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Genetics

Fabry disease: Isolation of a cDNA clone encoding human α-galactosidase A

(lysosomal hydrolases/glycolipids/λgt11 expression vector/synthetic oligonucleotides)

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ABSTRACT **Fabry disease is an X-linked inborn error of metabolism resulting from the deficient activity of the lysosomal hydrolase, α-galactosidase A (α-Gal A; α-D-galactoside galactohydrolase, EC 3.2.1.22). To investigate the structure, organization, and expression of α-Gal A, as well as the nature of mutations in Fabry disease, a clone encoding human α-Gal A was isolated from a λgt11 human liver cDNA expression library. To facilitate screening, an improved affinity purification procedure was used to obtain sufficient homogeneous enzyme for production of monospecific antibodies and for amino-terminal and peptide microsequencing. On the basis of an amino-terminal sequence of 24 residues, two sets of oligonucleotide mixtures were synthesized corresponding to adjacent, but not overlapping, amino acid sequences. In addition, an oligonucleotide mixture was synthesized based on a sequence derived from an α-Gal A internal tryptic peptide isolated by reversed-phase HPLC. Four positive clones were initially identified by antibody screening of 1.4×10^7 plaques. Of these, only one clone (designated λAG18) demonstrated both antibody binding specificity by competition studies using homogeneous enzyme and specific hybridization to synthetic oligonucleotide mixtures corresponding to amino-terminal and internal amino acid sequences. Nucleotide sequencing of the 5′ end of the 1250-base-pair *Eco*RI insert of clone λAG18 revealed an exact correspondence between the predicted and known amino-terminal amino acid sequence. The insert of clone λAG18 appears to contain the full-length coding region of the processed, enzymatically active α-Gal A, as well as sequences coding for five amino acids of the amino-terminal propeptide, which is posttranslationally cleaved during enzyme maturation.**

Fabry disease is an inborn error of glycosphingolipid metabolism that results from the defective activity of the lysosomal hydrolase α-galactosidase A (α-Gal A; α-D-galactoside galactohydrolase, EC 3.2.1.22) (1). The mature, active human enzyme is a homodimeric protein (subunit $M_r \approx 49{,}800$) (2, 3), which is encoded by a structural gene localized to a narrow region (q21–q22) on the X chromosome (4). Deficient α-Gal A activity results in the accumulation of its major glycosphingolipid substrate, globotriaosylceramide and related glycolipids with terminal α-galactosidic linkages (1, 5). Progressive substrate deposition, particularly in the plasma and vascular endothelium, leads to ischemia and infarction with early demise due to vascular disease of the heart, kidney, and/or brain (1).

Since the availability of a cDNA for α-Gal A would facilitate studies of the molecular basis of the disease, provide specific probes for heterozygote identification, and permit expression of large amounts of the enzyme for further structural characterization and therapeutic trials of enzyme replacement (6, 7), efforts were undertaken to isolate a cDNA encoding human α-Gal A. In this communication, we report the molecular cloning of a cDNA that apparently encodes the entire amino acid sequence of the mature enzyme, and we establish its authenticity by demonstrating correspondence between the nucleotide sequence and the amino-terminal amino acid sequence.

METHODS

Affinity Purification of Human α-Gal A. Homogeneous α-Gal A was purified from human lung by the method of Bishop and Desnick (2) with the following modifications. The post-Con A fraction was applied to the affinity support α-galactosylamine-Sepharose (α-GalNH_2-C_{12}-Sepharose) (2), and the bound enzyme was batch eluted with 0.4 M galactose. This step resulted in a 47-fold purification and eliminated unknown contaminants in the post-Con A fraction that inhibited activity. To completely eliminate the related lysosomal enzyme α-*N*-acetylgalactosaminidase (α-Gal B) (8, 9), the concentrated and desalted post-DEAE-cellulose α-Gal A fraction was applied to a 1.6 × 25 cm column of hydroxyapatite (Clarkson, Williamsport, PA) equilibrated in 1 mM sodium phosphate buffer, pH 7.0. α-Gal B activity was separately eluted by an initial 200-ml gradient (from equilibration buffer to 20 mM sodium phosphate, pH 5.5) and then α-Gal A activity was eluted with a 200-ml gradient (from the pH 5.5 buffer to 200 mM sodium phosphate, pH 7.0). After the second affinity chromatography step, trace contaminants were removed from the highly purified enzyme by HPLC using a gel permeation column (TSK 3000SW; Millipore, Milford, MA). The mobile phase was 10 mM Tris·HCl, pH 7.3, containing 0.2 M NaCl, and the flow rate was 1.0 ml/min. The fraction containing α-Gal A activity was dialyzed and concentrated by using a Micro-ProDiCon unit (Bio-Molecular Dynamics, Beaverton, OR).

