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Mapping of Aminoacylase-1 and β-Galactosidase-A to Homologous Regions of Human Chromosome 3 and Mouse Chromosome 9 Suggests Location of Additional Genes

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SUMMARY

Conserved linkage groups have been found on the X and autosomal chromosomes in several mammalian species. The identification of conserved chromosomal regions has potential for predicting gene location in mammals, particularly in humans. The genes for human aminoacylase-1 (ACY1, *N*-acylamino acid aminohydrolase, E.C.3.5.1.14), an enzyme in amino acid metabolism, and β-galactosidase-A (GLB1, E.C.3.2.1.23), deficient in G_{M1}-gangliosidosis, have been assigned to human chromosome 3. Using human-mouse somatic cell hybrids segregating translocations of human chromosome 3, expression of both ACY1 and GLB1 correlated with the presence of the p21→q21 region of chromosome 3. In a previous study, assignment of these genes to mouse chromosome 9 used mouse-Chinese hamster somatic cell hybrids, eliminating mouse chromosomes. To approximate the size of the conserved region in the mouse, experiments were performed with recombinant inbred mouse strains. An electrophoretic variant of ACY-1 in mouse strains was used to map the *Acy-1* gene 10.7 map U from the β-galactosidase locus. These data suggest that there is a region of homology within the p21→q21 region of human chromosome 3 and a segment of mouse chromosome 9. Since the mouse transferrin gene (*Trf*) is closely linked to the aminoacylase and β-galactosidase loci, we predict that the human transferrin (*TF*) gene is on chromosome 3.

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INTRODUCTION

Genes located on mammalian X chromosomes have been conserved during evolution, as postulated earlier by Ohno et al. [1]. This prediction has held up, as every mammalian species analyzed encodes the genes for hypoxanthine phosphoribosyl transferase, glucose-6-phosphate dehydrogenase, β-galactosidase, and phosphoglycerate kinase on their X chromosomes [2]. Recent evidence indicates that certain autosomal linkage groups are also conserved in such widely divergent species as man and mouse [2]. For example, genes located on the short arm of human chromosome 1 (*PGD*, *ENO1*, *AK2*, *GPD1*, and *PGM1*) are also linked on mouse chromosome 4 [3]. However, genes that are located on the same chromosome (syntenic) but not closely linked may not be syntenic in distantly related species. For example, the gene for peptidase C, located on the long arm of human chromosome 1, is not syntenic in *Mus musculus* with genes that in human are located on the short arm of chromosome 1.

We have mapped the genes for aminoacylase-1 (ACY1) (an enzyme for the degradation of acylated amino acids) [4] and β-galactosidase-A (GLB1), deficient in G_{M1}-gangliosidosis [5], to human chromosome 3. The homologous β-galactosidase gene has been mapped to mouse chromosome 9 by breeding experiments with inbred strains having a variant β-galactosidase [6]. In a study using mouse-Chinese hamster somatic cell hybrids, we assigned the homologous mouse gene for ACY-1 to mouse chromosome 9 [7], indicating that these regions may be homologous in man and mouse. To determine the extent of the homologous region, here we mapped both genes to a region of human chromosome 3 using somatic cell hybrids made from cells having translocations involving chromosome 3. In addition, we obtained the map distance between these genes in the mouse using recombinant inbred mouse strains and found them to be closely linked. Contained within the mouse *Bgs-Acy-1* linkage group is the gene for transferrin. The homology of these regions would predict that human transferrin will also map to human chromosome 3.

MATERIALS AND METHODS

Parental Cell Lines

Two human cell lines containing reciprocal translocations involving chromosome 3 were used in the production of somatic cell hybrids. GM194 has an X/3 translocation [46,X,t(X;3)(p28;q21)] ([8], A. de la Chapelle, personal communication, 1981, and fig. 1), and GM2808 has a translocation involving chromosomes 3 and 17 [46,XX,t(3;17)(p21;p13)] (fig. 2). The breakpoints of both translocations have been confirmed here.

