X-LINKED AND AUTOSOMAL GENES CONTROLLING MOUSE 
α-GALACTOSIDASE EXPRESSION

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Manuscript received July 15, 1977
Revised copy received October 25, 1977

ABSTRACT

Analysis of F2 and backcross animals has confirmed the X-chromosome linkage of Ags, the structural locus for mouse α-galactosidase. The position of Ags has been located in the X chromosome, 9 centimorgans from Mo, and the gene order is centromere-Hq-Bn-Ta-Mo-Ags. A variation in the developmental expression of α-galactosidase activity, inherited as an autosomal trait, has been characterized using recombinant inbred lines of mice. Among certain recombinant inbred lines, the variation appears to segregate as a single major locus.

THE enzyme α-galactosidase is derived from an X-linked gene in humans and mice. The first evidence for this came from the studies of KINT (1970), who showed that a deficiency of human α-galactosidase A is associated with Fabry's disease, a sex-linked metabolic disorder of glycolipid metabolism. Subsequently, somatic cell hybridization experiments suggested X-linkage of the enzyme in humans (GRZESCHIK et al. 1972; REBOURCET et al. 1975) and in mice (KOZAK, NICHOLS and RUDDLE 1975). Recently, a thermal stability variant for mouse α-galactosidase was found, and an analysis of the F1 generation provided direct evidence that the structural gene for α-galactosidase resides on the X chromosome (LUSIS and WEST 1976).

Some evidence, however, suggests that in mice an autosomal locus is involved in controlling the developmental appearance of α-galactosidase (LUSIS and PAIGEN 1975). Mice of certain inbred strains, including members of the C57-C58 family, have about a two-fold elevation in α-galactosidase activity in liver when compared with most other strains. The variation is enzyme specific, is restricted to liver, and becomes expressed only at a characteristic stage in development (about 25 days after birth). The genetics of this variation has proved difficult to investigate, since the difference in the parental activities is small and liver enzyme levels are relatively susceptible to nongenetic variation. Thus, it has not been possible to use conventional genetic crosses to separate backcross and F2 progeny into distinct phenotypic classes. Nevertheless, the results of such crosses are consistent with segregation at a single major autosomal locus with two alleles showing additive inheritance (LUSIS and PAIGEN 1975). The genetic

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analysis of this variation is of particular interest in clarifying the characteristics of genes involved in the developmental regulation of enzyme production (PAIGEN 1964; PAIGEN and GANSCHOW 1965; SCHWARTZ 1971; PAIGEN 1977).

We now report experiments extending these studies of the loci controlling α-galactosidase structure and activity in mice. We show that the thermal stability variation for α-galactosidase segregates as a single X-linked gene in backcross and F₂ animals, and we determine the location of this gene, designated Ags, on the X chromosome. The liver α-galactosidase activity variation was investigated using recombinant inbred lines of mice in order to clarify whether the variation is monogenic.

MATERIALS AND METHODS

(A) Mice

The thermolabile variant of α-galactosidase was discovered in a noninbred stock of Mus musculus molossinus. All the other mice tested in a survey of about 100 stocks have the more heat-stable form of the enzyme (LUSIS and WEST 1976). In the experiments described in this paper, we used M. m. molossinus, maintained in this laboratory, as the source of the Agsm allele, which codes for the thermolabile form of α-galactosidase. Inbred mice were obtained from the Jackson Laboratory, Bar Harbor, Maine (BALB/cBy, C3H/HeJ, C57BL/6By, C57BL/6J, DBA/2J, and BALB/cBy × C57BL/6By recombinant inbred lines) and from the Roswell Park production colony at West Seneca, New York (C3H/HeHa). We are grateful to DR. B. A. TAYLOR for providing C57BL/6J × DBA/2J and C57BL/6J × C3H/HeJ recombinant inbred lines, to DR. W. K. WHITTEN for Ta, and to DRS. J. T. NIELSEN and V. M. CHAPMAN for Ta-Mobr and Hq-Bn. Recombinant inbred lines are derived by inbreeding F₁ animals from a cross between two different inbred progenitor strains (BAILEY 1971).