Amino Acid Composition and Sequence Analyses. Two separate preparations of α-Gal A were analyzed by extrapolation of each amino acid concentration to its zero-time value from 24-, 48-, and 72-hr hydrolyses in 6 M HCl at 110°C. Performic acid oxidation was used for analysis of cysteine as cysteic acid and for methionine as the sulfone (10). Amino acid concentrations were determined in a Durrum model D-500 analyzer. The tryptophan concentration was obtained from the ratio of its absorbance to that of tyrosine by the spectrophotometric method of Edelhoch (11). Homogeneous α-Gal A was digested with trypsin treated with tosylphenylalanine chloromethyl ketone (12) or cleaved with cyanogen bromide (13), and the peptides were isolated by reversed-

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Abbreviations: α-Galase, α-Galactosidase; bp, base pair(s).

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phase HPLC (14). The amino acid sequences of the amino-terminal, tryptic and cyanogen bromide peptides were determined by automated gas-phase microsequencing and HPLC identification of the phenylthiohydantoin derivatives of the amino acids (15).

Antibody Screening of the λgt11 cDNA Library. Rabbit anti-human α-Gal A antibodies were produced against homogeneous enzyme and titrated as described (2). *Escherichia coli* and λgt11 proteins were immobilized on Sepharose 4B (Pharmacia) and used to absorb the antiserum (16). Optimum antibody concentrations and binding were determined by dot blot analysis with 1-μl aliquots of homogeneous α-Gal A (diluted with 1 mg of human serum albumin per ml of 25 mM sodium phosphate, pH 6.0) applied to nitrocellulose filter strips (17). The human liver cDNA λgt11 library was generously provided by T. Chandra and S. L. C. Woo, Baylor College of Medicine. This library, which contains approximately 1.4×10^7 independent clones, was plated at a density of 1×10^5 phage per 150-mm Petri dish and screened as described (16–18). After 4 hr of growth at 42°C, the plaques were overlaid for 2 hr at 37°C with dry 137-mm nitrocellulose filters that had been soaked in 10 mM isopropyl β-D-thiogalactoside (16). For antibody screening, nonfat dry milk (2.5%) was substituted for 3% gelatin or 10% fetal calf serum in all blocking and antibody incubation steps (19). Plaques expressing α-Gal A determinants were detected after overnight incubation of each filter with 10 ml of a 1:500 dilution of preabsorbed anti-α-Gal A antibodies followed by a 2-hr incubation with 10 ml of a 1:1000 dilution of peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad) as described (17).

Construction of Oligonucleotide Probes. Mixed oligonucleotide probes were synthesized on a Sam One Synthesizer (Biosearch, San Rafael, CA), using phosphotriester chemistry. Two sets of oligonucleotide mixtures, each containing the possible coding sequences for adjacent amino-terminal amino acid regions, as well as a mixture corresponding to the amino acid sequence of an internal tryptic peptide, were constructed. The oligonucleotide mixtures were purified by gel electrophoresis on 20% polyacrylamide/8 M urea gels and subsequently labeled at the 5′ end with [γ-^{32}P]ATP (5000 Ci/mmol; Amersham; 1 Ci = 37 Gbq) by using T4 polynucleotide kinase (Bethesda Research Laboratories) (20, 21).

