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# Assignment of the Gene for Acid β-Glucosidase to Human Chromosome 1

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SUMMARY

The structural gene for human acid β-glucosidase (GBA) has been assigned to chromosome 1 using somatic cell hybridization techniques for gene mapping. The human enzyme was detected in mouse RAG cell-human fibroblast cell hybrids by a sensitive double antibody immunoprecipitation assay using a mouse antihuman GBA antibody. No cross-reactivity between mouse β-glucosidase and human GBA or neutral β-glucosidase (GBN) was observed. Fifty-two primary, secondary, and tertiary man-mouse hybrid lines, derived from three separate fusion experiments, were analyzed for human GBA and enzyme markers for the human chromosomes. Without exception, the presence of human GBA in these hybrid clones was correlated with the presence of human chromosome 1 or its enzymatic markers, phosphoglucomutase 1 ($PGM_1$) and fumarate hydratase (FH). All other human chromosomes were eliminated by the independent segregation of GBA and their respective enzyme markers and/or chromosomes. Using a RAG × human fibroblast line with a mouse-human rearrangement of human chromosome 1, the locus for GBA was limited to the region 1p11 to 1qter.

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## INTRODUCTION

Two isozymes with β-glucosidase activity have been identified in normal human tissues using the artificial substrate, 4-methylumbelliferyl-β-D-glucopyranoside (4MU-β-GLU). The acid (E.C.2.3.1.45) and neutral (E.C.3.2.1.21) β-glucosidases (here designated GBA and GBN, respectively) have been differentiated by their relative pH optima [1–5], subcellular localization [2, 6], substrate specificities [2, 6, 7], sensitivities to anionic detergents and acidic phospholipids [6], affinity for concanavalin A [8, 9], and most recently by their differential electrophoretic migration on cellulose acetate gels [9]. The acid isozyme, a membrane-bound activity, has been shown to be deficient in the various subtypes of Gaucher disease: lysosomal storage diseases characterized by the accumulation of glucosyl ceramide [10]. To date, the chromosomal assignment of the structural gene for either of the human β-glucosidase isozymes has not been determined.

We report here the assignment of the structural gene for human GBA using human-rodent somatic cell hybrids. A sensitive immunoprecipitation assay was developed for the selective detection of the human enzyme in the presence of mouse β-glucosidase activity. Additional data for the regional assignment of the locus on chromosome 1 have been obtained from a hybrid clone containing a mouse-human chromosome 1 rearrangement.

## MATERIALS AND METHODS

### *Human and Mouse Parental Cells*

The parental lines used for hybridization were the mouse RAG cell line [11] and three human fibroblast lines (fetal lung, fetal liver, and fetal kidney) from different sources. Parental cells were grown in RPMI 1640 medium containing 10% fetal calf serum (Gibco, Grand Island, N.Y.) using standard tissue culture techniques.

### *Somatic Cell Hybrids*

Parental cells were fused by centrifugation through a 7%–50% polyethylene glycol gradient [12]. Heterokaryons were cultured in the hypoxanthine/aminopterin/thymidine selective medium [13]. Hybrid cells were then cloned using the technique of Ham and Puck [14]. After 10–20 passages, selected primary hybrid clones were recloned, giving rise to secondary clones, and for some hybrids, tertiary clones were recloned from subclones.

### *Solubilization of β-Glucosidase in Cell Extracts*

Early confluent cells were harvested by sequential exposure to 0.25% trypsin (Gibco) and 0.02% EDTA in 0.9% NaCl approximately 24 hrs after a change of medium [15]. Cells from one 75-cm$^2$ flask were suspended in 0.3 ml of distilled water and freeze/thawed five cycles in an acetone-dry ice slurry. A 0.2 ml aliquot was mixed with 0.7 ml 0.05 M sodium phosphate-citric acid buffer, pH 6.0, and 0.1 ml of 0.6% Triton X-100 and 1% crude sodium taurocholate (lot no. 6420410, Gallard and Schlesinger, Carle Place, N.Y.). The suspension was incubated for 15 min at 25° C and centrifuged for 30 min at 30,000 *g* at 4° C, and then the supernatant was removed for assay.

