BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **231,** 30–36 (1997)
ARTICLE NO. RC966031

Molecular Structure and Function of Rat CCAAT-Enhancer Binding Protein-Delta Gene Promoter

Tomikazu Fukuoka, Yutaka Kitami,[1] Katsuhiko Kohara, and Kunio Hiwada
The Second Department of Internal Medicine, Ehime University School of Medicine, Onsen-gun, Ehime 791-02, Japan

Received December 17, 1996

CCAAT-enhancer binding protein-delta (C/EBPδ) is a transcriptional nuclear factor, and belongs to basic region-leucine zipper class DNA binding proteins. One genomic clone containing a 12-kb sequence of the C/EBPδ gene was isolated from a rat genomic library, and a 2,056-bp fragment containing the 5′-flanking region was characterized. Sequence analysis of this fragment revealed that there were a TATA-like sequence (TAGAAAA) and many transcriptional regulatory elements. The transcription start site of the gene was determined by both primer extension analysis and riboprobe mapping. Both analyses indicated that the transcription start site was located at 31-bp downstream of the TATA-like sequence. Transient transfection experiments showed that the fragment cloned in this study was able to act as a functional promoter in rat vascular smooth muscle cells. The 5′-deletion analysis of this fragment revealed that the sequence spanning −235 through −82, which was designated as an upstream control element (UCE), remarkably increased a basal promoter activity of the C/EBPδ gene, and was also able to act as a promoter by itself. In addition, we also studied effects of the UCE on the heterologous gene promoter including rat α-actin gene promoter or SV40 virus promoter. Interestingly, the UCE specifically increased the promoter activity of the rat α-actin gene suggesting that the C/EBPδ gene may be positively controlled by the UCE via a cell-type or promoter-type specific manner.

CCAAT enhancer-binding protein (C/EBP) is a transcriptional nuclear factor, and belongs to basic region-leucine zipper class DNA binding proteins [1]. In mammalian species, C/EBP family consists of at least six unique members, C/EBPα, C/EBPβ (also known as LAP, NF-IL6, CRP2, and IL-6DPB), C/EBPδ (CELF, NF-IL-6β, and CRP3), C/EBPϵ (CRP1), C/EBPγ (Ig/EBP-1), and CHOP-10 (GADD153) [2-13]. Three members of this family, C/EBPα, C/EBPβ, and C/EBPδ, show a high level of amino acid homology within the DNA-binding domain [2]. Several studies suggest that C/EBPα and β are expressed highly in the liver, and play an important role in the establishment and maintenance of the cellular differentiation [14,15]. In contrast, C/EBPδ is expressed at an undetectable or minor level in normal tissues, and its expression is induced rapidly and drastically by treatment with lipopolysaccharide (LPS) or inflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor [11,16,17]. Therefore, C/EBPδ is thought to be an important factor to regulate the gene transcription of acute-phase reactive proteins such as third component of complement (C_3) gene, α_1-acid glycoprotein gene, and thiostatin gene [16,18].

Furthermore, we have previously reported that C/EBP family plays a main role in the transcriptional activity of the platelet-derived growth factor α-receptor gene (*pdgfr-α*) in cultured vascular smooth muscle cells (VSMC)[19]. Our preliminary study has also suggested that *pdgfr-α* transcription in VSMC is specifically induced by increasing C/EBPδ expression. Recently, another study has demonstrated that C/EBP family is an important transcriptional nuclear factor which controls NA^+/H^+ exchanger gene (*NHE-1*) expression in VSMC, and C/EBPδ can act as a main activator of *NHE-1* expression [20]. However, detailed regulatory mechanisms of the C/EBPδ gene in VSMC is unknown. In this study, we isolated the 5′-flanking region that contained a basic promoter region of the rat C/EBPδ gene, and analyzed its function to understand molecular mechanisms of C/EBPδ gene regulation in VSMC.

MATERIALS AND METHODS

Cloning of the rat C/EBPδ gene. A lambda DASH II rat genomic library (Stratagene, La Jolla, CA) obtained from testes of male Sprague-Dawley (SD) rats was screened by an EcoR I - BamH I fragment (~1.0 kb) of mouse C/EBPδ cDNA [2] as a probe. One

[1] To whom correspondence should be addressed. Fax: 81-89-964-1923. E-mail: kitamiyk@m.ehime-u.ac.jp.

The nucleotide sequence of the rat C/EBPδ gene reported in this paper has submitted to the GenBank with accession No. U44944.

