A Sequence-Ready BAC Contig of the $GABA_A$ Receptor Gene Cluster *Gabrg1–Gabra2–Gabrb1* on Mouse Chromosome 5

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The type-A receptors for the neurotransmitter GABA (γ-aminobutyric acid) are ligand-gated chloride channels that mediate postsynaptic inhibition. The functional diversity of these receptors comes from the use of a large repertoire of subunits encoded by separate genes, as well as from differences in subunit composition of individual receptors. In mammals, a majority of $GABA_A$ receptor subunit genes are located in gene clusters that may be important for their regulated expression and function. We have established a high-resolution physical map of the cluster of genes encoding $GABA_A$ receptor subunits α2 (*Gabra2*), β1 (*Gabrb1*), and γ_1 (*Gabrg1*) on mouse chromosome 5. Rat cDNA probes and specific sequence probes for all three $GABA_A$ receptor subunit genes have been used to initiate the construction of a sequence-ready contig of bacterial artificial chromosomes (BACs) encompassing this cluster. In the process of contig construction clones from 129/Sv and C57BL/6J BAC libraries were isolated. The assembled 1.3-Mb contig, consisting of 45 BACs, gives five- to sixfold coverage over the gene cluster and provides an average resolution of one marker every 32 kb. A number of BAC insert ends were sequenced, generating 30 new sequence tag sites (STS) in addition to 6 *Gabr* gene-based and 3 expressed sequence tag (EST)-based markers. STSs from, and surrounding, the *Gabrg1–Gabra2–Gabrb1* gene cluster were mapped in the T31 mouse radiation hybrid panel. The integration of the BAC contig with a map of loci ordered by radiation hybrid mapping suggested the most likely genomic orientation of this cluster on mouse chromosome 5: cen–*D5Mit151–Gabrg1–Gabra2–Gabrb1–D5Mit58*–tel. This established contig will serve as a template for genomic sequencing and for functional analysis of the $GABA_A$ gene cluster on mouse chromosome 5 and the corresponding region on human chromosome 4.

The sequence data described in this paper have been submitted to the GenBank/GSS data libraries under accession nos. AF156490 and AQ589406–AQ589436.

γ-Aminobutyric acid (GABA) is a potent inhibitory neurotransmitter in the central nervous system (CNS) that interacts with two different classes of GABA receptors: the ionotrophic $GABA_A$ receptor chloride channels (for review, see Rabow et al. 1995; Seeburg et al. 1990) and the recently cloned metabotropic G-protein-coupled $GABA_B$ receptors (Kaupmann et al. 1997, 1998).

$GABA_A$ receptors are multimeric membrane-spanning ligand-gated ion channels that admit chloride on binding of the neurotransmitter GABA (Bormann et al. 1987). Because GABA is the major inhibitory neurotransmitter of the CNS, modulation of receptor activity has profound implications for both brain function and therapy of various neuropsychiatric disorders. Drugs that alter the $GABA_A$ receptor channel activity, such as benzodiazepines, barbiturates, and steroids, have had important roles in the understanding and treatment of anxiety, sleep disorders, convulsive disorders, and epilepsy (for review, see Burt and Kamatchi 1991; Brooks-Kayal et al. 1998; Shiah and Yatham 1998).

Functional studies of the individual $GABA_A$ receptor genes have been hindered by a high structural diversity among the $GABA_A$ receptor subunits that assemble combinatorially to build different subtypes of $GABA_A$ receptors in various regions of the brain and the spinal cord. To date nineteen distinct subunit types (α1–α6, β1–β4, γ1–γ4, δ1, ϵ, ρ1–ρ3) have been identified and this isoform complexity is further complicated by the occurrence of alternative splicing and post-translational modifications (Wisden and Seeburg 1992). In the mammalian genome, many $GABA_A$ receptor subunit genes are organized as gene clusters on different chromosomes, with each of these clusters containing at least one gene of the α, β, and γ or ϵ class. In humans, five $GABA_A$ receptor subunit gene clusters have been described. The *GABRB2–GABRA1/GABRA6–GABRG2* cluster on human chromosome (HSA) 5q31.2–q35 (Kostrzewa et al. 1998) is homologous to the cluster on mouse chromosome (MMU) 11 (Garrett et al. 1997). Similarly, the *GABRB3–GABRA5–GABRG3* gene cluster, located close to the Prader-Willi/

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9:732–738 ©1999 by Cold Spring Harbor Laboratory Press ISSN 1054-9803/99 $5.00; www.genome.org

Angelman region on HSA 15q11–q13 (Glatt et al. 1997; Christian et al. 1998), corresponds to the *Gabrb3–Gabra5–Gabrg3* cluster located distal to the pink-eyed dilution (*p*) gene region on MMU 7 (Nakatsu et al. 1993; Culiat et al. 1994). An additional cluster, containing the *GABRA3*, *GABRB4*, and *GABRE2* subunit genes, has been mapped to human chromosome Xq28 (Levin et al. 1996; Wilke et al. 1997) and mouse chromosome X (Boyd et al. 1998). Two $GABA_A$ subunit genes, ρ1 and ρ2, expressed at a high level in the retina, have been shown to map to HSA 6q11–q14 and the corresponding region in the proximal portion of MMU 4 (Cutting et al. 1992).

