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Two human glutamate decarboxylases, 65-kDa GAD and 67-kDa GAD, are each encoded by a single gene

(γ-aminobutyric acid/insulin-dependent diabetes mellitus/chromosome 2q/chromosome 10p)

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ABSTRACT **We report the isolation and sequencing of cDNAs encoding two human glutamate decarboxylases (GADs; L-glutamate 1-carboxy-lyase, EC 4.1.1.15), GAD_{65} and GAD_{67}. Human GAD_{65} cDNA encodes a M_r 65,000 polypeptide, with 585 amino acid residues, whereas human GAD_{67} encodes a M_r 67,000 polypeptide, with 594 amino acid residues. Both cDNAs direct the synthesis of enzymatically active GADs in bacterial expression systems. Each cDNA hybridizes to a single species of brain mRNA and to a specific set of restriction fragments in human genomic DNA. *In situ* hybridization of fluorescently labeled GAD probes to human chromosomes localizes the human GAD_{65} gene to chromosome 10p11.23 and the human GAD_{67} gene to chromosome 2q31. We conclude that GAD_{65} and GAD_{67} each derive from a single separate gene. The cDNAs we describe should allow the bacterial production of test antigens for the diagnosis and prediction of insulin-dependent diabetes mellitus.**

γ-Aminobutyric acid (GABA) is the major known inhibitory neurotransmitter in the vertebrate brain and serves signaling and trophic functions in several nonneural tissues (for reviews, see refs. 1 and 2). In the pancreatic islets, for example, GABA produced in β cells contributes to the regulation of glucagon secretion by α cells (3).

The rate-limiting step in the synthesis of GABA is the decarboxylation of glutamate, a reaction catalyzed by glutamate decarboxylase (GAD; L-glutamate 1-carboxy-lyase, EC 4.1.1.15). Earliest information concerning the primary structure of GAD came from the sequence of a feline GAD cDNA (GAD_{67}; refs. 4 and 5). The deduced molecular weight of the GAD encoded by that cDNA is ≈67,000 (6–8). Recently, however, we reported the isolation of a rat cDNA that encodes a distinct GAD polypeptide (GAD_{65}) with a M_r of 65,000 (9, 10). In addition to having distinct sequences and molecular sizes, the two GADs differ in their intracellular distributions, their interactions with pyridoxal phosphate, and their pattern of expression in central neurons (refs. 9–12; S. Feldblum, and A.J.T., unpublished work).

An unexpected area of important GAD-related research has recently arisen—the discovery that GAD is the target of autoantibodies present in people who later develop insulin-dependent diabetes mellitus (IDDM, also called juvenile or type 1 diabetes; ref. 13). Using rat cDNAs in a bacterial expression system to produce GAD_{65} and GAD_{67}, we have detected autoantibodies to both forms of GAD years before the onset of clinical diabetes (14). The human GAD cDNAs reported here‡‡ should, therefore, make it possible to develop a sensitive predictive test for IDDM, by using bacterially produced human GADs as antigens.

Our sequence data also suggest that autoimmunity to GAD may also play a role in the pathogenesis of IDDM because a 24-amino acid residue segment of human GAD_{65} shares 10 identities and nine similarities with the P2-C protein of Coxsackie virus, an agent often suggested as an environmental triggering agent of IDDM (11, 15–17). Autoimmunity in IDDM may thus arise by "molecular mimicry" between GAD and a viral polypeptide (18). If GAD is, indeed, involved in pathogenesis, as suggested by the early appearance of autoantibodies, possibly the sequence information reported here could be used to devise immunosuppressive strategies to block the function of specific major histocompatibility and T-cell receptor molecules and, thus, prevent or delay IDDM onset.

