

# Genomic organization of $\alpha_1$ and $\beta_1$ subunits of the mammalian soluble guanylyl cyclase genes

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The structures of the genes encoding the  $\alpha_1$  and  $\beta_1$  subunits of murine soluble guanylyl cyclase (sGC) were determined. Full-length cDNAs isolated from mouse lungs encoding the  $\alpha_1$  (2.5 kb) and  $\beta_1$  (3.3 kb) subunits are presented in this report. The  $\alpha_1$  sGC gene is approximately 26.4 kb and contains nine exons, whereas the  $\beta_1$  sGC gene spans 22 kb and consists of 14 exons. The positions of exon/intron boundaries and the sizes of introns for both genes are described. Comparison of mouse genomic organization with the Human Genome Database predicted the exon/intron boundaries of the human genes and revealed that human and mouse  $\alpha_1$  and  $\beta_1$  sGC genes have similar structures. Both mouse genes are localized on the third chromosome, band 3E3-F1, and are separated by a fragment that is 2% of the chromosomal length. The 5' untranslated regions of  $\alpha_1$  and  $\beta_1$  subunit genes were subcloned into luciferase reporter constructs, and the functional analysis of promoter activity was performed in murine neuroblastoma N1E-115 cells. Our results indicate that the 5' untranslated regions for both genes possess independent promoter activities and, together with the data on chromosomal localization, suggest independent regulation of both genes.

nitric oxide | chromosomal localization

Nitric oxide-dependent signal transduction is associated with a number of important physiological processes, including smooth muscle relaxation (1), platelet aggregation (2), neurotransmission (3), cellular differentiation (4), and apoptotic cell death (5–7). Soluble guanylyl cyclase (sGC), a NO-stimulated hemoprotein, which converts GTP to cyclic guanosine monophosphate, is a key element in these processes.

sGC is a heterodimer consisting of  $\alpha$  and  $\beta$  subunits (8), which are encoded by separate genes (9). A heme prosthetic group is crucial for the stimulation of the enzyme by NO (10, 11). The enzyme has been purified from various animal tissues (10, 12, 13), and corresponding cDNAs were cloned from various vertebrate species, including rat (14, 15), human (16), bovine (17, 18), and fish (19). At least two isoforms for each subunit of the enzyme have been identified in various species, prompting a recent revision of the nomenclature of sGC subunits (20). Although isoforms for both subunits were detected at the mRNA level in various tissues and were found to have an overlapping tissue distribution, until recently only the  $\alpha_1/\beta_1$  heterodimeric enzyme has been isolated from native sources. However, Russwurm and coworkers (21) described an additional  $\alpha_2/\beta_1$  heterodimer, which was shown previously to be catalytically active *in vitro*. Alternatively spliced transcripts for both human  $\alpha$  (22) and  $\beta$  (23) subunits also have been reported. mRNA for the human  $\alpha_1$  subunit undergoes alternative splicing, resulting in several mRNA species that are N-terminally truncated. The  $\alpha_{21}$  subunit, an alternatively spliced variant of  $\alpha_2$ , has been detected in several mammalian cell lines and tissues at the mRNA level (24), and a  $\beta_1$  cDNA splice variant has been detected in humans (25).

Evidence that sGC activity is regulated both at the protein and mRNA levels has begun to emerge. Several groups have reported that such treatments as forskolin, dibutyryl-cAMP or 3-isobutyl-

1-methyl xanthine (26), endotoxin and/or IL-1 $\beta$  (27), NO-donating compounds (28), and nerve growth factor (29) affect the sGC mRNA levels in various cell types. There is also evidence that levels of sGC mRNA expression are subject to developmental regulation (30). Ongoing work in our laboratory, to be reported elsewhere, clearly demonstrates hormonal regulation of the mRNA levels for  $\alpha_1$  and  $\beta_1$  sGC subunits.

Despite an increasing interest in genetic aspects of sGC regulation, relatively little is known about the genes or the promoter regions of mammalian sGCs. Only the genomic organization of sGC for such distant species as the *Anopheles* mosquito (31) and Medaka fish (32) has recently been described.

Here we report the cDNA cloning, chromosomal localization, and structure of the mouse genes for the  $\alpha_1$  and  $\beta_1$  subunits of sGC and a comparative analyses with the human sGC genes, using the Human Genome Database (National Center for Biotechnology Information, NCBI). This information furthers our understanding of the mechanisms of sGC regulation through alternative splicing and/or modulation of mRNA levels.