(B) Genetic crosses

The X linkage of Ags was confirmed by continuing the reciprocal crosses between M. m. molossinus and C3H/HeJ (previously described by Lusis and West 1976) to obtain F₂ and backcross progeny. Three crosses were used to locate Ags in the X chromosome, as shown in Table 1. Four morphological X-linked markers, tabby (Ta), brindled (Mob'), harlequin (Hq) and bent-tail (Bn), were used in these experiments, but as all the crosses were constructed in the same way, only cross 1 will be described. Ta Ags*/+Agsh females were mated to (M. m. molossinus × C3H/HeJ)F₁ males (+Agsm/Y). Female Ta Ags*/+Agsh offspring, recognized by their coat patterns, were mated with C3H/HeHa (+Agsh/Y) males and the male progeny were classified for Ags and Ta as indicated in Table 1. Brain homogenates were used to classify mice for Ags. Only male progeny were used to determine linkage relationships. The male progeny from cross 2 were classified at about 10 days because Mobr/Y males normally die before weaning age. Some of the male progeny from cross 3 were also classified at this age, but all the others in crosses 1 and 3 were classified at about 70 days of age. The age of the mice did not significantly alter the heat stability of α-galactosidase. Thus, Agsm*/Y males could be distinguished from Agsh/Y males both at 10 and 70 days. Occasionally, the heat stability of α-galactosidase was ambiguous; if so, the samples were assayed a second time, and if the ambiguity remained, they were excluded from the experiment. For this reason, 3 of the 371 male progeny were not included in the calculations shown in Table 1. The thermostability of α-galactosidase in all linkage experiments segregated with brain α-galactosidase activity levels (see Figure 2A). Although thermostability alone was used in typing the animals for Ags, an examination of the activity remaining in homogenates after heat treatment (for example, see Figure 2B) was in some cases useful in confirming the boundary between the AGS-M and AGS-H types in heat stability plots.
TABLE 1

Recombination between Ags and four X-linked morphological markers in three genetic crosses

<table>
<thead>
<tr>
<th>Cross 1</th>
<th>Ta Ags(^h)/+ \times Ags(^m)♀ × + Ags(^h)/Y♂</th>
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</thead>
<tbody>
<tr>
<td>Genotype of male progeny</td>
<td>Number*</td>
</tr>
<tr>
<td>Ta Ags(^h)</td>
<td>17</td>
</tr>
<tr>
<td>+ Ags(^m)</td>
<td>50</td>
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<tr>
<td>Ta Ags(^m)</td>
<td>1</td>
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<tr>
<td>+ Ags(^h)</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
</tr>
</tbody>
</table>

Recombination:
Ta-Ags 9/76 = 11.8 ± 3.7%

<table>
<thead>
<tr>
<th>Cross 2</th>
<th>Ta Mo(^br) Ags(^h)/+ + Ags(^m)♀ × + + Ags(^h)/Y♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype of male progeny</td>
<td>Number†</td>
</tr>
<tr>
<td>Ta Mo(^br) Ags(^h)</td>
<td>86</td>
</tr>
<tr>
<td>+ + Ags(^m)</td>
<td>109</td>
</tr>
<tr>
<td>Ta Mo(^br) Ags(^m)</td>
<td>9</td>
</tr>
<tr>
<td>+ + Ags(^h)</td>
<td>11</td>
</tr>
<tr>
<td>Ta + Ags(^m)</td>
<td>7</td>
</tr>
<tr>
<td>+ Mo(^br) Ags(^h)</td>
<td>3</td>
</tr>
<tr>
<td>Ta + Ags(^h)</td>
<td>0</td>
</tr>
<tr>
<td>+ Mo(^br) Ags(^m)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>225</td>
</tr>
</tbody>
</table>

Recombination:
Ta-Mo 10/225 = 4.4 ± 1.4%
Ta-Ags 30/225 = 13.3 ± 2.3%
Mo-Ags 20/225 = 8.9 ± 1.9%