MATERIALS AND METHODS

Preparation of cDNA Probes. The probe for the identification of human GAD_{67} cDNA clones was feline GAD_{67} cDNA, which is 2266 base pairs (bp) long (4, 5). The probe for the isolation of human GAD_{65} cDNA was a 180-bp fragment from the coding region of rat GAD_{65} cDNA (10). Probes were labeled by random priming (19).

cDNA Cloning and Sequencing. Several overlapping human GAD_{67} cDNA clones were isolated from an oligo(dT)-primed, 22-week human fetal brain λgt11 library. The GAD_{67} cDNA clone with the longest insert (2644 bp) was designated hGAD 2.7. Another GAD_{67} cDNA clone, hGAD 0.4, contained an insert of 350 bp and a 3′ stretch of adenine residues. A cDNA that spanned the gap of 605 bp between these two cDNAs (in the 3′ noncoding region) was derived by using the PCR with primers located near the 3′ end of hGAD 2.7 insert and the 5′ end of hGAD 0.4 insert.

Our initial human GAD_{65} cDNAs, isolated from a human hippocampus cDNA library in λ-ZapII (Stratagene), lacked the 5′ end of the mRNA sequence. Anchored PCR (20), with a template copied with Moloney murine leukemia virus reverse transcriptase (BRL) from the poly(A)$^+$ RNA of adult human cerebral cortex, yielded a 151-bp product.

For sequencing, human GAD cDNAs and their exonuclease III deletions were subcloned into Bluescript SK plasmid (Stratagene) and transformed into *Escherichia coli* strain DH5α. Both DNA strands were sequenced by the dideoxy-

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Abbreviations: GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; IDDM, insulin-dependent diabetes mellitus; nt, nucleotide(s).

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‡‡The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M81882 and M81883).

nucleotide chain-termination method of Sanger *et al.* (21) by using a Pharmacia T_7 sequencing kit.

Bacterial Expression of Human GADs. The coding regions of human GAD_{67} and GAD_{65} cDNA were subcloned in frame into *Bam*HI sites of pET-5C (22) and pGEX-3X (Pharmacia) vector. The recombinant pET-5C plasmids were transformed into *E. coli* BL21(DE3), and the recombinant pGEX-3X plasmids were transformed into *E. coli* JM101. Extracts of transformed, isopropyl β-D-thiogalactoside-induced bacteria were assayed for GAD activity (23) and for protein with a Bio-Rad protein assay. The bacterial lysates were examined by immunoblotting with monospecific anti-GAD antibodies, as described (10, 11, 24). Fusion proteins produced from pGEX-3X recombinants were purified by glutathione-Sepharose 4B (Pharmacia) (25).

Northern (RNA) Blotting. Total RNA was extracted from human fetal brain (22 weeks) and from adult cerebellum (26). Poly(A)$^+$ RNA was fractionated by electrophoresis in formaldehyde/agarose, transferred to Biotrans nylon membranes (ICN), and hybridized to labeled human GAD_{67} or GAD_{65} cDNA, as described (10).

Preparation and Southern Blotting of Human Genomic DNA. Human genomic DNAs were isolated from peripheral blood lymphocytes (27), digested with *Bgl* II, *Eco*RI, *Hin*dIII, *Sst* I, and *Xba* I, and analyzed by Southern blot hybridization to full coding length human GAD_{67} or GAD_{65} cDNAs. For hybridization at high stringency, blots were hybridized at 42°C in 5× standard saline citrate (SSC)/50% formamide and then washed in 0.1× SSC. Hybridization at low stringency was done with 35% instead of 50% formamide, followed by washes in 2× SSC.

Genomic GAD DNA Cloning. We used standard plaque and colony hybridization methods to isolate cloned genomic DNA from bacteriophage and cosmid libraries (28). Human GAD_{67} genomic DNA clones were isolated from both cosmid (pcos2EMBL) and λ phage (Charon 4A) libraries obtained from H. Lehrach (Imperial Cancer Research Fund, London) and T. Maniatis (Harvard University), respectively. We obtained three overlapping cosmid clones and five λ phage clones. Screening a human genomic DNA pWE15 cosmid library (29) yielded six overlapping GAD_{65} cosmid clones.