Characterization of Positive Clones. Antibody-positive clones were subjected to competition studies with α-Gal A-absorbed antiserum to demonstrate binding specificity. Polyclonal anti-α-Gal A antibodies were preincubated with an excess of α-Gal A [36,000 units/ml of antiserum] at 37°C for 60 min, and then at 4°C for 15 hr prior to incubation with filters containing plaques expressing α-Gal A determinants. In addition, to compare the inserts from antibody-selected clones, phage DNA was isolated (22), digested with *Eco*RI, and electrophoresed in 0.7% agarose gels.

To identify cDNA insert fragments that hybridized to synthetic oligonucleotide probes, DNA from antibody-positive clones was digested with *Eco*RI in combination with *Hae* III, *Hinf*I, *Msp* I, *Taq* I, *Alu* I, *Sau*3AI, or *Fnu*DII (International Biotechnologies or New England Biolabs) and electrophoresed in agarose gels. DNA was transferred to nylon membranes (Zetabind transfer media; AMF Cuno, Meriden, CT) by the method of Southern (23). The membranes were incubated in 6× SSPE/5× Denhardt's solution/0.5% $NaDodSO_4$ (1× SSPE = 0.15 M NaCl/10 mM NaH_2PO_4/1.0 mM EDTA, pH 7.4; and 1× Denhardt's solution = 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin), then hybridized in the same solution containing 0.23 ng of each oligonucleotide species in the mixture per ml ($1–4 \times 10^6$ cpm/ml). Incubation and hybridization were performed at 5°C below the melting temperature, t_m. The membranes were washed after hybridization at the t_m for the sequence in the mixture with the lowest t_m (59°C for probes 1A and 1B; 33°C for probes 2A and 2B; 46°C for probe mixture 3) with three changes of 6× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0).

The *Eco*RI insert from λAG18 was subcloned in pBR322 and designated pAG18 (21). The amino-terminal coding cDNA sequence was obtained initially from the pBR322 subclone by primer extension using synthetic oligonucleotide mixture 2B as described by McGraw (24) and was confirmed by the method of Maxam and Gilbert (20).

RESULTS

Purification and Amino Acid Composition of Human α-Galase A. Table 1 summarizes the purification scheme used to obtain sufficient homogeneous α-Gal A from human lung for amino acid composition and sequencing of amino-terminal, cyanogen bromide, and tryptic peptides. The affinity method (2) was modified to eliminate trace contaminants, including α-Gal B. The purified enzyme was homogeneous by $NaDodSO_4$ gel electrophoresis (Fig. 1 *Inset*). Amino acid composition analyses of two independent enzyme preparations were consistent with a subunit molecular weight of 41,800 (Table 2). After treatment of the enzyme with *N*-glycanase (Genzyme, Boston, MA), the molecular weight of the denatured, deglycosylated monomeric enzyme was estimated to be 41,400 (Fig. 2). From these results, it was estimated that the mature enzyme consists of ≈370 amino acid residues.

Amino Acid Sequencing and Oligonucleotide Synthesis. Microsequencing of the unblocked mature enzyme provided an amino-terminal sequence of 24 residues (Fig. 3). In addition, two cyanogen bromide and five tryptic peptides were sequenced, providing amino acid sequence data for a total of 101 residues, about 27% of the mature enzyme (data not shown). Oligonucleotide mixtures were constructed to include the possible codon combinations predicted from two adjacent, but nonoverlapping, regions of the amino-terminal amino acid sequence, as well as to a sequence from an

Table 1. Purification of α-Gal A from 8 kg of human lung

Step	Total activity, units × 10^6	Specific activity, units/mg	Purification fold	Yield, %
Crude extract	57.0	120	1	100
Con A-Sepharose	38.6	6,190	52	68
Batch α-GalNH_2-C_{12}-Sepharose	54.0	295,000	2,460	95
DEAE-cellulose	38.6	568,000	4,730	68
Hydroxyapatite	37.7	869,000	7,240	66
Gradient α-GalNH_2-C_{12}-Sepharose	21.4	4,480,000	37,300	38
Gel permeation HPLC	17.5	5,000,000	41,700	31

Units of activity are nmol of 4-methylbelliferyl α-D-galactoside hydrolyzed per hr at 37°C. Protein was determined by the fluorescamine procedure (25). The fluorescamine protein values are one-half the dry weight or Lowry values for homogeneous enzyme.

