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G_{M1}-Gangliosidosis: Chromosome 3 Assignment of the β-Galactosidase-A Gene (βGAL_A)

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Abstract—*The structural gene (βGAL_A) coding for lysosomal β-galactosidase-A (EC 3.2.1.23) has been assigned to human chromosome 3 using man–mouse somatic cell hybrids. Human β-galactosidase-A was identified in cell hybrids with a species-specific antiserum to human liver β-galactosidase-A. The antiserum precipitates β-galactosidase-A from human tissues, cultured cells, and cell hybrids, and recognizes cross-reacting material from a patient with G_{M1} gangliosidosis. We have analyzed 90 primary man–mouse hybrids derived from 12 separate fusion experiments utilizing cells from 9 individuals. Enzyme segregation analysis excluded all chromosomes for βGAL_A assignment except chromosome 3. Concordant segregation of chromosomes and enzymes in 16 cell hybrids demonstrated assignment of βGAL_A to chromosome 3; all other chromosomes were excluded. The evidence suggests that G_{M1} gangliosidosis is a consequence of mutation at this βGAL_A locus on chromosome 3.*

INTRODUCTION

The G_{M1} gangliosidoses are fatal inherited lysosomal storage disorders associated with deficiency of β-galactosidase activity (1, 2). Human tissues contain two major β-galactosidase isozymes (1–5). β-Galactosidase-A is optimally active between pH 3.5 and pH 4.5 and is localized in lysosomes. The other major isozyme is optimally active near pH 6.5 and is referred to as "neutral" β-galactosidase. In patients with G_{M1} gangliosidosis, β-galactosidase-A is deficient while the neutral β-galactosidase appears to be unaffected

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(6–8). Evidence for a structural alteration of β-galactosidase-A in patients with G_{M1} gangliosidosis has been provided by the identification of enzymatically inactive, immunologically cross-reacting material (8–10) and by the altered properties of residual enzyme activity (6). Chromosome assignment of a structural gene for β-galactosidase-A would be useful in genetic counseling for this autosomal recessive disorder.

We have applied somatic cell genetic techniques to assign the structural gene encoding β-galactosidase-A to human chromosome 3. A species-specific antiserum to human liver β-galactosidase-A was utilized to analyze human–mouse interspecific cell hybrids segregating the gene for human β-galactosidase-A. The antiserum was prepared in rabbits using partially purified β-galactosidase from human liver (8). This purified β-galactosidase preparation can be designated as β-galactosidase-A by the criteria of pH optimum, mode of interaction with the inhibitor *N*-bromoacetyl-β-D-galactosylamine (4), and activity towards the natural substrates G_{M1}-ganglioside and asialo-G_{M1} ganglioside (11). The rabbit antiserum precipitates β-galactosidase-A from human tissues and cultured fibroblasts, and recognizes CRM in liver from a patient with G_{M1} gangliosidosis (8). This antiserum does not react with the human neutral β-galactosidase nor with mouse β-galactosidase, and can be used as a specific reagent for identification of the product of the human β-galactosidase-A structural gene. The evidence reported here for assignment of a β-galactosidase gene to chromosome 3 is supported by the observations of Bruns et al. (12, 13) using man–Chinese hamster somatic cell hybrids. A preliminary report of these data has appeared (14).

MATERIALS AND METHODS

Human and Mouse Parental Cells. Fibroblasts or leukocytes from nine individuals were used for constructing cell hybrids. They were WI-38 fibroblasts (ATCC CCL 75), AnLy fibroblasts [46, X, t(X; 9)(q12; p24)] (15) CaVa leukocytes [46, X, t(X; 22)(q22; q13)] (15), AlTr leukocytes [46, X, t(X; 5)(q22; q35)] (16), SH 421 fibroblasts (17), DUV fibroblasts [46, X, t(X; 15)(p11; q11)] (18, 19), JoSt fibroblasts [46, XY, t(7; 9)(q22; p24)] (20), and GM 1006 and GM 654 fibroblasts (Human Genetic Mutant Cell Repository) (21). The mouse parental cell lines were RAG ($HPRT^-$), LM/TK^-, LTP ($HPRT^-$, TK^-), and A9 ($HPRT^-$) (21, 22). Mouse cells were maintained on Dulbecco's growth medium (GIBCO) or Ham's F10 medium (GIBCO).