## Materials and Methods

**Isolation of a cDNA Clone for Mouse sGC $\alpha_1$  Subunit.** A mouse lung  $\lambda$  Triplex cDNA library (CLONTECH) was screened by hybridization using a 1.3-kb rat sGC $\alpha_1$  cDNA fragment obtained by PCR using *Taq* polymerase (GIBCO) and the oligonucleotide primers 5'-<sup>91</sup>TGCACTTCAGAGAACCTTG-3' and 5'-<sup>520</sup>CTCCACCTTGTAGACATCCA-3' (superscript indicates position of codon at which the primers start). Six positive clones were identified from approximately  $1 \times 10^6$  independent phage plaques. Positive clones were subsequently purified, sequenced bidirectionally for positive clone identification, and analyzed by using DNASTAR software (DNASTAR, Madison, WI). After analysis, the clone was defined as mouse  $\alpha_1$  sGC and submitted to the NCBI database (accession no. AF297082). This clone was used in all subsequent experiments and alignments in this article.

**Isolation of Genomic Clones for Mouse sGC $\alpha_1$  and  $\beta_1$  Subunits.** A bacterial artificial chromosome (BAC) high-density membrane mouse library was purchased from Genome Systems (St. Louis). The hybridization was performed overnight at 46°C in standard hybridization solution (33). A random primer-labeled  $\alpha$ -<sup>32</sup>P-dCTP-labeled cDNA fragment (0.9 kb) for the  $\beta_1$  sGC probe was generated by reverse transcription-PCR from a total RNA preparation from murine neuroblastoma N1E-115 cells, using

Abbreviations: sGC, soluble guanylyl cyclase; NCBI, National Center for Biotechnology Information; BAC, bacterial artificial chromosome;  $\beta$ -gal,  $\beta$ -galactosidase.

Data deposition: The sequences reported in this paper have been deposited in the NCBI database (accession nos. AF297082 and AF297083).

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the oligonucleotides 5'-<sup>3</sup>GACACCATGTACGGTTTCGTG-3' and 5'-<sup>243</sup>CCCTTCCTTGCTTCTCAGTAC-3' (superscript indicates the base pairs upstream of the start codon or the position of the codon at which the primers start).

The membranes then were rehybridized with an  $\alpha_1$  sGC cDNA probe (1.3 kb containing coding sequence) under the same conditions. Positive BAC clones were identified by using the manufacturer's procedure and purchased from Genome Systems. A BAC plasmid purification kit (CLONTECH) was used for BAC DNA isolation from bacterial culture. BAC DNA was subjected to restriction and Southern blot hybridization analysis (33) using the same hybridization probes to confirm isolation of positive clones (data not shown).

**Determination of Boundaries and Sizes of Introns.** Based on the  $\alpha_1$  and  $\beta_1$  sGC cDNA sequences, sequencing oligonucleotide primers were designed to determine the genomic structure of each subunit. All sequencing analyses were performed at the Molecular Core Sequencing Facility at the University of Texas-Houston Medical School on an ABI Prism 377 DNA sequencer with the DigDye Terminator cycle sequencing kit (Applied Biosystems). Primers positioned in the exons of both subunits were used to determine the intron sizes by PCR with Pfu-Turbo DNA polymerase (Stratagene) from BAC DNA templates. PCR conditions were: (i) melting step at 95°C for 1 min, (ii) primer annealing at 55°C for 1 min, and (iii) extension step at 72°C for 3 min, repeated for 35 cycles. PCR products were separated by electrophoresis on 1% agarose gels.

**3' Rapid Amplification of cDNA End (RACE) of Mouse sGC $\beta_1$  Subunit.** Poly(A)<sup>+</sup> RNA was purified from lung tissue of CD57 mice by using an mRNA extraction kit (Dyna). Determination of the 3' end of  $\beta_1$  sGC mRNA was performed by using a SMART RACE cDNA Amplification kit (CLONTECH). In brief, the first-strand cDNA synthesis was achieved by incubating the poly(A)<sup>+</sup> RNA with a 3' cDNA-specific primer and Superscript Reverse Transcriptase (GIBCO) for 1.5 h at 42°C. Next a "touchdown" PCR to amplify the fragment was executed by using an oligonucleotide 5'-<sup>258</sup>CTGCTACAAGCATTGCCTAGACGGACG-3' (superscript indicates the base pairs downstream of the stop codon where the primer starts), specific to the 3' end of the published  $\beta_1$  sGC sequence, and the universal primer mixture that recognizes the modified 3' end of cDNA. PCR conditions were as suggested by the manufacturer (CLONTECH). PCR products were subcloned into pCR 2.1-Topo vector (Invitrogen), and both strands were sequenced for verification.