0006-291X/97 $25.00

genomic clone containing a 12-kb sequence of the rat C/EBPδ gene was isolated, and a 2,056-bp genomic region that contained the fragment spanning −1,991 through +65 of the rat C/EBPδ gene was excised from this clone by double-digestion with Sac II and EcoR I. After conversion of the Sac II-end to a blunt end using T_4 DNA polymerase I (Takara, Tokyo), this fragment was subcloned into the Hinc II - EcoR I site of pBluescript II KS(+) vector (Stratagene). The resultant plasmid was designated as pKS-1,991, and an entire sequence of the inserted DNA fragment was determined on both strands.

Cell culture and RNA isolation. VSMC were isolated from the thoracic aorta of male SD rats by the method described previously [21]. For the isolation of RNA, cells were seeded in 100-mm dishes at a density of 1×10^6 cells per dish, and were cultured in Dulbecco's modified Eagle's medium with 10% heat-inactivated fetal calf serum at 37 °C in a humidified atmosphere of 95% air/5% CO_2. Isolation of total cellular RNA was carried out with cells at subconfluency using the method described previously [22]. Further purification of poly $(A)^+$ RNA was performed with oligo(dT)-Latex (Oligotex^{TM}-dT30 Super; Takara).

Primer extension analysis and riboprobe mapping. A reverse complement primer (5′-TGGGCCAGGGTGTGCCGCGTGCTGGG-3′) corresponding to the nucleotide position +30 to +55 of the C/EBPδ gene (Fig. 1) was synthesized for primer extension analysis. The 5′-end of the primer was labeled with [γ-^{32}P]ATP using T_4 polynucleotide kinase (Takara), and was hybridized with 2 μg of poly$(A)^+$ RNA isolated from VSMC or 2 μg of yeast tRNA. Primer extension was carried out with 50 U of avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI), and final products were analyzed on a 6% sequencing gel containing 8M urea. In addition, riboprobe mapping was also carried out to confirm the result obtained from primer extension analysis. For synthesis of a sense or an antisense RNA probe, pKS-235, which was one of 5′-deletions of pKS-1,991 (show plasmid construction of 5′-deletions used for promoter assays), was linearized by digestion with Xho I or Sac I, respectively. Using MAXIscript *In Vitro* Transcription Kit (Ambion Inc., Austin, TX), an [α-^{32}P]CTP-labeled sense (378 n.t.) or antisense (362 n.t.) probe was synthesized with T3 or T7 phage RNA polymerase, respectively. According to manufacture's specifications of RPA II Kit (Ambion Inc.), riboprobe mapping was carried out. Briefly, each labeled RNA probe was incubated with 2 μg of poly$(A)^+$ RNA for 3 min at 90 °C, and then was allowed to hybridize for 8 h at 42 °C. After digestion with RNase, digested samples were analyzed on a 6% sequencing gel containing 8M urea.

Plasmid construction of 5′-deletions used for promoter assays. Serial 5′-deletions of C/EBPδ gene promoter were prepared by using Discrete Delete Exo III-Mung Bean Nuclease Deletion Kit (Epicentre Biotech., Madison, WI). Briefly, pKS-1,991 was digested with Sac II and BamH I, and the linearized DNA was shortened by an exposure to exonuclease III for increasing length of reaction time. After digestion with mung bean nuclease, shortened fragments were ligated back into pBluescript KS(+). Six 5′-deletions, which had the nucleotide sequence starting at the position −1,298, −906, −509, −235, −82, or −55, were selected, and were designated as pKS-1,298, pKS-906, pKS-509, pKS-235, pKS-82, or pKS-55, respectively. After the inserted DNA fragment was excised from pKS-1,991 through pKS-55 by double-digestion with Xho I and Sac I, each fragment was ligated into the same restriction site of the promoterless firefly-luciferase expression vector, pGL3-Basic (pGLB) (Promega). Finally, resultant plasmids were designated as pGL-δ1 through pGL-δ7 as shown in Fig. 3. In addition, pGL3-Promoter (pGLP) (Promega) which has a SV40 virus promoter in front of a firefly-luciferase cDNA was used as a positive control vector.

Construction of α-actin gene promoter and upstream control element of C/EBPδ gene. Genomic region of the rat α-actin gene, which was able to act as a functional promoter in the rat smooth muscle cells [23], was cloned with PCR from male SD rat genomic DNA. A 553-bp sequence that contained the promoter region spanning −525 through + 28 [23] was amplified using specific primers (forward primer: 5′-GAAGATCTTCGAGAGCAGAGCAGAGGAATGCAG-3′, and reverse primer: 5′-CCCAAGCTTCTCCACCTGGGTGGGTGGT-GTC-3′). After digestion with Xho I and Hind III, the DNA fragment was subcloned into the same restriction site of pGLB, and the resultant plasmid was designated as pGLA. In addition, an upstream control element (UCE) of C/EBPδ gene promoter located between pGL-δ5 and pGL-δ6 was also amplified with PCR using specific primers (forward primer: 5′-CGGGGTACCAGGCTCAGACCGCTAAGT-AGG-3′, and reverse primer: 5′-CCGCTCGAGCGCGCACGCCCC-GCCCCGG-3′). The amplified PCR fragment was digested with Kpn I and Xho I, and was ligated into the same restriction site of pGLB, pGLA, and pGLP. Resultant plasmids were designated as pGLB(+), pGLA(+), and pGLP(+), respectively.