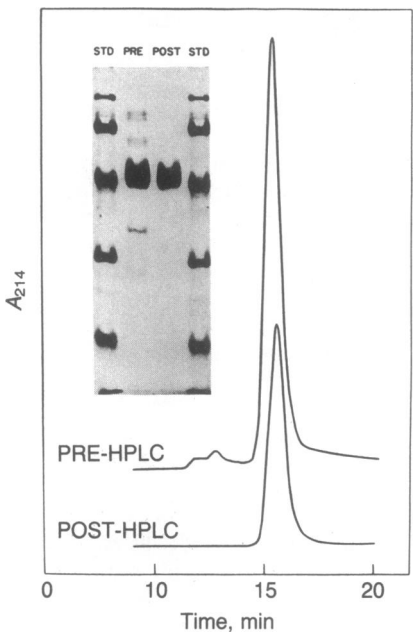

FIG. 1. HPLC purification of human lung α-Gal A. Upper tracing, elution profile of 2.8 μg of enzyme purified by the gradient affinity step. Lower tracing, elution profile of 1.3 μg of enzyme purified by HPLC. (*Inset*) 10% $NaDodSO_4$ gels (26) of the pre- and post-HPLC purified α-Gal A. Each enzyme sample contained approximately 40 μg of protein. The molecular weight standards were phosphorylase b, 94,000; bovine serum albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; and soybean trypsin inhibitor, 20,100.

internal tryptic peptide (Fig. 3). Oligonucleotides 1A and 1B were 23-mers (mixtures of 64 and 128 oligonucleotide species, respectively), corresponding to amino acid residues 11 through 18. Oligonucleotides 1A and 1B differed in that they were specific for leucine codons $UU\frac{A}{G}$ and CUN, respectively. Oligonucleotide mixtures 2A and 2B were each composed of four different 14-mers and corresponded to amino acid residues 19 through 23. The complexity of these mixtures was reduced by selecting G for the first nucleotide of the codon for leucine, based on the frequency (94%) of its human codon

Table 2. Amino acid composition of human α-Gal A

Amino acid residue	Moles residue per mole subunit		
	Prep. 1	Prep. 2	Average integral no.
Asx	41.2	45.7	44
Thr	16.1	13.4	15
Ser	24.2	21.7	23
Glx	38.9	35.8	37
Pro	19.1	18.4	19
Gly	28.9	29.8	29
Ala	25.8	27.2	27
Val	19.2	13.7	16
Met	7.4	8.7	8
Ile	15.3	18.8	21
Leu	36.3	37.7	37
Tyr	15.9	14.3	15
Phe	14.9	14.3	15
His	7.0	9.0	8
Lys	16.1	15.1	16
Arg	18.7	15.5	17
Cys	9.1	9.6	9
Trp	14.9	13.4	14

*Based on a subunit molecular weight of 41,800. These results provide an estimate of 370 amino acid residues per α-Gal A subunit.

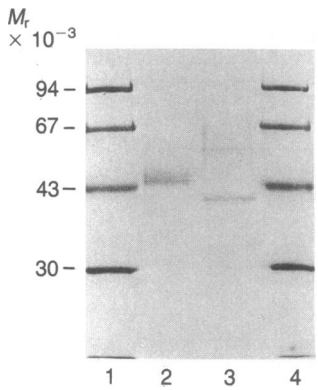

FIG. 2. *N*-Glycanase digestion of human lung α-Gal A. Lanes 1 and 4, 1.0 μg each of the same standards as in Fig. 1. Lane 2, 0.40 μg of purified α-Gal A. Lane 3, 0.34 μg of deglycosylated α-Gal A. The enzyme (0.8 μg) was boiled for 3 min in 0.5% $NaDodSO_4$ and 0.1 M 2-mercaptoethanol and then digested overnight at 30°C with 10 units of *N*-glycanase per ml in 50 mM sodium phosphate, pH 8.6/5 mM EDTA/6% (vol/vol) Nonidet P-40 in 35 μl. $NaDodSO_4$ gel electrophoresis was as described for Fig. 1. The deglycosylated α-Gal A migrated with an apparent M_r of ≈41,800; the other bands in lane 3 were from the *N*-glycanase.

usage (27). Oligonucleotide mixture 3 was composed of 96 different 17-mers corresponding to an internal tryptic peptide sequence (Fig. 3).