<table>
<thead>
<tr>
<th>Cross 3</th>
<th>Hq Bn Ags(^h)/+ + Ags(^m)♀ × + + Ags(^h)/Y♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype of male progeny</td>
<td>Young†</td>
</tr>
<tr>
<td>Hq Bn Ags(^h)</td>
<td>4</td>
</tr>
<tr>
<td>+ + Ags(^m)</td>
<td>14</td>
</tr>
<tr>
<td>Hq Bn Ags(^m)</td>
<td>1</td>
</tr>
<tr>
<td>+ + Ags(^h)</td>
<td>3</td>
</tr>
<tr>
<td>Hq + Ags(^m)</td>
<td>1</td>
</tr>
<tr>
<td>+ Bn Ags(^h)</td>
<td>0</td>
</tr>
<tr>
<td>Hq + Ags(^h)</td>
<td>0</td>
</tr>
<tr>
<td>+ Bn Ags(^m)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
</tr>
</tbody>
</table>

Recombination:
Hq-Bn 4/67 = 6.0 ± 2.9%
Hq-Ags 17/67 = 25.4 ± 5.3%
Bn-Ags 13/67 = 19.4 ± 4.8%

* About 70 days old.
† About 10 days old.
(C) Assays and α-galactosidase heat inactivation

α-Galactosidase was assayed fluorometrically at pH 4.3, using 4-methylumbelliferyl-α-D-galactoside as the substrate, as previously described (Lusis and Paigen 1976). One unit of activity is that amount of enzyme that will hydrolyze 1 μmol of substrate per hr at 37°C.

Temperature inactivation was performed at 53°C in 0.02 M imidazole, pH 7.4, using brain homogenates, as previously described (Lusis and West 1976). The percentage activity remaining sometimes differed slightly between experiments; this is probably due to small differences in inactivation conditions. Although the heat stability variation for α-galactosidase is expressed in all tissues examined, brain homogenates were used because the coefficient of variation of α-galactosidase activity is considerably lower in brain than in other tissues, and the kinetics of α-galactosidase heat inactivation are nearly first order using brain homogenates. The fact that α-galactosidase does not show first-order kinetics of heat inactivation is probably due to microheterogeneity resulting from covalent modification and aggregation of the enzyme (Lusis and Paigen 1976).

Protein was determined according to Lowry et al. (1951).

RESULTS

(A) Segregation pattern of Ags

We have previously reported a heat-labile, structural variant for α-galactosidase in mice (Lusis and West 1976). Under the conditions of heat inactivation that we employed, the enzyme from M. m. molossinus retains about 30% of its initial activity after heating, while the enzyme from C3H/HeJ and all other strains tested retains about 55% of its activity. In a cross between M. m. molossinus and C3H/HeJ, F1 male progeny had enzyme stabilities similar to their female parent, while F1 female progeny had enzyme stabilities intermediate between the parents (Lusis and West 1976). This pattern of inheritance is consistent with X linkage, since F1 males derive an X chromosome from only their female parent, while F1 females derive an X chromosome from each parent.

X linkage of α-galactosidase has now been confirmed using backcross and F2 matings. Figure 1 shows the thermal stability of brain α-galactosidase for progeny from the two types of F2 crosses and two of the four possible backcrosses. In each cross, the male progeny resembled either M. m. molossinus or C3H in enzyme stability. In three of the crosses, the ratio of males in the two classes did not differ significantly from the expected ratio of 1:1 (for the cross in Figure 1A, $x^2 = 1.09, P = 0.3$; Figure 1B, $x^2 = 0.29, P = 0.75$; Figure 1D, $x^2 = 0.04, P = 0.85$). In one of the F2 crosses (described in Figure 1C), an unexpectedly high fraction of M. m. molossinus-type males was obtained ($x^2 = 8.05, P < 0.01$); the explanation for this result is not known but may be due to the small sample size. The female progeny from these matings were distributed among the parental and intermediate enzyme stability types expected for an X-linked locus. However, it was not possible to sort the female progeny into distinct phenotypic classes, since the intermediate and parental enzyme stability types had overlapping distributions. These segregation patterns can be explained by assuming the parental genotypes shown in Figure 1. The results indicate that the heat stability variation for α-galactosidase segregates as a single X-linked locus.
Figure 1.—Segregation of α-galactosidase heat stability in four crosses. Brain homogenates were diluted in 0.02 M imidazole (pH 7.5) and incubated at 53° for 3 hr, after which α-galactosidase activity was determined. Data are expressed as percent α-galactosidase activity remaining after 3 hr at 53°. Each oval symbol represents one mouse: open symbols represent females and closed symbols represent males. The genotypes assume X linkage of Ags: h = Ags^h; m = Ags^m.