**Chromosomal Localization of Mouse sGC $\alpha_1$  and  $\beta_1$  Subunits.** Chromosomal localization of  $\alpha_1$  and  $\beta_1$  sGC genes was performed by Genome Systems using fluorescence *in situ* hybridization. Briefly, purified BAC DNA for each clone, containing the genomic sequence of  $\alpha_1$  and  $\beta_1$  sGC, was labeled with digoxigenin dUTP by nick-translation. The labeled probe was combined with sheared mouse DNA and hybridized to normal metaphase chromosomes derived from mouse embryo fibroblast cells in a solution containing 50% formamide, 10% dextran sulfate, and 2 $\times$  SSC. The hybridization was detected by using fluorescent antidigoxigenin antibodies followed by counterstaining with 4',6-diamidino-2-phenylindole. In addition, a probe specific for the telomeric region of chromosome 3 was cohybridized with each clone to verify specific labeling of the telomere to chromosome 3. Specific measurements identifying the hybridization signal between the heterochromatic-euchromatic boundary to the telomere of chromosome 3 indicated the band location of each clone on mouse chromosome 3.

**Cloning of Luciferase Plasmid Constructs.** To create the plasmid constructs containing the 5' upstream regions of  $\alpha_1$  and  $\beta_1$  sGC

extended to the first identified exon for each gene, DNA fragments were obtained by PCR using the specific genomic clones as templates and Pfu Turbo DNA Polymerase (Stratagene). Positive-strand oligonucleotide primers for each construct were: 1.6 kb,  $\alpha_1$  5'-<sup>1901</sup>GTCAGTGTACAGACCTGAAGATGCTG-3' and 1.4 kb,  $\beta_1$  5'-<sup>1528</sup>CTCTCTGTGTGTGAGAGAGAG-3' (superscript indicates the base pairs upstream of the start codon). Each of these positive-strand oligonucleotide primers contained a *KpnI* restriction site linker sequence at the 5' end. The negative-strand primers were: 5'-<sup>104</sup>CATGATGCGATCACAGGAGGC-3' for the  $\alpha_1$  construct and 5'-<sup>105</sup>CGCCCGAGCCTAGGAAGCAG-3' for the  $\beta_1$  construct (superscript indicates the base pairs upstream of the start codon). Each of the negative-strand primers contained a *BglII* restriction site linker sequence at the 5' end. After restriction digestion of the ends, the PCR fragments were directionally cloned into the luciferase reporter vector pGL3-Basic (Promega) between the *KpnI* and *BglII* restriction sites upstream of the luciferase gene.

**Transfection and Detection of Luciferase Activity.** N1E-115 mouse neuroblastoma cells were maintained in DMEM with 4 mM L-glutamine, 4.5 g/liter glucose, 1% penicillin-streptomycin mixture, and 10% FBS (HyClone). Cells were transiently transfected with each ( $\alpha_1$  and  $\beta_1$  sGC) luciferase plasmid construct by using Fugene-6 transfection reagent (Roche Molecular Biochemicals). Cultures were incubated in the presence of Fugene and DNA (1  $\mu$ g) for 48 h and assayed for luciferase activity by using a luciferase reporter assay (Promega). Cells were cotransfected with a  $\beta$ -galactosidase ( $\beta$ -gal) construct (cytomegalovirus- $\beta$ -gal, 1/5 of the concentration of sGC constructs) and assayed for  $\beta$ -gal activity in the N1E cell lysates to normalize the transfection efficiency between cell groups (not shown).

## Results

**Cloning of the Mouse  $\alpha_1$  sGC Subunit cDNA.** The cDNA for mouse  $\alpha_1$  subunit of sGC was not previously isolated and reported. We isolated six clones by screening a mouse cDNA library (CLONTECH) using a rat cDNA sequence as a probe. The clone containing the longest insertion was sequenced and analyzed for the presence of the ORF encoding the  $\alpha_1$  sGC subunit. The sequence comparison of isolated cDNA demonstrated 93.3% and 83.9% homology with rat and human  $\alpha_1$  cDNA, respectively, confirming that the isolated clone indeed encodes mouse  $\alpha_1$  sGC.