Transient transfection and luciferase assay. For the transfection experiment, VSMC were seeded in 60-mm dishes at a density of 5×10^5 cells per dish 24 h before transfection. Transient transfection was performed with cells at approximately 70% confluency by the method of DEAE-dextran described previously [19]. Five micrograms of promoter/firefly-luciferase cDNA fusion vectors were cotransfected into VSMC together with 5 μg of pRL-CMV vector (Promega), which has a cytomegalovirus promoter in front of a renilla-luciferase cDNA, and cells were incubated for an additional 48 h. Luciferase assays were carried out according to manufacture's specifications of Dual-Luciferase Reporter Assay System (Promega), and the activity of renilla-luciferase was used to normalize for variation in transfection efficiency. After sequential quantitation of firefly and renilla luciferase activities in cell lysates, promoter activity of each construct was finally expressed as firefly/renilla luciferase activity ratio.

RESULTS

Isolation of the 5′-flanking region of rat C/EBPδ gene and determination of the transcription start site. One genomic clone containing a 12-kb sequence of the rat C/EBPδ gene was isolated from approximately 1×10^6 clones of a rat genomic library. Southern blot and partial sequence analyses of the clone revealed that the rat C/EBPδ gene lacks introns, and has a single large exon. This exon contained an entire sequence of the open reading frame encoding a protein of 268 amino acids. Since a Sac II - EcoR I fragment of this clone contained a partial sequence of the exon (65 bp) and a 5′-flanking region (1,991 bp), an entire nucleotide sequence of this DNA fragment was determined. Southern blot analysis of SD rats genomic DNA using the Sac II - EcoR I fragment as a probe revealed that the clone obtained in this study was a single copy gene (data not shown). Fig. 1 shows a 2,056-bp genomic sequence of the rat C/EBPδ gene that contained the region spanning −1,991 through +65. The nucleotide sequence is numbered beginning with the first base (+1, marked by asterisk) of the initiator codon (ATG). To determine the transcription start site of the C/EBPδ gene, both primer extension analysis and riboprobe mapping were carried out. In Fig. 2(a), primer extension products from poly $(A)^+$ RNA isolated from VSMC (lane 2) were identified as a single major band (marked by arrow) corresponding to the guanine residue (G)