Chromosomal Localization. Heparinized whole blood from a karyotypically normal male subject (46XY) was cultured at 37°C for 72 hr in medium/0.2% phytohemagglutinin. Chromosomes preparations were made by using standard prometaphase techniques (30). Fluorescence *in situ* hybridization was done as described (31), with biotinylated GAD_{65} cosmid and GAD_{67} bacteriophage probes.

RESULTS

Human GAD_{67} and GAD_{65} cDNAs Resemble the Corresponding Rat GAD cDNAs. Human GAD_{67} cDNA contains an open reading frame of 1782 nucleotides (nt) (594 codons), beginning with the first ATG start codon at nt 551–553 and ending with the TAA stop codon at nt 2333–2335. The open reading frame (Fig. 1) encodes a polypeptide of 594 amino acid residues, with a calculated M_r of 67,000. Within the coding region, the nucleotide sequence of human GAD_{67} cDNA is 93% identical to the corresponding sequence in feline cDNA and 91% identical to that of rat cDNA. At the amino acid level, human and rat GAD_{67}s are 97% identical, whereas human and feline GAD_{67}s are 98% identical (Table 1).

Our human GAD_{67} cDNA extends 1267 nt into the 3′ noncoding region and contains a poly(A) tail. A polyadenylation consensus sequence (AATAAA) lies 20–25 bases 5′ to the poly(A) tract. In the noncoding regions present in both rat and human GAD_{67} cDNAs, we find 74% identity in the 3′ untranslated region (8).

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hGAD67 MASSTPSSSATSSNAGADPNTTNLRPTTYDTWCGVAHGCTRKLGLKICGFLQRTNSLEEK  60
rGAD67         P

hGAD67 SRLVSAFRERQSSKNLLSCENSDRDARFRRTETDFSNLFARDLLPAKNGEEQTVQFLLEV 120
rGAD67           A          PG            Q

hGAD67 VDILLNYVRKTFDRSTKVLDFHHPHQLLEGMEGFNLELSDHPESLEQILVDCRDTLKYGV 180
rGAD67

hGAD67 RTGHPRFFNQLSTGLDIIGLAGEWLTSTANTNMFTYEIAPVFVLMEQITLKKMREIVGWS 240
rGAD67                                                        I

hGAD67 SKDGDGIFSPGGAISNMYSIMAARYKYFPEVKTKGMAAVPKLVLFTSEQSHYSIKKAGAA 300
rGAD67 N                                                    H

hGAD67 LGFGTDNVILIKCNERGKIIPADFEAKILEAKQKGYVPFYVNATAGTTVYGAFDPIQEIA 360
rGAD67                       L     D     F  L

hGAD67 DICEKYNLWLHVDAAWGGGLLMSRKHRHKLNGIERANSVTWNPHKMMGVLLQCSAILVKE 420
rGAD67                              S

hGAD67 KGILQGCNQMCAGYLFQPDKQYDVSYDTGDKAIQCGRHVDIFKFWLMWKAKGTVGFENQI 480
rGAD67

hGAD67 NKCLELAEYLYAKIKNREEFEMVFNGEPEHTNVCFWYIPQSLRGVPDSPQRREKLHKVAP 540
rGAD67                                                  E      R

hGAD67 KIKALMMESGTTMVGYQPQGDKANFFRMVISNPAATQSDIDFLIEEIERLGQDL 594
rGAD67
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FIG. 1. Deduced amino acid sequence of human GAD_{67}. The putative pyridoxal phosphate-binding site is underlined. Amino acid residues of rat GAD_{67} that differ from those of human GAD_{67} are shown below the human sequence; a dot indicates that there is no corresponding amino acid residue.

