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Molecular dissection of mouse soluble guanylyl cyclase α_1 promoter

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Abstract

Soluble guanylyl cyclase (sGC) is the only known receptor for nitric oxide (NO) and is downregulated in aging and hypertension. Little is known about sGC gene transcriptional regulation. In order to characterize the sGC transcriptional system, we cloned and sequenced the 5′ flanking region of mouse sGC α_1 gene (AY116663). Structurally, it is a non-canonical TATA-less promoter that we mapped to chromosome 3 with many putative regulation sites for Sp-1, NF-κB, and AP-1 transcription factors amongst others, and two $(TG:CA)_n$ dinucleotide microsatellites near the transcriptional start point. The cloned upstream sequence produced a 5-fold increase in luciferase activity in Cos7, HeLa, NIH3T3, and 293 cells as well as in mouse VSMC-like kidney mesangial cells. In the latter cell type, we showed that sGC α_1 promoter activity was dependent on the presence of its 5′ unstranslated region (5′UTR).

Keywords: Soluble guanylyl cyclase; Promoter; Dinucleotide microsatellites; Transcriptional regulation; UTR regions

Nitric oxide (NO), one of the smallest and most ubiquitous signaling molecules, is involved in a number of physiological and pathophysiological reactions [1,2]. The most important pathway whereby NO modifies cell physiology is through activation of sGC and subsequent induction of cGMP production, which in turn activates cGMP-dependent mechanisms (i.e., ion channels and kinases).

The most abundant form of sGC is a heterodimer composed of two subunits α and β of which four types exist (α_1, β_1, α_2, and β_2) [3]. Structurally, each subunit has a C-terminal cyclase catalytic domain, a central dimerization region, and an N-terminal heme-receptor. This last portion constitutes the NO-binding domain and represents the least conserved region of the protein [4]. Cloning and expression experiments have demonstrated that both α and β subunits are required for sGC activity [5,6]. In their homodimer form, both α and β subunits are inactive [7]. Soluble GC has been considered as a cytosolic receptor for many years; nevertheless, Zabel et al. [8] have recently shown that sGC in its active form associates with the plasma membrane when intracellular Ca^{2+} levels increase.

In the last decade, studies have elucidated the localization and structure of the genes coding for sGC subunits. The chromosomal location of sGC genes has been determined in rat [9], mouse [10], and humans [11]. In all cases, subunits α_1 and β_1 are co-localized on the same locus, and α_2 and β_2 lie on separate chromosomes. How sGC gene expression is modulated under certain conditions and disease states such as hypoxia [12], hypertension [13–15], inflammatory responses [16], and aging [13,17] is still unexplored. Only recently Sharina et al. [18] have shown a detailed description of the human sGC β_1 promoter. In this report for the first time, the regulatory sequences for the sGC α_1 expression were scrutinized. Therefore, we cloned and characterized the mouse sGC α_1 promoter region and the promoter activity in vascular smooth muscle-like kidney mesangial (GM6) cells and other cell lines. In addition, the present study presents an in silico analysis of potential

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transcription factors binding sites, which may be involved in the control sGC gene expression. An evolutionary comparison of mouse sGC promoter with its rat and human orthologs is also presented.

Materials and methods

DNA recombinant and molecular biology techniques. Recombinant DNA techniques were performed according to Sambrook et al. [19]. Most of the enzymes were purchased from NE Biolabs, MA. Competent DH5 α *Escherichia coli* cells were obtained from Invitrogene, CA. pGL-3 promoterless vector (Promega, Madison, WI) was used for promoter characterizations. TOPO TA cloning kit was used for PCR product cloning. Sequencing was performed at the University of Miami DNA core Facility using an ABI PRISMA sequencer.

Cell culture. Gm6 mesangial cell line was a kind gift from Dr. Alessia Fornoni (University of Miami) and used between passages 15 and 22 for transfection experiments. All cell lines including NIH3T3, Cos7, and HeLa were cultured in complete medium (Dulbecco's modified Eagle's medium supplemented with 20% F12, 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin) at 37 °C and 5% CO_2. Media and reagents were purchased from Invitrogen Life Technologies, CA.

Cloning of the sGC α_1 promoter. The upstream region of sGC α_1 gene and part of its 5′UTR (168 bp) were amplified with the primers 5′GTCAGTGTCAGACCTGAAGATGCTG3′ and 5′ CATGATGC GATCACAGGAGGC3′ using genomic DNA from 4-month-old C57BL/6 mouse aortas as a template [10]. PCR was carried out with HiFi Platinum *Taq* polymerase (Invitrogene Life Technologies, CA) and 50 pmol of each primer, with an annealing temperature of 61 °C and 35 cycles. The 1614-bp amplicon was size fractionated on a 1% agarose gel, excised, and directly inserted into the TOPO 2.1 cloning vector (Invitrogen Life Technology, CA). Proper insertion and orientation were verified by direct sequencing from both directions before cloning into the *Kpn*I/*Eco*RI sites of pGL3 basic vector (Promega, MA) containing the luciferase gene (luc), termed pCTS 1614.