Somatic Cell Hybrids. Human and mouse cells were fused in monolayers or in suspension with inactivated Sendai virus or with polyethylene glycol (15, 23), Hybrid clones were selected and maintained on HAT (hypoxanthine/aminopterin/thymidine) selection medium which is

basically Dulbecco's modified Eagle's medium (GIBCO) with 10% fetal calf serum and antibiotics (22). Primary cell hybrid clones were analyzed from 12 separate fusion experiments. The hybrid sets, preceded by the number of primary clones used in this study, were 27 JSR (JoSt × RAG), 18 ICR (GM 1006 × RAG), 14 ICL (GM 1006 × LM/TK$^-$), 11 WIL (WI-38 × LTP), 15 REX (CaVa × RAG), 8 DUM (DUV × RAG), 7 RAS (SH 421 × RAG), 6 MAR (GM 654 × RAG), 4 ALR (AnLy × RAG), 4 ATR (AlTr × RAG), 1 ICA (GM 1006 × A9), and 1 DUA (DUV × A9). An additional seven primary and nine secondary clones from some of these hybrid sets were used in chromosome studies.

Hybrid Cell Extracts. Cells from confluent monolayers were homogenized in 0.05 M Tris buffer, pH 7.5 (22), at a concentration of 70–80 × 10^6 cells/ml. Electrophoresis was carried out on the 30,000*g* supernatant of the cell homogenate. Prior to centrifugation, the homogenates were rapidly frozen in liquid N_2 and thawed at 37°C, five times, to increase the recovery of β-galactosidase activity in the supernate.

Enzyme Assay. β-Galactosidase was assayed in cell extracts using 5 mM *p*-nitrophenyl-β-D-galactopyranoside (Sigma) as substrate in 0.1 M NaCl, 0.1 M sodium acetate buffer, pH 5.0 (24). One unit of β-galactosidase activity catalyzes the hydrolysis of 1 μmol of this substrate per hour at 37°C.

Antiserum to Human β-Galactosidase. β-Galactosidase-A was prepared from human liver obtained at autopsy. The enzyme was purified 300× as previously described (25). This partially purified preparation was subjected to electrophoresis on polyacrylamide gels (diameter 1.5 cm.) in Tris/glycine buffer, pH 8.1 (24). The enzyme was localized by staining the gel for 15 min at 37°C with 5-bromo-4-chloro-3-indoyl-β-D-galactoside (Fig. 1). The additional purification obtained by this step can be estimated from protein-stained gels to be roughly tenfold. The portion of the gel containing active enzyme was removed, frozen, and homogenized with a Polytron homogenizer in 0.14 M NaCl. This preparation, suspended in Freund's adjuvant, was used to immunize a rabbit. The resulting antiserum completely precipitates β-galactosidase-A from liver extracts, and produces an immunoprecipitate which is enzymatically active.

Antiserum to Mouse β-Galactosidase. This was prepared using purified mouse liver enzyme as previously described (24). Mouse β-galactosidase is a predominantly lysosomal enzyme with optimal activity at acidic pH.

Analysis of β-Galactosidase in Cell Hybrids. The presence of human β-galactosidase-A in hybrid cell extracts was analyzed by double immunodiffusion on agar slides using the antiserum to human β-galactosidase-A. Slides were prepared with 0.8% agar in phosphate-buffered saline (26). For routine typing, cell extracts were diluted to a concentration between 2.5 and 9.0 units of β-galactosidase per ml. A

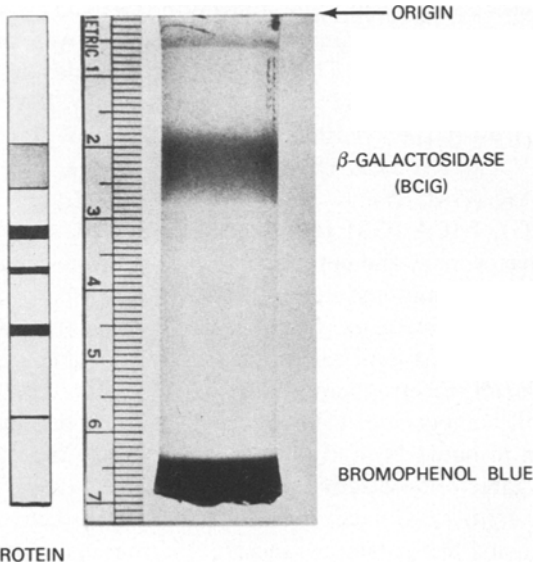

Fig. 1. Purification of human liver β-galactosidase-A by polyacrylamide gel electrophoresis. 8 mg of 300-fold purified enzyme was subjected to electrophoresis at pH 8.1 for 120 min. β-Galactosidase was detected with 5-bromo-4-chloro-3-indoyl-β-*D*-galactoside (BCIG). The region of the gel containing enzyme activity was removed and used as antigen for preparation of the antiserum employed for testing cell hybrids.