In humans, the *GABRA2*, *GABRG1*, and *GABRB1* genes have been mapped to HSA 4p12–p13 (Buckle et al. 1989; Kirkness et al. 1991; Wilcox et al. 1992). Furthermore, somatic cell hybrid analysis has indicated that the *GABRA4* gene maps to the same cluster (McLean et al. 1995). The murine orthologs, *Gabra2* and *Gabrb1* subunit genes, have been localized to the central portion of MMU 5, whereas the *Gabra4* subunit gene has been assigned to proximal MMU 7 (Danciger et al. 1993). We have previously placed the murine *Gabrb1* locus on a long-range restriction map, 3 Mb proximal to the dominant spotting locus (*W*) encoded by the proto-oncogene c-*Kit* (Nagle et al. 1995). To gain insight into the genomic organization of the $GABA_A$ receptor gene cluster on mouse chromosome 5, we have constructed a sequence-ready bacterial artificial chromosome (BAC) contig of 1.3 Mb. This contig has been anchored to other chromosome 5 loci using radiation hybrid (RH) mapping, and the transcriptional orientation of two $GABA_A$ receptor subunit genes, *Gabra2* and *Gabrb1*, has been determined. This high-resolution physical map will provide the basis for functional characterization and sequencing of genes located in this cluster.

RESULTS

The contig spanning the $GABA_A$ receptor genes in the central portion of mouse chromosome 5 was generated in the following steps: (1) initial hybridization screen; (2) STS content mapping; (3) chromosome walk using selected STSs generated from BAC ends; and (4) fine mapping by fingerprinting and Southern blot analysis. To initiate the construction of a BAC contig, we used *Gabra2* and *Gabrg1* rat cDNA probes and a mouse *Gabrb1* cDNA clone to screen two 129/Sv BAC libraries. We isolated 27 BAC clones, and 23 were confirmed to correspond to $GABA_A$ receptor genes by dot-blot colony assays and by Southern blot analysis. The BAC-insert sizes were determined by pulsed field gel electrophoresis (PFGE) following a *Not*I digestion of BAC DNA.