Generation of recombinant plasmids using portions of the sGC α_1 promoter and its 5′UTR. A set of genetic constructs was made by combining portions of the cloned sGC upstream region with or without its 5′UTR to the luciferase gene. The sGC α_1 5′UTR region was isolated from mouse aortic total RNA by One Step RT-PCR (Invitrogen Life Technology, CA) with the following primers: 5′CTCAG AGCCGCGGGTTTCTCACACACCGCC3′ and 5′CCTGCAGACC ATGGTGTTCCTGGAGCTTGGCTGG3′ (annealing temperature at 58 °C, 35 cycles). The resulting 277-bp fragment containing the sGCα 5′UTR was cloned into pCR 2.1 and subsequently sequenced before further insertion into *Sac*I/*Nco*I sites of pGL-3 to generate pCTS-UTR. Meanwhile, the 1518-bp *Kpn*I/*Sac*II fragment containing the upstream region of sGC α_1 gene was transferred to pBlueScrip SK (+) (pBS-A1) to facilitate further cloning steps. Then, a *Kpn*I/*Sac*I region from pBS-A1 was introduced into pGL-UTR to yield pCTS 1715. Finally, pCTS 1453 was made by insertion of the 1518-bp fragment from pBS-A1 into the *Kpn*I/*Sma*I pGL-3 vector.

Co-transfections. Transient co-transfection was performed using the Lipofectamine 2000 (Invitrogene Life Technologies, CA) according to manufacturer's instructions. Briefly, 20,000 cells were seeded onto 24-well plates one day before transfection. Complete medium was removed, and cells were washed twice with PBS and then incubated in 0.5 ml of serum-free transfection medium containing 1 μg of the respective promoter-luc constructs, and 0.5 μg of pSV-β-galactosidase control vector (Promega, Madison, WI), which served as a control for transfection efficiency. After 2 h, 1 ml of complete medium was added and then cultured for an additional 48 h. All transfections were carried out in triplicate and the experiments were independently repeated at least three times.

Reporter gene assay. Determination of the luciferase (luc) and β-galactosidase (β-gal) enzymatic activities was performed on supernatants of lysed cells in Reporter Lysis Buffer (Promega, Madison, WI). Luc activity was measured using the luc assay system according to manufacturer's instructions (Promega, Madison, WI). The transfection efficiency was evaluated by determination of the β-gal activity using Galactosidase Enzyme Assay Systems (Promega, Madison, WI) according to the instruction manual. Data are presented as means of triplicates with variations among replicates less than 5%.

Bioinformatics. Vector NTI (Informax, Bethesda, USA) was used with computational DNA sequence analysis and cloning strategies. Contig assembly of the sGC α_1 sequences was conducted with Contig Express (Informax, Bethesda, USA) and BLAST was used to identify and retrieve orthologous sGC α_1 promoters from mouse, rat, and human genomes from project *Ensembl* at The Wellcome trust Sanger Institute (www.ensembl.org). The sGC α_1 upstream sequence was analyzed for the presence of repeat regions and transposable elements, using Repeat Masker (Smit and Green http://repeatmasker.genome.washington.edu/cgi-bin/RepeatMasker). Confirmation of Pol II promoter and recognition of the transcriptional start site were conducted with Promoter Scan II and Neural Network Promoter Prediction (Reese MG, Harris NL, Eeckam FH http://www.fruitfly.org/seq_tools/promoter.html). In silico analysis for transcription factor (TF)-binding sites was performed with MatInspector professional and Transfact 4 matrixes [20]. In addition to the mouse, human, and rat sGC α_1 promoters were compared for TF binding sites. The curves of levels of identity were calculated and plotted by VISTA computer package [21] for comparative genomic studies.

Results

Cloning the 5′-flanking region of sGC α_1 and analysis of the putative transcription factor binding sites

In order to characterize the sGC α_1 transcriptional system, we sequenced the 5′-flanking region of sGC α_1 gene and its first exon (AY116663, see Fig. 1). The transcriptional start site was located at the 1434-bp by pairwise blasting of sGC α_1 5′-flanking region with the complete mRNA sequence (AF297082) cloned by Sharina et al. [10] using RACE-PCR. Blasting the sequence of sGC α_1 upstream region into the *Ensembl* mouse genome, we found that sGC α_1 gene was located on chromosome 3 adjacent to the sGC β_1 subunit gene, which was confirmed using the NCBI mouse genome database. Scanning the sequence with the Novel Neural Network Algorithms for Improved Eukaryotic Promoter Site Recognition did not reveal any TATA box in close proximity to the transcription initiation site. Moreover, there were neither obvious consensus initiator elements nor downstream promoter elements. Using Repeat Masker, we were able to identify two dinucleotide $(TG:CA)_n$ microsatellite repeats that we codenamed MCI and MCII.