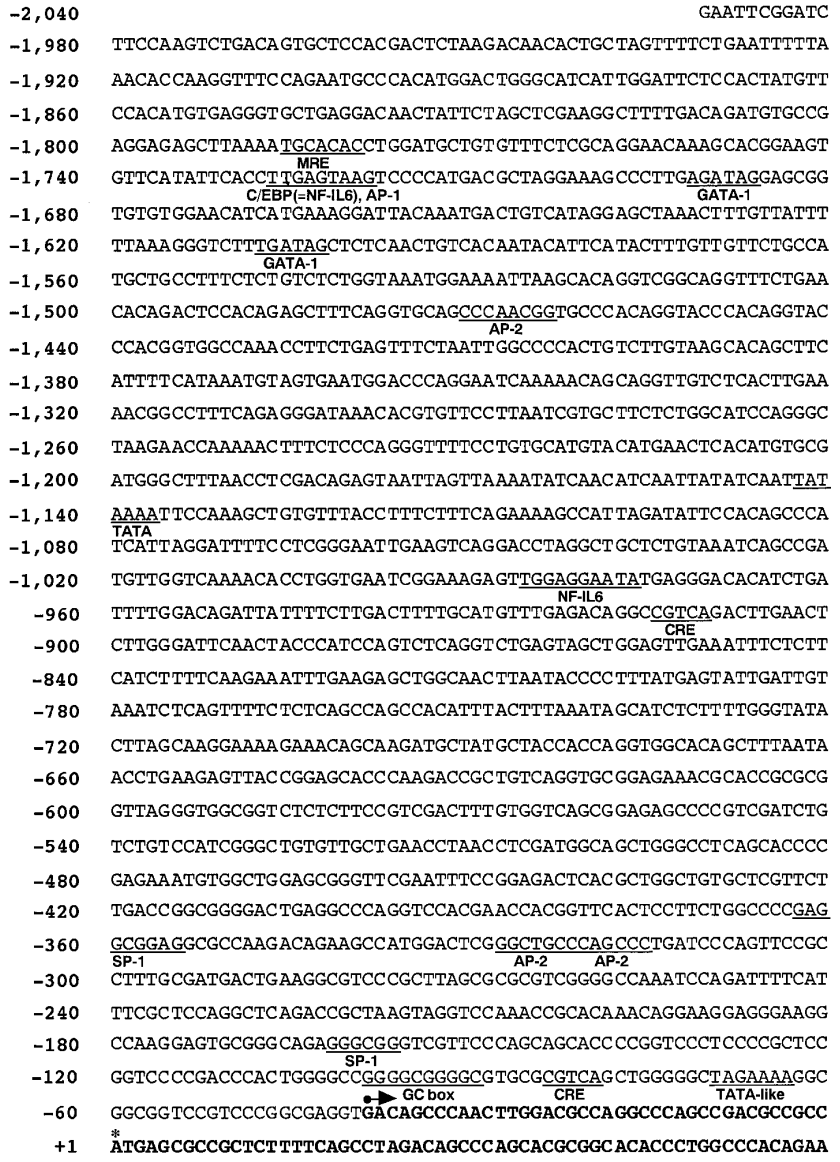

FIG. 1. The sequence of the promoter region and its upstream *cis*-acting elements for the rat C/EBPδ gene. The transcription start site determined by both primer extension analysis and riboprobe mapping is indicated by an arrow, and the exon is shown in bold-faced letters. The nucleotide sequence is numbered beginning with the first base (+1, marked by asterisk) of the initiator codon (ATG). Underlined sequences show notable regulatory *cis*-acting elements found in the 5′-flanking region of the C/EBPδ gene. TATA-like indicates a variant of TATA box; CRE, cAMP responsive element; SP-1, SP-1 element; AP-2, AP-2 enhancer element; NF-IL6, binding site for nuclear factor for IL-6; TATA, TATA box; GATA-1, GATA-1 box; C/EBP, enhancer core sequence for CCAAT-enhancer binding proteins; AP-1, protein kinase C-responsive element; and MRE, metallothionein responsive element.

which was located at 39 bp upstream of the initiator codon. To confirm the result obtained from primer extension analysis, riboprobe mapping was also performed. In Fig. 2(b), a 362-n.t. length of antisense RNA probe (lane 3) was protected from RNase, and migrated as a 104-n.t. length of band after hybridization with the poly(A)$^+$RNA isolated from VSMC (lane 4) in complete agreement with the result obtained from primer extension analysis.

Cis-acting elements in the 5′-flanking region of C/EBPδ gene. In particular with the 5′-flanking region of the rat C/EBPδ gene, a TATA-like sequence (TAGAAAA), which was a variant of TATA box, was found within a 40-bp upstream region of the transcription start site (Fig. 1). A computer-assisted search was performed for exact matches with well-defined transcriptional *cis*-acting elements. Notable sequences in the 5′-flanking region of the C/EBPδ gene that perfectly

