Cell-specific Transcriptional Regulation of Human Leukotriene B₄ Receptor Gene

By Kazuhiko Kato,*§ Takehiko Yokomizo,**† Takashi Izumi,**† and Takao Shimizu*‡

From the *D department of Biochemistry and M olecular Biology, Faculty of M edicine, The U niversity of Tokyo, Tokyo 113-0033, Japan; **C ore R esearch for E volutional S cience and T echnology (C R EST), Japan S cience and T echnology C orporation, Tokyo 113-0033, Japan; and the ‡ P harmaceutical R esearch C enter, M ejii S eika K aisha, Limited, Yokohama 222-8567, Japan

Abstract

Leukotriene B₄ (LTB₄) is a lipid mediator that activates leukocytes and is involved in host defense and inflammation. BLT1, a high-affinity receptor for LTB₄ (originally termed BLT), is expressed exclusively in inflammatory cells and is inducible in macrophages upon activation. The mechanisms of tissue-specific expression and induction of BLT1 are important for the understanding of mechanism of onset and the potential treatment of inflammatory disorders. Here, we report the genomic structure and a promoter analysis of the human BLT1 gene, with an emphasis on the mechanism of cell-specific transcription. No TATA or CAAT elements exist around the transcription initiation sites, but a GC-rich sequence is observed in this region. A reporter gene assay revealed that a region ~80 basepair upstream from the initiator sequence is required for the basal transcription of the BLT1 gene. Sp1 was found to be a major activator of basal transcription by electrophoretic mobility shift assays and site-directed mutagenesis. The CpG sites of the BLT1 promoter region were highly methylated in BLT1-nonexpressing cells, but not methylated in BLT1-expressing cells. Further, methylation of this region in vitro inhibited the promoter activity to ~15% of the control. Thus, methylation at CpG sites in the promoter region is important for cell-specific transcription of the BLT1 gene. The promoter region of the BLT1 gene is localized within the open reading frame (ORF) of the BLT2 gene, which encodes a low-affinity receptor for LTB₄ (Yokomizo, T., K. Kato, K. Terawaki, T. Izumi, and T. Shimizu. 2000. J. Exp. Med. 192:421–431). To our knowledge, this is the first example of “promoter in ORF” in higher eukaryotes.

Key words: leukotriene B₄ receptor • inflammation • methylation • Sp1 • THP-1 cell

Introduction

Leukotriene B₄ (LTB₄),¹ a metabolite of arachidonic acid, is a potent lipid mediator. LTB₄ mainly activates leukocytes, leading to chemotaxis, degranulation, and production of superoxide anions, thus playing important roles in host defense (1, 2). LTB₄ is also related to inflammatory diseases such as rheumatoid arthritis (3), bronchial asthma (4), psoriasis (5), ulcerative colitis (6), and postischemic tissue injuries (7, 8). These actions of LTB₄ are mediated by a specific cell surface receptor, leukotriene B₄ receptor (BLT). Human BLT1 cDNAs were isolated (9, 10), and its orthologues were obtained from mouse (11, 12), rat (13), and guinea pig (14, 15). Human BLT1 mRNAs are expressed abundantly in leukocytes, and to a lesser degree in spleen and thymus. In mice, BLT1 mRNA is expressed in activated peritoneal macrophages in rats (13). Therefore, the transcription of the BLT1 gene appears to be tightly regulated in a cell- and tissue-specific manner, and is inducible by various stimuli. To elucidate the mechanism of the BLT1 expression, we analyzed the structure, promoter region, and regulation of cell-specific transcription of the human BLT1 gene. During the course of analysis of the BLT1 gene, we identified a putative open reading frame

Address correspondence to Takao Shimizu, Department of Biochemistry and M olecular Biology, Faculty of M edicine, The U niversity of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan. Phone: 81-3-3802-2925; Fax: 81-3-3813-8732; E-mail: tshimizu@m.u-tokyo.ac.jp.