MatInspector with Transfact 4 matrixes program was used to identify putative transcription factor binding sites in the 5′ flanking region shown in Fig. 1. Seven GC boxes for Sp1 and two sites for NF-κB were identified. GC boxes serve to modulate basal transcription of the core promoter and operate as enhancer sequences. We have also identified consensus sequences for c-Est-1,

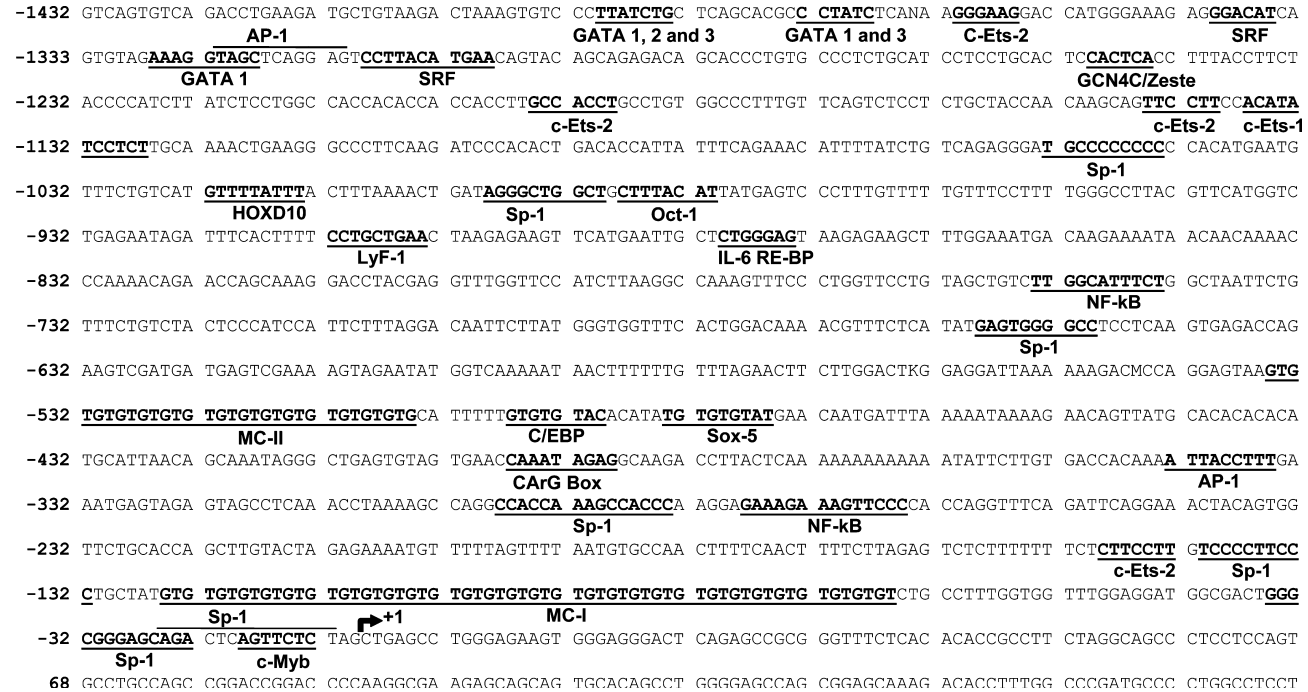

Fig. 1. Genomic nucleotide sequence of the 5′ flanking region of the mouse sGC α_1 gene. Two $(TG:CA)_n$ dinucleotide microsatellites (MCI and MCII) and putative transcription factor binding sites are underlined. The transcription initiation site is indicated with an arrow and designated as +1.

Ap-1, and GATA 1 among others. Comparison of the 5′ flanking sequence of sGC α_1 promoter with its rat and human orthologous sequences showed regions with some nucleotide homology within the first −1700 bp of the sGC α_1 genes (Fig. 2). Another area of conserved sequences was located between −2000 and −2500 bp. Global alignments showed a 56% degree of homology between mouse and rat sequences, but only a 42% between mouse and human. Rat sequence was closer to humans with a 45% of homology. Detection of common transcription factor binding sites between human and rodent sequences using rVISTA analysis revealed 15 conserved motifs (Fig. 3). All these transcription factor binding sites were found at nearby position in all analyzed species and are follows: ATPlal regulatory element protein 6; CCAAT/enhancer-binding protein α; c-ets-1; CRX; general initiation sequences 2 and b; c-Myb; paired box gene 2; protein inhibitor of activated stat y2, 5A, and 6; CCAAT/enhancer-binding protein β; engrailed 1; E1A-binding protein; and SRY-BOX 10.

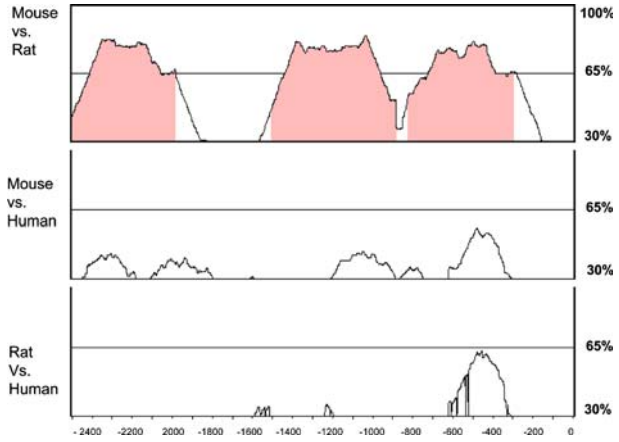

Fig. 2. Two-way nucleotide comparisons between mouse and rat, mouse and human, and rat and human sGC α_1 promoter regions. It is evident that nucleotide homology regions are being conserved in the first −1700 bp of the sGC α_1 genes. Another area of conservation sequences was located between −2000 and −2500 bp. Global alignments showed a 56% of homology between mouse and rat sequences, but only a 42% between mouse and human. Rat and human had 45% homology. The curves of levels of identity were calculated and plotted by VISTA computer package for comparative genomic studies. The window size was 50 bp with a resolution set at 30 bp. Highly conserved regions (above 65%) are highlighted (gray shadow).