Human GAD_{65} cDNA encodes a polypeptide of 585 amino acid residues that has a calculated M_r of 65,000 (Fig. 2). In the coding region, human GAD_{65} is 89% identical to rat GAD_{65} cDNA at the nucleotide level, and the deduced amino acid sequences of the two proteins are 96% identical (Table 1). For both GAD_{65} and GAD_{67} cDNAs, anchored PCR and subsequent sequencing of the cloned PCR products revealed no other ATG triplets 5′ to the methionine codons indicated in Figs. 1 and 2.

Comparing the deduced amino acid sequences of human GAD_{67} and GAD_{65}, we found that the two are 65% identical (Table 1). Counting the conservative changes in amino acid residues, the overall similarity of the two proteins is 81%. The greatest density of different amino acid residues in the two proteins is in the 100 amino acid residues nearest to the amino terminal, as is also the case for rat GAD_{67} and GAD_{65} (10). Both GAD_{65} and GAD_{67} contain the tetrapeptide sequence Asn-Pro-His-Lys, which has been identified as the pyridoxal phosphate-binding site in porcine dihydroxyphenylalanine (DOPA) decarboxylase (32).

Human GAD_{65} and GAD_{67} cDNAs Direct the Bacterial Synthesis of Enzymatically Active GADs. To test the functional properties of the polypeptides encoded by human GAD_{65} and GAD_{67} cDNAs, we produced human GAD_{65} and human GAD_{67} polypeptides in the pET-5C bacterial expression system (22). Each of these bacterial products is recognized by the appropriate monospecific antibodies in immunoblots: our K-2 antibody recognizes bacterially produced GAD_{67} (with only slight reactivity to bacterially produced GAD_{65}), and the GAD-6 monoclonal antibody recognizes only bacterially expressed GAD_{65} (Fig. 3). The estimated molecular weights of these bacterially expressed GADs are

Table 1. Interspecies comparisons of GAD nucleotide and amino acid sequences

	Sequence identity (nucleotides/amino acids), %				
	Human GAD_{65}	Human GAD_{67}	Rat GAD_{65}	Rat GAD_{67}	Feline GAD_{67}
Human GAD_{65}		63/65	89/96	63/66	64/66
Human GAD_{67}			63/65	91/97	93/98
Rat GAD_{65}				63/65	65/66
Rat GAD_{67}					90/97

Nucleotide sequence comparisons are limited to coding regions of the GAD cDNAs. Rat and feline sequences were taken from refs. 5, 8, and 10. First number is % identity at nucleotide level; second number is % identity at amino acid level.

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hGAD65 MASPGSGFWSFGSEDGSGDSENPGTARAWCQVAQKFTGGIGNKLCALLYGDAEKPAESGG  60
rGAD65                  P                                    S

hGAD65 SQPPRAAARKAACACDQKPCSCSKVDVNYAFLHATDLLPACDGERPTLAFLQDVMNILLQ 120
rGAD65  VTS   T  V  T          P G     L          E

hGAD65 YVVKSFDRSTKVIDFHYPNELLQEYNWELADQPQNLEEILMHCQTTLKYAIKTGHPRYFN 180
rGAD65                                            T

hGAD65 QLSTGLDMVGLAADWLTSTANTNMFTYEIAPVFVLLEYVTLKKMREIIGWPGGSGDGIFS 240
rGAD65

hGAD65 PGGAISNMYAMMIARFKMFPEVKEKGMAALPRLIAFTSEHSHFSLKKGAAALGIGTDSVI 300
rGAD65            L   Y           V

hGAD65 LIKCDERGKMIPSDLERRILEAKQKGFVPFLVSATAGTTVYGAFDPLLAVADICKKYKIW 360
rGAD65                  V

hGAD65 MHVDAAWGGGLLMSRKHKWKLSGVERANSVTWNPHKMMGVPLQCSALLVREEGLMQNCNQ 420
rGAD65                  N                                    S

hGAD65 MHASYLFQQDKHYDLSYDTGDKALQCGRHVDVFKLWLMWRAKGTTGFEAHVDKCLELAEY 480
rGAD65                                                     I

hGAD65 LYNIIKNREGYEMVFDGKPQHTNVCFWYIPPSLRTLEDNEERMSRLSKVAPVIKARMMEY 540
rGAD65                              FV      V

hGAD65 GTTMVSYQPLGDKVNFFRMVISNPAATHQDIDFLIEEIERLGQDL 585
rGAD65
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FIG. 2. Deduced amino acid sequence of human GAD_{65}. See legend for Fig. 1.