### *β-Glucosidase Assay*

β-Glucosidase activity was assayed with the fluorogenic substrate, 4MU-β-GLU (RPI, Elk Grove Village, Ill.). The reaction mixture contained the substrate solution (5.0 mM 4MU-β-

GLU, 60 μl; 0.2 M sodium phosphate-citric acid buffer, pH 6.0, 30 μl; 0.12% Triton X-100; and 1% crude sodium taurocholate, 10 μl) and solubilized cell extract (20 μl). The reaction mixture was incubated for 15 min at 37°C and then terminated with 4.0 ml of 0.085 M glycine-carbonate buffer, pH 10.0. The liberated 4-methylumbelliferone was quantitated in a Turner model 111 fluorometer (G.K. Turner, Palo Alto, Ca.). One U of enzymatic activity represented 1 pmol of 4MU-β-GLU hydrolyzed per hr at 37°C.

*Purification of Human GBA*

GBA was highly purified from human placenta essentially by the method of Shafit-Zagardo and Turner [16]. Placental tissue was homogenized in 2 vol of distilled water and centrifuged for 30 min at 10,000 *g*. All steps were carried out at 4°C. The pellet was washed in distilled water, resuspended in a volume of the extraction buffer (0.05 M phosphate-citric acid, pH 6.0, containing 0.06% Triton X-100 and 0.1% crude sodium taurocholate) equal to three times the original weight of tissue, and was centrifuged as above. The solubilized extract was first precipitated with 33% ammonium sulfate, and then the supernatant was precipitated with 55% ammonium sulfate [17]. The pellet was resuspended in 0.06% Triton X-100 and then dialyzed overnight against 5.0 mM sodium phosphate-citric acid buffer, pH 4.0, containing 5.0 mM 2-mercaptoethanol (2-ME) and 5.0 mM EDTA. The dialyzed solution was extracted with n-butanol by the gradual addition of 20% (v/v) n-butanol with rapid stirring at 2°C. After mixing 30 min, the extract was centrifuged (10,000 *g*, 30 min) and the lower aqueous layer removed and dialyzed against binding buffer (0.05 M sodium phosphate-citric acid, pH 6.5, containing 0.02% Triton X-100). The enzyme was applied to an affinity column of dextran sulfate (Pharmacia, Piscataway, N.J.) bound to Sepharose 4B (Pharmacia) using a lysine spacer [18]. The enzyme was eluted with crude sodium taurocholate (3 mg/ml) in binding buffer. Fractions containing GBA activity were pooled, diluted 1:1 with distilled water, and adjusted to pH 4.5 with 0.5 M citric acid. n-Butanol extraction was performed as above. The sample was dialyzed against 0.1 M sodium citrate buffer, pH 5.0, containing 2% (v/v) n-butanol, 5.0 mM EDTA, and 5.0 mM 2-ME. The dialyzed preparation was applied to an octyl-Sepharose column (Pharmacia) [19], and the bound enzyme was eluted with 80% (v/v) ethylene glycol. Fractions containing GBA activity were concentrated (PM10, Amicon, Lexington, Mass.) and dialyzed in sucrose ultracentrifugation buffer (0.15 M phosphate-citric acid buffer, pH 6.0, containing 0.02% Triton X-100, 5.0 mM EDTA, 1.0 mM 2-ME). The enzyme was layered on linear sucrose gradients (5%–20%; w/w). Tubes were centrifuged in a Beckman model L5-75 ultracentrifuge at 82,000 *g* in a SW-27.1 rotor for 48 hrs at 4°C to a final $\omega^2 t$ of $1.1 \times 10^{12}$ rad$^2$/second. Fractions (0.5 ml) containing GBA were concentrated (Amicon, PM10), dialyzed overnight against the ultracentrifugation buffer containing 25% glycerol, and then stored at −20°C. The final enzyme preparation had a specific activity of $2.7 \times 10^8$ U/mg protein and was free of various lysosomal hydrolase activities including acid α-glucosidase, β-hexosaminidase B, and β-glucuronidase.