Screening Human Liver cDNA Expression Library. Monospecific rabbit anti-human α-Gal A antibodies recognized only α-Gal A in immunoprecipitations of [^{35}S]methionine-labeled fibroblast proteins (data not shown). No cross-reactivity was found with the related enzyme, α-Gal B, at antibody concentrations up to 4000 times the concentration required to precipitate α-Gal A. With peroxidase-conjugated goat anti-rabbit IgG, 0.2 ng of enzyme protein was reliably detected on nitrocellulose dot blots.

A human liver cDNA λgt11 expression library containing 1.4×10^7 independent clones was screened for α-Gal A by using the antibody detection method of Young and Davis (16, 18) as modified by deWet *et al.* (17). Four antibody-positive clones (λAG2, -14, -15, and -18) were isolated (Fig. 4*A*) and plaque-purified. Each clone was plated in duplicate on filters and separately tested with antibody and antibody absorbed

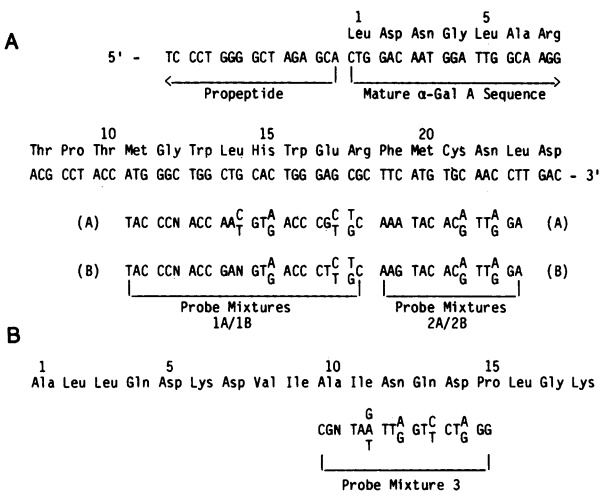

FIG. 3. Amino-terminal peptide (*A*) and internal tryptic peptide (*B*) sequences of human α-Gal A used to construct the synthetic oligonucleotide probe mixtures. The DNA sequence for the amino-terminal region of clone pAG18 (*A*) was colinear with the amino acid sequence.

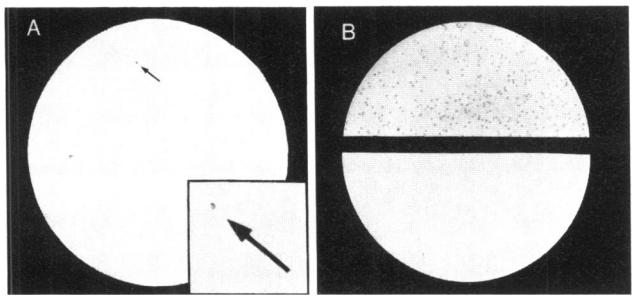

FIG. 4. Isolation of λgt11 clones containing human α-Gal A cDNA sequences. Clones were plated on *E. coli* Y1090 cells, overlaid with isopropyl thiogalactoside-impregnated nitrocellulose filters, probed with rabbit anti-human α-Gal A antibody, and detected with peroxidase-conjugated goat anti-rabbit IgG antibody. (*A*) Antibody-positive signal in a background of 100,000 negative clones, magnified 3× in *Inset*. (*B*) The upper semicircle shows plaque-purified positive clone λAG18 spread at a density of 1000 plaques per plate and incubated with anti-α-Gal A. The lower semicircle shows clone λAG18 plated at the same density and incubated with anti-α-Gal A after absorption with an excess of α-Gal A.

with an excess of homogeneous α-Gal A. Of the four clones, one (λAG15) did not show antibody binding specificity in competition with purified enzyme. Fig. 4*B* shows positive signals for purified clone λAG18 and no detectable signals for the same clone tested with enzyme-absorbed antibody.