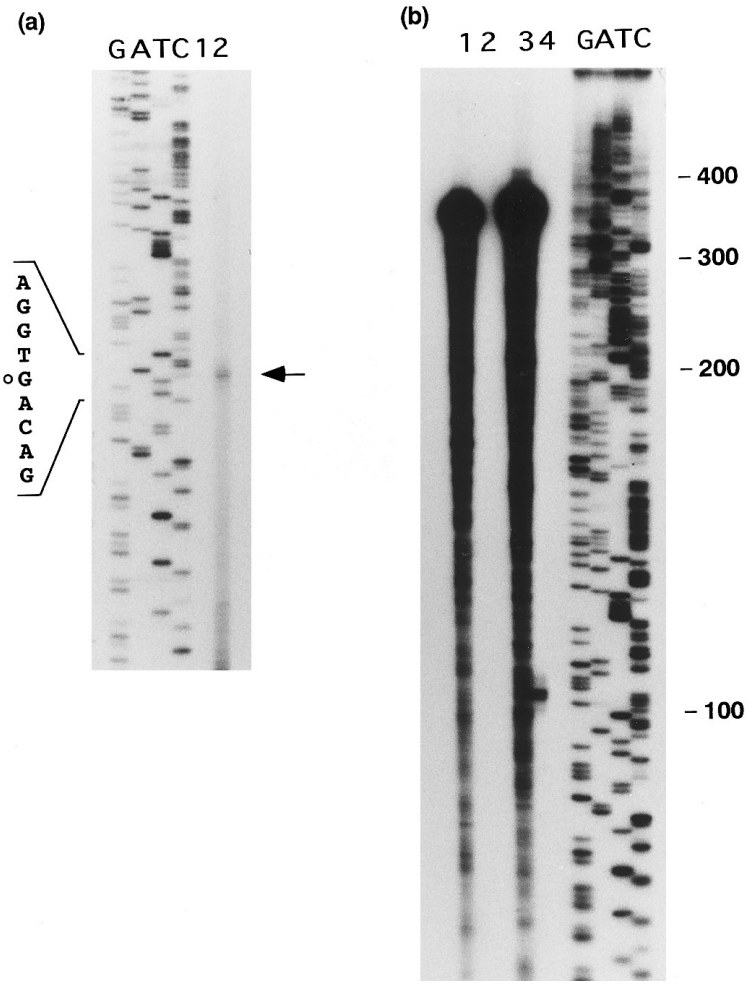

FIG. 2. Determination of the transcription start site of the rat C/EBPδ gene by primer extension analysis and riboprobe mapping. (a) Primer extension analysis. A 26-mer synthetic oligonucleotide primer corresponding to the nucleotide position +30 to +55 (in Fig. 1) was labeled with [γ-^{32}P]ATP, and hybridized with 2 μg of poly(A)$^+$RNA isolated from VSMC or 2 μg of yeast tRNA for 1 h at 58 °C. Primer extension was carried out with 50 U of avian myeloblastosis virus reverse transcriptase for 1 h at 42°C, and final products were resolved on a 6% polyacrylamide sequencing gel before autoradiography. Lanes G, A, T and C indicate sequence ladders obtained from pKS-1,991 as a double-stranded template DNA using the same primer; lane 1, primer extension from yeast tRNA; and lane 2, primer extension from poly(A)$^+$ RNA isolated from SD rats-derived VSMC. Arrow indicates the guanine residue (G) corresponding to a transcription start site of the C/EBPδ gene. (b) Riboprobe mapping. An [α-^{32}P]CTP-labeled sense or antisense probe was synthesized with T7 or T3 phage RNA polymerase, respectively. Each RNA probe was incubated with 2 μg of poly(A)$^+$ RNA isolated from VSMC for 5 min at 80 °C, and then allowed to hybridize at for overnight 58 °C. After digestion with RNase, digested samples were resolved on a 6% polyacrylamide sequencing gel before autoradiography. Lane 1, a 362-n.t. of sense RNA probe; lane 2, RNase-digested samples after hybridized with a sense RNA probe (none); lane 3, a 378-n.t. of antisense RNA probe; and lane 4, RNase-digested samples after hybridization with an antisense RNA probe (104 n.t.). Lanes G, A, T and C indicate unrelated sequence ladders used as a size marker.

match consensus sequences included two cAMP responsive elements (CRE) found at positions −84 and −915 bp; one GC box at −99 bp; two SP-1 elements at −162 and −363 bp; three AP-2 enhancer elements at −323, −328 and −1,471 bp; two binding sites for nuclear factor for IL-6 (NF-IL6) at −986 and −1,727 bp; one TATA box at −1,140 bp; two GATA-1 boxes at −1,608 and −1,693 bp; one enhancer core sequence for C/EBP which was overlapped with both NF-IL6 and protein kinase c-responsive element (AP-1) at −1,727 bp; and one metallothionein responsive element (MRE) at −1,786 bp, respectively.

Basal promoter activity of the 5′-flanking region of C/EBPδ gene. Transient transfection experiments were carried out to assess a basal promoter activity of the 5′-flanking region of the C/EBPδ gene. Promoter activity was measured by the ability to drive firefly-luciferase cDNA expression in VSMC derived from SD rats. To normalize for variation in transfection efficiency, firefly-luciferase activity was corrected by