*Antiserum to Human GBA*

BALB/C mice (Jackson, Bar Harbor, Maine) were immunized with four biweekly intraperitoneal injections of 5 μg of purified enzyme in Freund's complete adjuvant. When 2,000 U of GBA activity from human fibroblast extracts were titrated against increasing amounts of the antiserum, optimal precipitation of GBA was obtained with 2 μl of antiserum and 20 μl of rabbit antimouse Ig. The precipitated enzyme-antibody complex retained catalytic activity and was quantitated by the standard enzymatic assay for β-glucosidase as described below. The antiserum selectively precipitated human GBA; no β-glucosidase activity was precipitated from mouse, rat, or Chinese hamster fibroblasts. In addition, to determine the specificity of the immunoprecipitation assay for GBA, partially purified GBN from human liver [9] was used in place of fibroblast cell extracts. No GBN activity was detected in the immunoprecipitate. Assays of the fibroblast enzyme-antibody complex for the presence of other enzymes

including β-glucuronidase, β-hexosaminidase B, α-glucosidase at pH 4.0 and 6.0, β-galactosidase at pH 4.5 and 7.0, α-L-arabinosidase, and β-D-xylosidase [9] proved negative, thus further demonstrating the specificity of the antibody for human GBA.

*Standard Immunoprecipitation Assay for Human GBA*

Cell extracts from parental lines of individual hybrid clones were assayed for β-glucosidase activity; 2,000 U were added to each assay tube. The volume was brought to 200 μl with the immunoprecipitation buffer (0.2 M sodium phosphate-citric acid buffer, pH 6.0, containing 0.05% Triton X-100, 10.0 mM 2-ME, 1.0 mM EDTA, and 1.0 mg/ml bovine serum albumin [BSA]). Anti-GBA antibody (2 μl) was added and the mixture incubated for 30 min at 37°C. Rabbit antimouse immunoglobulin was then added (20 μl) and the mixture incubated for 1–2 hrs at 37°C. Blanks contained 2 μl of normal mouse serum instead of the anti-GBA antiserum. Samples were centrifuged in 1.0 ml conical polypropylene tubes at 27,000 *g* for 20 min in a Sorvall RC-2B centrifuge, and the supernatant was retained and assayed for β-glucosidase activity. The pellet was washed with 0.5 ml of immunoprecipitation buffer and centrifuged as above. The pellet was assayed for β-glucosidase activity with the following modifications: 100 μl of substrate solution (see above) was added directly to the pellet and incubated for 1 hr at 37°C. The reaction mixture was stopped with 1.0 ml of 0.085 M glycine-carbonate buffer, pH 10.0, the mixture transferred to a 10 × 75 mm glass test tube, and fluorescence determined. For each hybrid clone, the background fluorescence in the blank was subtracted and the activity in the immunoprecipitate expressed as the percent of total activity recovered in supernatant and pellet.

*Determination of the Human Chromosomal Constitution of Hybrid Clones*

Marker enzymes for specific human chromosomes were determined by established electrophoretic methods [20–27]. Metaphase spreads were prepared [28], and the chromosomes were banded to distinguish mouse from human chromosomes with the Giemsa 11 technique [29] and subsequently destained and banded with quinacrine hydrochloride fluorescence [30]. Human chromosomal constitution of individual hybrid clones was based on enzyme marker data and/or cytogenetic analysis in which at least 30% of the metaphase spreads contained a specific chromosome. Human GBA immunoprecipitation assays, marker enzyme electrophoreses, and cytogenetic analyses were performed on cell hybrids harvested from the same passage.

## RESULTS

### *Specificity of Antihuman GBA in Hybrid Clones*

Table 1 shows the specificity of the immunoprecipitation assay for human GBA. Using the standard immunoprecipitation assay, purified human placental GBA activity was depleted from the supernatant and 35% of the total recovered activity was present in the immunoprecipitate. Similarly, GBA activity in solubilized human fibroblast extracts was precipitated by the antiserum; typically 30%–45% of the total recovered activity was precipitated. In the absence of the anti-GBA antibody and/or the rabbit antimouse Ig, no GBA activity was detected in the immunoprecipitate. Partially purified GBN from human liver was not immunoprecipitated, nor was any β-glucosidase activity precipitated from mouse (RAG), rat, or Chinese hamster fibroblasts. In addition, the immunoprecipitate showed no enzymatic activity when assayed for the presence of other glycosidases including α-L-arabinosidase, β-galactosidase at pH 4.5 and 7.0, α-glucosidase at pH 4.0 and 6.0, β-glu-