Abbreviations used in this paper: EMSA, electrophoretic mobility shift assay; GPCR, G protein-coupled receptor; LTB₄, leukotriene B₄; ORF, open reading frame; RACE, rapid amplification of cDNA ends; UTR, untranslated region.
Materials and Methods

Cell Culture and Isolation of \( N \)ude Cells. THP-1 (human monocytic leukemia cell line), U937 (human histiocytic lymphoma cell line), and HL-60 (human leukemia cell line) cells were maintained in RPMI 1640 medium. HeLa (human cervical cancer cell line) and HepG2 (human hepatoma-derived cell line) cells were maintained in DME. All media were supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 \( \mu \)g/ml streptomycin. Poly(A)\(^+\) RNA was isolated using a QuickPrep 

PCR using the primer 3 (5\( \text{GAGCCGGGCAGGGCACATCCTCAGTACGAGG-3} \)) and the 5\( \text{AAACACCTAGTGAGG-3} \)) and 3\( \text{MS-1 (5\text{-GCCACCGTCGTGCACTAGTACGGGIAAAGATGTAGTGC-3} \text{relative to the adenosine of first methionine; see Fig. 2 A). PCR} \)

RNase extraction.

Isolation of the BLT1 Gene. A human lymphocytes genomic library (Strategene) was screened by plaque hybridization with the BLT1 ORF labeled with 32\( \text{P}\)dCTP. The positive genomic clone was subcloned into pBlueScript SK(−) and sequenced with an ABI 373 automated sequencer.

5’ Rapid Amplification of cDNA Ends (RACE). The plasmid, pRL-CMV (Promega; 0.1 \( \text{g of protein were in-}\)

cubated in 20 \( \text{mL of binding buffer (10 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM MgCl}\), 0.5 mM EDTA, 0.5 mM DTT, 50 \( \mu\text{g/mL poly(dI-dC) poly(dI-dC), and 4% glycerol)} \text{with or with-}\)

out a cold competitor (a 200-fold molar excess). For supershift assays, 1 \( \mu\text{g of anti-Sp1 antibody or rabbit IgG (Santa Cruz Bio-}\)

technology, Inc.) was incubated at room temperature for 10 min. The DNA probe (10,000 cpm) labeled with \( \gamma\text{-[32P]ATP} \) was added, and the samples were incubated at room temperature for 20 min. Reaction mixtures were separated in a 4% polyacryla-

mide gel and autoradiographed to an X-ray film.

Site-directed Mutagenesis. Mutagenesis of the putative Sp1 site in pl (−123/91) was introduced using a QuikChange™ Site-

directed Mutagenesis Kit (Stratagene) according to the manu-

facturer’s instructions. The primer used were MS-1 (5\( \text{-GCCTTG-GCGAAGCTGAAAGACAGCCGCGAGCCG-3} \), from −66 to −33 relative to the adenosine of the initiation mutant

sites are shown as bold letters in the primer sequence) and

MRS-1 (5\( \text{-CGCCTGCGCGGTCTGTTCTGCAGTTCCAGG-3} \), from −33 to −66).

Genomic Southern Blot Analysis. 10 \( \mu\text{g of genomic DNAs were digested by Hpal I or MspI, and then digested by EcoRI. Re-}\)

action mixtures were separated in 1% agarose gels and trans- 

ferred to nylon membranes (Amersham Pharmacia Biotech). The membranes were incubated with 32\( \text{P}\)dCTP labeled DNA probes (see Fig. 5 C) at 65°C overnight, and washed with 0.1× SSC, 0.1% SDS at 65°C. The washed membrane was autoradio-

graphed to an X-ray film.