Selection and Counterselection of Somatic Cell Hybrids

GM194 was fused with RAG ($HPRT^-$) mouse cells using polyethylene glycol [9]. Twenty-four hybrid clones, designated XTR, were isolated on HAT medium [10]. For cells to grow on HAT medium, hypoxanthine phosphoribosyl transferase (HPRT), whose gene is located in the q26→q28 region of the human X chromosome, must be supplied by the human genome. In the case of GM194, the X/3 translocation chromosome (Xpter→Xq28:: 3q21→3qter) contained the selectable HPRT gene. To select against the X/3 translocation chromosome, three hybrids were subcloned in 8-azaguanine [11].

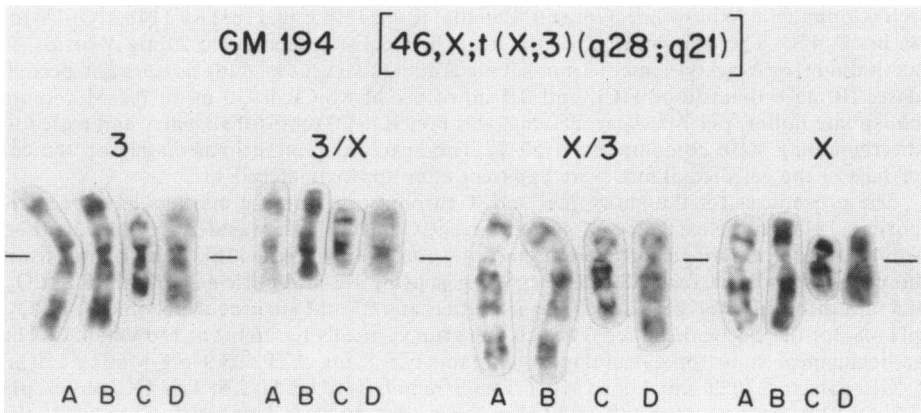

FIG. 1.—Partial karyotype of GM194. Shown are the normal chromosome 3 and the translocation chromosomes abstracted from the karyotype of four cells (*A*, *B*, *C*, and *D*). All other chromosomes were normal in appearance. The apparent variation in size in each group of chromosomes is due to differences in magnification.

Hybrid clones were also made with GM2808, a human fibroblast containing a 3/17 translocation, and mouse LM/TK$^-$ cells. Thymidine kinase (TK), encoded by a gene on human chromosome 17 (q21→q22), is essential for hybrid growth on HAT medium. Therefore, the nine primary clones isolated on HAT contained either a normal chromosome 17 or the 17/3 translocation chromosome (3pter→3p21::17p13→17qter).

Karyotypic Analysis of Hybrid Clones

Human chromosomes were identified in hybrid cells by the Giemsa-trypsin staining technique [12].

Detection of Enzymes

The procedures for detecting human β-galactosidase-A, described in Shows et al. [5], was a double immunodiffusion assay using human specific anti-β-galactosidase-A. ACY1 was detected by bioautography [4], a method that uses bacteria to detect the location of an enzyme in a gel, or by histochemical staining [13]. Enzymes were separated on 12% starch

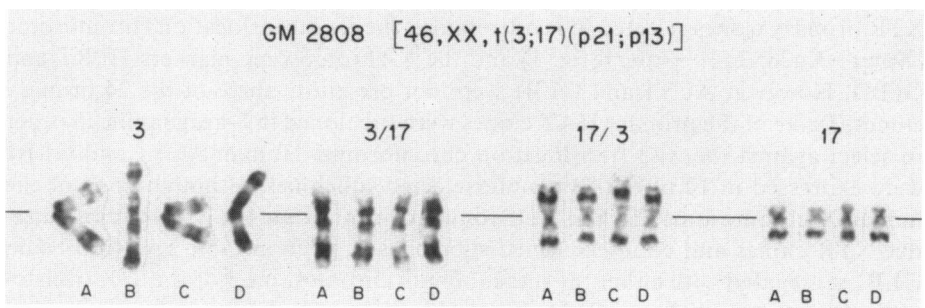

FIG. 2.—Partial karyotype of GM2808. Shown are normal chromosome 3 and 17 and the 3/17 and 17/3 translocation chromosomes from the karyotype of four different cells (*A*, *B*, *C*, and *D*). All other chromosomes appeared normal.