Table 1. Primers and PCR Conditions for STSs and Genes in the Contig

BAC end / gene probe	Locus	Accession No.	Forward primer	Reverse primer	T_{Ann} (°C)	size (bp)
241N21.SP6	*D5Buc6*	AQ589407	5'-AAT CAT TGT CCC GAA ATC CC-3'	5'-GAT GAT ATG AGC AGC ATG GC-3'	55	214
337O19.T7	*D5Buc7*	AQ589414	5'-CAT CAG GCC TCA CAT GAG TAA TCC-3'	5'-GTG ATT GCT GTT TTA TTC AAT AGG-3'	58	207
344L2.T7	*D5Buc8*	AQ589416	5'-TAC TTT GGG AGG TGA TTG CC-3'	5'-ATG AAA TGG TGA GGC TCC AG-3'	55	194
3'-*Gabrg1*	*Gabrg1*	AF156490	5'-TCC CTA ACA CCT TTA ACA ATG AGC-3'	5'-ATA CTG TGA GAA TTA TAG TTG TCC-3'	58	325
5'-*Gabrg1*	*Gabrg1*	AF156490	5'-GGC TTC CCC AGG TCT CCA TGC TGG-3'	5'-TAT CCG CCC TTC CCT CCA GGA CCC-3'	59	145
350I23.SP6	*D5Buc9*	AQ589417	5'-TTT GAA GTT TGG CAG AGA AAG-3'	5'-CAG CCA TTG CAT TTG ATG TC-3'	58	204
241N21.T7	*D5Buc10*	AQ589408	5'-TGC TCT TTA TTG GCA TCA CC-3'	5'-TCT TTT TGT CCA AAG AAT TAT GC-3'	55	154
337O19.SP6	*D5Buc11*	AQ589413	5'-TCC TCA GTT GTT TGG CAT TAT GCC-3'	5'-TTT ACT CAC TCT TTT AGT AAA GGC-3'	58	212
437P3.SP6	*D5Buc12*	AQ589435	5'-AAG CTT TGG CCT GTT CCT ACT AGC-3'	5'-GCA GGA TTA GAA GTT GGT TCA TCC-3'	58	211
344L2.SP6	*D5Buc13*	AQ589415	5'-TGA ATA TTG CAG TGG ATG GC-3'	5'-CAA GCA ACC TTG CTA TGC AG-3'	58	183
441I19.T7	*D5Buc14*	AQ589427	5'-AAC TTA GAG CCT GGT GTG TGG-3'	5'-AGG CAA AAT CCC ACC AAA G-3'	60	151
3'-*Gabra2*	*Gabra2*	M86567	5'-TTG TAC AGT CTG ACT AAT AAC TGC-3'	5'-TGA AAC CCA CTT TAA ACT AGT TCC-3'	58	203
536J3.T7	*D5Buc15*	AQ589436	5'-AGC CAT GTG GAT CAC TGT TTC-3'	5'-GCC ATA TAT GCA TAG TGA ACC TG-3'	57	218
5F3.T7	*D5Buc16*	AQ589434	5'-GTG TGA TAA CAG TTT TAT CAA AGG-3'	5'-CCC ATT TCA CTA AAT GTA GAG TGC-3'	58	151
5F3.SP6	*D5Buc17*	AQ589433	5'-AAA GGC ATC TAC ATA TAA TTC AGG-3'	5'-ATG TAG AGT GCT TTG TGA CAA AGG-3'	58	220
253K12.T7	*D5Buc18*	AQ589411	5'-TCA GAC CTC CTG CTT TCA TGC TGG-3'	5'-ACA TTG TAA TCA CTG CAA AAG ACG-3'	58	227
5'-*Gabra2*	*Gabra2*	M86567	5'-AAA TTG AGC ACA TGC AAT GTA TGG-3'	5'-CTA AGC CGA TTA TCA TAA CCA TCC-3'	59	1700
37L14.T7	*D5Buc19*	AQ589420	5'-GTC TCC ACT CAG AGC TGT TAG TCC-3'	5'-TCC ACG GTT ACT TTT CGT CAT GCC-3'	58	223
432N14.T7	*D5Buc20*	AQ589426	5'-TTG TAG TTA TTA ATA CTC TAC TCC-3'	5'-AAG AGA AGT ATT TCT CAG AGG-3'	55	290
D5Mit305	*D5Mit305*	-	5'-AAG ATG GGA AAA TCA GGG ATG-3'	5'-AAA TGT TCC CTT CAT TTT CTT CC-3'	55	106
351F9.T7	*D5Buc21*	AQ589419	5'-AGG CTG ATG TTG AAA CCT GC-3'	5'-CCA ATG CTC TAG AAA GCC AGG-3'	55	155
381P13.SP6	*D5Buc22*	AQ589421	5'-GGA AGA AGG GAG GAT TCA GC-3'	5'-AGG CAG CTT TTC CTA ATC CC-3'	55	172
449H5.SP6	*D5Buc23*	AQ589428	5'-TGT CACAGC AGA AAC CTT GC-3'	5'-AAC CAG GAA ACG GAC AAA TG-3'	57	311
IMAGE:1069176	*D5Buc24e*	AA792909, AQ589428	5'-AAC CCA TAT CTC ATT CTG TGG AGG-3'	5'-AGG CCG TGT GTG AGC AGC TCC TGG-3'	59	195
5'-*Gabrb1*	*Gabrb1*	U14418	5'-TCC TCC TCC TCT TCT TCC TTC TCC-3'	5'-CCT CAT CTA CTA TGC ACT GAG TGG-3'	58	180
388L8.SP6	*D5Buc25*	AQ589423	5'-TGT GTA TCA TCC CAT GTC AAG ATC-3'	5'-ATG GCT ATT CCT GGC GGT CAC C-3'	59	138
IMAGE:1958590	*D5Buc26e*	AI272450, AQ589418	5'-CTG TGA TAA TCC TGG TGG GC-3'	5'-TTG CCT TTC TGT CGT AGC TG-3'	55	154
Gabr Rep1	*D5Buc27*	AQ589406	5'-ATT CTA CCT GGT TCT GCG TAG TCC-3'	5'-AGG GGA TCA CAC AGA TCT CCA ACC-3'	58	615
388L8.T7	*D5Buc28*	AQ589424	5'-ATG TGA GAG CCA GGT TAT GGA TCC-3'	5'-GTA AAG AGG TTT AGA TCA TGA AGG-3'	59	257
556B6.SP6	*D5Buc29*	AQ589431	5'-GTG TTT TGT GTG TTC AGC CG-3'	5'-TCA AAA GTC TCC AGC GTG TG-3'	60	276
3'-*Gabrb1*	*Gabrb1*	U14418	5'-GAG GTA AGA GAT TCA GCC TTC CAG-3'	5'-CCA GGG TAA CTG AGA AAG ACT GC-3'	58	230
Brain EST MDB0818	*D5Buc30e*	R74668, AQ589431	5'-TTA ATG GGA AAT GTC TGC CAT GGG-3'	5'-CTG GAA TGA TTG AAA ATG TAA TGG-3'	58	164
381P13.T7	*D5Buc31*	AQ589422	5'-GCC GAA GCT GAA AAG ATG AG-3'	5'-GGG AAC TGC AGA GTT CAA GC-3'	55	185
249L8.SP6	*D5Buc32*	AQ589409	5'-CAG AAA TGT GGG AGG AGA GG-3'	5'-ATA AAT GCA GGG GTG GTC TG-3'	57	186
503D21.T7	*D5Buc33*	AQ589430	5'-CGA TTC TTC TGA CTC AGC CC-3'	5'-TGT CAC TGG CAT CTG CCT AC-3'	56	263
393M12.SP6	*D5Buc34*	AQ589425	5'-CAG CCT CTG TTT TAC TCG TTC ACC-3'	5'-ATA AGC ATG TCA GTA TTG AAG TGC-3'	58	247
249L8.T7	*D5Buc35*	AQ589410	5'-CCT TTG GTT TTC GCA ATC TC-3'	5'-CAC TCC ATT TCC CCC ATT C-3'	57	196
556B6.T7	*D5Buc36*	AQ589432	5'-TAC TGA ACC CTT GCC TGT CC-3'	5'-AAA GAA AAT CCA TGC GGT TG-3'	60	233
335K24.T7	*D5Buc37*	AQ589412	5'-AGA ACA TCA TCT TTT AAC TTC ACT AGG-3'	5'-CAG CCA ATT TCA TTT TTA TAG ATT CC-3'	55	176
503D21.SP6	*D5Buc38*	AQ589429	5'-ACA GGA GTT TCA GGG GAC AG-3'	5'-TTG CAA ATC CCC AAG AAA AC-3'	58	299
Gabra4	*Gabra4*	AF090373	5'-TGA TAT ATA TGT CAC CAG CTT TGG-3'	5'-GTT ATG GAG ACA GAT TTC TTT CC-3'	58	850