**Isolation of 3' End Fragment of  $\beta_1$  sGC cDNA.** The NCBI database contains a 2.3-kb cDNA sequence for mouse  $\beta_1$  sGC (GenBank accession no. AF020339). Northern analysis of mouse lung total RNA performed in our laboratory showed a 4-kb transcript for  $\beta_1$  sGC (data not shown). To find the missing portion of the mRNA for  $\beta_1$  sGC, a 3' rapid amplification of cDNA end analysis was performed on the mouse lung mRNA preparation (see *Materials and Methods*). The first cDNA strand was generated by using a primer located upstream of the known 3' end of mouse cDNA and the oligo(dT) adaptor primer. A 1-kb fragment was successfully isolated. Sequence analysis of this fragment indicated that it contained the expected 70-bp region identical to the known mouse 3' end of  $\beta_1$  cDNA followed by a 956-bp novel sequence containing a conservative consensus for the polyadenylation signal and poly(A) stretch. The sequence was highly homologous to the rat 3' end of  $\beta_1$  cDNA (data not shown). This allowed us to conclude that the complete 3' untranslated region for  $\beta_1$  sGC cDNA had been isolated. The full cDNA sequence of mouse  $\beta_1$  sGC subunit was submitted to the NCBI database (accession no. AF297083).

**Genomic Organization of Mouse  $\alpha_1$  and  $\beta_1$  sGC Genes.** Three overlapping BAC clones were isolated for each of the sGC genes by

**Table 1. Exon-intron splice junctions of the  $\alpha_1$  and  $\beta_1$  sGC genes**

	Splice donor*	Size of intron, kb	Splice acceptor
<b><math>\alpha_1</math> sGC</b>			
Intron 1	cat <sup>-103</sup> g/GTGGGTTTCGCTCAGC	>2.0	TCCACTGCTCATAG/gt gct
Intron 2	cca <sup>85</sup> gag/GTGAGTGTCTCCC	5.5	TCTTTTCTTTCCAG/tgt gag
Intron 3	aac <sup>106</sup> ag/GTAAGCTAAGTTACC	2.2	TTAATTATTTCCAG/g aaa
Intron 4	gca <sup>126</sup> g/GTAATAAATAAACT	1.9	CTGTGTGCTTGCAG/gtg ccc
Intron 5	tca <sup>362</sup> agg/GTAAGGAAAATGTAA	3.0	CCTTTCCTTGCAG/gtt atg
Intron 6	tac <sup>524</sup> aag/GTAGGGAAGGTGGAA	4.2	TATATTGTATCTAG/gtg gag
Intron 7	atc <sup>572</sup> aag/GTA AGGCCGTGACTT	4.4	TGTTTTGCCTTTCAG/atg cga
Intron 8	tac <sup>624</sup> ag/GTATGGATGGCACTA	2.8	TAAATTGTCTCAG/g tta
<b><math>\beta_1</math> sGC</b>			
Intron 1	acc <sup>1</sup> atg/GTGAGTGCTGTGACG	0.5	TCTCTGCCCTTTCAG/tac ggt
Intron 2	atc <sup>26</sup> aa/GTAAGTGAACAGCC	2.5	TCCATTTTCTTTCCAG/a aaa
Intron 3	ctc <sup>60</sup> a/GTAGGTTGAAAAC	2.4	CATCTACAAAACAG/ac ctc
Intron 4	tttg <sup>98</sup> cag/GTGAGATGTTTCGAG	0.8	CTGTGCTACTACAG/aac ctc
Intron 5	atg <sup>164</sup> aag/GTAGTGTTCACCCG	1.1	CCATTGACACTAG/gtg att
Intron 6	ccc <sup>241</sup> cag/GTAAAATGCACAG	1.1	TTTCTGTGCTTTCAG/ctc cag
Intron 7	agc <sup>280</sup> aag/GTAAGCAAGAACC	1.0	CCTTCTGTTTAAAG/gaa ggg
Intron 8	cca <sup>325</sup> ag/GTAACAACTTTTAA	3.0	CTCTGTGTGACAG/t gtg
Intron 9	gac <sup>391</sup> ac/GTAAGCAAGGGAG	1.5	CTAATTTCCACAG/a ttg
Intron 10	tac <sup>470</sup> aag/GCAAGTCTTCATGG	2.5	TGTGTCCACCTAG/gtg gaa
Intron 11	ggt <sup>517</sup> cag/GTGAGTAAATAAAT	0.4	CTTTGCTTCTGCAG/ata aca
Intron 12	tac <sup>569</sup> ag/GTGAGGAGGGAAAT	0.3	CTCATGACTTTCAG/g tgt
Intron 13	acg <sup>611</sup> gag/GTATGGCTCATTAG	1.1	TCGACCCATTTAAG/gaa aca