10-μl aliquot was placed in the center well and serial dilutions of the antiserum were placed in the peripheral wells. Immunodiffusion proceeded for 60–72 h in a moist chamber at room temperature. The slides were washed with phosphate-buffered saline, pH 6.6, for 24 h at room temperature with at least three changes of buffer. Slides were routinely stained for 15–50 min at 37°C with a saturated solution of 4-methylumbelliferyl-β-D-galactopyranoside (Koch-Light) in 0.1 M NaCl, 0.1 M sodium acetate buffer, pH 5.0, or in 0.1 M citrate/phosphate buffer pH 4.5. Fluorescence of the product 4-methylumbelliferone was enhanced with NH_3 vapor, and precipitin bands were viewed with longwave UV light. Extracts from cells containing human βGAL_A produced fluorescent immunoprecipitin bands. In some cases, slides were stained with 5-bromo-4-chloro-3-indoyl-β-D-galactoside (BACHEM) in 0.1 M NaCl, 0.1 M sodium acetate buffer (27). By this method we can detect a minimum of 2.3×10^{-3} units of human β-galactosidase-A using the fluorescent substrate, or 4.5×10^{-3} units with 5-bromo-4-chloro-3-indoyl-β-D-galactoside.

$ACON_M$ in Cell Hybrids. Hybrid cell extracts were tested for the presence of human mitochondrial aconitase ($ACON_M$) by vertical starch gel electrophoresis. Because human mitochondrial aconitase from human

fibroblasts and from human–mouse cell hybrids is very labile, cell extracts that had been frozen only once were used. Extracts of WI-38 fibroblasts or human heart or kidney were used as the human aconitase standard. Electrophoresis was performed in 15% Electrostarch gels for 17 h at 170 V (constant). The electrode buffer was 0.1 M Tris, 0.02 M trisodium citrate, adjusted with about 0.17 M HCl to pH 7.4. The gel buffer was a 1/10 dilution of the electrode buffer. Aconitase activity was visualized using an agarose overlay in 0.5 M Tris HCl, pH 8.0, buffer with 0.046 M *cis*-aconitic acid, 1 mM NADP, 0.5 mM MTT, 0.5 mM PMS, 0.01 M $MgCl_2$, and 0.35 units/ml of isocritic dehydrogenase (Sigma type IV, from pig heart) adapted from Slaughter et al. (28). Specific enzyme staining was achieved at 37°C for 1 h. This electrophoresis system is also suitable for the resolution of the cytoplasmic anodal form of aconitase (29).

RESULTS

Expression of Human β-Galactosidase in Cell Hybrids. Cell hybrid and parental cell lines were tested by immunoprecipitation with the two species-specific antisera (Fig. 2). Extracts from some hybrid cell lines contained β-galactosidase which was precipitated by the antiserum to human β-galactosidase-A (Fig. 2A). We can estimate the minimal contribution of human β-galactosidase-A to the total β-galactosidase activity of each extract, since a positive immunoprecipitate contains a minimum of 2.3×10^{-3} units. The extracts used here contained a total of 25×10^{-3} to 90×10^{-3} units of β-galactosidase. Therefore, in cell lines which are scored positive, a minimum of 2.3/90 or 2.6% of the total β-galactosidase is contributed by the human enzyme. Hybrid cell lines were designated as negative for human β-galactosidase-A if they did not produce an enzymatically active precipitin band when tested with the antiserum to human β-galactosidase. All hybrid cell lines produced a precipitin band when tested with antiserum to mouse β-galactosidase (Fig. 2B). Artificial mixtures of human and mouse β-galactosidase were precipitated by both antisera (Fig. 2).