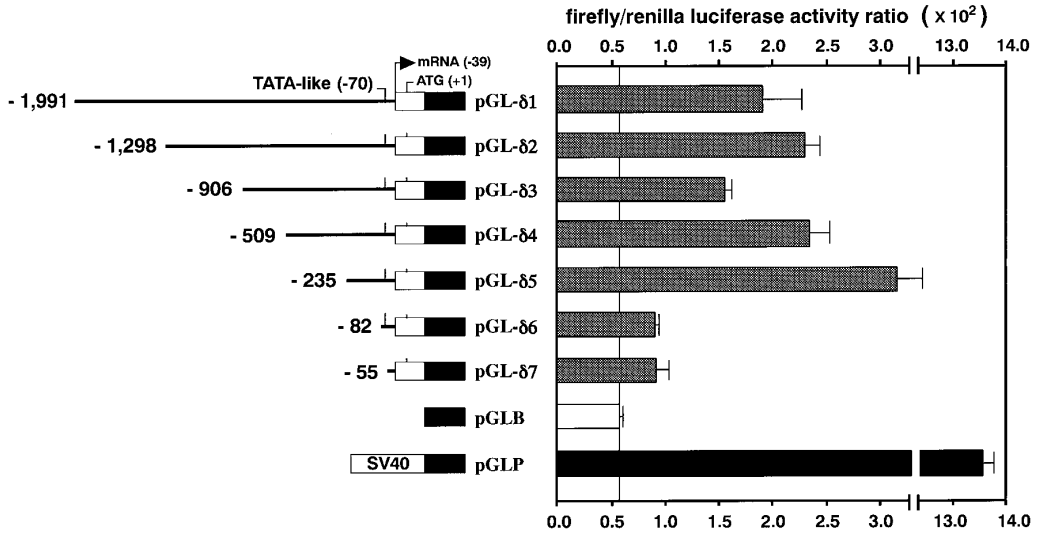

FIG. 3. Construction of C/EBPδ gene promoter/firefly-luciferase fusion vectors and their luciferase activities in VSMC derived from SD rats. Progressive 5′-deletions, pGL-δ1 through pGL-δ7 had nucleotide sequences starting at the position −1,991, −1,298, −906, −509, −235, −82, or −55 upstream from the first base of the initiator codon (ATG), respectively. Each luciferase construct was transiently cotransfected into VSMC derived from SD rats together with pRL-CMV vector using the method of DEAE-dextran. After 48 h, promoter activities of these constructs were assessed by their abilities to drive firefly-luciferase cDNA expression after normalizing with the renilla-luciferase activity. Finally, the promoter activity of each construct was expressed as firefly/renilla luciferase activity ratio. Data are expressed as means + SE of four separate assays.

renilla-luciferase activity, and then promoter activity was expressed as firefly/renilla luciferase activity ratio (Fig. 3). The longest construction of promoter/luciferase fusion vectors, pGL-δ1, consistently drove the luciferase activity to a level 3-fold higher over that seen in the promoterless construct, pGLB. In addition, promoter activities of six 5′-deletions, pGL-δ2 through pGL-δ7, were also determined by the luciferase assay. While 5′-deletions between pGL-δ2 through pGL-δ5 showed a significant promoter activity compared with pGLB, the promoter activity was drastically decreased between pGL-δ5 and pGL-δ6 to almost equal level with pGLB. On the other hand, the promoter activity did not change between pGL-δ6 and pGL-δ7.

Functional analysis of the region between pGL-δ5 and pGL-δ6. The 5′-deletion analysis indicates that a154-bp fragment between pGL-δ5 and pGL-δ6 (designated as a UCE) has an important role in the promoter activity of the rat C/EBPδ gene. To assess it's ability as a promoter or transcriptional regulatory element, this fragment was ligated into the promoterless luciferase vector or in front of the heterologous gene promoter (Fig. 4). The pGLB(+), which was inserted a UCE into pGLB, showed a remarkable promoter activity to a level 22-fold higher than that seen in pGLB. Moreover, pGLA(+), which had a UCE in front of a rat α-actin gene promoter of pGLA, showed a 5-fold increase in the promoter activity compared with pGLA. In contrast, pGLP(+), which had a UCE in front of a SV40 virus promoter of pGLP, did not show a remarkable change (only a 1.2-fold increase) in the promoter activity compared with pGLP.

DISCUSSION

Although genomic DNA sequences of mouse [6] and human [11] C/EBPδ genes have been also reported, 5′-flanking regions of these sequences were very limited (only a 90-bp region for mouse and a 120-bp region for human), and the determination of the transcription start site was just performed by primer extension analysis. In contrast, the 5′-flanking region of the rat C/EBPδ gene reported herein contained approximately 2,000-bp region upstream of the initiation codon, and its transcription start site was determined by both primer extension analysis and riboprobe mapping. Besides, we have also demonstrated that the 5′-flanking region of the gene contained several transcriptional *cis*-acting elements as shown in Fig. 1, and was actually active as a promoter in VSMC using the transient transfection experiment as shown in Fig. 3. Several studies have demonstrated that the expression of the C/EBPδ gene is regulated by treatment with LPS or inflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor [11,19,20]. Furthermore, studies based on the promoter analysis of the C/EBPα gene indicate that the C/EBPα gene is regulated by C/EBPα itself via an auto-regulatory pathway [24]. Present study has demonstrated that several responsive elements for LPS