\*The positions of codons at which the introns interrupt the coding sequence are indicated, except for the first intron of  $\alpha_1$  sGC gene, where it indicates the base pairs upstream of the start codon.

separate screening of a BAC mouse genomic library (Genome Systems) by using probes containing a 1.3-kb fragment of the coding sequence for mouse  $\alpha_1$  sGC and a 0.9-kb N-terminal fragment of the coding mouse  $\beta_1$  sGC sequence (see *Materials and Methods*). Southern analysis of isolated clones with probes specific for the 5' and 3' cDNA fragments for both sGC subunits confirmed that at least two of three clones for each subunit contained a genomic fragment that hybridized with both 5' and 3' probes from the  $\alpha_1$  and  $\beta_1$  cDNAs (data not shown). Genomic sequences isolated during screening have 99% and 100% homology in coding regions with the murine  $\alpha_1$  and  $\beta_1$  cDNAs, respectively, confirming successful isolation of the genes for these two isoforms and subunits. Comparison of the coding sequences for the  $\alpha_1$  sGC subunit gene with previously cloned cDNA revealed seven mismatches in codons 49 (TAC→GAC), 52 (GAG→GAA), 319 (AAC→AGC), 343 (AAC→AAT), 445 (GAA→GAG), 487 (ATC→ACC), and 690 (GTA→ATA). Of the seven codons, four of the replacements (codons 49, 319, 487, and 690) introduce different amino acid residues in the final protein sequence. The source for the BAC genomic library (Genomic Systems) used in our analyses differed from that of the cDNA library (CLONTECH), suggesting that the inconsistency in these sequences reflect DNA polymorphism between different strains of mice (i.e., 129/SvJ I vs. 200 BALB/c, respectively).

The positions of the exon/intron boundaries were identified by sequencing using oligonucleotide primers located within the coding sequences of each gene (Table 1). Intron sizes were estimated by using PCR (see Table 1, Fig. 1) and for introns 1, 2, 10, 11, and 12 of  $\beta_1$  sGC by complete sequencing. Although the size of intron 1 for sGC $\alpha_1$  was not determined, partial sequencing suggests it is more than 2 kb.

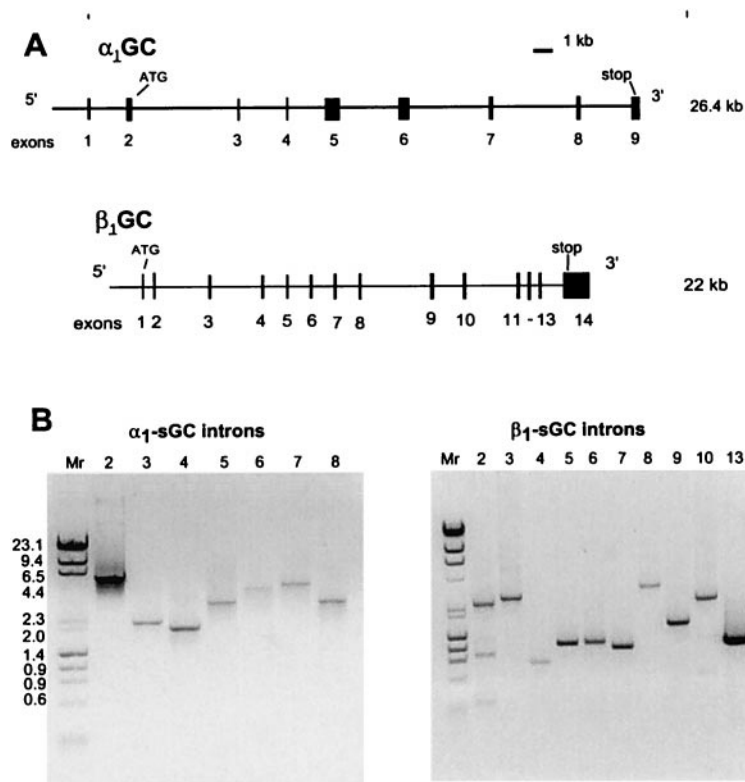
The  $\alpha_1$  sGC gene encompasses at least 26.4 kb and includes nine exons and eight introns, whereas the  $\beta_1$  sGC gene contains 14 exons and 13 introns and spans 22 kb (see Fig. 1). Start codons are positioned in the second and first exons of the  $\alpha_1$  and  $\beta_1$  genes, respectively. The GT/AG donor/acceptor consensus was maintained in all introns for both genes, except for intron 10 in