gels (Connaught, Willowdale, Ontario, Canada) using TEB buffer, pH 8.6 [14], at 270 V for 16 hrs at 4°C. The agar overlay for the histochemical stain contained 20 mg *N*-formyl-L-methionine (or *N*-acetyl-L-methionine), 6 mg crude snake venom, 6 mg horseradish peroxidase, 10 mg o-dianisidine HCl, and 0.5 ml of 0.1 M $MnCl_2$ in 10 ml of 0.1 M sodium phosphate buffer, pH 7.0. Agar, 250 mg, was boiled in 20 ml distilled water and added to the remaining stain components at 50°C. The agar was poured immediately on the cut surface of the gel slice. Bands were apparent after approximately 1 hr.

The procedures for the autosomal and X chromosome enzyme markers have been reported [15] with the exception of galactokinase. Galactokinase was detected by a method similar to that described for adenine phosphoribosyl transferase [16]. Electrophoresis of homogenates was performed in 12% starch gels using 1.2 mM citric acid, 6 mM K_2HPO_4, 0.5 mM dithiothreitol, pH 6.8, for the gel buffer; and 27 mM citric acid, 160 mM K_2HPO_4, pH 6.8, for the electrode buffer. The gels were run vertically for 16 hrs at 150 V at 4°C. The radiochemical stain for galactokinase contained 29.8 mg ATP, 23.9 mg $MgCl_2$, 150 μl [^{14}C]D-galactose (0.26 μmol of sp act 56.5 mCi/mmol) in 10.0 ml 0.2 M Tris-HCl buffer, pH 7.2. DE81 paper was saturated with the radioactive mixture and placed in contact with the gel. The reaction was allowed to proceed for 2 hrs at room temperature. The DE81 paper was then washed in a Buchler funnel by vacuum with 8 liters of water to remove any unconverted [^{14}C]galactose. The DE81 paper was dried and exposed to X-ray film for 2 days.

Recombinant Inbred Mouse Strains

BXD [17] and AKXL [18] recombinant inbred mouse strains were obtained from the Jackson Laboratory, Bar Harbor, Me. Kidney homogenates were prepared and scored for several genetic markers as reported [19, 20].

RESULTS

Somatic Cell Hybrids

Two human cell lines containing reciprocal translocations involving chromosome 3 were used in the production of somatic cell hybrids: GM194 [46,X,t(X;3)(p28;q21)] and GM2808 [46,XX,t(3;17)(p21;p13)]. These cell lines were obtained from the Mutant Human Cell Repository, Camden, NJ. GM194 was fused with RAG ($HPRT^-$) mouse cells, and 24 hybrid clones (the XTR series) were isolated on HAT medium. Somatic cell hybrids were karyotyped by Giemsa-trypsin banding in order to follow the segregation of the chromosome 3 translocations. The XTR primary clones selected in HAT retained the X/3 translocation chromosome (Xpter→Xq28::3q21→3qter) (fig. 1) and the X-chromosome markers HPRT and G6PD. However, ACY1 and GLB1 were not present in three of the 24 primary clones. Three of the primary HAT clones were subcloned in 8-azaguanine in order to select against the X/3 translocation chromosome. Human ACY1 and GLB1 were expressed in 12 of the 23 counterselected subclones, although none of the human X-chromosome markers were found. Chromosome analysis of representative XTR clones and counterselected subclones is given in table 1A. ACY1 and GLB1 segregated with either the intact human chromosome 3 or the 3/X translocation chromosome (3pter→3q21::Xq28→Xqter) (fig. 1). These data indicate that the *ACY1* and *GLB1* genes are located in the pter→q21 region of chromosome 3 (fig. 3).