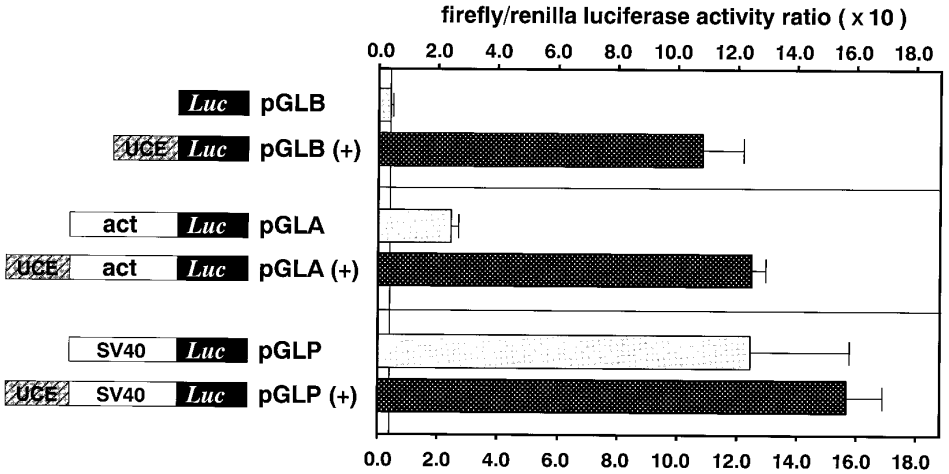

FIG. 4. Effects of a UCE of the C/EBPδ gene on the promoter activity of heterologous genes. A 553-bp sequence that contained the promoter region spanning −525 through +28 was subcloned into pGLB, and the resultant plasmid was designated as pGLA. A 154-bp fragment between pGL-δ5 and pGL-δ6, which was designated as a UCE, was ligated into pGLB or in front of the promoter of pGLA or pGLP. Resultant plasmids were designated as pGLB(+), pGLA(+), and pGLP(+), respectively. Act and SV40 indicate a 553-bp sequence of rat α-actin promoter and SV40 virus promoter, respectively. All constructs were transiently transfected into VSMC derived from SD rats, and the promoter activity was assessed in the same manner mentioned in Fig. 3. Data are expressed as means + SE of four separate assays.

or inflammatory cytokines were found in the 5′-flanking region of the rat C/EBPδ gene. Particularly, this region contained an enhancer core sequence for C/EBP at nucleotide position −1,727 bp, which is overlapped with a NF-IL6 (also known as a C/EBPβ) or a CRE, and a binding site for NF-IL6 at nucleotide position −986 bp, suggesting that C/EBPδ gene transcription may be regulated by C/EBPδ itself or other C/EBP members such as C/EBPα and β. However, more detailed studies are needed to clarify the regulatory network in the C/EBP family.

Sequence alignments of mouse and human C/EBPδ genes have revealed that a TATA-like sequence (TAGAAAA) was commonly found within 40-bp upstream of their transcription start sites. In the present study, we have demonstrated that the TATA-like sequence was also located at 31-bp upstream of the transcription start site of the rat C/EBPδ gene. This indicates that the TATA-like sequence is a common or conservative feature of the C/EBPδ gene, and is able to act as a core promoter for interaction with the RNA polymerase II transcription complex such as a TATA box. However, transient transfection experiments of 5′-deletions reported herein revealed that there was no significant difference in the promoter activity between pGL-δ6 and pGL-δ7 (Fig. 3), indicating that the promoter activity of the TATA-like sequence is very weak in VSMC, and that the sequence mainly acts as an initiator to determine the transcription start site of the C/EBPδ gene.

On the other hand, the promoter activity was drastically increased between pGL-δ5 and pGL-δ6, and the region between these constructs (designated as a UCE) contained GC-rich sequences including a GC box and a SP-1 element suggesting that this region may be interacted with the TATA-like sequence and modulate the basal promoter activity of the C/EBPδ gene. Moreover, in Fig. 4, pGLB(+) which had a UCE in front of a luciferase cDNA showed a remarkable promoter activity to a level 22-fold higher than that seen in pGLB. This indicates that the UCE spanning −235 through −82 is able to accelerate the basal promoter activity, and is also able to work as a strong promoter by itself. In addition, we have studied effects of the UCE on heterologous gene promoters including α-actin gene promoter and SV40 virus promoter using chimeric plasmid constructs. Genomic region of rat α-actin gene promoter used in this study was able to act as a functional promoter, and was specifically active in rat smooth muscle cells [23]. Interestingly, the UCE of the C/EBPδ gene was able to increase the promoter activity seen only in rat α-actin gene promoter (pGLA(+) vs. pGLA) but not in SV40 promoter (pGLP(+) vs. pGLP). These results suggest that the transcription of the C/EBPδ gene may be positively controlled by the UCE via a cell-type or promoter-type specific manner.