Promoter activity of the 5′-flanking sequence of sGC α_1 gene

In order to test promoter activity, the 5′-flanking region of sGC α_1 gene was inserted into the pGL-3 vector upstream of the luciferase gene to yield pCT-1453. As compared to promoterless pGL-3 plasmid, the resulting construct increased luciferase activity 3.5 times in 293 cells, 7.7 times in HeLa cells, and 3.7 times in Cos7 cells. When pCT-1453 was introduced in NIH3T3 fibroblast and in Gm6 the luciferase activity increased 7.9 and 2.8 times, respectively (Fig. 4). Soluble GC α_1 promoter showed a moderate activity in both Cos7 and Gm6 cells when compared to CMV promoter-driven luciferase

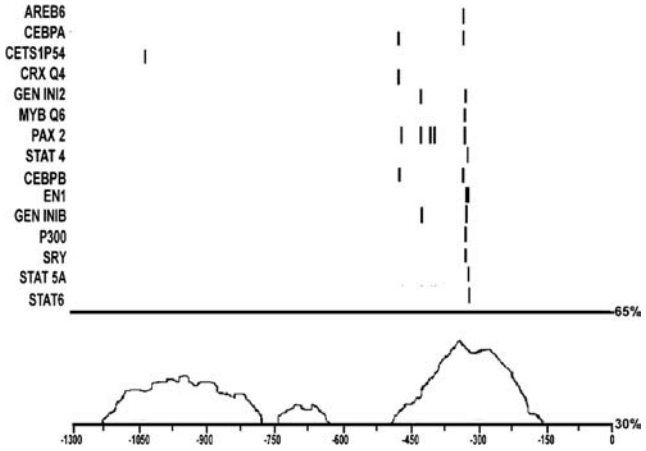

Fig. 3. Mouse and human rVISTA analysis for detection of conserved transcription factor binding sites in the sGC α_1 gene promoters. We found at nearby position the following group of conserved transcription factors binding sites: ATPlal regulatory element protein 6 (AREB6); CCAAT/enhancer-binding protein α (CEBPA); c-ets-1 (CETS1P54); CRX (CRX Q4); general initiation sequences 2 and b (GEN INI2 and GEN INIB); c-Myb (MYB Q6); paired box gene 2 (PAX2); protein inhibitor of activated stat y 2, 5A, and 6 (STAT 2, 5A, and 6); CCAAT/enhancer-binding protein β (CEBPB); engrailed 1 (EN1); E1A-binding protein (P300); and SRY-BOX 10 (SRY). Note that most of these putative sites lie within 200 bp segment of the core promoter. The upstream sequence for human promoter was obtained from *Ensembl* from the Wellcome Trust Sanger Institute (www.ensembl.org). Only the conserved transcription factor binding sites were represented. Core similarity used was 1 and matrix similarity >0.87.

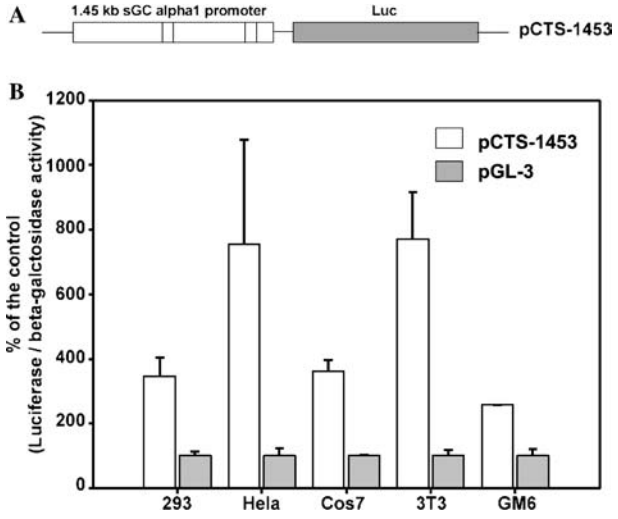

Fig. 4. Promoter activity of a 1.6-kb sGc α_1 promoter as depicted by driving luciferase reporter gene expression. (A) Genetic construct. (B) Promoter activity after transient transfection into different cell lines. The sGC α_1 promoter activity (open bars) and the activity of a promoterless control plasmid (pGL-3, filled bars) are shown. Note that the sGC α_1 promoter had greater activity in human cervix adenocarcinoma cells (HeLa) and in mouse fibroblast cell line (NIH3T3). This promoter was also active in human (293T), monkey (Cos-7), and mouse (GM6) cells. Bars represent the mean of three individual experiments ± SD. The pCTS-1453 plasmid was consistently co-transfected with pSV-β-galactosidase control vector for normalization purposes. Promoter activities in all experimental groups were significantly higher than the control plasmid (p values <0.01; two-tail t test for unequal variances).

activity (data not shown). Overall, these data clearly demonstrated the presence of sGC α_1 promoter within the cloned 1.6 kb DNA fragment.