TABLE 1

LOCALIZATION OF *ACY1* AND *GLB1* TO THE p21→q21 REGION OF HUMAN CHROMOSOME 3

A) Segregation of ACY1 and GLB1 with the 3/X translocation chromosome

Hybrid	ACY1	GLB1	HPRT, G6PD, PGK	3	X/3	3/X	X
HAT selected:							
XTR-1	+	+	+	−	+	+	−
XTR-3	+	+	+	+	+	+	+
XTR-4	+	+	+	+	+	+	+
XTR-18	+	+	+	+	+	+	+
XTR-22	−	−	+	−	+	−	−
8-azaguanine counterselected:							
XTR-1BSAgA	+	+	−	−	−	+	−
XTR-1BSAgD	+	+	−	−	−	+	+
XTR-1BSAgE	+	+	−	−	−	+	−
XTR-3BSAgA	+	+	−	+	−	−	(7)
XTR-3BSAgE	+	+	−	−	−	+	−
XTR-3BSAgH	+	+	−	−	−	+	(4)

B) Segregation of ACY1 and GLB1 with the 3/17 translocation chromosome

Hybrid	ACY1	GLB1	GALK	3	3/17	17/3	17
TSL-1	+	+	+	+	−	−	+
TSL-2	+	+	+	−	(4)	+	−
TSL-4	−	−	+	−	−	−	+
TSL-5	−	−	+	−	−	−	+
TSL-6	+	−	+	(7)	−	+	−
TSL-6D	−	−	+	−	−	+	−
TSL-6F	−	−	+	−	−	+	−
TSL-8	+	*	+	−	+	−	+

NOTE: A clone is positive for a human chromosome if there is one copy in at least 10% of the cells. This value corresponds with the proportion of human chromosomes needed to produce a detectable marker enzyme. Nos. in parentheses indicate the percentage of cells that have a particular chromosome if the value is less than 10%. A) Segregation of ACY1 and GLB1 with the 3/X translocation chromosome (3pter→3q21::Xq28→Xqter). Hybrids on HAT retained the X/3 translocation. Those hybrids counterselected in 8-azaguanine were selected for the loss of the X/3 translocation chromosome. The segregation of the 3/X, 3, and inactive X chromosomes was followed by karyotypic analysis. The expression of both ACY1 and GLB1 correlated with the presence of either a normal chromosome 3 or a 3/X translocation chromosome. B) Segregation of ACY1 and GLB1 with the 3/17 translocation chromosome (17 pter→17p13::3p21→3qter). The expression of ACY1 and GLB1 was correlated to human chromosome content using samples made at the same cell passage. Both genes segregated with the 3/17 translocation chromosome.

* Not enough β-galactosidase present to assay [5].

To restrict the regional location of *ACY1* and *GLB1* to a smaller region, TSL hybrids segregating the pter→p21 region of chromosome 3 were analyzed (figs. 2 and 3). All nine hybrids retained the human *TK* gene, located on chromosome 17, because of the TK deficiency of the rodent parent. In addition, these nine hybrids expressed human galactokinase (GALK), which is also coded by a gene on chromosome 17 [21]. However, the *TK* and *GALK* genes can be contributed to the hybrid by either a normal chromosome 17 or the 17/3 translocation chromosome (3pter→3p21::17p13→17qter) (fig. 2). Karyotyping the hybrid cells was necessary to determine which chromosome was present in a hybrid clone. As shown in table