$\beta_1$  sGC, where the donor site was GC. The sequences that flank the exon/intron boundaries in the  $\alpha_1$  and  $\beta_1$  sGC genes are presented in Table 1.

**Chromosomal Localization.** BAC clones containing  $\alpha_1$  and  $\beta_1$  genes were used for chromosomal localization by fluorescence *in situ* hybridization analysis as described in *Materials and Methods*. A total of 80 metaphase cells were analyzed for each genomic clone with 71 and 72 chromosomes exhibiting specific labeling for  $\alpha_1$  and  $\beta_1$  sGC genes, respectively (Fig. 2). Both genes colocalized to mouse chromosome 3. The  $\alpha_1$  sGC gene positioned at 44% and  $\beta_1$  sGC at 46% of the distance from the heterochromatic-euchromatic boundary to the telomere of chromosome 3, which corresponds to band 3E3-F1.

**Analysis of the Promoter Activity of 5' Regions for  $\alpha_1$  and  $\beta_1$  sGC Genes in the N1E-115 Cell Line.** Murine neuroblastoma N1E-115 cells were selected for promoter analysis as host cells because expression of sGC was previously shown in this cell line (34); 1.6 kb and 1.4 kb of the 5'-flanking regions extended until the first identified exons of the  $\alpha_1$  and  $\beta_1$  sGC subunit genes were subcloned upstream of the luciferase gene of the pGL3-Basic luciferase reporter vector (Promega). The constructs were transiently transfected in N1E-115 cells (see *Materials and Methods*). Luciferase activity generated by each of the constructs was compared with the activity of the promoterless pGL3-basic plasmid. The upstream regions of the  $\alpha_1$  and  $\beta_1$  sGC genes demonstrated different transcriptional activity in N1E-115 cells (Fig. 3). The relative level of luciferase activity for the  $\beta_1$  sGC construct was 4.6-fold higher when compared with the control, whereas the  $\alpha_1$  sGC construct activity was only 2-fold higher than the control promoterless plasmid.

**Prediction of the Organization of the Human sGC Genes.** cDNAs for mouse and human  $\alpha_1$  and  $\beta_1$  subunits were compared with the Human Genome Database. We identified a clone containing putative genomic regions for both human  $\alpha_1$  and  $\beta_1$  subunits