Role of the 5′UTR sequence in the transcriptional modulation of sGC α_1 gene expression

To determine whether the sGC α_1 5′UTR sequence affects promoter function, we cloned, sequenced (AY170858), and generated a series of recombinant constructs based on the pGL-3 vector (as shown in Fig. 5): *pCTS-UTR*, 5′UTR region alone; *pCTS-1453*, promoter without 5′UTR; *pCTS-1614*, promoter with half 5′UTR; and *pCTS-1715*, promoter with the entire 5′UTR. The results showed that the promoter activity was dependent on the presence of 5′UTR region upstream of the luciferase gene. Interestingly, the 5′UTR diminished sGC α_1 gene promoter efficiency (1.6-fold less) in Cos7 cells, which could be explained by the absence of species and/or tissue specific regulatory factors (Fig. 5). In contrast, the same 5′UTR region enhanced promoter activity in Gm6 (3.3-fold) providing evidence that this region has a regulatory role in sGC α_1 gene expression. In the absence of other promoter sequences, this 5′UTR sequence alone showed little or no promoter activity in any of the assayed cells.

The similarity of this 5′UTR sequence with its human and rat homologs was assessed using ClustalW software for multisequence alignment. Mouse 5′UTR sequence (280 bp) was smaller than its rat (456 bp) and human (522 bp) orthologs. There was 52% similarity between mouse and rat and 31% similarity between mouse and human within this region. The similarity between mouse, rat, and human is higher in the regions closer to the translational ATG, while similarity is significantly lower at the start of the 5′UTR region (Fig. 6).

Discussion

So far, little has been known about the mechanisms involved in transcriptional regulation of the sGC genes in humans or other species. This is an original study to molecularly dissect the upstream region of the mouse sGC α_1 gene. Herein, we cloned 1.6 kb of the 5′ flanking region of sGC α_1 gene and its first exon, and the cloned DNA fragment was shown to have promoter activity in various cell lines.

This TATA-less promoter has no initiator consensus around the transcription start point or downstream promoter elements described elsewhere for other promoters [22]. However, it contains several putative

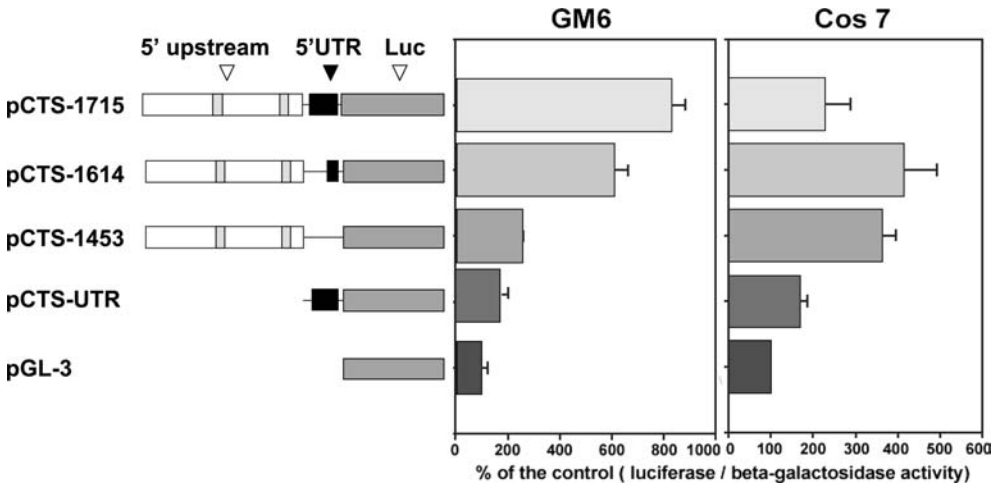

Fig. 5. Importance of the 5′UTR region on sGC α_1 promoter activity. Four different genetic constructs, with a full (pCTS-1715), half (pCTS-1615) or no (pCTS-1453) 5′UTR region, were made. This 5′UTR seems to increase luciferase activity in GM6 cells but not in Cos7. Bars represent the mean of three independent experiments ± SD.

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Rat       1 --------------------------GAATTCCGGATTACAGACACGTATGTCATATGGAT
Mouse     1 ------------------------------------------------------------
Human     1 CCCTTATGGCGATTGGGCGGCTGCAGAGACCAGGACTCAGTTCCCCTGCCCTAGTCTGAG

Rat      36 GTTGGAGATTTGAACTCAGGTCCTCATGTTTGGGTGGTAAGTGGTTCTAACCACTGAGCT
Mouse     1 ------------------------------------------------------------
Human    61 CCTAGTGGGTGGGACTCAGCTCAGAGTCAGTTTTCAGAAGCAGGTTTCAGTTGCAGAGTT

Rat      96 GTCTCACCAGTCTTCAGTGGGGTACCCACACTGACCACTCT--CCGCCCACTCCTGAAATG
Mouse     1 ------------------------------------------------------------
Human   121 TTCCTACACTTTTCCTGCGCTAGAGCAGCGAGCAGCCTGGAACAGACCCAGGCGGAGGAC