TABLE 1

SPECIFICITY OF THE IMMUNOPRECIPITATION ASSAY FOR HUMAN GBA

| ENZYME SOURCE | ANTI-GBA ANTIBODY | RABBIT ANTIMOUSE Ig | % TOTAL ACTIVITY β-GLUCOSIDASE RECOVERED | |
|---|---|---|---|---|
| | | | Supernatant | Pellet |
| Purified human placenta GBA | + | + | 65 | 35 |
| Human fibroblast extract | + | + | 72 | 28 |
| Human fibroblast extract | + | − | 100 | ND* |
| Human fibroblast extract | − | + | 100 | ND |
| Human fibroblast extract | NMS† | + | 100 | ND |
| Partially purified human liver ' GBN‡ | + | + | 100 | ND |
| RAG fibroblast extract | + | + | 100 | ND |
| RAT fibroblast extract | + | + | 100 | ND |
| Chinese hamster fibroblast extract | + | + | 100 | ND |

* ND = not detectable.
† NMS = normal mouse serum.
‡ GBN was partially purified from human liver as described [9] and used as the β-glucosidase source in this assay.

curonidase, β-hexosaminidase B, and β-D-xylosidase. Thus, the antiserum had specificity only for the human GBA isozyme.

For detection of human GBA in hybrid clones, the sensitivity of the competitive immunoprecipitate assay for human GBA was determined in the presence of mouse β-glucosidase activity. Figure 1 shows that the antihuman GBA antibody permitted the sensitive and specific precipitation of human GBA in mixtures containing varying percentages of human and mouse fibroblast extracts. The assay was linear over the entire range of 0 to 2,000 U of the human enzyme and allowed detection of human GBA in man-mouse hybrid clones.

For each set of immunoprecipitation assays, diploid human fibroblast extracts were used as controls. A mean value of 37.8% (range = 30.6%–44.9%, no. = 12) of total recovered β-glucosidase activity was found in the human control immunoprecipitates. On the basis of gene dosage, it is estimated that homogeneous hybrid lines carrying a single human chromosome coding for GBA would have about 1/3 of the total recovered β-glucosidase activity in the immunoprecipitate compared with control diploid human fibroblasts. Clones that are not homogeneous for the GBA structural gene would contain fewer molecules of human GBA. Only clones containing a human chromosome 1 in at least 30% of the metaphases studied were considered positive for that chromosome. Therefore, the maximal percent immunoprecipitated activity expected in a heterogeneous clone with 30% of the metaphase spreads containing human chromosome 1 would be 30% times the maximal

% immunoprecipitated activity in diploid human fibroblasts (37.8) times 1/3 to adjust for gene dosage in the hybrid cell, that is, 0.3 × 37.8% × 1/3 = 3.78% of total recovered activity. Thus, hybrids were scored positive for human GBA when the % of immunoprecipitated GBA was greater than 4% of total recovered activity (or greater than 10% of human control activity precipitated [see table 3]).

*Segregation Analysis of Human GBA in Cell Hybrids*

Segregation of human GBA and the enzyme markers for the human chromosomes in primary and secondary hybrid clones is shown in table 2. Table 3 shows the human chromosome complements and the immunoprecipitation data in representative secondary or tertiary clones selected for the presence or absence of human GBA. Based on these analyses, all human chromosomes except chromosome 1 were excluded from gene assignment for human GBA. The segregation of human GBA activity in the hybrids demonstrated 100% concordant expression of the enzyme with $PGM_1$, FH, and the intact chromosome 1. All the other chromosomes had a discordant frequency for GBA ranging from 0.26 to 0.75 and, therefore, could be eliminated.