Segregation Analysis of Human β-Galactosidase-A in Cell Hybrids. β-Galactosidase-A and 26 enzyme markers, encoded by genes on all human chromosomes except 3 and Y, were examined in a series of cell hybrids from 12 separate fusion experiments utilizing cells from 9 individuals and 4 different mouse cell lines. Concordant segregation of β-galactosidase-A and another enzyme marker would be indicated by the concurrent presence or absence of both. Discordant segregation would be characterized by the expression of either β-galactosidase-A or the other enzyme marker, but not both. β-Galactosidase-A and marker enzymes were always tested on extracts from the same cell passage. The Y chromosome could be excluded for

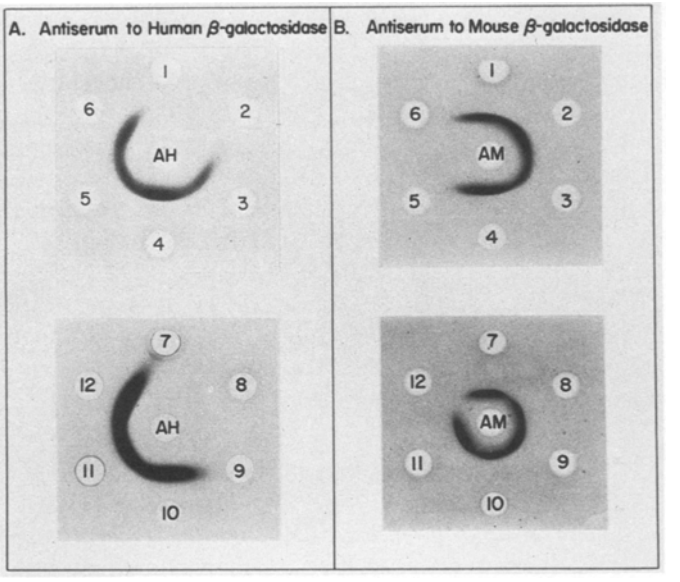

Fig. 2. Immunodiffusion analysis of β-galactosidase in cell hybrids. Center wells contained either antiserum to human β-galactosidase-A (AH, left) or antiserum to mouse β-galactosidase (AM, right). Immunodiffusion was carried out as described in text. Ten microliters of each sample containing the indicated units of β-galactosidase was stained with 5-bromo-4-chloro-3-indoyl-β-*D*-galactoside. 1. RAG, mouse tissue culture cell extract, 50×10^{-3} u (units); 2. mouse kidney extract, 40×10^{-3} u; 3. mixture: mouse kidney extract (2) and human liver extract (5); 4. JSR-13, cell extract of positive hybrid, 50×10^{-3} u; 5. human β-galactosidase, purified from normal human liver, 40×10^{-3} u; 6. WI-38, human fibroblast cell extract, 45×10^{-3} u; 7. RAG, mouse tissue culture cell extract, 50×10^{-3} u; 8. JSR-4, cell extract of negative hybrid, 70×10^{-3} u; 9. ICL-11, cell extract of negative hybrid, 54×10^{-3} u; 10. REX-49, cell extract of positive hybrid, 74×10^{-3} u; 11. JSR-3, cell extract of positive hybrid, 86×10^{-3} u; 12. WI-38, human fibroblast cell extract, 45×10^{-3} u.

chromosome assignment since female human cells contained the enzyme. β-Galactosidase-A segregated discordantly with all enzyme markers tested, with a discordancy frequency ranging from 0.16 to 0.73 (Table 1). All chromosomes except chromosome 3 were thus excluded from gene assignment by these results.

While this work was in progress, the assignment of β-galactosidase-A to human chromosome 22 was reported (30) based on electrophoretic and immunological analyses of human–Chinese hamster cell hybrids segregating an X/22 translocation. Human aconitase mitochondrial form ($ACON_M$) has previously been assigned to chromosome 22 (31–33), and we have confirmed this observation (34). Human $ACON_M$ migrates to a different position from the rodent enzyme by starch gel electrophoresis and can be visualized in fresh homogenates from hybrid cells (Fig. 3). We analyzed $ACON_M$ in our hybrid

Table 1. Segregation of βGAL_A and Enzyme Markers for 22 Human Chromosomes[a]