Rat     154 CCCTTTGTTCTCCCCATC-CCTACGCTATCTCTGAGAGCTCACCCCGATACCCTTCTGTT
Mouse     1 ------------------------------------------------------------
Human   181 ACCTGTGGGGGAGGGAGCGCCTGGAGGAGCTTAGAGA------CCCCAGCCGGGCGTGAT

Rat     213 -CTCACACA----CAGCCTT-CCAGGCG-TCTCTC----AG-TGCCTGCCAGCCG-----
Mouse    17 TCTCACACA----CCGCCTT-CTAGGCA-GCCCTCCTCCAG-TGCCTGCCAGCCGGA--C
Human   235 -CTCAC-CATGTGCGGATTTGCGAGGCGCGCCCTGG---AGCTGCTAGAGATCCGGAAGC

Rat     256 ---ACCCCAAGG----CGAAGG-AGCATGCAGTGCTCAGCCCGGGGAGTCCTCG-AAGCG
Mouse    68 CGGACCCCAAGG----CGAAG--AGCA-GCAGTGCACAGCCTGGGGAGCCAGCG-GAGCA
Human   290 ACAGCCCCGAGGTGTGCGAAGCCACCAAG-ACTGCG--GCTCTTGGAGAAAGCGTGAGCA

Rat     307 AAGA--CACC------TGCA-CCGGA-TGC-CCCTG--GCCTC--CCG--TGACCGCATC
Mouse   120 AAGA--CACC------TTTGGCCCGA-TGC-CCCTG--GCCTC--CTG--TGATCGCATC
Human   347 GGGGGCCACCGCGGTCTCCGGCCTGTCTGCACCCTGTCGCCTGAGCTGCCTGACAGTGAC

Rat     350 A-TGA-TGCTGGGCCACTCGTGTCTTTGAGTCAGTAGAAGCAGATCTTCATCAGTC-CAC
Mouse   164 A-TGGGTGCTGGGTCACTCCTGTCCTTGAGTCAGTAGAAGCAGATCTTCATCAGTC-CAC
Human   407 AATGACATCCCAGTTACCAGTGTCCTTGAATTGATAGTGGCTTCTGTTTGTCAGTCTCAT

Rat     407 ATCAACAC--CGGCTAATAAGGAGGAAACCACTGCC----AAGCTCCAGGAACACC----
Mouse   222 ATCAACAC--CAGCTAGTCAGAAGGAAGCCACTGCC----AAGCTCCAGGAACACCATGT
Human   467 ATAAGAACTACAGCTCATCAGGAGGAGATCGCAGCAGGGTAAGAGACACCAACACC----

Rat         --------
Mouse   276 TCTGCAGG
Human       --------
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Fig. 6. Comparison of the mouse sGC α_1 5′UTR sequence with its mouse and human homologs. Mouse 5′UTR sequence (280 bp) is smaller than its rat (456 bp) and human (522) orthologs. Within this region, there is a 52% similarity between mouse and rat and 31% between mouse and human. The similarity between mouse, rat, and human scores higher in the regions closer to the translational ATG. The alignment was created using ClustalW and Blosum 62 substitution matrix scoring, manually edited, and shadowed with BOXSHADE. The shading is to a 50% consensus, with the black boxes indicating invariant bases.

GC-rich boxes or motifs for Sp1 (a zinc finger transcription factor), known to play a role in regulation of genes lacking a functional TATA box and thus, we believe to be important for the promoter activity and regulation of this gene. An example of this is the human androgen receptor gene that has a TATA-less promoter

and in which Sp1 is an essential transcription factor that binds to a GC-box [23]. We described three Sp1 sites located within the core promoter of sGC α_1, bordering one of the two $(TG:CA)_n$ microsatellites, with the two first Sp1 motifs organized in tandem. Interestingly, we found that both $(TG:CA)_n$ repeats in the sGC 5′-flanking sequence are only at −28 and −538 bp from the transcription start point, which makes these microsatellites likely to participate in sGC gene transcription regulation. Dinucleotide repeats like the ones we identified in the sGC 5′-flanking region have the potential to generate alternative DNA structures such as Z-DNA, H-DNA, and cruciform DNA [24]. Dinucleotide repeats have been shown to also play the role of gene silencers or gene enhancers [24–27].

Although the expression of the sGC genes has been shown to be affected in hypertension [13–15], aging [13,17], inflammatory responses [16], and hypoxia [12], the regulation per se of these genes is poorly understood. Inflammation may indeed play a role in sGC α_1 gene regulation since we found several putative sites for NF-κB, AP-1, and IL-6 RE-BP in the 5′ flanking region. Furthermore, lipopolysaccharides were shown to modulate sGC levels and activity [16]. Those factors along with Sp1 could play a role in the regulation of sGC α_1 with advance in age through changes in nuclear redox environment as illustrated in aging and breast cancers [28].