*Regional Localization of GBA on Chromosome 1*

A hybrid cell line ($R/KidA_{10}$) with a human-mouse rearrangement involving human chromosome 1 was used to further localize the gene for GBA. As shown in figures 2 and 3, the chromosomal rearrangement was cytogenetically defined as human 1pter→p11 to a mouse chromosome by Giemsa 11 and Q-banding. Consistent with the cytogenetic analysis, the hybrid line was positive for human chromosome 1 short-arm markers, enolase 1 ($ENO_1$), $PGM_1$, and α-fucosidase (FUCA), and was negative for the long-arm markers: peptidase C (PEPC) and FH. Representative gels for $ENO_1$, $PGM_1$, and PEPC are shown in figure 4. The absence of

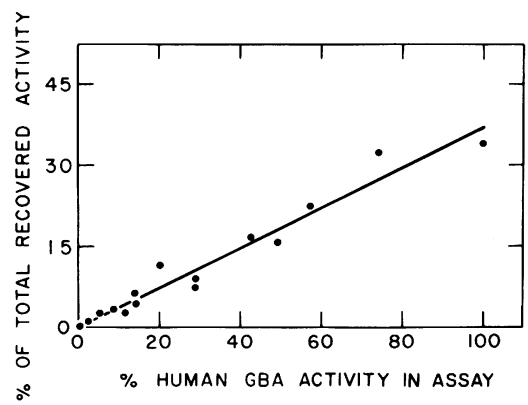


FIG. 1.—Immunoprecipitation of human GBA in mixtures of parental mouse and human fibroblast extracts. Total amount of GBA activity (2,000 U) in the assay was the same in all cases; however, the mixtures contained the indicated percentage of human fibroblast extract. See MATERIALS AND METHODS for details.

TABLE 2

SEGREGATION OF HUMAN CHROMOSOME MARKERS AND HUMAN GBA IN PRIMARY AND SECONDARY SOMATIC CELL HYBRIDS

| | | CONCORDANT | | | | DISCORDANT | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | +/+ | | −/− | | +/− | | −/+ | | |
| ENZYMES* | CHROMOSOME | P† | S‡ | P | S | P | S | P | S | FREQUENCY DISCORDANT |
| $PGM_1$, FH | 1 | 14 | 19 | 8 | 11 | 0 | 0 | 0 | 0 | 0.00 |
| $MDH_1$ | 2 | 1 | 0 | 3 | 0 | 7 | 1 | 0 | 1 | 0.69 |
| $GLB_1$ | 3 | 6 | 7 | 0 | 0 | 2 | 0 | 4 | 4 | 0.43 |
| $PGM_2$ | 4 | 3 | 4 | 1 | 0 | 3 | 3 | 3 | 1 | 0.56 |
| HEX B | 5 | 3 | 8 | 1 | 4 | 3 | 2 | 4 | 3 | 0.43 |
| $SOD_2$, $ME_1$ | 6 | 3 | 4 | 4 | 0 | 7 | 0 | 4 | 1 | 0.52 |
| GUSB | 7 | 1 | 2 | 0 | 0 | 5 | 0 | 2 | 0 | 0.70 |
| GSR | 8 | 0 | 4 | 5 | 1 | 2 | 3 | 2 | 2 | 0.47 |
| $AK_1$, $AK_3$, $ACON_1$ | 9 | 14 | 4 | 2 | 4 | 0 | 4 | 7 | 0 | 0.31 |
| $GOT_1$ | 10 | 3 | 0 | 1 | 1 | 4 | 1 | 4 | 0 | 0.64 |
| LDHA | 11 | 1 | 1 | 5 | 4 | 7 | 5 | 2 | 2 | 0.59 |
| LDHB, PEPB | 12 | 7 | 5 | 5 | 3 | 4 | 0 | 2 | 1 | 0.26 |
| ESD | 13 | 4 | 3 | 0 | 1 | 2 | 2 | 4 | 0 | 0.50 |
| NP | 14 | 3 | 5 | 1 | 5 | 4 | 1 | 3 | 0 | 0.36 |
| HEX A, MPI | 15 | 5 | 7 | 1 | 0 | 5 | 2 | 5 | 6 | 0.58 |
| PGP | 16 | 2 | 4 | 3 | 3 | 2 | 2 | 3 | 0 | 0.37 |
| GLUA, GALK | 17 | 0 | ... | 4 | ... | 2 | ... | 1 | ... | 0.75 |
| PEPA | 18 | 7 | 4 | 2 | 1 | 1 | 0 | 5 | 1 | 0.33 |
| GPI | 19 | 10 | 8 | 5 | 9 | 1 | 5 | 2 | 3 | 0.26 |
| ADA | 20 | 5 | 1 | 4 | 2 | 4 | 2 | 1 | 1 | 0.40 |
| $SOD_1$ | 21 | 9 | 3 | 1 | 1 | 0 | 0 | 6 | 3 | 0.39 |
| $ACON_2$ | 22 | 4 | 0 | 2 | 3 | 2 | 3 | 4 | 0 | 0.50 |
| G6PD | X | 2 | ... | 1 | ... | 2 | ... | 3 | ... | 0.63 |