Oligonucleotide Hybridization of Antibody-Selected cDNA Inserts. The insert size of each clone was analyzed by *Eco*RI digestion and agarose gel electrophoresis. Fig. 5*C* shows that clone λAG18 had a single 1250-base-pair (bp) insert, while clone λAG15 contained several *Eco*RI insert fragments. The 1250-bp insert of λAG18 was sufficiently long to include the 1110 bp predicted for the coding region of the 370 amino acid subunit of the mature enzyme. Southern blot analysis of this gel showed strong hybridization with both oligonucleotide mixtures 1A plus 1B and 2A plus 2B to the 1250-bp λAG18 *Eco*RI restriction fragment (Fig. 5 *A* and *B*) but not to fragments from λAG15 (Fig. 5 *A* and *B*, lane 3) or to inserts from λAG2 or λAG14 (data not shown). Digestion of λAG18 with *Alu* I revealed a 470-bp fragment that hybridized to oligonucleotide mixture 2B but not to mixture 2A (Fig. 5 *C* and *D*), demonstrating the specificity of the melting temperature used to discriminate between a perfect match and a one-base mismatch with these two probe mixtures. Double digestion with *Eco*RI and *Alu* I resulted in a 142-bp fragment that hybridized to combined oligonucleotide mixtures 1A and 1B and to mixture 2B (data not shown). In addition, the *Eco*RI insert of clone λAG18 hybridized specifically (data not shown) with oligonucleotide mixture 3, which was derived from an α-Gal A internal tryptic peptide.

Colinearity of the λAG18 Nucleotide Sequence and α-Gal A Amino-Terminal Amino Acid Sequence. The λAG18 *Eco*RI insert was subcloned in pBR322 to facilitate nucleotide sequencing by both the chemical and enzymatic methods (20, 24, 28). The DNA sequence included a 5′ coding region that predicted an amino acid sequence with perfect correspondence to the known amino-terminal sequence (Fig. 3). In addition, this sequence included 17 nucleotides of the propeptide.

DISCUSSION

A cDNA clone encoding the lysosomal hydrolase α-Gal A was isolated from a human liver cDNA library constructed in the λgt11 expression vector. This clone was identified as one of approximately 1.4×10^7 screened by using monospecific polyclonal antibodies and synthetic oligonucleotide probes. A modification of our purification procedure for α-Gal A permitted the preparation of highly specific antiserum and

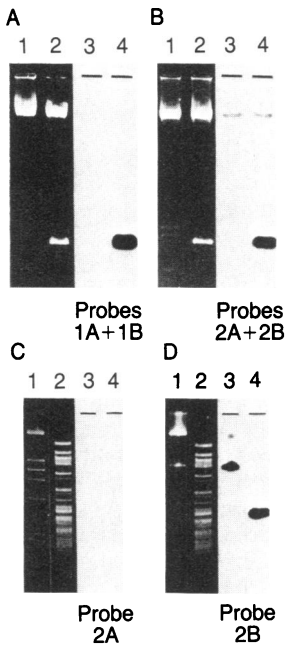

FIG. 5. Hybridization of ^{32}P-labeled oligonucleotide probe mixtures with Southern blots of λAG18 restriction digests. (*A*) Lanes 1 and 2, agarose gel (0.7%) of *Eco*RI-digested λAG15 and λAG18, stained with ethidium bromide. Lanes 3 and 4, Southern blots of the digested DNA in lanes 1 and 2 probed with combined oligonucleotide mixtures 1A and 1B. (*B*) The same as in *A*, only hybridized with probes 2A and 2B. (*C*) Agarose gel (1.7%) of digested λAG18 cDNA. Lane 1, *Hae* III-digested phage φX174 DNA standards (New England Biolabs) plus *Eco*RI-digested λAG18. Lane 2, *Alu* I-digested λAG18. Lanes 3 and 4, Southern blot of lanes 1 and 2 incubated with oligonucleotide mixture 2A. (*D*) Lane 1, *Eco*RI-digested λAG18. Lane 2, as in *C* lane 2. Lanes 3 and 4, Southern blot of lanes 1 and 2 incubated with oligonucleotide mixture 2B.

made it possible to obtain accurate amino acid sequence information for the amino-terminal region, as well as for internal cyanogen bromide and tryptic peptides. The clone containing α-Gal A cDNA was initially detected as an antibody-positive clone. Its identity was confirmed by demonstrating (*i*) the specificity of antibody binding by competition with highly purified α-Gal A, (*ii*) selective and specific hybridization of synthetic oligonucleotide probes corresponding to both amino-terminal and internal amino acid sequences, and (*iii*) colinearity between predicted and known amino-terminal amino acid sequence.