≈68,000 and 66,000, reflecting the additional 14 amino-terminal amino acid residues in the pET-5C constructs. Bacterial lysates of bacteria that produce GAD_{65} and GAD_{67} have GAD activities that are 200 and 50 times as great as extracts of host bacteria that contain the nonrecombinant vector (Table 2).

E. coli transformed with pGEX-3X + GAD_{67} or pGEX-3X + GAD_{65} also produce enzymatically active fusion proteins (Table 2). Some of each fusion protein is soluble, so the fusion GADs can be purified to near homogeneity by affinity chromatography on glutathione-Sepharose.

GAD_{65} and GAD_{67} Derive from Two mRNAs, Which Derive from Two Different Genes. Northern blots with poly(A)$^+$ RNA extracted from fetal and adult human brain showed that the GAD_{67} cDNA probe hybridized to a 3.7-kilobase (kb) mRNA, whereas the GAD_{65} cDNA probe hybridized to a 5.7-kb mRNA (Fig. 4). Although the sizes, immunoreactivities, and enzymatic activity of bacterially produced GAD_{65} and GAD_{67} indicate that our cDNAs contain full coding sequences, sizes of the corresponding mRNAs suggest our GAD_{65} cDNA sequence lacks ≈3.3 kb of the noncoding region, whereas that of GAD_{67} cDNA lacks only ≈100 bp. We observed no cross hybridization of the GAD_{65} probe to a 3.7-kb RNA or of the GAD_{67} probe to a 5.7-kb RNA.

Human GAD_{65} cDNA and human GAD_{67} cDNA hybridize to a different specific set of restriction fragments from human genomic DNA. For example, nine *Eco*RI fragments (11.5, 5.6, 5.4, 4.5, 3.7, 3.5, 2.4, 1.5, and 1.3 kb) hybridize to a human GAD_{65} probe, whereas five *Eco*RI restriction fragments (5.7, 5.0, 3.7, 2.3, and 2.1 kb long) hybridize to a human GAD_{67} probe (Fig. 5).

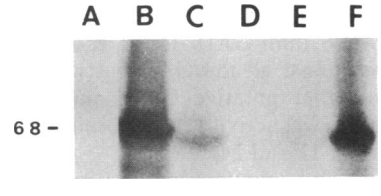

FIG. 3. Immunoblots of extracts from bacteria engineered to produce human GADs. Lanes A and D: extracts of bacteria transformed with pET-5C vector. Lanes B and E: pET-5C + GAD_{67}. Lanes C and F: pET-5C + GAD_{65}. Lanes A–C: K-2 polyclonal antibody, specific for GAD_{67}. Lanes D–F: GAD-6 monoclonal antibody, specific for GAD_{65}. In several such experiments with bacteria engineered to express human GAD cDNAs, bacterially produced human GAD_{67} had an estimated M_r of 68,000–69,000, reflecting the additional 11 codons of the gene *10* protein and the 3 linker codons. Bacterially produced human and rat GAD_{65} consistently had slightly greater electrophoretic mobilities, reflecting their smaller molecular size.

Table 2. GAD activity of bacterially expressed proteins

Sample	GAD activity, nmol of $^{14}CO_2$ per mg of protein/hr
Bacterial lysate from bacteria transformed with	
pET-5C vector	8
pET-5C + GAD_{65}	1626
pET-5C + GAD_{67}	450
pGEX-3X vector	17
pGEX-3X + GAD_{65}	203
pGEX-3X + GAD_{67}	74
Purified fusion protein	
GST-GAD_{65}	3244
GST-GAD_{67}	5071

GST, glutathione-*S*-transferase.