SYNTENIC RELATIONSHIPS OF HUMAN CHROMOSOME 3 AND MOUSE CHROMOSOME 9

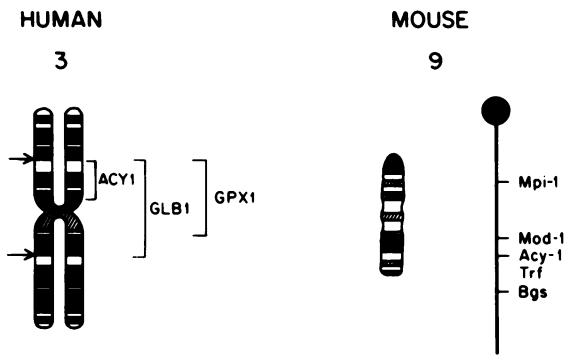

FIG. 3.—Comparison of the human and mouse homologous regions. *ACY1* is located in the p21→q12 region of human chromosome 3 and most likely the p21 band. *GLB1* is located in the p21→q21 region, and *GPX1* in the p13→q12 region. *Arrows* indicate the breakpoint of GM2808 in the short arm of chromosome 3 and of GM194 in the long arm. The mouse chromosome banding pattern is shown *on the left*, and the linkage map *on the right*.

1B, ACY1 was expressed in a clone (TSL-8) that contained the 3/17 translocation (17pter→17p13::3p21→3qter) (fig. 2). However, due to technical limitations of the assay [5], we were unable to score GLB1 in this hybrid. Two hybrid clones (TSL-6D and TSL-6F) that had the 17/3 translocation chromosome and lacked both a normal chromosome 3 and the 3/17 translocation were negative for the expression of ACY1. Since GLB1 was not expressed in these two hybrid cells, *GLB1* probably is located on the 3/17 translocation chromosome also. These data in combination with the previous data indicate that the *ACY1* gene is located in the p21→q21 region of human chromosome 3 and suggest, also, that the *GLB1* gene is in the same region of human chromosome 3.

Recombinant Inbred Mouse Strains

We have previously shown with mouse-Chinese hamster hybrids segregating mouse chromosomes that *Acy-1* is located on mouse chromosome 9 [7]. The gene complex for β-galactosidase, *Bgs*, is also on mouse chromosome 9 [6], suggesting that this linkage has been conserved throughout evolution. To determine the actual map distance between *Bgs* and *Acy-1*, *Acy-1* was mapped using recombinant inbred mouse strains.

Recombinant inbred (RI) mouse strains are the result of crossing two progenitor inbred mouse strains. The progeny of this mating are then inbred to yield new strains. Recombinant inbred strains are characteristically homozygous for a set of chromosome regions contributed randomly from the original inbred strains [22]. Since the combination of C57BL/6J and DBA/2J as well as the AKR/J and C57L/J inbred strains have distinguishable electrophoretic phenotypes of ACY-1 [4] (table 2), the RI strains BXD (C57BL/6J × DBA/2J) [17] and AKXL (AKR/J × C57L/J) [18] were used in mapping *Acy-1*. The mouse chromosome 9

TABLE 2

SEGREGATION OF ACY-1 IN RECOMBINANT INBRED MOUSE STRAINS

No. strains	MOD-1		LTW-3	KFO-1	ACY-1		FV-2		BGS
BXD (C57BL/6J × DBA/2J)									
8	B		B	B	B		B		B
3	D		D	D	D		D		D
1	D	X	B	B	B		B		B
1	D		D	D	D	X	B		B
1	B		B	B	B	X	D		D
1	B		B	B	B		B	X	D
1	B	X	D	D	D	X	B		B
1	D	X	B	B	B		B	X	D
2	B		B	B	B	X	D		B
AKXL (AKR/J × C57L/J)									
11			AK	AK	AK		AK		AK
5			L	L	L		L		L
1			L	L	L		L	X	AK
2			AK	AK	AK	X	L		L