* Enzyme markers were performed by starch or cellulose acetate gel electrophoresis as described [20–27].
† P = primary.
‡ S = secondary.

detectable GBA immunoprecipitable activity in this hybrid eliminated assignment of the GBA structural gene to the region 1pter→p11.

## DISCUSSION

The structural gene for human GBA has been localized to chromosome 1 using somatic cell hybridization techniques and a double antibody immunoprecipitation assay specific for the human GBA isozyme. This is the first time a chromosomal assignment has been designated for this enzyme. In all 52 hybrid clones examined, 100% concordant expression of human GBA and the enzymatic markers for chromosome 1, $PGM_1$ and FH, was observed (table 2). Cytogenetic analysis of these hybrid lines also demonstrated segregation of GBA with an intact chromosome 1 (table 3). All other human chromosomes showed discordancy for GBA as determined by either enzyme marker or cytogenetic analyses.

TABLE 3

SEGREGATION OF HUMAN GBA AND HUMAN CHROMOSOMES IN RAG × HUMAN FIBROBLAST SECONDARY AND TERTIARY HYBRID CLONES

| CELL LINE | HUMAN GBA | | | CHROMOSOMES | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % Immunoprecipitate* | % human control† | Pos/Neg‡ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| Human fibroblast | 37.8 | 100.0 | | | | | | | | | | | | | | | | | | | | | | | |
| RAG | 0.0 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | |
| RAG × $F_2$ Liver: | | | | | | | | | | | | | | | | | | | | | | | | | |
| R/$F_2$Li IB | 14.7 | 38.8 | + | + | − | + | + | − | + | − | − | + | + | − | − | − | + | − | − | + | + | − | + | + | + |
| R/$F_2$Li IC | 17.0 | 44.9 | + | + | − | + | − | + | + | − | − | + | − | + | + | + | − | − | + | − | − | + | − | + | − |
| R/$F_2$Li IE | 8.7 | 23.0 | + | + | − | − | − | − | − | − | − | − | − | + | + | − | − | + | − | − | + | + | − | − | − |
| R/$F_2$Li IF | 5.0 | 13.2 | + | + | − | + | + | + | + | − | − | + | + | − | − | − | − | + | + | − | + | − | − | + | + |
| R/$F_2$Li IG | 13.7 | 36.2 | + | + | − | + | − | + | + | − | − | + | − | − | + | − | − | + | + | − | − | − | + | + | − |
| R/$F_2$Li $F_3$ | 2.4 | 6.3 | − | − | − | − | − | − | + | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − |
| RAG × $F_3$ Lung: | | | | | | | | | | | | | | | | | | | | | | | | | |
| R/$F_3$Lu $E_{21}G$ | 7.3 | 19.3 | + | + | − | + | − | + | + | − | − | − | − | − | + | + | − | − | − | − | − | − | − | − | − |
| R/$F_3$Lu $E_2H$ | 0.9 | 2.3 | − | − | − | − | − | + | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | + | − |
| R/$F_3$Lu $E_{14}$ | 2.9 | 7.6 | − | − | − | − | + | − | + | − | − | − | − | + | + | − | + | − | + | − | − | − | − | + | + |
| RAG × $F_3$ Kidney: | | | | | | | | | | | | | | | | | | | | | | | | | |
| R/$F_3$Kid $A_1$ | 7.3 | 19.3 | + | + | − | − | − | + | − | − | − | + | − | − | − | + | − | + | − | − | − | + | − | + | + |
| R/$F_3$Kid $A_4$ | 7.5 | 19.8 | + | + | − | + | − | + | − | − | + | + | − | − | − | − | − | + | + | − | − | + | − | + | − |
| R/$F_3$Kid $A_{12}$ | 5.6 | 14.8 | + | + | − | − | − | − | − | − | + | − | + | − | + | − | − | + | + | + | + | + | − | + | + |
| R/$F_3$Kid $Q_1$ | 13.7 | 36.2 | + | + | − | + | − | + | − | − | − | − | − | − | + | + | + | − | − | + | + | + | − | + | − |
| R/$F_3$Kid $Q_3$ | 7.4 | 19.5 | + | + | − | + | − | + | − | − | + | − | − | − | + | − | − | + | + | − | − | − | − | + | + |
| R/$F_3$Kid $A_3$ | 0.0 | 0.0 | − | − | − | + | − | + | + | − | − | − | − | − | − | + | − | − | − | + | − | − | − | + | − |
| R/$F_3$Kid $U_3$ | 0.5 | 1.3 | − | − | − | + | + | + | − | − | + | + | − | − | − | − | − | + | + | + | + | + | − | + | + |