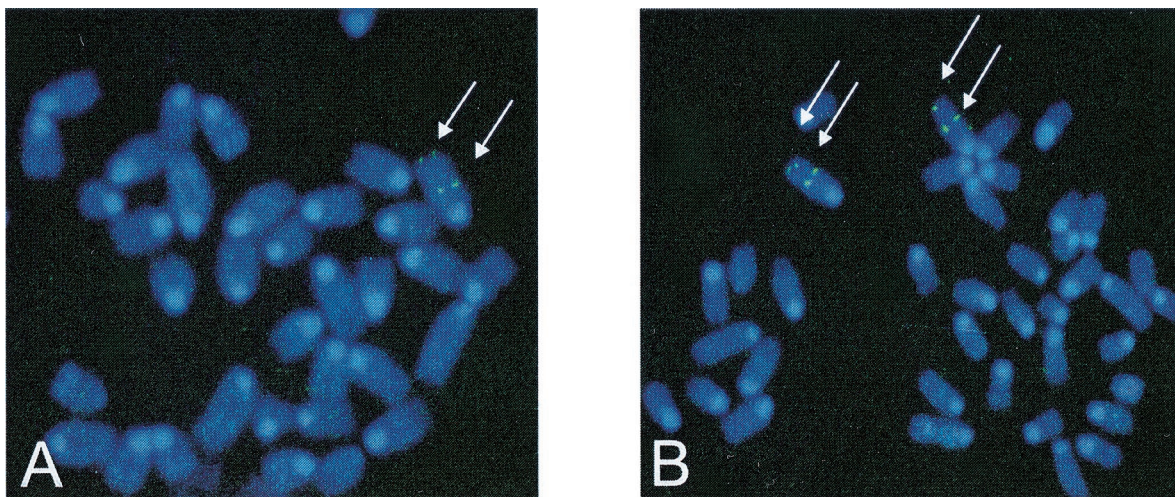


**Fig. 1.** Genomic structure and size of introns of murine  $\alpha_1$  and  $\beta_1$  sGC. (A) Exon-intron distribution of murine  $\alpha_1$  and  $\beta_1$  sGC genes (drawn to scale). Exons are represented by black boxes. (B) Estimation of the intron sizes by PCR using primers located in cDNA sequences flanking the introns (see *Materials and Methods*). The amplified DNA products were separated on 1% agarose gel and stained with ethidium bromide. The molecular mass markers (mixture of Lambda DNA-*Hind*III digest and  $\phi$ X174 DNA-*Hae*III digest, New England Biolabs) are shown at the left of each panel.

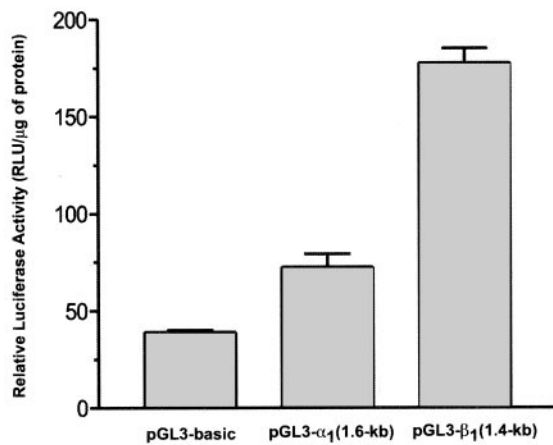
(clone AC021433), which is ascribed to chromosome 2. Exons 3–9 of the human  $\alpha_1$  gene and all exons of the human  $\beta_1$  gene were identified in this genomic fragment. The comparison of predicted exon-intron boundaries and donor/acceptor sites from the human genes with those of the mouse genes revealed that they are identical in both species with the exception of intron 4 (donor and acceptor sites) and intron 9 (acceptor site) of the  $\beta_1$  subunit.

### Discussion

Despite the significant role sGC plays in numerous physiological processes, little is known about the genomic organization of the genes for this enzyme in mammalian species. Recently, the genomic organization for the  $\alpha_1$  and  $\beta_1$  subunits of sGC in Medaka fish (32) and the  $\beta$  subunit of sGC in mosquito (*Anopheles gambiae*) were reported (31). Tandem genomic or-



**Fig. 2.** Chromosomal localization of murine  $\alpha_1$  and  $\beta_1$  sGC genes. DNA from BAC clones containing genomic regions of  $\alpha_1$  and  $\beta_1$  sGC genes was labeled with digoxigenin dUTP and hybridized to normal metaphase chromosomes derived from mouse embryo fibroblast cells (for details see *Materials and Methods*). Cohybridization of  $\alpha_1$  sGC and  $\beta_1$  sGC probe with a probe specific for telomeric region on chromosome 3 is shown on A and B, respectively. Arrows indicate the sites of specific hybridizations.



**Fig. 3.** Promoter activity of 5' flanking regions of  $\alpha_1$  and  $\beta_1$  sGC in N1E-115 cells. Summary of luciferase expression assays of  $\alpha_1$  and  $\beta_1$  sGC putative promoter regions in murine neuroblastoma N1E-115 cells. Luciferase activity was assayed as described in *Materials and Methods*. Values were normalized by using a  $\beta$ -gal construct (cytomegalovirus- $\beta$ -gal) cotransfections and the total protein concentration of each group. The values represent the normalized means  $\pm$  SD of three different experiments.