The other interesting finding from the analysis of sGC α_1 upstream region was the presence of p300/CBP binding sites conserved between rodents and human. Those factors are multifunctional transcriptional co-activators that can influence different physiologic processes, including but not limited to: cell growth, proliferation, and differentiation [29]. This may infer a relationship between p300/CBP and the known functions of sGC in control of cell proliferation and differentiation. The presence of other motifs for transcription factors such as GATA-1, c-Est-2, and c-Myb suggests a developmental modulation of sGC α_1 from embryogenesis to senescence. This assumption is further supported by data whereby injection of Medaka fish embryos with antisense oligonucleotide specific for sGC α_1, α_2, and β_1 resulted in abnormalities in the central nervous system, including defects in the formation of forebrain, eye, and otic vesicles [30]. As of today and to our knowledge, there is not a single report on sGC gene knockout in vertebrates.

The comparison of the 2.4-kb mouse sGC α_1 promoter sequence in genome databases revealed a low degree of similarity between rodents and human. This evolutionary distance may indicate differential regulation of sGC α_1 transcription between species. Key regulatory elements of mouse, rat, and human sGC α_1 genes may lie between −600 and −300 bp since enough homology was found in this particular area. At a distance farther than −700 bp, the similarity among rat, mouse, and human sequences is almost totally lost.

In this report, we have also examined whether 5′UTR sequence contributes to the promoter activity that drives sGC α_1 gene activity. Indeed, our data clearly show that the promoter activity was dependent on the presence of 5′UTR region upstream of the reporter gene. On one hand, this 5′UTR sequence was able to reduce sGC α_1 gene promoter efficiency in Cos7 cells, which could be explained by species and/or tissue specific regulatory factors. On the other hand, the same 5′UTR region had some enhancer activity in mouse kidney mesangial cells (smooth muscle cell-like cells). The 5′UTR sequences in eukaryotic genes are known to affect both transcription and post-transcription mRNA processing. Indeed, these structures can adopt various conformations from simple linear DNA tail to complicated structures such as hairpin [31].

In summary, we presented herein the sequence and the analysis of the sGC α_1 promoter, and identify a number of putative transcription factor binding sites. We have shown that this promoter is transcriptionally active in mouse and in human cells alike. This is a primary step that will allow further scrutiny of key transcription sites and candidate transcription factors in sGC gene regulation. The identification of two dinucleotide microsatellite repeats may prove to be important since these microsatellites were shown to play a role as enhancers or repressors in other genes. Equally important is that these microsatellites may be targets for mutations under different metabolic conditions or aging [32].

References

[1] F. Murad, Nitric oxide signaling: would you believe that a simple free radical could be a second messenger, autacoid, paracrine substance, neurotransmitter, and hormone? Recent Prog. Horm. Res. 53 (1998) 43–59, discussion 59–60.
[2] K.L. Davis, E. Martin, I.V. Turko, F. Murad, Novel effects of nitric oxide, Annu. Rev. Pharmacol. Toxicol. 41 (2001) 203–236.
[3] S. Andreopoulos, A. Papapetropoulos, Molecular aspects of soluble guanylyl cyclase regulation, Gen. Pharmacol. 34 (2000) 147–157.
[4] J.W. Denninger, M.A. Marletta, Guanylate cyclase and the NO/cGMP signaling pathway, Biochim. Biophys. Acta 1411 (1999) 334–350.
[5] Y. Kamisaki, S. Saheki, M. Nakane, J.A. Palmieri, T. Kuno, B.Y. Chang, S.A. Waldman, F. Murad, Soluble guanylate cyclase from rat lung exists as a heterodimer, J. Biol. Chem. 261 (1986) 7236–7241.
[6] C. Harteneck, D. Koesling, A. Söling, G. Schultz, E. Böhme, Expression of soluble guanylate cyclase: catalytic activity requires two subunits, FEBS Lett. 272 (1990) 221–223.
[7] U. Zabel, C. Hausler, M. Weeger, H.H. Schmidt, Homodimerization of soluble guanylyl cyclase subunits: dimerization analysis using a glutathione *S*-transferase affinity tag, J. Biol. Chem. 274 (1999) 18149–18152.

[8] U. Zabel, C. Kleinschnitz, P. Oh, P. Nedvetsky, A. Smolenski, H. Muller, P. Kronich, P. Kugler, U. Walter, J.E. Schnitzer, H.H. Schmidt, Calcium-dependent membrane association sensitizes soluble guanylyl cyclase to nitric oxide, Nat. Cell Biol. 4 (2002) 307–311.

[9] M. Azam, G. Gupta, W. Chen, S. Wellington, D. Warburton, R.S. Danziger, Genetic mapping of soluble guanylyl cyclase genes: implications for linkage to blood pressure in the Dahl rat, Hypertension 32 (1998) 149–154.

[10] I.G. Sharina, J.S. Krumenacker, E. Martin, F. Murad, Genomic organization of alpha1 and beta1 subunits of the mammalian soluble guanylyl cyclase genes, Proc. Natl. Acad. Sci. USA 97 (2000) 10878–10883.

[11] G. Giuili, N. Roechel, U. Scholl, M.G. Mattei, G. Guellaen, Colocalization of the genes coding for the alpha 3 and beta 3 subunits of soluble guanylyl cyclase to human chromosome 4 at q31.3–q33, Hum. Genet. 91 (1993) 257–260.

[12] D. Li, N. Zhou, R.A. Johns, Soluble guanylate cyclase gene expression and localization in rat lung after exposure to hypoxia, Am. J. Physiol. 277 (1999) L841–L847.