Chromosome	Enzyme	βGAL_A/enzyme marker				Frequency discordant
		Concordant		Discordant		
		+/+	−/−	+/−	−/+	
1	AK-2, PEPC	31	25	7	8	0.21
2	IDH_S	39	17	2	9	0.16
3						
4	PEPS	10	12	8	9	0.44
5	HEX_B	30	11	9	13	0.35
6	ME_S	35	24	12	3	0.20
7	GUS	36	18	10	12	0.29
8	GSR	2	6	4	7	0.73
9	AK-1	9	30	37	11	0.55
10	GOT_S	41	14	7	17	0.30
11	LDH_A	33	22	9	13	0.28
12	PEPB	43	14	7	9	0.22
13	ESD	32	15	5	15	0.30
14	NP	47	15	4	22	0.30
15	MPI, IDH_M	36	26	13	8	0.25
16	APRT	26	18	6	10	0.27
17	TK[b]	8	0	0	17	0.68
18	PEPA	43	18	7	18	0.29
19	GPI	41	17	7	19	0.31
20	ADA	40	24	8	18	0.29
21	SOD_S	41	14	6	24	0.35
22	$ACON_M$	6	5	4	8	0.52
X	HPRT, G6PD, PGK	46	10	2	21	0.29

[a] Analyses of βGAL_A and each enzyme marker were performed on the same cell extracts of primary hybrid clones. Enzyme markers were determined by starch gel electrophoresis as previously described (36–38). Enzyme symbols have been presented (39).

[b] Mouse TK^-–human hybrids require TK, assigned to human chromosome 17 (40), for growth on HAT selection medium. Human β-galactosidase-A was scored in WIL and ICL human–mouse hybrids to determine its linkage relationships to chromosome 17.

lines in order to test the assignment of the βGAL_A gene to chromosome 22. We did not observe cosegregation of $ACON_M$ and β-galactosidase-A in our hybrids (Table 1); there were 12 discordant lines in the 23 lines analyzed. Another recent report (35) has assigned a β-galactosidase gene to human chromosome 12. The method was based on the different pH optima of the human and mouse "acid" β-galactosidases. Our results for peptidase B, an enzyme marker encoded by a gene on chromosome 12 (Table 1), are inconsistent with this assignment since we observed discordancy in 16 out of 73 hybrid lines examined. Assignment of the β-galactosidase-A gene to chromosomes 12 or 22 is also inconsistent with the chromosome analysis of our hybrid lines discussed below.

Segregation of β-Galactosidae-A in Cell Hybrids with an X/22 Translocation. To further test the assignment of β-galactosidase-A to chromosome

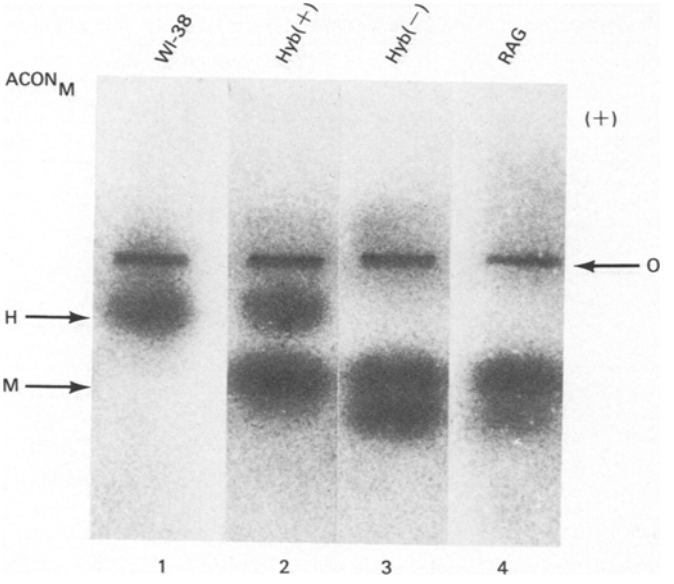

Fig. 3. Electrophoretic phenotypes of mitochondrial aconitase ($ACON_M$) in cell hybrid and parental cells. This chromosome 22 enzyme marker was detected after starch gel electrophoresis and specific histochemical staining.

Channel 1: Human WI-38 extract. Channel 2: REX-18, cell extract of human $ACON_M$-positive hybrid showing both rodent and human bands. Channel 3: ICR-9, cell extract of human $ACON_M$-negative hybrid showing only the mouse enzyme. Channel 4: RAG, mouse cell culture extract.