ganization and evidence of directly coordinated transcription for  $\alpha_1$  and  $\beta_1$  subunits in Medaka fish suggested the possibility of a similar mechanism of expression of sGC subunits in all vertebrates. Coexpression of both  $\alpha$  and  $\beta$  subunits in transfected cells is required for enzyme activity (15). Furthermore, the  $\alpha_1$  and  $\beta_1$  sGC genes have been localized to the same chromosome in rat and human (35, 36). Coexpression of both  $\alpha_1$  and  $\beta_1$  subunits is obligatory for enzyme activity in rat lung (8, 9, 14, 15) and human cerebral cortex, cerebellum, and lung (20). However, the ratio of expression levels for both subunits varies in a tissue-dependent manner (20), suggesting that the regulation of expression for these subunits is not tightly coordinated as was suggested for Medaka fish. Here, we report that mouse  $\alpha_1$  and  $\beta_1$  sGC genes map to the third chromosome (see Fig. 2). However, they are separated from each other by an extended region comprising 2% of the total chromosomal length. This finding excludes the possibility of tandem organization and directly coordinated transcription as proposed for the fish genes. This conclusion is supported by the independent ability of the 5' flanking regions of the  $\alpha_1$  and  $\beta_1$  sGC genes to drive transcription (see Fig. 3). However, the trans-coordinated transcriptional regulation by the same factors is possible.

The rat  $\alpha_1$  and  $\beta_1$  genes map to chromosome 2 and are closely linked to the particulate guanylyl cyclase isoform locus and quantitative trait locus, which have been associated with salt-sensitive hypertension in Dahl rats (36). Thus far, no direct connection of the genomic loci identified for  $\alpha_1$  and  $\beta_1$  sGC to hypertension in mouse or human has been demonstrated (37). Here we show that both mouse genes colocalize on chromosome

3 in the area corresponding to band 3E3-F1. The  $\alpha_1$  and  $\beta_1$  subunits are colocalized with Muc1, Pklr, Ntrk1, CD1, and Fcfr1 loci located at 44.8–46.5 positions of chromosome 3. The chromosomal positioning of mouse sGC genes will stimulate the search for diseases associated with genomic aberrations in this region in mice.

Probing of the Human Genome Database with the mouse and human cDNA sequences identified the clone AC021433, which contains eight exons of the human  $\alpha_1$  gene and 14 exons of the human  $\beta_1$  gene. This fragment is ascribed to human chromosome 2. Although this fragment is missing the first two exons of the  $\alpha_1$  gene, this is the most probable candidate for the locus of the human  $\alpha_1$  and  $\beta_1$  genes. In a previous report (35),  $\alpha_3$  and  $\beta_3$  subunits of human sGC (later confirmed to be  $\alpha_1$  and  $\beta_1$  sGC genes; ref. 20) were colocalized to human chromosome 4 at q31.1-q33, which represents some discrepancy with recent results. However, previously reported mapping of the chromosomal position for  $\alpha_1$  and  $\beta_1$  of sGC were made by using cDNA for corresponding isoforms as probes for the localization (35). Considering the high level of homology between coding regions of both sGC subunits in various isoforms and, in addition, the existence of regions of extensive homologies (in the catalytic domain, for example) to the particulate guanylyl cyclase family, the use of cDNA for chromosomal localization could result in ambiguities and errors.

The transcriptional regulation of the expression of sGC has not been previously examined. Recently, evidence to support altered expression of mRNA of sGC subunits has emerged. In primary rat pulmonary artery smooth muscle cells, prolonged NO treatment leads to decreased NO-stimulated sGC activity and mRNA levels (28). sGC levels rise in unborn rat pulmonary artery, beginning at approximately 20 days of gestation, and mRNA, protein, and activity remain elevated at least 8 days after birth (30). Decreased rates of sGC transcription also have been suggested in other models after NO treatment and administration of cAMP-elevating agents (24, 25). Furthermore, nerve growth factor administration to rat PC-12 cells results in decreased steady-state levels of sGC  $\alpha_1$  and  $\beta_1$  mRNA. In a related study in our laboratory, we found that estrogen treatment decreases  $\alpha_1$  and  $\beta_1$  sGC mRNA levels in rat uterus (to be reported elsewhere). The precise mechanisms underlying these effects on sGC in specific tissues are largely unknown. Our findings on the activity of putative promoter regions demonstrate different transcriptional activity for both subunits, suggesting the potential for finely tuned regulation. These data represent the initial studies on the transcriptional regulation of mouse sGC genes and will be continued in our laboratory. Considering the increased interest in sGC function and multiple reports concerning the functional significance of the splice variants for both subunits (ref. 22 and references therein), our results should help to gain insights on the genetic basis of regulation of this important enzyme.

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