[13] S. Kloss, A. Bouloumie, A. Mulsch, Aging and chronic hypertension decrease expression of rat aortic soluble guanylyl cyclase, Hypertension 35 (2000) 43–47.

[14] H. Ruetten, U. Zabel, W. Linz, H.H. Schmidt, Downregulation of soluble guanylyl cyclase in young and aging spontaneously hypertensive rats, Circ. Res. 85 (1999) 534–541.

[15] J.F. Ndisang, R. Wang, Age-related alterations in soluble guanylyl cyclase and cGMP pathway in spontaneously hypertensive rats, J. Hypertens. 21 (2003) 1117–1124.

[16] C.E. Pedraza, M.A. Baltrons, M.T. Heneka, A. Garcia, Interleukin-1beta and lipopolysaccharide decrease soluble guanylyl cyclase in brain cells: NO-independent destabilization of protein and NO-dependent decrease of mRNA, J. Neuroimmunol. 144 (2003) 80–90.

[17] C. Ibarra, P.I. Nedvetsky, M. Gerlach, P. Riederer, H.H. Schmidt, Regional and age-dependent expression of the nitric oxide receptor, soluble guanylyl cyclase, in the human brain, Brain Res. 907 (2001) 54–60.

[18] I.G. Sharina, E. Martin, A. Thomas, K.L. Uray, F. Murad, CCAAT-binding factor regulates expression of the beta1 subunit of soluble guanylyl cyclase gene in the BE2 human neuroblastoma cell line, Proc. Natl. Acad. Sci. USA 100 (2003) 11523–11528.

[19] J. Sambrook, E.F. Fritsh, T. Maniatis, Molecular Cloning. A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, USA, 1989.

[20] K. Quandt, K. Frech, H. Karas, E. Wingender, T. Werner, MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data, Nucleic Acids Res. 23 (1995) 4878–4884.

[21] G.G. Loots, I. Ovcharenko, L. Pachter, I. Dubchak, E.M. Rubin, rVista for comparative sequence-based discovery of functional transcription factor binding sites, Genome Res. 12 (2002) 832–839.

[22] T.W. Burke, J.T. Kadonaga, The downstream core promoter element, DPE, is conserved from *Drosophila* to humans and is recognized by TAFII60 of *Drosophila*, Genes Dev. 11 (1997) 3020–3031.

[23] P.W. Faber, H.C. van Rooij, H.J. Schipper, A.O. Brinkmann, J. Trapman, Two different, overlapping pathways of transcription initiation are active on the TATA-less human androgen receptor promoter. The role of Sp1, J. Biol. Chem. 268 (1993) 9296–9301.

[24] S. Rothenburg, F. Koch-Nolte, F. Haag, DNA methylation and Z-DNA formation as mediators of quantitative differences in the expression of alleles, Immunol. Rev. 184 (2001) 286–298.

[25] H. Funke-Kaiser, A. Thomas, J. Bremer, S.D. Kovacevic, K. Scheuch, J. Bolbrinker, S. Theis, J. Lemmer, A. Zimmermann, F.S. Zollmann, S.M. Herrmann, M. Paul, H.D. Orzechowski, Regulation of the major isoform of human endothelin-converting enzyme-1 by a strong housekeeping promoter modulated by polymorphic microsatellites, J. Hypertens. 21 (2003) 2111–2124.

[26] H. Hamada, M. Seidman, B.H. Howard, C.M. Gorman, Enhanced gene expression by the poly(dT–dG)·poly(dC–dA) sequence, Mol. Cell. Biol. 4 (1984) 2622–2630.

[27] Y.H. Chen, S.J. Lin, M.W. Lin, H.L. Tsai, S.S. Kuo, J.W. Chen, M.J. Charng, T.C. Wu, L.C. Chen, Y.A. Ding, W.H. Pan, Y.S. Jou, L.Y. Chau, Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients, Hum. Genet. 111 (2002) 1–8.

[28] J. Quong, S. Eppenberger-Castori, D. Moore III, G.K. Scott, M.J. Birrer, W. Kueng, U. Eppenberger, C.C. Benz, Age-dependent changes in breast cancer hormone receptors and oxidant stress markers, Breast Cancer Res. Treat. 76 (2002) 221–236.

[29] H.M. Chan, N.B. La Thangue, p300/CBP proteins: HATs for transcriptional bridges and scaffolds, J. Cell Sci. 114 (2001) 2363–2373.

[30] T. Yamamoto, Y. Yao, T. Harumi, N. Suzuki, Localization of the nitric oxide/cGMP signaling pathway-related genes and influences of morpholino knock-down of soluble guanylyl cyclase on medaka fish embryogenesis, Zool. Sci. 20 (2003) 181–191.

[31] J.P. Rodrigues, M. Rode, D. Gatfield, B.J. Blencowe, M. Carmo-Fonseca, E. Izaurralde, REF proteins mediate the export of spliced and unspliced mRNAs from the nucleus, Proc. Natl. Acad. Sci. USA 98 (2001) 1030–1035.

[32] N.A. Yamada, J.M. Parker, R.A. Farber, Mutation frequency analysis of mononucleotide and dinucleotide repeats after oxidative stress, Environ. Mol. Mutagen 42 (2003) 75–84.