After the initial screening and selection of antibody-positive clones, efforts were directed to establishing their authenticity. Competition experiments with antiserum that was absorbed with an excess of homogeneous α-Gal A demonstrated the α-Gal A specificity of the fusion protein expressed by three of the four positive clones, and they eliminated from further analyses the clone reactive with nonspecific antibodies in the rabbit antiserum. Nonetheless, investigators experienced in screening expression vector libraries have detected, purified, and even sequenced nonauthentic clones, which cross-reacted with highly purified monospecific antibodies (29). Therefore, our strategy was designed to further characterize the antibody-positive clones with oligonucleotide mixtures corresponding to amino-terminal and internal peptide amino acid sequences. The availability of sufficient affinity-purified α-Gal A facilitated accurate amino acid sequencing; a critical residue in our sequence differed from that reported (30). On Southern blot analysis, the *Eco*RI insert from clone λAG18 strongly hybridized to three different synthetic oligonucleotide mixtures. Two of the oligonucleotide probes corresponded to

adjacent amino-terminal amino acid sequences, which were separated by only one nucleotide. Hybridization of both amino-terminal probe mixtures to the same 142-bp *Alu* I fragment, and the fact that a probe corresponding to an internal peptide sequence hybridized to the *Eco*RI insert, further established the identity of this clone. The subsequent demonstration of colinearity between the predicted and known amino-terminal amino acid sequence confirmed this clone's authenticity.

The 1250-bp insert of λAG18 could encode a sequence of 416 amino acids. Molecular weight estimates of mature α-Gal A, before and after *N*-glycanase treatment, indicated that the mature glycoprotein contained ≈370 amino residues and ≈15% carbohydrate. Thus, clone λAG18 is of sufficient length to include the entire coding sequence of the mature enzyme (i.e., 1110 bp).

With the notable exception of rodent β-glucuronidase (31, 32), lysosomal hydrolases are present at relatively invariant levels in all tissues studied, and no methods are known that lead to dramatic increases or decreases in their synthesis. Thus, cloning strategies for preparing libraries or cDNA probes from selected tissues or from tissues treated and untreated with hormones or other selective enrichment regimens are not available for the cloning of human lysosomal genes. Also, lysosomal hydrolases are present at very low levels in terms of percent of total cellular protein. For example, α-Gal A constitutes approximately 0.002% of total cellular protein in human lung, the source used for enzyme purification. Furthermore, the fact that lysosomal hydrolases turn over slowly in tissues (33, 34) leads one to predict that the corresponding mRNAs will be present at disproportionately lower concentrations in poly(A)$^+$ mRNA preparations used to make cDNA libraries. Indeed, the cDNA clone corresponding to another lysosomal hydrolase, α-fucosidase, is among the rarest cloned to date, with an mRNA abundance of 0.002% and a protein concentration of 0.01% of liver protein (27). In spite of these difficulties, a variety of recombinant DNA techniques have made it possible to obtain cDNA clones for human α-Gal A and other human lysosomal hydrolases (35–38).

The molecular cloning of human α-Gal A cDNA will be useful for obtaining cDNA clones that include the entire prepropeptide coding region as well as possible 5′ and 3′ untranslated regions, for characterization of genomic organization and for elucidation of the molecular defect(s) in unrelated patients with Fabry disease. In addition, this clone, which apparently contains the complete coding sequence for the processed, enzymatically active enzyme, and other cDNA clones coding for the complete prepro sequence, may be useful for the production of human α-Gal A. Previous clinical trials indicated that enzyme replacement therapy may be a feasible approach for the treatment of patients with Fabry disease (6, 7). Microbial and mammalian systems are available for the synthesis of the human enzyme (e.g., 39, 40), thus overcoming the primary obstacle to this mode of therapy.

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