Under low-stringency hybridization and washing conditions, Southern blots show that cDNAs for human GAD_{65} and GAD_{67} hybridize to the same respective restriction fragments seen under high-stringency conditions. No additional bands hybridized to GAD_{65} or GAD_{67} cDNAs (Fig. 5).

Hybridization with GAD_{65} and GAD_{67} cDNAs identified bacteriophage and cosmid clones from human genomic libraries. Sizes of all the genomic DNA fragments that hybridize with each GAD cDNA are identical with those in the respective cosmids. Cosmids encoding human GAD_{65} and human GAD_{67} sequences do not cross-hybridize with GAD_{67} and GAD_{65} cDNAs, supporting the conclusion that the two GADs derive from separate genes.

The Genes Encoding GAD_{65} and GAD_{67} Lie on Different Chromosomes. Thirty metaphases with clearly hybridized probe signal and good chromosome morphology were analyzed for each clone. GAD_{65} cosmid DNA consistently hybridized to chromosome 10p11.23; GAD_{67} bacteriophage DNA hybridized to 2q31 (Fig. 6).

DISCUSSION

The cDNAs we describe here contain the full coding regions of human GAD_{65} and GAD_{67}: in genetically engineered

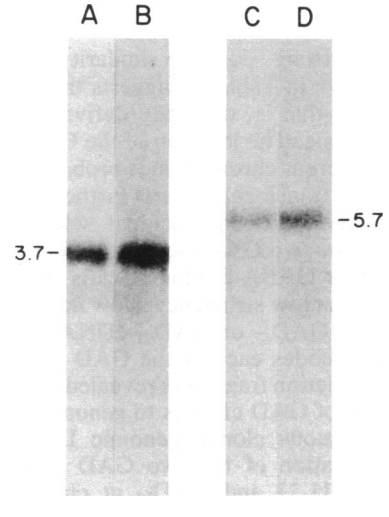

FIG. 4. Northern blots of brain RNA. Lanes A and C: 2 μg of poly(A)$^+$ RNA extracted from human fetal brain. Lanes B and D: 2 μg of poly(A)$^+$ RNA from human adult cerebellum. Lanes A and B: hybridization to human GAD_{67} cDNA. Lanes C and D: hybridization to GAD_{65} cDNA. Numerals are molecular sizes in kb, determined from marker lanes (not shown).

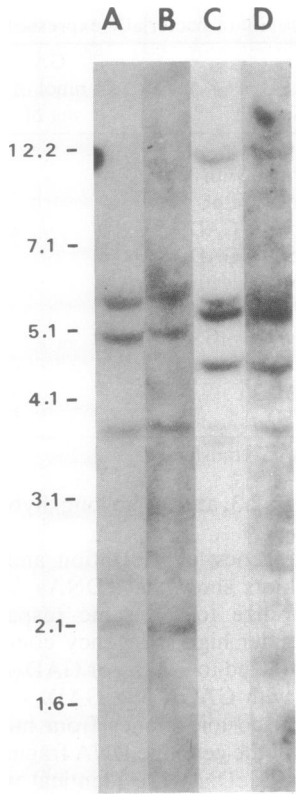

FIG. 5. Southern blots to human genomic DNA. Human genomic DNA was completely digested with *Eco*RI. Lanes A and B: hybridization to human GAD_{67} cDNA. Lanes C and D: hybridization to human GAD_{65} cDNA. Lanes A and C were under high-stringency hybridization and washing; lanes B and D were under low-stringency hybridization.