The locus determining α-galactosidase thermal stability has been designated Ags (Lusis and West 1976), with the allele Ags^m determining the heat-labile form found in M. m. molossinus and the allele Ags^h determining the heat-stable form found in C3H and all other strains examined. Ags is probably the structural gene for α-galactosidase, since the thermal stability variation is expressed in all tissues examined and is retained after partial purification of the enzyme (Lusis and West 1976).

Associated with the α-galactosidase thermal stability variation is a small variation in enzyme activity levels. M. m. molossinus mice, carrying the Ags^m allele,
have about 30% lower $\alpha$-galactosidase activity levels in brain than strains carrying the $Ags^{h}$ allele, and in genetic crosses enzyme activity in brain segregates with enzyme stability (Figure 2a). This activity difference was observed in both young (10-day-old) and adult mice and is apparently systemic, since $Ags^{m}$ mice also have similarly reduced enzyme activity levels in other organs, including liver and kidney (data not shown). An increased separation of the $AGS-M$ and $AGS-H$ types can be achieved using enzyme activity levels after heat treatment of homogenates, rather than enzyme stability alone (compare Figure 1 and Figure 2b). Although the factors contributing to the activity variation are not yet understood, this improved separation could be useful in future studies of X-linked gene expression and X-chromosome inactivation.

**Figure 2.**—Relationship between brain $\alpha$-galactosidase activity and heat stability in *M. m. mollossinus* x C3H backcross and F$_1$ animals. The brain $\alpha$-galactosidase activity of male progeny from the four crosses shown in Figure 1 are plotted. *M. m. mollossinus* mice (carrying the $Ags^{m}$ allele) contained about 2.2 units of $\alpha$-galactosidase activity/g brain while C3H/HeJ mice (carrying the $Ags^{h}$ allele) contained about 3.0 units/g brain. Panel (A) shows the activity levels before heat treatment of homogenates. Panel (B) shows the activity levels after heat treatment of homogenates. Each symbol represents a single backcross or F$_1$ animal. Open symbols represent mice carrying the $Ags^{m}$ structural allele; closed symbols represent mice carrying the $Ags^{h}$ structural allele.
(B) Location of Ags in the X-Chromosome

The genotypes of the male progeny from three crosses using X-linked morphological markers are shown in Table 1. The results from crosses 2 and 3 provide gene orders of Ta-Mo-Ags and Hq-Bn-Ags respectively. The location of Ags in the X chromosome, estimated from each of the three crosses, is shown diagrammatically in Figure 3. The three crosses agree well, and the estimate of recombination between Ta and Ags from the combined data of crosses 1 and 2 is 13 ± 2%.

The deficiency of Bn/Y males in cross 3 is probably due to fetal or perinatal death (HULING, EICHER and COLEMAN 1973), although the incomplete penetrance of the Bn allele (GREEN 1966) may have resulted in some misclassification of Bn/Y males as +/Y. However, the location of Ags in the X chromosome is clear, even if this cross is ignored.

(C) Liver α-galactosidase activity variation

Adult mice of certain inbred strains, including members of the C57-C58 family, typified by C57BL/6, have a 2-fold elevation in α-galactosidase activity in liver. Previous analyses, involving conventional genetic crosses, have suggested that a single major autosomal locus, with two alleles showing additive inheritance, is involved in the liver activity variation. However, since segregation into distinct phenotypic classes was not obtained in backcross and F₂ progeny, these conclusions remain uncertain (LUSIS and PAIGEN 1975). An approach that has proved useful for the analysis of certain complex biochemical traits is the use of recombinant inbred lines of mice (BAILEY 1971; SWANK and BAILEY 1973; TAYLOR et al. 1975), and we have employed this approach in examining the liver α-galactosidase activity variation.