Effect of Methylation at CpG Sites on the Promoter Activity. 20 \( \mu\text{g of the plasmid pl (−123/91) was digested by KpnI and HindIII, and the insert was purified from a 2% agarose gel. This fragment was in-}\)

culated with or without SssI methylase (6 U/\( \mu\)g DNA; N\text{ew England BioLabs, Inc.) at the presence of 16 \( \mu\)/M 5′-adenosylmethionine. After incubation at 37°C for 3 h, the DNA fragment was ethanol precipitated, followed by ligation to the pGL3 basic vector at 16°C for 16 h. Aliquots of the ligated DNA were ethanol precipitated and transfected into HeLa cells, and the luciferase assay was performed.

Results

Isolation of the BLT1 Gene.

10\(^6\) genomic clones were screened using the human BLT1 ORF as a probe, and we obtained one positive clone (LambdaNOK) containing the BLT1 gene. The map of this clone is shown in Fig. 1. The human BLT1 gene is 5.5 kb in length and consists of three exons. Most of the 5′ untranslated region (UTR) of either the HL-5 or HL-1 clone (10) is present on exon I and exon II, respectively. The BLT1 ORF is found on exon III, and is not interrupted by any introns. All the exon/intron junctions obeyed the rule of GT/AG consensus sequence (data not shown; 18).

5′ RACE Analysis. When nested PCR was performed using an oligonucleotide specific to the HL-5 sequence (primer 3; Fig. 2 A), several amplified fragments were observed (data not shown). Sequence analysis of these fragments revealed three transcription initiation sites (Fig. 2 B, asterisks). However, using a primer specific to the HL-1 clone, no specific fragment was observed (data not shown).

Nucleotide sequences of the putative promoter region are shown in Fig. 2 B. One of the transcription initiation sites is highly homologous to the initiator sequence
Neither a TATA box nor a CAAT box was observed near these transcription initiation sites, but a high GC content was observed in this region. A search for binding sites for transcription factors revealed consensus sequences for NFκB (20), USF (21), AP-1 (22), AP-4 (23), and Sp1 (reference 24; Fig. 2B).

**Promoter Activity and ORF in the Promoter Region.**

The promoter activity was determined by transfecting the 5′ flanking region–luciferase gene fusion plasmids to THP-1 and HeLa cells. THP-1 cells express BLT1, whereas HeLa cells do not (see Fig. 5D). Significant promoter activity was observed in both cell lines transfected with the constructs containing the region between 21091 and 276 relative to the adenosine residue of the initiator sequence (Fig. 3). This activity was decreased markedly when the region between 276 and 233 was deleted. No activity was detected using p(2491) in both cells. These results suggest that the region from 276 to 233 is crucial for the basal transcription of the BLT1 gene. Surprisingly, another ORF homologous to BLT1 overlapped the promoter region. This ORF was shown to encode the second receptor of LTB4, which is described in detail in an accompanying paper (16).

**Electrophoretic Mobility Shift Assays and Site-directed Mutagenesis.**

By luciferase assay, the region from 276 to 233 was found to be an important cis element in the basal transcription of the BLT1 gene. Between 276 and 233, there is a consensus sequence for Sp1 binding (Fig. 2B). Electrophoretic mobility shift assay (EMSA) was performed to determine whether Sp1 binds to this region. Several DNA-protein complexes were observed in nuclear extracts of both THP-1 and HeLa cells (Fig. 4A, lanes 2 and 8). The upper two bands disappeared when an excess of unlabeled 276–233 (a 200-fold molar excess) was added (Fig. 4A, lanes 3 and 9), showing that these bands are specific for this region. However, an excess of oligonucleotide mutated at this putative Sp1 binding site (5′-GAAACAG-3′ at -52 to -47; mutation sites are shown as bold letters) did not compete away the binding (Fig. 4A, lanes 4 and 10). Coincubation with anti-Sp1 antibody resulted in a supershift (Fig. 4A, lanes 6 and 12), but control IgG did not give this response (Fig. 4A, lanes 5 and 11). These results show that Sp1 binds to the BLT1 promoter region and activates the basal transcription.