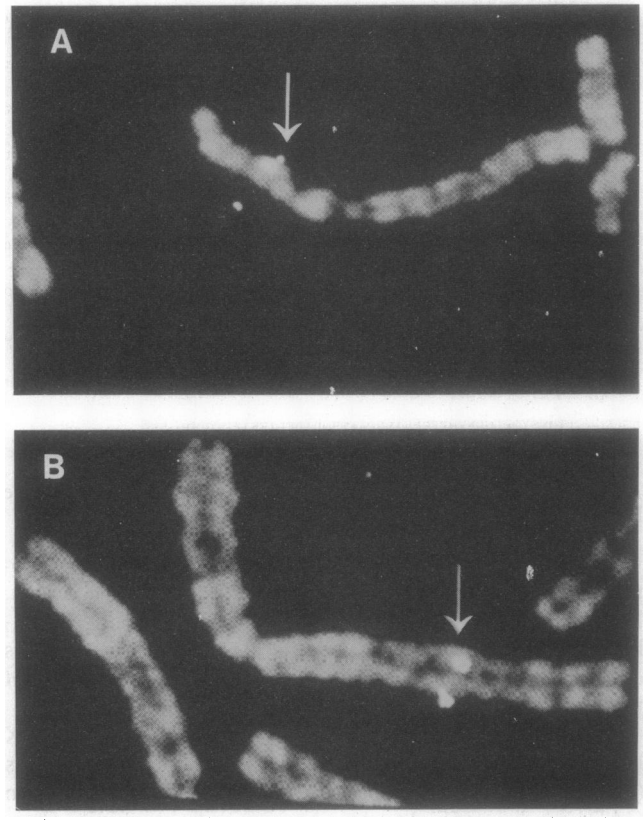

FIG. 6. Chromosomal localization of GAD_{65} and GAD_{67} (confocal microscope images). (*A*) *In situ* hybridization to a GAD_{65} cosmid. Arrow, GAD_{65} hybridization signal at p11.23 band of human chromosome 10. (*B*) *In situ* hybridization to a GAD_{67} bacteriophage DNA. Arrow, GAD_{67} hybridization signal at q31 band of human chromosome 2.

bacteria, each cDNA directs the synthesis of polypeptides with the immunoreactivity and enzymatic activity of the corresponding brain GAD.

The nucleotide sequences and the predicted amino acid sequences of human GAD_{65} and GAD_{67} are similar to those of rat and feline counterparts (Table 1). Even *Drosophila* GAD shows considerable sequence identity to the mammalian GADs (10, 33). These extensive sequence identities suggest that GAD structure has been subject to intense selective pressure during phylogeny.

Similarly, the strong sequence similarity between GAD_{65} and GAD_{67} (shown in Table 1) suggests that the two GADs are homologous—that is, that they derive from a common ancestral GAD gene. The location of the GAD_{65} and GAD_{67} genes on two different chromosomes probably resulted from gene duplication, translocation, and further sequence divergence, apparently under independent selective pressures, consistent with the two GADs serving different functions in neurons and other GABA-producing cells (1, 10, 11).

Southern blots at low stringency show no additional bands that hybridize to GAD_{67} or GAD_{65} cDNA, suggesting that only one gene encodes each of the GAD cDNAs. Furthermore, all the restriction fragments revealed in Southern blots by hybridization of GAD cDNAs to genomic DNA are also present in contiguous cloned genomic DNA. The actual chromosomal location of the two GAD genes, GAD_{65} at chromosome 10p11.23 and GAD_{67} at chromosome 2q31, provides additional markers in these regions. We have reported (34) an *Msp* I polymorphism in the GAD_{67} gene, but no polymorphisms have yet been found for the GAD_{65} gene. Of the inherited diseases for which the chromosomal location of the disease-causing gene is known, none maps to either of these two sites. So neither GAD-encoding gene can be considered a candidate for the site of the genetic lesion in any mapped genetic disorder.