To facilitate further analysis, we selected gene-specific primers for the three $GABA_A$ genes (Table 1). Whereas nucleotide sequence of full-length cDNAs was available for the mouse *Gabra2* and *Gabrb1* subunit genes (Table 1; Wang et al. 1992; Kamatchi et al. 1995), we obtained partial sequence for the mouse *Gabrg1* gene by screening an olfactory bulb cDNA library with a rat *Gabrg1* probe (Table 1). For each gene, 5′ and 3′ PCR assays were developed and used for BAC contig construction. Among 23 positive BAC clones, 13 BACs were selected for nucleotide sequence analysis of the insert ends (Fig. 1). The nonrepetitive insert end sequences provided 19 new STSs (Table 1). STS mapping using all available markers revealed that we had isolated three independent groups of BACs corresponding to the *Gabra2*, *Gabrb1*, and *Gabrg1* subunit gene regions with no overlaps between the three groups of clones.

To comply with the mouse genome initiative that has designated the C57BL/6J genome as a reference strain for genomic sequencing, further BAC isolation in the $GABA_A$ cluster on mouse chromosome 5 was performed by screening a C57BL/6J BAC library. To efficiently convert a 129/Sv clone collection into a C57BL/6J BAC contig, we selected 10 STSs for library screening. Sixteen new C57BL/6J BACs were sized and tested for STS and probe content and precisely positioned to the regions already covered by the 129/Sv BACs. In addition, STSs corresponding to the BAC ends were used to isolate clones that joined the *Gabra2* and *Gabrg1* groups of BACs, showing that these two genes map within an interval of 370 kb. Furthermore, BAC clones isolated with the *D5Mit305* marker filled the gap between the *Gabra2* and *Gabrb1* BACs. The *D5Mit305* marker was the only simple sequence length