* % total recovered activity in immunoprecipitate.

† GBA activity in immunoprecipitate of hybrids expressed as % activity immunoprecipitated from human fibroblast controls.

‡ Hybrids scored as positive had ≥ 10% control human parental fibroblast GBA activity in immunoprecipitates.

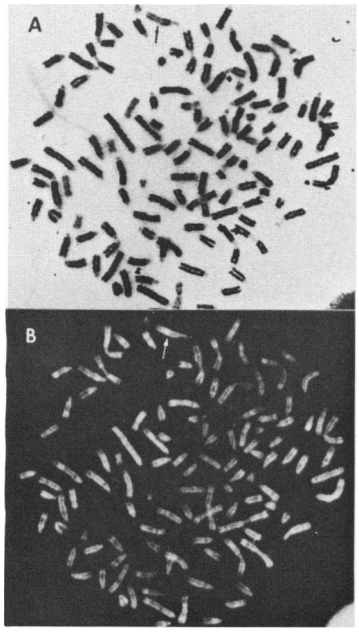


FIG. 2.—Metaphase chromosome spreads showing the human 1(p11→pter)/mouse chromosome translocation carried in the mouse-human hybrid line R/KidA$_{10}$. *A*, Giemsa 11 banding; *B*, quinacrine fluorescent banding. The translocation chromosome is indicated by *arrow*.

The assignment of GBA to chromosome 1 is strengthened by the following factors. First, the somatic cell hybrids used were derived from the fusion of a mouse RAG cell line with human fibroblasts from three different individuals. This excludes a potential source of error arising from a single fusion containing a large number of

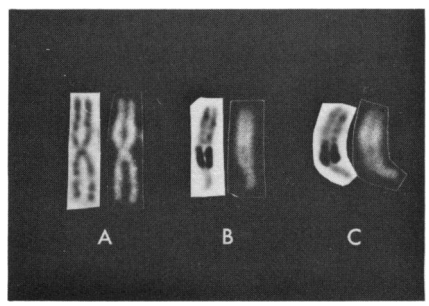


FIG. 3.—Chromosome 1 from metaphase spreads of normal human cells and the R/KidA$_{10}$ hybrid clone carrying the mouse-human chromosome 1 (p11→pter) translocation. *A*, Normal human chromosome 1 banded by Giemsa 11 and quinacrine, respectively; *B* and *C*, the translocated chromosome banded with Giemsa 11 and quinacrine, respectively. The darkly staining mouse chromosomal material is detected by the Giemsa 11 stain while the quinacrine banding showed the two fluorescent bands indicative of the p region of human chromosome 1.