(Figure 3.—Diagram showing localization of Ags in the mouse X chromosome, derived from the three crosses shown in Table 1. The positions of Hq, Bn, Ta and Mo in the X chromosome, taken from GREEN (1966) and ISAACSON, STEWART and FALCONER (1974), are shown in the top line. The data from our three crosses are shown below. For each cross the position of Ags is shown relative to the position of the closest marker, and the standard errors for the estimates are indicated by the shaded boxes. From cross 1, Ags could be located 11.8 map units to either side of Ta, but the results from crosses 2 and 3 place Ags to the right of Ta.)

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MOUSE α-GALACTOSIDASE GENES

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Preliminary to examining the levels of α-galactosidase in recombinant inbred lines, the enzyme levels in progenitor strains (C57BL/6J, C57BL/6By, DBA/2J, C3H/HeJ and BALB/cBy) and their F₁ progeny were determined (Figure 4 and Table 2). In each of these groups, male and female animals had similar liver α-galactosidase activity levels, and F₁ progeny had activity levels intermediate between the parental types. Since male F₁ progeny derive their X chromosomes from their mothers, the primary determinants for liver α-galactosidase activity variation are not sex linked. Furthermore, there were no differences in liver enzyme activities between males and females in either F₁ or backcross generations derived from reciprocal crosses involving C57BL/6J (high liver activity) and CBA/J (low liver activity) (Lusis and Paigen 1975; Lusis unpublished). It should be noted that the mean liver α-galactosidase activity of the F₁ progeny in each cross was slightly below the mean of the parental types (Table 2).

Recombinant inbred lines derived from strains DBA/2J and C57BL/6J fall into two groups with respect to α-galactosidase liver activity (Figure 5 and Table 2). Eight of the lines have activities similar to C57BL/6J, and 12 of the lines have activities similar to, or slightly higher than, that of DBA/2J. A comparable distribution of activities is found among recombinant inbred lines derived from BALB/cBy and C57BL/6By (Figure 5 and Table 2). The distribution of α-galactosidase activities among recombinant inbred lines derived from C57BL/6J and C3H/HeJ is less clearly bimodal, with most lines having activity levels intermediate between the progenitor strains (Figure 5). The results of these
### TABLE 2