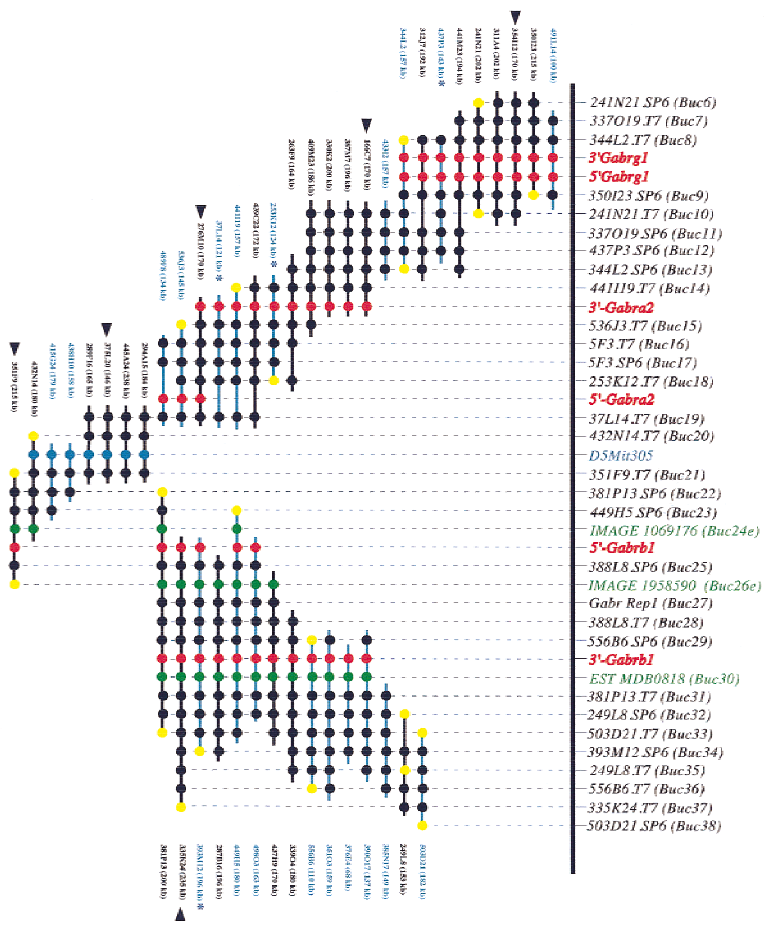

Figure 1 A BAC-based STS/EST-content map of the *Gabr* gene cluster on mouse chromosome 5 (oriented with centromeric end at *left* and telomeric end at *right*). The relative positions of mapped STSs and ESTs are indicated at *top*, including the corresponding loci names (*D5Buc*). The isolated BAC clones are shown as horizontal lines with circles along the lines indicating positive STS hits. The STSs were developed from BAC insert ends (black), known genes (red), ESTs (green), and an SSLP marker *D5Mit305* (blue). When an STS corresponds to a clone insert end, a yellow circle is present at the end of the clone from which it was derived. BAC clones were isolated from the C57BL/6J library (black lines), from the 129/Sv library (blue lines), or from the Research Genetics CITB library (*). The size of each BAC as determined by PFGE analysis is indicated. C57BL/6J BAC clones selected to represent the minimal tiling path are indicated by black arrowheads. The map is displayed with equal spacing between STS/EST markers and the depicted clones together span a distance of ~1300 kb. The orientation of the 5′- and 3′-*Gabrg1* markers with respect to surrounding STSs on the contig could not be determined.

polymorphism (SSLP) marker among 15 markers assigned to the 41 cM interval on the composite genetic map (Kozak and Stephenson 1998) that mapped to the 1.3 Mb region covered by the BAC contig.

The established BAC contig contains 45 BAC clones, covers a physical distance of about 1.3 Mb, and provides ordering information for 40 new markers. Among these are 29 STSs designed from BAC-insert ends, 5′- and 3′-specific sequence markers developed from $GABA_A$ receptor subunit cDNAs, and three new ESTs that were found by sequence homology of BAC ends to mouse EST cDNA clones (Table 1). Overall, this results in an average spacing of 1 marker per 32 kb within the contig and a five- to sixfold coverage with independent BAC clones. Because of the uneven distribution of STSs within the contig, the number of "hits" per BAC clone varies from 3 to 15. Fourteen C57BL/6J-derived BAC clones were selected for fingerprinting of *Eco*RI-digested DNA (data not shown). The pattern of *Eco*RI restriction fragments further confirmed the order of clones established by STS content mapping. This analysis identified six BAC clones that represent a minimal tiling path (Fig. 1). The STS content mapping using 5′ and 3′ gene-specific primer pairs and Southern blot analysis determined the transcriptional orientation of the *Gabra2* and *Gabrb1* genes with respect to contig ends and thus, with respect to each other. The two genes are transcribed in opposite directions (Fig. 1).

Finally, a genomic PCR assay specific for the *Gabra4* subunit gene was developed from a partial mouse cDNA sequence (Table 1). Primers were chosen from the portion of the gene encoding the amino-terminal extracellular domain (amino acids 72–166) yielding a 280-bp amplicon in brain cDNA and a genomic PCR product of 1.2 kb. A PCR assay using these primers was performed to test for the presence of the *Gabra4* gene on the contig (data not shown). We found no evidence that the *Gabra4* gene is located within this $GABA_A$ cluster. We have not discounted the possibility that *Gabra4* could be located close to, but not within, the 1.3-Mb region covered by the contig.