NOTE: Recombinant inbred mouse strains have been produced by the repeated inbreeding of C57BL/6J × DBA/2J (BXD) [17] and AKR/J × C57L/J (AKXL) [18]. The strains that result from these crosses contain chromosomes that have recombined the genotypes of both parents. The "X" indicates where crossing over has taken place in a given strain. By following the segregation of ACY-1 with other markers in these mice, it is possible to locate precisely the gene on the mouse map. B = C57BL/6J phenotype, D = DBA/2J phenotype, AK = AKR/J phenotype, and L = C57L/J phenotype.

markers assayed in these strains were malic enzyme (MOD-1) [23], the two-dimensional gel markers (LTW-3) [19] and KFO-1 [20], Friend murine erythroleukemia virus susceptibility (FV-2) [19], and β-galactosidase (BGS) [24]. Recombination has taken place between these loci on chromosome 9 in the RI strains chosen, making it possible to estimate the map distances between *Acy-1* and other markers.

The results of analyzing 38 RI mouse strains are given in table 2. ACY1 segregated with no recombination with the markers KFO-1 and LTW-3. The distance between the β-galactosidase and ACY1 genes was calculated to be 10.7 ± 6.2 map U [25]. A diagrammatic representation of the mouse map for chromosome 9 is given in figure 3.

DISCUSSION

In this study, homology between human chromosome 3 and mouse chromosome 9 was demonstrated. Although it is not possible to determine the map distance between human *ACY1* and *GLB1* genes with current technology, we localized both genes to the p21→q21 region of chromosome 3 using cell hybrids segregating human chromosomes involving chromosome 3. Other laboratories have been involved in regionally localizing the gene for ACY1 in man. Kit et al. [13], using cell hybrids containing a rearranged chromosome 3, found that ACY1 segregated with the pter→p12 region of chromosome 3. Combining these results with our data, *ACY1* lies within the p21→p12 region of chromosome 3. Another

group, Voss et al. [26], has placed *ACY1* in the p21→pter region. These data suggest that the chromosomal breaks seen in the study by Voss et al. and the one reported here must have overlapping regions in common. Therefore, *ACY1* is very likely within the p21 band of chromosome 3. The current data would suggest that the GLB1 gene is close to *ACY1*. In mouse, *Acy-1* and *Bgs* are closely linked and approximately 10.7 ± 6.2 map U apart.

The evidence for conservation of homologous regions has been mounting [2]. In areas of autosomes where the location of several gene markers is known in one species, markers that are close together have been found to be conserved in other species. The best example is the group of genes located on the short arm of human chromosome 1 [3]. The *PGM-PGD-GPD* linkage was demonstrated both in humans and on the homologous mouse chromosome 4. Other human genes assigned to the distal region of chromosome 1, *ENO1*, *AK2*, and *FUCA*, were later found to be located on chromosome 4 in mouse [3, 27].

Noting this observation, we predict that genes located in the conserved region of mouse chromosome 9 will be located on human chromosome 3. The gene for transferrin, *Trf*, has been mapped very close to *Acy-1* in mouse [19]. The human transferrin gene, *TF*, however, has proved elusive in Mendelian mapping studies, although it has been linked to *CHE1*, the pseudocholinesterase-1 locus [28]. Conversely, genes on human chromosome 3 may be located in the corresponding region of mouse chromosome 9. The gene for glutathione peroxidase-1, *GPX1*, also has been assigned to the p13→q12 region of chromosome 3 [29]. Although the region under consideration is large, possibly the mouse genes for GPX and CHE1 will also map to chromosome 9.

Comparison of mammalian linkage groups may predict the location of several human genes. This is especially important in mapping genes that are not expressed in cultured cells or are not highly polymorphic in the human population. Our conjecture that transferrin is located on human chromosome 3 can be tested: the gene for transferrin can be cloned by recombinant DNA techniques and could be used to detect transferrin-specific DNA sequences in human-rodent hybrids. This new methodology has already resulted in the mapping of the human genes for insulin [30], growth hormone [31], interferon [32], and proopiocortin (ACTH) [33].

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