In conclusion, we isolated the 5′-flanking region of the rat C/EBPδ gene, and characterized its promoter activity in VSMC derived from SD rats. Structural analysis of the gene revealed that C/EBPδ promoter region had a TATA-like sequence and many regulatory *cis*-acting elements including a UCE. Functional promoter analysis indicates that the UCE spanning −235 through −82 is able to drive and accelerate a basal promoter activity of the C/EBPδ gene in VSMC.

ACKNOWLEDGMENTS

We thank Dr. Steven L. McKnight (Tularik Inc., South San Francisco, CA) for the generous gift of mouse C/EBPδ cDNA. This work was supported in part by a Grant-in-Aid of Scientific Research from the Ministry of Education, Science, Culture and Sports, Japan (No. 08457210, No. 08670797).

REFERENCES

1. Landschulz, W. H., Johnson, P. F., and McKnight, S. L. (1988) *Science* **240,** 1759–1764.
2. Cao, Z., Umek, R. M., and McKnight, S. L. (1991) *Genes & Dev.* **5,** 1538–1552.
3. Landschulz, W. H., Johnson, P. F., Adashi, E. Y., Graves, B. J., and McKnight, S. L. (1988) *Genes & Dev.* **2,** 786–800.
4. Descombes, P., Chojkier, M., Lichtsteiner, S., Falvey, E., and Schibler, U. (1990) *Genes & Dev.* **4,** 1541–1551.
5. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T., and Kishimoto, T. (1990) *EMBO J.* **9,** 1897–1906.
6. Williams, S. C., Cantwell, C. A., and Johnson, P. F. (1991) *Genes & Dev.* **5,** 1553–1567.
7. Poli, V., Mancini, F. P., and Cortese, R. (1990) *Cell* **63,** 643–653.
8. Chang, C.-J., Chen, T.-T., Lei, H.-Y., Chen, D.-S., and Lee, S.-C. (1990) *Mol. Cell. Biol.* **10,** 6642–6653.
9. Roman, C., Platero, J. S., Shuman, J., and Calame, K. (1990) *Genes & Dev.* **4,** 1404–1415.
10. Kageyama, R., Sasai, Y., and Nakanishi, S. (1991) *J. Biol. Chem.* **266,** 15525–15531.
11. Kinoshita, S., Akira, S., and Kishimoto, T. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1473–1476.
12. Ron, D., and Habener, J. F. (1992) *Genes & Dev.* **6,** 439–453.
13. Cleutjens, C. B. J. M., van Eekelen, C. C. E. M., van Dekken, H., Smit, E. M. E., Hagemeijer, A., Wagner, M. J., Wells, D. E., and Trapman, J. (1993) *Genomics* **16,** 520–523.
14. Lin, F.-T., MacDougald, O. A., Diehl, A. M., and Lane, M. D. (1993) *Proc. Natl. Acad. Sci. USA.* **90,** 9606–9610.
15. Friedman, A. D., and McKnight, S. L. (1990) *Genes & Dev.* **4,** 1416–1426.
16. Juan, T. S.-C., Wilson, D. R., Wilde, M. D., and Darlington, G. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2584–2588.
17. Alam, T., An, M. R., and Papaconstantinou, J. (1992) *J. Biol. Chem.* **267,** 5021–5024.
18. Baumann, H., Morella, K. K., Campos, S. P., Cao, Z., and Jahreis, G. P. (1992) *J. Biol. Chem.* **267,** 19744–19751.
19. Kitami, Y., Inui, H., Uno, S., and Inagami, T. (1995) *J. Clin. Invest.* **96,** 558–567.
20. Kolyada, A. Y., Johns, C. A., and Madias, N. E. (1995) *Am. J. Physiol.* **269,** C1408–C1416.
21. Inui, H., Kitami, Y., Kondo, T., and Inagami, T. (1993) *J. Biol. Chem.* **268,** 17045–17050.
22. Kitami, Y., Hiwada, K., and Kokubu, T. (1989) *J Hypertens* **7,** 727–731.
23. Blank, R. S., McQuinn, T. C., Yin, K. C., Thompson, M. M., Takeyasu, K., Schwartz, R. J., and Owens, G. K. (1992) *J. Biol. Chem.* **267,** 984–989.
24. Legraverend, C., Antonson, P., Flodby, P., and Xanthopoulos, K. G. (1993) *Nucl. Acid. Res.* **21,** 1735–1742.