Nucleotide sequence analysis was performed on 40 BAC ends. The overall percentage of repetitive sequence detected using RepeatMasker in these BAC end-sequences was 17.5%. Three BAC ends contained sequences with high homology to ESTs (Table 1), but no homology to any known gene in the GenBank database. Expression analysis using RT–PCR confirmed that these are indeed transcribed sequences, expressed in several tissues, such as liver, spleen, testis, kidney, lungs and brain (data not shown).

To integrate the *Gabrg1–Gabra2–Gabrb1* BAC contig with the existing map of the mouse chromosome 5, we mapped several STSs and chromosome 5 SSLP markers using the mouse whole-genome RH panel. The commonly used T31 panel consists of 100 hybrid cell lines generated with a 3000-rad dose and has been shown to have a retention frequency of 27.6% (McCarthy et al. 1997). In contrast to genetic mapping, which requires markers with SSLP polymorphism between inbred strains used to generate the cross, PCR-based RH mapping requires markers that are present in mouse and absent in hamster DNA, or alternatively, that the amplicons detected in these DNAs are of different size. This makes RH mapping useful as an aid to anchor clone contigs on the chromosome relative to markers previously mapped or ordered along the chromosome. It also enables quick verification of chromosomal position of BAC clones containing members of large gene families.

To determine the orientation of our BAC contig, we mapped the following STSs: 253K12.T7(*D5Buc18*), 473P3.SP6(*D5Buc12*), and an EST developed from the 3′-UTR of the *Gabrb1* gene in the T31 RH panel. We also included the SSLP marker *D5Mit305* placed on the contig between *Gabra2* and *Gabrb1* (Fig. 1). PCR analysis of each marker was performed twice and consensus vector scores (Fig. 2) were entered in a data file containing scores for >50 loci along mouse chromosome 5 (L. Tarantino, C. Otmani, T. Wiltshire, A. Lengeling, and M. Bucan, unpubl.). Pairwise analysis of the data

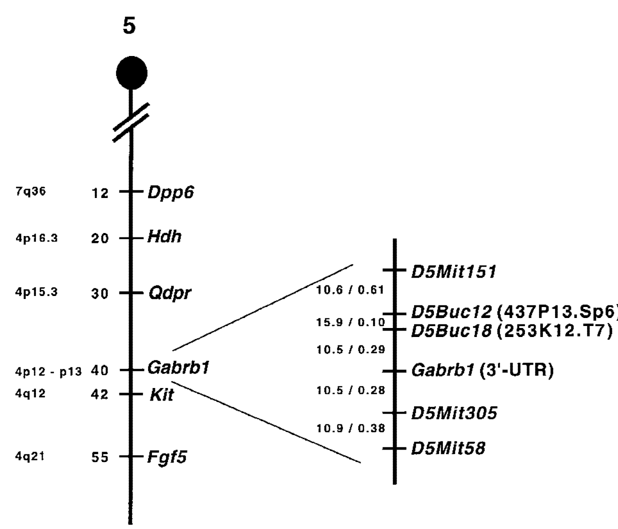

Figure 2 RH map of central mouse chromosome 5. The genetic map of mouse chromosome 5 with some selected loci and their position in cM (from the centromere) shown for orientation (Kozak and Stephenson 1998). Corresponding regions of homology in the human genome are denoted (http://www3.ncbi.nlm.nih.gov/Omim/Homology). The RH map of central mouse chromosome 5 from this work is shown in the enlarged area. Indicated are the positions of *D5Mit* markers and new *D5Buc* markers isolated from BAC clones with their specified insert end in brackets (Table 1). Pairwise lod scores and distances in centirays are indicated between neighboring loci.The RH mapping data (vector scores) have been deposited at the European Bioinformatics Institute (http://www.ebi.ac.uk/RHdb/index.html).

gave a single linkage group for the three $GABA_A$ receptor loci and several SSLP markers (*D5Mit151*, *D5Mit305*, and *D5Mit58*) localized in the central portion of mouse chromosome 5 (Fig. 2). This analysis confirmed the location of the BAC contig on mouse chromosome 5. Furthermore, using Map Manager QT, we calculated the relative marker order along the chromosome by minimizing the number of occurred breaks and determined the most likely order: cen–*D5Mit151*–*D5Buc12* (*Gabrg1*)–*D5Buc18* (*Gabra2*)–*Gabrb1*–*D5Mit58*–tel (Fig. 2).