The existence of separate genes for GAD_{65} and GAD_{67} explains much of the long discussed heterogeneity of brain GAD (1, 35, 36). It is possible, however, that other GAD genes may exist, but conclusive data have not yet been reported, although Huang *et al.* (37) have reported a candidate mouse GAD cDNA. This cDNA encodes an M_r 83,000 polypeptide; the deduced amino acid sequence is unrelated to GAD_{65} or GAD_{67}. The enzymatic activity of the fusion protein encoded by this cDNA, however, suggests the involvement of enzymes other than GAD: subtraction of the $^{14}CO_2$ produced by bacteria containing the nonrecombinant expression vector gives a ratio of $^{14}CO_2$ to ^{14}C-labeled GABA of less than the expected 1:4. Furthermore, the monospecific antibodies GAD-6 (anti-GAD_{65}) and K-2 (anti-GAD_{67}) together remove almost all the GAD activity from brain homogenates, so other putative GADs must not contribute much to overall GAD activity in the brain (D.L.K., unpublished work; refs. 11 and 24).

GAD activity and immunoreactivity are also present in nonneural tissues, including pancreatic islets, testis, and oviduct (2, 38, 39–42). Testis, for example, contains immunoreactive GAD_{67} as well as GAD_{67} mRNA (32, 40). But the GAD_{67} mRNAs in the testis have multiple sizes, all different from the 3.7-kb brain mRNA, and presumably derived from the single GAD_{67} gene by alternative splicing or alternative polyadenylylation (40, 42). The oviduct, like the testis, contains multiple GAD mRNAs, but these derive principally from the GAD_{65} gene (40, 42). Direct evidence for alternative splicing of a GAD_{67} transcript, however, is thus far available only in the developing rat brain, where alternative splicing

leads to an in-frame stop codon and a truncated GAD polypeptide (43).

The discovery that GAD is the earliest known autoantigen during the development of IDDM has renewed interest in molecular identity of pancreatic β cell GAD (13). For example, Cram *et al.* (41) have recently reported the sequences of cloned PCR products apparently derived from GAD_{67} RNAs of human brain and pancreatic islets. These two PCR products differ in 45/540 nt, of which 7 changed the encoded amino acid residue. Their brain GAD PCR product differed from our human GAD_{67} cDNA sequence at only one (silent) of the GAD_{67} nucleotide residue. Because our data demonstrate that there is but a single GAD_{67} gene, the two sequences of Cram must reflect either alternative use of closely related exons or PCR-derived sequence differences. Interestingly, the amino acid sequence of the GAD_{67} of Cram most closely resembles rat brain GAD_{67}, with differences at only three positions (6, 10, 41)

The human GAD cDNAs we report here should allow the production of antigens for the routine detection of GAD autoantibodies. The presence of GAD autoantibodies is the earliest known predictor of IDDM and is also diagnostic of stiff-man syndrome (13, 14, 39, 44–47). While bacterially produced GADs have so far proved immunologically identical to pancreatic and brain GADs, further work will be necessary to learn how islet cell GADs may be modified posttranslationally and how they become associated with the synapse-like vesicles within β cells (39, 48).

The GAD_{65} sequence reported here contains an intriguing sequence match between amino acid residues 250–273 and a segment of the P2-C polypeptide of Coxsackie virus, a long-suspected etiologic agent in IDDM (11, 14). These two 24-amino acid residue segments contain 10 identities and nine similarities (conservative changes), suggesting a role for molecular mimicry in the pathogenesis of IDDM, although no experimental data are yet available to support this hypothesis. The primary structures of the two GADs and the ability to program both prokaryotic and eukaryotic cells to produce them should, thus, not only lead to a reliable method for diagnosing and predicting IDDM but should also accelerate the search for pathogenic mechanisms.

Note. While this paper was under review, Karlsen *et al.* (49) reported the sequence and chromosomal location of GAD_{65} cDNA from a human islet cell library, and Michelsen *et al.* (50) reported the sequence of GAD_{67} cDNA from a rat islet cell library.

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