22, human cells carrying an X/22 translocation [(X, 46, t(X; 22)(q22: q13)] were fused with mouse cells to form cell hybrids. In this translocation, the selectable X-linked hypoxanthine phosphoribosyltransferase (HPRT) gene has been translocated to the q terminus of chromosome 22. Thus, the 22/X chromosome (22pter → 22q13 :: Xq22 → Xqter) possesses X-linked *HPRT* and glucose-6-phosphate dehydrogenase (*G6PD*) (15), and the X/22 chromosome (Xpter → Xq22 :: 22q13 → 22qter) possesses the X-linked marker phosphoglycerate kinase (*PGK*). Hybrid clones maintained on HAT selection medium require the 22/X chromosome for growth (15). In these hybrids the segregation of β-galactosidase-A was independent of the HPRT and G6PD (22/X) markers (Table 2). In hybrids tested for the phosphoglycerate kinase gene located on X/22, β-galactosidase-A also segregated independently of the enzyme and chromosome marker. When hybrid clones were counterselected on 8-azaguanine-supplemented medium, only those that had not retained the *HPRT* locus on 22/X could proliferate. In these clones β-galactosidase-A segregated independently of HPRT and G6PD. The same independent segregation was observed in X/22 hybrids growing on

Table 2. Segregation of βGAL_A and X-Linked Markers in t(X; 22) Translocation Hybrids[a]

A. Primary hybrid clones growing on HAT-supplemented medium

		βGAL_A				βGAL_A	
		+	−			+	−
HPRT, G6PD (22/X)	+	10	5	PGK (X/22)	+	1	0
					−	6	6

B. Hybrid clones counterselected on 8-azaguanine-supplemented medium

		βGAL_A				βGAL_A	
		+	−			+	−
HPRT, G6PD (22/X)	−	2	2	PGK (X/22)	+	0	0
					−	2	2

[a]Segregation of βGAL_A and X-linked enzyme markers hypoxanthine phosphoribosyltransferase (HPRT), glucose-6-phosphate dehydrogenase (G6PD), and phosphoglycerate kinase (PGK) in t(X; 22) translocation hybrids. βGAL_A segregated independently of the enzyme markers for both the 22/X and X/22 chromosomes. All hybrids growing on HAT must retain the 22/X marker; hybrids growing on 8-azaguanine must lose the 22/X marker (15).

8-azaguanine-supplemented medium. These results (Table 2) demonstrate that the gene for β-galactosidase-A is not located on chromosome 22.

Chromosome Assignment of β-Galactosidase-A. The presence of human chromosomes 3, 12, and 22 in 11 hybrid cell lines as well as five additional hybrid lines involving the X/22 translocation was examined (Table 3). The other human chromosomes were also analyzed and were observed to segregate discordantly with β-galactosidase-A (data not included). In these hybrids β-galactosidase-A segregated with chromosome 3. β-Galactosidase-A did not cosegregate with peptidase B (PEPB) and chromosome 12 (35), nor with $ACON_M$ and chromosome 22 (30). In REX hybrids segregating the X/22 and 22/X translocation chromosomes, β-galactosidase-A failed to cosegregate with these marker chromosomes. When hybrid line REX 11 BSH, which retained β-galactosidase-A and chromosomes 3, 22, and 22/X, was counterselected on 8-azaguanine-supplemented medium and cloned (REX 11 BSAg), β-galactosidase-A and chromosome 3 were retained but chromosomes 22 and 22/X were lost. These direct cytogenetic data confirm our assignment of β-galactosidase-A to chromosome 3 in man.

Three of the hybrid lines used in the chromosome studies retained chromosome 3 in only a small proportion of cells. Hybrids JSR 15, JSR 24, and REX 12 retained chromosome 3 in 9%, 8%, and 7% of the hybrid cells, respectively. The first two of these were positive for human β-galactosidase-A in our test, while the third was negative. We believe that the low frequency of human chromosome 3 in these lines is close to the lower limit of sensitivity of our test system, which requires that 2.6% of the total β-galactosidase be of human origin. In REX 12, for example, only 7% of the cells contain a copy of

Table 3. Segregation of βGAL_A, PEPB, $ACON_M$ and Human Chromosomes 3, 12, 22, and X/22 and 22/X[a]