DISCUSSION

In this report we present a sequence-ready BAC contig spanning the cluster of genes encoding $GABA_A$ receptor subunits *Gabra2*, *Gabrb1*, and *Gabrg1* located in the central portion of mouse chromosome 5. The established contig covers ~1.3 Mb, as determined by the sizes of BACs corresponding to a minimal tiling path. The gene order of the three subunit genes on mouse chromosome 5 is the same as in all clusters that are composed of three subunits genes (β–α–γ/ϵ) and as observed in the human chromosome 15 cluster, α and β subunits are transcribed in opposite directions (Greger et al. 1995). In addition to the genes encoding $GABA_A$ subunits, this physical map includes three ESTs encoding genes of unknown function, with a widespread tissue expression pattern, and with no corresponding or mapped human ESTs. Although chromosomal localization for several $GABA_A$ receptor clusters has been determined in the mouse and human genome, a high-resolution physical map is only available for the $GABA_A$ clusters on human chromosomes 15 and 5 (Christian et al. 1998; Kostrzewa et al. 1998). Comparative sequence analysis of coding and noncoding regions of $GABA_A$ receptor genes, both within a cluster and between different clusters, in the mouse and humans may provide important information concerning the complex regulation of gene expression of members of this large gene family. For example, comprehensive expression analysis indicates overlapping expression of *Gabra2*, *Gabrb1*, and *Gabrg1* in several regions of the brain, such as the neocortex, hippocampus, basal nuclei, amygdala, and red nucleus of the midbrain (for a summary, see Rabow et al. 1995). Comparative physical mapping and sequencing will shed light on the mechanisms involved in the tandem gene duplication events and transpositions that led to the clustered organization of the $GABA_A$ receptor genes. Further studies concerning the presence of an additional α subunit (α_4) on human chromosome 4 (McLean et al. 1995), and apparent absence of the mouse ortholog in the immediate vicinity of the α_2 subunit gene in the corresponding cluster on mouse chromosome 5, may indicate a dynamic evolution of this gene family. Furthermore, the presence or absence of nonrelated genes dispersed among the gene-family members in other clusters will provide useful insight into the timing of rearrangements during the evolution and origin of these genes in different species.

In the mouse, the *Gabrg1*–*Gabra2*–*Gabrb1* cluster is located proximal to the cluster of classical developmental mutations—dominant spotting (*W*) and patch (*Ph*), which are caused by mutations or chromosomal rearrangements in the tyrosine kinase receptor genes *Kit* and *Pdgfra* (Reith and Bernstein 1991). In the human genome, orthologous genes are located in the centromeric portion of chromosome 4, with the *GABRA2*, *GABRB1*, and *GABRG1* loci mapped to the short arm (4p12–p13), and the *KIT*–*PDGFRA* cluster on the long arm (4q12–q13) (http://www3.ncbi.nlm.nih.gov/Omim/Homology/). Sequence analysis of the region between the two clusters in the mouse should aid in determining sequences surrounding the centromere of human chromosome 4.

The construction of this contig coincides with the launching of an initiative to generate a working draft of the mouse genome sequence by 2003 (Battey et al. 1999). This effort will employ a random strategy for selection of clones that will not involve extensive mapping efforts and construction of sequence-ready BAC contigs prior to sequencing. In the initial phase, however, established contigs such as this, spanning the $GABA_A$ cluster, will provide a useful template for the generation of long stretches of contiguous genomic sequence in the mouse. A common C57BL/6J BAC library has been designated as the reference library in this sequencing effort (http://bacpac.med.buffalo.edu/mouse_bac.html). Our data add to the initial evaluation of the high quality of this library, its uniform coverage, and a large average insert size (197 kb). Although the comparative sequence analysis of the $GABA_A$ clusters will provide important information concerning functional domains in the coding and noncoding regions, BAC clones containing individual $GABA_A$ subunit genes will provide immediate resources for functional studies.

METHODS

Isolation and Processing of BAC Clones

BAC clones were isolated by hybridization of probes to high-density library filters from three different BAC libraries: 129/Sv (Research Genetics, Huntsville, AL), RPCI-22 129/SvEvTACfBr, and RPCI-23 C57BL/6J BAC libraries (K. Osoegawa, M. Tateno, and P. de Jong, in prep.; for more information, see http://bacpac.med.buffalo.edu/mouse_bac.html). BAC libraries were initially screened with probes from rat *Gabra2* cDNA (Khrestchatisky et al. 1991), rat *Gabrg1* cDNA (M. Khrestchatisky and A. Tobin, unpubl.), and the mouse *Gabrb1* cDNA (Nagle et al. 1995), and subsequent screenings used STSs generated from BAC-end sequences. All radioactive labeling of probes used standard random-primed methods (Feinberg and Vogelstein 1983).