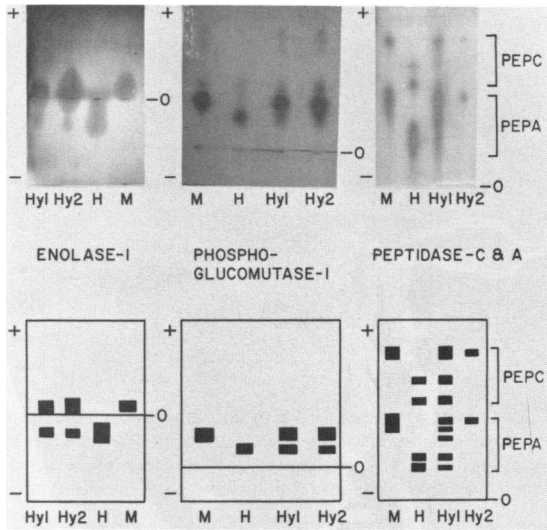


FIG. 4.—Starch gel electrophoresis of enzymatic markers for chromosome 1. Fibroblast extracts of mouse (*M*), human (*H*), R/Lung $E_{21}$ (*Hy1*), and R/Kid$A_{10}$ (*Hy2*) were electrophoresed and stained for enzymatic activity of various chromosome 1 enzyme markers. The mouse-human hybrid line, Hy1, which carries an intact human chromosome 1, demonstrated both mouse and human isozymes for all the chromosome 1 markers tested. The Hy2 hybrid line, which carries the chromosome 1 (p11→pter)/mouse translocation, was positive for the short-arm markers, $ENO_1$ and $PGM_1$, and negative for the long-arm marker, PEPC. These results confirmed the cytogenetic data. Isozyme pattern of each gel is shown diagrammatically *below*.

hybrid subclones [31]. Second, only the GBA isozyme is expressed in human fibroblast cell lines [32], thereby eliminating the possibility of precipitating the human GBN isozyme. In support, partially purified hepatic GBN was not precipitated by the antiserum. Third, the antibody to human GBA was prepared in BALB/C mice, which decreased the probability of any cross-reactivity between the human and mouse isozymes. In fact, no cross-reactivity between the mouse, rat, or Chinese hamster fibroblast β-glucosidase and the human enzyme was observed. Furthermore, the immunoprecipitation assay proved to be a sensitive and reliable method to detect human GBA activity in the presence of the mouse isozyme.

In addition to assigning the structural gene for human GBA to chromosome 1, the gene locus has been further localized using a hybrid line with a human chromosome 1 (pter→p11)/mouse chromosome translocation. Enzyme marker analyses substantiated the cytogenetic data, as $ENO_1$, FUCA, and $PGM_1$, which are all within the translocated region 1pter→p11, were present in this hybrid line, while PEPC and FH (localized at 1q25→1q42 and at q42→qter, respectively) were absent. No GBA activity was detected in this hybrid, indicating that the translocated segment of chromosome 1 did not have the locus for GBA. Thus, the region on human chromosome 1 to which the structural locus for GBA has been localized, 1p11→qter, is illustrated in figure 5.

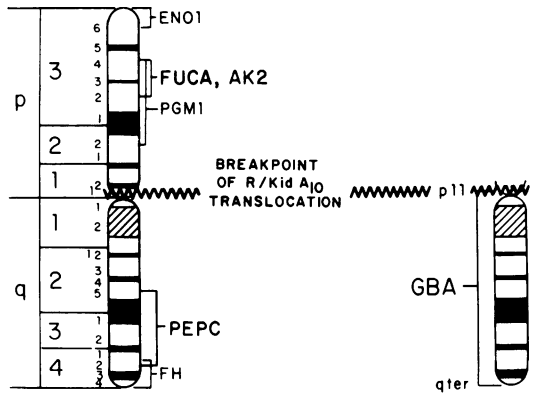


FIG. 5.—Regional assignment of human GBA to chromosome 1. Human chromosome 1 and its enzymatic markers are shown diagrammatically *on the left*. Location of the breakpoint of the translocation carried in the hybrid line R/Kid$A_{10}$ is designated by *broken line*. The structural gene locus for GBA has been mapped to the region 1p11→qter and is depicted diagrammatically *on the right*.

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