**Methylation of CpG Sites in the Promoter Region.**

There are many CpG sites surrounding the promoter region of the BLT1 gene. However, an excess of oligonucleotide mutated at this putative Sp1 binding site (5′-GAAACAG-3′ at -52 to -47; mutation sites are shown as bold letters) did not compete away the binding (Fig. 4A, lanes 4 and 10). Coincubation with anti-Sp1 antibody resulted in a supershift (Fig. 4A, lanes 6 and 12), but control IgG did not give this response (Fig. 4A, lanes 5 and 11). These results show that Sp1 binds to the GC box at -52/-47 in the BLT1 promoter region. Moreover, a lower band was supershifted when an Sp3 antibody was coincubated (data not shown). To investigate the effect of Sp1 binding in the promoter activity, site-directed mutagenesis was performed. In both THP-1 and HeLa cells, the luciferase activity of the mutated construct was decreased to ~25% of the wild-type (Fig. 4B). These results indicate that Sp1 binds to the BLT1 promoter region and activates the basal transcription.

**Methylation of CpG Sites in the Promoter Region.**

There are many CpG sites surrounding the promoter region of the BLT1 gene. However, an excess of oligonucleotide mutated at this putative Sp1 binding site (5′-GAAACAG-3′ at -52 to -47; mutation sites are shown as bold letters) did not compete away the binding (Fig. 4A, lanes 4 and 10). Coincubation with anti-Sp1 antibody resulted in a supershift (Fig. 4A, lanes 6 and 12), but control IgG did not give this response (Fig. 4A, lanes 5 and 11). These results show that Sp1 binds to the GC box at -52/-47 in the BLT1 promoter region. Moreover, a lower band was supershifted when an Sp3 antibody was coincubated (data not shown). To investigate the effect of Sp1 binding in the promoter activity, site-directed mutagenesis was performed. In both THP-1 and HeLa cells, the luciferase activity of the mutated construct was decreased to ~25% of the wild-type (Fig. 4B). These results indicate that Sp1 binds to the BLT1 promoter region and activates the basal transcription.

**Methylation of CpG Sites in the Promoter Region.**

There are many CpG sites surrounding the promoter region of the BLT1 gene. However, an excess of oligonucleotide mutated at this putative Sp1 binding site (5′-GAAACAG-3′ at -52 to -47; mutation sites are shown as bold letters) did not compete away the binding (Fig. 4A, lanes 4 and 10). Coincubation with anti-Sp1 antibody resulted in a supershift (Fig. 4A, lanes 6 and 12), but control IgG did not give this response (Fig. 4A, lanes 5 and 11). These results show that Sp1 binds to the GC box at -52/-47 in the BLT1 promoter region. Moreover, a lower band was supershifted when an Sp3 antibody was coincubated (data not shown). To investigate the effect of Sp1 binding in the promoter activity, site-directed mutagenesis was performed. In both THP-1 and HeLa cells, the luciferase activity of the mutated construct was decreased to ~25% of the wild-type (Fig. 4B). These results indicate that Sp1 binds to the BLT1 promoter region and activates the basal transcription.
the BLT1 gene. To determine the methylation state at these CpG sites, Southern blotting was performed using genomic DNAs from various cell lines. Although both MspI and HpaII recognize and digest 5'-CCGG-3' sequences, MspI but not HpaII can cleave these sequences when the second cytosine residue is methylated. Capitalizing on this difference, methylation in the promoter region was detected. Using probe A, a 5' flanking region of the promoter, a band of 1.1 kb was detected by MspI digestion in all the cells examined (Fig. 5 A). However, this band of 1.1 kb was detected only in HL-60 and U937 cells by HpaII digestion, whereas a partial digested band of 2.5 or 5.5 kb was detected in HLa and THP-1 cells or HLa cells, respectively. In HLa and HepG2 cells, neither the 1.1 kb nor the 2.5 kb band was observed, whereas longer bands (~3.0 kb and 7.0 kb, respectively) were detected (Fig. 5 A). Similar results were observed in Southern blotting using probe B, a sequence 3' to the promoter region (Fig. 5 B). Considering the size of the detected bands, the region surrounding the BLT1 promoter is not methylated in U937 and THP-1 cells, and is almost completely methylated in HLa and HepG2 cells. In HL-60 cells (both