Segregation of α-galactosidase liver activity among recombinant inbred lines

<table>
<thead>
<tr>
<th>Line</th>
<th>α-galactosidase activity (units/g liver) mean ± S.E. (n)</th>
<th>Bgt allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) C57BL/6J × DBA/2J lines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>10.6 ± 0.3 (20)</td>
<td>B</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>5.5 ± 0.1 (20)</td>
<td>D</td>
</tr>
<tr>
<td>(C57BL/6J × DBA/2J)F₁</td>
<td>6.9 ± 0.2 (20)</td>
<td>—</td>
</tr>
<tr>
<td>line 19</td>
<td>4.6 ± 0.3 (4)</td>
<td>B</td>
</tr>
<tr>
<td>23</td>
<td>4.8 ± 0.7 (2)</td>
<td>B</td>
</tr>
<tr>
<td>11</td>
<td>5.2 ± 0.7 (6)</td>
<td>B</td>
</tr>
<tr>
<td>16</td>
<td>5.7 ± 0.3 (6)</td>
<td>B</td>
</tr>
<tr>
<td>21</td>
<td>6.3 ± 0.3 (6)</td>
<td>D</td>
</tr>
<tr>
<td>24</td>
<td>6.5 ± 0.3 (6)</td>
<td>B</td>
</tr>
<tr>
<td>13</td>
<td>7.0 ± 0.8 (4)</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>7.0 ± 0.3 (9)</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>7.3 ± 0.8 (5)</td>
<td>B</td>
</tr>
<tr>
<td>29</td>
<td>7.4 ± 0.3 (5)</td>
<td>B</td>
</tr>
<tr>
<td>8</td>
<td>7.6 ± 0.3 (6)</td>
<td>D</td>
</tr>
<tr>
<td>27</td>
<td>7.9 ± 0.3 (6)</td>
<td>B</td>
</tr>
<tr>
<td>18</td>
<td>9.3 ± 0.5 (7)</td>
<td>D</td>
</tr>
<tr>
<td>9</td>
<td>10.1 ± 0.5 (4)</td>
<td>B</td>
</tr>
<tr>
<td>25</td>
<td>10.1 ± 0.5 (3)</td>
<td>D</td>
</tr>
<tr>
<td>22</td>
<td>10.6 ± 0.5 (5)</td>
<td>D</td>
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<tr>
<td>28</td>
<td>10.6 ± 0.3 (6)</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>11.1 ± 0.6 (2)</td>
<td>D</td>
</tr>
<tr>
<td>14</td>
<td>12.3 ± 1.1 (10)</td>
<td>B</td>
</tr>
<tr>
<td>20</td>
<td>13.0 ± 0.7 (5)</td>
<td>D</td>
</tr>
<tr>
<td>(b) C57BL/6J × C3H/HeJ lines</td>
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<tr>
<td>C57BL/6J</td>
<td>12.0 ± 0.8 (12)</td>
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<tr>
<td>C3H/HeJ</td>
<td>5.3 ± 0.2 (11)</td>
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<tr>
<td>(C57BL/6J × C3H/HeJ)F₁</td>
<td>7.8 ± 0.3 (13)</td>
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<tr>
<td>line 18</td>
<td>5.3 ± 0.7 (4)</td>
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</tr>
<tr>
<td>7</td>
<td>7.1 ± 0.2 (8)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7.6 ± 0.3 (5)</td>
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<tr>
<td>8</td>
<td>7.7 ± 0.6 (5)</td>
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<td>7.7 ± 0.6 (5)</td>
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<tr>
<td>12f</td>
<td>8.4 ± 0.4 (6)</td>
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<td>4</td>
<td>8.9 ± 0.5 (7)</td>
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<td>2</td>
<td>9.7 ± 1.1 (3)</td>
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<tr>
<td>3</td>
<td>10.4 ± 0.5 (7)</td>
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<tr>
<td>9</td>
<td>10.6 ± 0.1 (7)</td>
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<tr>
<td>6</td>
<td>10.7 ± 1.4 (6)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>11.4 ± 0.1 (2)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11.5 ± 0.8 (4)</td>
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</tbody>
</table>
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TABLE 2—Continued

<table>
<thead>
<tr>
<th>Line</th>
<th>α-galactosidase activity (units/g liver) mean ± S.E. (n)</th>
<th>Bgt allele</th>
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<td>(c) BALB/cBy x C57BL/6By lines</td>
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<tr>
<td>C57BL/6By</td>
<td>10.9 ± 0.5 (10)</td>
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<tr>
<td>BALB/cBy</td>
<td>5.7 ± 0.2 (10)</td>
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</tr>
<tr>
<td>(BALB/cBy x C57BL/6By)F₁</td>
<td>7.4 ± 0.3 (12)</td>
<td></td>
</tr>
<tr>
<td>line D</td>
<td>5.1 ± 0.3 (4)</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>6.0 ± 0.5 (4)</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>6.2 ± 0.7 (4)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>7.3 ± 0.5 (4)</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>8.1 ± 0.6 (4)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>11.1 ± 0.3 (4)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>12.5 ± 0.5 (4)</td>
<td></td>
</tr>
</tbody>
</table>

Only males from recombinant inbred lines were used. All animals were sacrificed at about 70 days of age. The distribution of Bgt alleles (C57BL/6J carries Bgtb and DBA/2J carries Bgt(b)) is from the data of MEISLER (1976). The differences in activity levels of C57BL/6J parental animals, in parts (a) and (b) above, are nongenetic in origin, and are typical of variation normally observed between experiments. Possible sources of such variation include season, diet, and small differences in dissection procedures and homogenization conditions.

