidase mRNA—Since the differences in rates of β-glucuronidase synthesis might be due to either differences in β-glucuronidase mRNA levels or to differences in the efficiency of mRNA translation, we have measured mRNA concentrations directly by hybridization to a β-glucuronidase specific single-stranded probe. The results (Table III) indicate that, for a given tissue, there is little variation among the *GUS* haplotypes tested. Thus the systemic regulator, *Gus-u*, does not appear to affect mRNA levels but rather the efficiency of mRNA translation.

Aside from the question of genetic differences, the data in Table III also show that the amount of β-glucuronidase mRNA, expressed as a fraction of total RNA, varies little from one tissue to another. At first this seems to contrast with the substantial tissue-specific variation in enzyme activity (Table I), but it should be noted that enzyme activity was expressed as units/g of tissue whereas β-glucuronidase mRNA was expressed as ng/g of total RNA. When β-glucuronidase mRNA is expressed per mass of tissue, the tissue-specific variation follows the same general pattern as enzyme activity. For example, mouse strains *B6*, *B6.CS*, and *B6.CL* yielded 40, 41, and 37 pg of β-glucuronidase mRNA/g of brain while they yielded 962, 435, and 515 pg/g of liver.

**TABLE II**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relative rate of synthesis</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days⁻¹ × 10⁶</td>
<td>units/g liver</td>
</tr>
<tr>
<td><em>B6</em></td>
<td>3.62 (±0.06)</td>
<td>25.2 (±1.2)</td>
</tr>
<tr>
<td><em>B6.CS</em></td>
<td>2.34 (±0.10)</td>
<td>10.2 (±0.6)</td>
</tr>
<tr>
<td><em>B6.CL</em></td>
<td>2.04 (±0.14)</td>
<td>7.89 (±0.48)</td>
</tr>
</tbody>
</table>

**TABLE III**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Brain*</th>
<th>Liver*</th>
<th>Kidney*</th>
<th>Liver (8-day)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/g</td>
<td>ng/g</td>
<td>ng/g</td>
<td>ng/g</td>
</tr>
<tr>
<td><em>B6</em></td>
<td>66</td>
<td>96</td>
<td>94</td>
<td>117</td>
</tr>
<tr>
<td><em>B6.CS</em></td>
<td>71</td>
<td>92</td>
<td>114</td>
<td>106</td>
</tr>
<tr>
<td><em>B6.CL</em></td>
<td>53</td>
<td>88</td>
<td>82</td>
<td>101</td>
</tr>
</tbody>
</table>

*The mean (±S.E.) of 3 independent RNA preparations each derived from 4–7 adult female mice.

One RNA preparation per strain each using 8 mice 8 days of age.

**DISCUSSION**

The *Gus*CS and *Gus*CL haplotypes of the β-glucuronidase gene complex each contain a new allele of the β-glucuronidase structural gene, *Gus-s*; the new alleles are designated *Gus-s*CS and *Gus-s*CL. Compared to the *Gus-s* standard allele the product of the *Gus-s*CS allele behaves as though it carries one additional negative charge on each monomer, exhibits decreased thermolability, and lacks at least one of the antigenic determinants common to other β-glucuronidase allozymes. The product of the *Gus-s*CL allele also appears to carry one additional negative charge on each monomer but exhibits increased thermolability when compared to the standard allozyme. The amino acid substitutions affecting thermolability appear to be independent of those affecting charge. It is interesting that both the *CS* and *CL* allozymes show an elevated activation energy for the denaturation reaction (compared to other allozymes) but differ in their absolute rates of denaturation at the same temperature. Thus, both the activation energy and absolute rate can be used to detect amino acid substitutions. Although the data has not been presented,
studies with F1 hybrids have confirmed that, as with other structural gene differences, these phenotypes are expressed codominantly.

The two new alleles, together with the three already described in the literature (1, 7), plus one additional allele that has one additional positive charge/monomer, bring the total of presently distinguishable structural alleles ofβ-glucuronidase to six. These include four thermolability classes and three electrophoretic types in various combinations. It is not necessary to assume that each allele represents a novel amino acid replacement; the properties of some alleles could represent the result of intramolecular recombination between mutant sites.

The CS and CL allozymes had the same activity/molecule as the B enzyme when this was evaluated by determining the amount of enzyme protein present in an immune precipitate containing a fixed number of enzyme activity units. The CS allozyme, however, appears to lack an antigenic determinant that is present in all other allozymes. This was apparent in rocket immunoelectrophoresis and was confirmed by quantitative titration curves with Sepharose-bound antibody. It required more anti-GUS A antibody to precipitate a given number of CS enzyme units than did to precipitate an equivalent number of CL or B enzyme units.