	Enzymes			Chromosomes[b]				
Hybrid clone	βGAL_A	PEPB	$ACON_M$	3	12	22	22/X	X/22
JSR 6	+	+	+	12/21	4/21	6/21		
JSR 6C	+	−	−	16/20	0/20	0/20		
JSR 6D	+	+	+	13/24	2/24	3/24		
JSR 15	+	+	+	2/22	4/22	2/22		
JSR 17	−	+	−	0/53	44/53	0/53		
JSR 17F	+	+	−	22/22	15/22	0/22		
JSR 24	+	+	−	2/24	9/24	0/24		
JSR 24C	+	−	−	4/21	3/21	0/21		
JSR 24D	+	−	−	11/16	0/16	0/22		
ATR 13	+	+	−	6/9	6/9	0/9		
ATR 8 BSAg	−	+	+	0/23	3/23	6/23		
REX 12	−	−	+	2/30	0/30	0/30	26/30	0/30
REX 57 BSH	−	−	+	0/14	0/14	0/14	12/14	0/14
REX 18	+	−	+	12/21	0/21	2/21	1/21	0/21
REX 11 BSH	+	−	+	12/26	0/26	2/26	24/26	0/26
REX 11 BSAg	+	−	−	11/18	0/18	0/18	0/18	0/18

[a] Determination of β-galactosidase-A and the electrophoretic markers PEPB and $ACON_M$ were performed on the same cell passage as the chromosome determinations. All hybrids were maintained on HAT medium except for ATR 8 BSAg and REX 11 BSAg which were grown on 8-azaguanine-supplemented medium. REX 11 BSAg is a subclone of REX 11 BSH. Hybrids with symbols ending in capital letters are subclones.

[b] Numerator equals the number of metaphases containing the human chromosome; denominator equals number of metaphases scored.

human chromosome 3 while all the cells presumably contain at least two copies of the mouse β-galactosidase structural gene. Thus, approximately 3.5% of the β-galactosidase structural genes in these cells are human. If protein production is roughly proportional to gene dosage in these cell lines, then the human β-galactosidase in this line would approach the lower limit of sensitivity of our assay. We believe that this accounts for the single case of discordancy between β-galactosidase-A and human chromosome 3 in our study.

DISCUSSION

We have examined independent man–mouse somatic cell hybrids derived from human fibroblasts or leukocytes from nine individuals and four mouse mutant cell lines. Potential errors arising from the use of a single human line or large numbers of hybrid subclones were thus excluded. Human β-galactosidase-A was identified in cell hybrids with a species-specific antiserum by double immunodiffusion followed by histochemical staining of the immunoprecipitate.

Our segregation analysis of somatic cell hybrids is consistent with an assignment of the β-galactosidase-A locus to human chromosome 3. Analysis

of enzyme markers in 90 independent cell hybrids demonstrated lack of synteny for βGAL_A and all human chromosomes except 3 and Y. The Y chromosome was excluded since female parental cells contain this enzyme. Chromosome analysis of primary and secondary clones demonstrated concordant segregation of β-galactosidase-A and chromosome 3. Assignment of a β-galactosidase locus to chromosome 3 has also been observed by Bruns et al. (12, 13) using electrophoretic analysis of human-Chinese hamster cell hybrids. The β-galactosidase studied by Bruns et al. (12, 13) appears also to be β-galactosidase-A, since acidic conditions were used for detection of enzyme activity.

Assignment of the β-galactosidase gene to two other human chromosomes based on cell hybrid analysis has been recently reported. DeWit et al. (30) reported assignment to chromosome 22. They used an X/22 translocation [46, X, t (X; 22)(q23; q13)] segregating in human–Chinese hamster hybrids and reported the assignment of a β-galactosidase locus to the q13 → qter region of human chromosome 22. Our synteny analyses demonstrated discordant segregation between β-galactosidase-A, and $ACON_M$ which is coded by a locus on chromosome 22. Chromosome analysis was also inconsistent with assignment of β-galactosidase-A to chromosome 22. Analysis of β-galactosidase-A in hybrids with a human X/22 translocation also demonstrated discordant segregation with the 22/X and X/22 chromosomes in cells grown in HAT selection medium or in 8-azaguanine counterselection medium.

The second assignment of a β-galactosidase gene, to chromosome 12, was recently reported by Rushton and Dawson (35), who used their different pH optima to distinguish human and mouse β-galactosidase in cell hybrids. Our data are also inconsistent with this assignment.

Since there are multiple human isozymes with β-galactosidase activity, it remains possible that chromosomes 12 or 22 might be required for the isozymes detected in other laboratories. Since our antiserum was prepared against an enzyme that has been well characterized as β-galactosidase-A, we conclude that the structural gene (βGAL_A) is located on chromosome 3 in man.

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