Experiments did not differ when α-galactosidase activity was expressed as a function of protein weight, rather than wet liver weight.

Using the C57BL/6J x DBA/2J recombinant inbred lines, the segregation of liver α-galactosidase activity has been compared with the segregation of other

![Graph](image-url)

**Figure 5.**—Liver α-galactosidase activity among recombinant inbred lines. Data from Table 2 are plotted; progenitor strain activity levels are shown using arrows.
markers (B. A. Taylor, personal communication; a list of these markers has been published by Taylor et al. 1975). However, linkage of the autosomal \( \alpha \)-galactosidase activity locus has not been observed. Strain C57BL/6J shows a developmental variation for the enzyme \( \beta \)-galactosidase remarkably similar to the \( \alpha \)-galactosidase variation (Paigen et al. 1976; Meisler 1976). The variation for both enzymes is restricted to liver and results in a doubling of enzyme activity (relative to most other strains) between 20 and 50 days after birth. Nevertheless, it is clear from the C57BL/6J \( \times \) DBA/2J recombinant inbred lines that the locus controlling liver \( \beta \)-galactosidase activity, \( Bgt \), segregates independently of liver \( \alpha \)-galactosidase activity (Table 2).

**DISCUSSION**

The seven gene products known to be \( X \) linked in the mouse are also linked to the human \( X \) chromosome (Table 3). This strongly supports Ohno's theory of \( X \)-chromosome homology (Ohno 1967, 1973), and it may soon be possible to determine whether the order of \( X \)-linked genes has been conserved during mammalian evolution, as Ohno (1973) suggested. Clearly some rearrangements have occurred, as the mouse \( X \) chromosome is acrocentric, whereas the human \( X \) is metacentric. The gene orders for the mouse and human \( X \)-chromosomes are shown in Figure 6. The mouse \( X \)-chromosome map is based on genetic studies, whereas the human \( X \)-chromosome map is derived from somatic cell hybridization studies. The relative positions of the centromere and the genes for PGK and \( \alpha \)-GAL have been determined for both the mouse and human \( X \)-chromosomes, and it is possible to compare gene orders, but not the precise locations, of the genes given by the two mapping methods (Lyon 1976). Comparative mapping of other \( X \)-linked genes will be of interest to test whether the distal end of the mouse \( X \) chromosome is homologous to the long arm of the human \( X \) chromosome.

The loci responsible for the liver \( \alpha \)-galactosidase activity variation do not appear to be \( X \) linked. A previously reported genetic cross between C57BL/6J

![Figure 6](attachment:mouse_x_chromosome_map.png)  
*Figure 6.—Genetic map of the mouse \( X \) chromosome (from Womack 1976 and from data of Nielsen and Chapman 1977) and somatic cell genetic map of the human \( X \) chromosome (from Shows 1977). The gene symbols are shown above the mouse \( X \) chromosome and the gene products are given below each chromosome. (See Table 3 for explanation of abbreviations for the gene products). The position of the centromere is shown by a closed circle.*
### TABLE 3