In addition to carrying novel alleles of the structural locus the [Gus]c and [Gus]d haplotypes appear to carry mutations in a systemic regulatory locus. Mice carrying either haplotype possess half or less of the β-glucuronidase activity of B mice in several tissues throughout development. As summarized in Table IV, the reduction in enzyme activity is only partially accounted for by reduced rates of β-glucuronidase synthesis. Comparing the ratios of activity to rate of synthesis suggests that there is an additional reduction in enzyme activity that results from higher turnover rates for the CS and CL allozymes. The reduced rates of protein synthesis were not a consequence of reduced levels of β-glucuronidase mRNA, suggesting that the mRNAs coded by Gus-s+a and Gus-s+a are translated with lower efficiency than the mRNA coded by Gus-s+a.

A uniform effect on enzyme activity among different cell types and at various stages of development defines a locus as a systemic regulator and distinguishes it from either a temporal gene, which is tissue and time specific in its expression, or a hormonal response regulator, which acts only in responsive cell types (1). Independent evidence for the presence of a systemic regulatory gene or genes in the β-glucuronidase complex has been obtained in a detailed study of the cis versus trans action of the regulatory differences seen between [Gus]d and [Gus]a or [Gus]b haplotypes (9). [Gus]a proved to differ at a new cis-acting site that acted equally in all tissues examined to reduce enzyme activity to about 30% of the levels seen in [Gus]a or [Gus]b. Superimposed on this in strains carrying [Gus]d were developmental changes determined by a trans-acting regulatory site equivalent to the previously described Gus-t site. The cis-acting systemic regulator was designated Gus-u and has properties similar to those described here for the M. m. castaneus-derived haplotypes. Because of this phenotypic similarity, the systemic regulator causing the reductions of β-glucuronidase activity displayed by [Gus]c and [Gus]d mice has been considered another allele of Gus-u and is designated Gus-u’. By comparison, [Gus]b includes the Gus-u’ allele, and both [Gus]a and [Gus]b include the Gus-u” allele. We use this nomenclature because it is a convenient notation for designating phenotypic determinants and it is consistent with past usage (1, 9). It will require additional molecular studies to decide whether Gus-u’ represents a single or multiple mutational difference from Gus-u”, whether all natural variants of systemic regulation of β-glucuronidase share the same mechanism, and whether the DNA sequences determining Gus-u and Gus-s are necessarily distinct entities or may overlap.

Gus-u is quite analogous in its properties to the Bgl-s systemic regulator linked to the β-galactosidase structural gene (31) and the Lw systemic regulator linked to the amino-levulinate dehydratase structural gene (32, 33). In each case the regulatory element is closely linked to the structural gene, determines the rate of enzyme synthesis, and shows additive inheritance. The Gus-u” allele acts cis (9) as do the Bgl-s alleles; the cis versus trans action of Lw alleles is unknown. Although F1 heterozygotes between Gus-u” and Gus-u” animals exhibit additive inheritance, there is insufficient evidence to determine whether these alleles act cis or trans. If the Gus-u’ site or Gus-u site, we expect that it acts cis.

The β-glucuronidase mRNA concentration in liver was about 20% higher in 8-day-old animals than in adults. This difference was reflected in the developmental profile of liver β-glucuronidase. There were, however, no significant differences in the shape of the developmental curves for β-glucuronidase when mice carrying the B, CS, and CL haplotypes were compared. We conclude that these haplotypes all carry the same allele of the Gus-t temporal gene located in the [Gus] complex. The androgen inducibility of β-glucuronidase in kidney does differ among these haplotypes and has been reported elsewhere (8).

From our present information the phenotypic properties of mice carrying the [Gus]c and [Gus]d haplotype suggest that in this case the genetic structure of the [Gus] complex is Gus-s+a, t+a, u+a, r+a, and that the genetic structure of the [Gus]d haplotype is Gus-s+a, t+a, u+a, r+a. The genotype of [Gus]d is Gus-s+a, t+a, u+a, r+a, and is the standard to which other haplotypes of [Gus] are compared.

The finding of novel structural and regulatory alleles in the β-glucuronidase gene complex of M. m. castaneus provides additional insights to our understanding of β-glucuronidase gene expression. From this and from other recent studies identifying regulatory variants that do affect β-glucuronidase mRNA concentration (8, 21, 33, 34) it is becoming clear that β-glucuronidase is regulated at multiple levels and that different regulatory loci in the [Gus] gene complex can exert their effects by independent mechanisms.

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REFERENCES

New Haplotypes of the β-Glucuronidase Gene