All BACs isolated were arrayed as colony dot blots in a 96-well format. BACs were grown overnight in 100 µl of Luria broth (LB)/chloramphenicol, spotted onto nylon filters, and then grown for 8 hr on LB agar plates. Filters were processed using alkaline lysis and Proteinase K/Sarkosyl treatment (see http://www.resgen.com/depts/rnd/rapid.html).

BAC DNA was prepared by standard alkaline lysis methods (Sambrook et al. 1989) from 5 ml of overnight culture and resuspended in 40 µl of TE buffer. Miniprep DNA (5 µl) was digested immediately in a total volume of 20 µl with 5 units of *Not*I enzyme (New England Biolabs, Inc., Beverly, MA) for 2 hr at 37°C. Samples were loaded on a 1% agarose gel in 0.5% Tris-borate–EDTA (TBE) and subjected to PFGE (Bio-Rad CHEF DR II) for 16 hr at 6 V/cm, 15°C with a switching interval from 5 sec to 15 sec. BAC insert sizes were assigned from ethidium bromide-stained gels using AlphEase software and an Alpha-Imager 2000 gel-documentation system (Alpha Innotech, San Leandro, CA). *Eco*RI digests of freshly prepared miniprep DNA were also used to fingerprint clones according to the methods of Marra et al. (1997). Clone overlap analysis was carried out manually.

Sequencing of BAC-Insert Ends

BAC DNA for sequencing was prepared from 200 ml of overnight culture according to the modified protocol for BACs using P100 midi-prep columns (Qiagen, Inc., Valencia, CA). Automated dideoxy-terminator cycle sequencing was carried out with SP6 and T7 primers on BAC DNA (2 µg of DNA in a 20-µl reaction) using ABI Big Dye Terminator sequencing chemistry with Taq FS polymerase from Applied BioSystems (Foster City, CA). Reaction products were purified by G50 spin columns and analyzed on ABI 377 automated sequencers (DNA Sequencing Facility, Department of Genetics, University of Pennsylvania, Philadelphia).

Development of New STSs and Marker Content Mapping

BAC end sequence was assessed for development of new STS markers. To determine rodent specific and low complexity repeats, nucleotide sequences were analyzed using RepeatMasker (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker). Primer 3.0 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) was used for selection of PCR primers. STS content mapping of BACs was determined by hybridization of specific probes to colony dot blots. PCR was performed with diluted mini-prep BAC DNA in 15-µl reactions consisting of 1× buffer (20 mM Tris-HCl at pH 8.3, 50 mM KCl, and 2.5 mM $MgCl_2$), 0.2 mM each dNTP, 1 µM each STS primer, and 0.5 unit of *Taq* polymerase (Roche, Indianapolis, IN) under the following conditions: 94°C for 30 sec, annealing for 30 sec (temperatures listed in Table 1), 72°C for 30 sec, for 35 cycles.

Screening of cDNA Libraries

The rat *Gabrg1* cDNA probe (1200-bp *Eco*RV fragment) was used to screen an arrayed mouse olfactory bulb cDNA library (Resource Center/Primary Database of the German Human Genome Project, Max Planck Institute for Molecular Genetics, Berlin-Charlottenburg, Germany). Clone UCDMp608P0343Q2 was isolated, sequenced, and used to design 5′- and 3′-specific PCR assays for the mouse *Gabrg1* gene.

RH Mapping

T31 RH panel DNAs (Research Genetics, Huntsville, AL) were diluted to 3 ng/µl and 3 µl of each cell hybrid clone DNA was used in PCRs. PCR reagents and conditions were previously described. Primers were initially tested on mouse and hamster DNA controls, prior to the analysis of 100-cell hybrid lines. PCR amplicons were run on 2% agarose gels and the presence or absence of PCR fragments were scored. For each marker the T31 RH panel was typed twice. Data analysis was performed with Map Manager QTb 27 ppc. Distances between neighboring loci (in centirays) were calculated with the RH2PT function of the RH Map program (Lunetta et al. 1996). The RH mapping data (vector scores) have been deposited at the European Bioinformatics Institute (http://www.ebi.ac.uk/RHdb/index.html).

ACKNOWLEDGMENTS

We thank Michel Khrestchatisky and Allan Tobin for the rat *Gabrga2* and *Gabrg1* cDNA probes, Lisa Tarantino and Ali Alavizadeh for help with the RH mapping analysis, Ariel Soiffer for the technical assistance in the initial phase of this project, Nadja Pohl for assistance with the cDNA screen, Lisa Stubbs and Mark LaLande for discussions on gene order in $GABA_A$ clusters, and Tom Ferraro and Lisa Tarantino for comments on the manuscript. These studies were supported by grants from the National Institutes of Health (HD 28410 and MH 57855).

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Received April 26, 1999; accepted in revised form June 10, 1999.