**Biochemical markers in mouse and human X chromosomes**

<table>
<thead>
<tr>
<th>X-linked character</th>
<th>Human references</th>
<th>Mouse references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>Childs et al. (1958)</td>
<td>Epstein (1969)</td>
</tr>
<tr>
<td>E.C. 1.1.1.49 (G6PD)</td>
<td></td>
<td>Chapman and Shows (1976)</td>
</tr>
<tr>
<td>Hypoxanthine phosphoribosyltransferase</td>
<td>Segmiller, Rosenbloom and Kelly (1967)</td>
<td>Epstein (1972)</td>
</tr>
<tr>
<td>E.C. 2.4.2.8 (HPRT)</td>
<td></td>
<td>Chapman and Shows (1976)</td>
</tr>
<tr>
<td>E.C. 2.7.2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorylase kinase (PHK)</td>
<td>Huljing and Fernandes (1969)</td>
<td></td>
</tr>
<tr>
<td>E.C. 2.7.1.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Galactosidase (α-GAL)</td>
<td>Kint (1970); Gezeschik et al. (1972);</td>
<td>Kozak, Nichols and Ruddle (1975)</td>
</tr>
<tr>
<td>E.C. 3.2.1.22</td>
<td>Reboucet et al. (1975)</td>
<td>Lusis and West (1976)</td>
</tr>
<tr>
<td>Ornithine carbamoyltransferase (OCT)</td>
<td>Scott et al. (1972); Ricciuti,</td>
<td>DeMars et al. (1976)</td>
</tr>
<tr>
<td>E.C. 2.1.3.3</td>
<td>Gelehrter and Rosenberg (1976)</td>
<td></td>
</tr>
<tr>
<td>Androgen receptor protein (ARP)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(high liver activity) and CBA/J (low liver activity) suggested that liver activity is determined primarily by a single autosomal locus showing additive inheritance (Lusis and Paigen 1975). In addition, male and female F₁ progeny from crosses between C57BL/6 and several other strains did not differ in liver α-galactosidase activity (Figure 4, Table 2). Somatic cell hybridization studies suggested that human α-galactosidase is a dimer containing identical subunits (Rebourcet et al. 1975). Thus, by analogy it is unlikely that there is a second α-galactosidase structural gene that is autosomal. Therefore, the autosomal locus or loci controlling mouse liver activity probably regulates the expression of the X-linked Ags structural locus. The existence of regulatory loci for specific proteins in eukaryotes that are unlinked to the structural genes for those proteins has been suggested by several recent studies (for example, Boubelik et al. 1975; Schwartz 1976; Doane and Abraham 1976; Arst, 1976; for discussion see Paigen 1977).

Since in conventional genetic crosses, liver α-galactosidase activity does not segregate into distinct phenotypic classes (Lusis and Paigen 1975), we have examined the liver activity variation in recombinant inbred lines. In at least one series of recombinant inbred lines (C57BL/6J × DBA/2J), segregation into distinct groups, resembling the progenitor strains, was obtained. The simplest model for this pattern of inheritance is segregation of a single major locus with two alleles showing additive inheritance. This is consistent with the results of conventional crosses, and the ratio of animals in the two groups (12:8) is not significantly different from the ratio of 1:1 predicted by such a model ($\chi^2 = 0.80$, $P = 0.4$). However, alternative models involving multiple loci cannot be ruled out. The range of activity levels within each of the two groups is relatively broad, and several of the lines in the low (resembling DBA/2J) liver activity group appear to have activity levels slightly higher than the DBA/2J progenitor strain. Also, many of the C57BL/6J × C3H/HeJ recombinant inbred lines have liver activity levels intermediate between the progenitor strains. Segregation at minor loci in addition to a major locus controlling liver activity could explain these inheritance patterns. We propose the provisional symbol Tag (temporal α-galactosidase) for the major gene controlling the developmental expression of α-galactosidase activity in liver.

In conclusion, the variation in mouse α-galactosidase thermostability is controlled by a single X-linked locus, Ags, located in the X chromosome 9 centimorgans from Mo. Ags is probably the structural gene for α-galactosidase, and the thermal stability variant, Ags<sup>+</sup>, should be useful for studies of X-chromosomal expression in the mouse. The gene is expressed in all adult tissues examined (Lusis and Paigen 1976), probably as early as the four-cell stage in preimplantation mouse embryos (Adler, West and Chapman 1977). A single major locus controlling liver α-galactosidase activity seems to segregate among certain recombinant inbred lines; this agrees with results from previous genetic crosses. Thus, although the α-galactosidase structural gene resides on the X chromosome, the enzyme activity levels in liver are apparently controlled by a separate autosomal locus.
We wish to thank Drs. B. A. Taylor and W. K. Whitten, both from the Jackson Laboratory, Bar Harbor, Maine, J. T. Nielsen, from the Institute of Genetics, University of Aarhus, Denmark and V. M. Chapman, of this department, for kindly supplying mice. We also thank Drs. K. Paigen and V. M. Chapman for reading the manuscript and for much helpful discussion. This work was supported, in part, by a grant from the Public Health Service.

LITERATURE CITED


Corresponding editor: D. BENNETT