**Figure 3.** Serial deletion mutant analysis of the BLT1 promoter. Promoter activities are shown as the luciferase activity relative to that of the pGL3 basic vector (a promoterless vector). The activities of THP-1 cells (white bars) and HLa cells (black bars) are shown as the mean ± SD from three independent experiments performed in triplicate.

**Figure 4.** SPl binds and activates the BLT1 promoter. (A) EMSAs were performed with 32P-labeled −76/−33 using nuclear extracts prepared from THP-1 and HLa cells. Two DNA–protein complexes were detected in both cells (lanes 2 and 8; shown as b and c). They were competed by 200-fold molar excess of unlabeled −76/−33 WT, lanes 3 and 9, and not by 200-fold molar excess of unlabeled −76/−33 with mutations in the GC box (−52/−47) (lanes 4 and 10). The band b was supershifted by incubation with 1 μg of anti-Sp1 antibody (lanes 6 and 12; shown as a) but not by the control IgG (lanes 5 and 11). (B) The effect of mutagenesis of Sp1 binding site on the promoter activities. Luciferase activities of the wild-type construct, p(−123/−91) (white columns) and the construct mutated at GC box (black columns) are indicated as mean ± SD from three independent experiments performed in triplicate. Mutation sites are shown in bold at right.
differentiated and nondifferentiated), this region appears to be partially methylated. Northern blotting of these cell lines showed that HL-60, U937, and THP-1 cells express BLT1 mRNA, whereas HeLa and HepG2 cells do not (Fig. 5D). These results led us to the conclusion that methylation inhibits BLT1 transcription. Thus, the effect of methylation on the promoter activity was investigated. The insert of p(–123/+91) was treated with SssI methylase, which methylates cytosine residues at the CpG sites, and a luciferase assay was performed. The activity of methylated construct was decreased to ~15% of that of the unmethylated construct (Fig. 6), supporting the conclusion that methylation of the CpG sites inhibits the BLT1 promoter activity.

**Discussion**

LTB$_4$ is a potent activator of granulocytes, eosinophils, and macrophages. The biological activities of LTB$_4$ are comparable to those of IL-8 and FMLP, so-called “classical chemoattractants” (25). LTB$_4$ is unique because it is a lipid mediator biosynthesized from membrane phospholipids by the actions of phospholipase A$_2$ (26, 27), 5-lipoxygenase, and LTA$_4$ hydrolase (2, 28). The actions of LTB$_4$ are believed to be mediated by a specific cell surface receptor, BLT (29). Mice overexpressing human BLT1 (originally termed BLT) showed enhanced granulocyte accumulation in skin microabscesses and lungs after ischemia-reperfusion induced tissue injury (30), confirming the pathophysiological importance of LTB$_4$ in vivo.

![Figure 5](https://example.com/fig5.png)

**Figure 5.** Methylation of CpG sites in the BLT1 promoter region. (A and B) Genomic DNAs isolated from various cell lines were digested with HpaII or MspI, followed by digestion with EcoRI. The digested DNAs were electrophoresed in 1% agarose gels, transferred to nylon membranes, and blotted with (A) probe A and (B) probe B. The positions of the probes A and B are shown in C. HL-60 (RA) means HL-60 cells differentiated by 1 μM retinoic acid for 48 h. (C) Methylation sites and genomic organization. (D) Northern blotting of various cells for BLT1 ORF. 3 μg of poly(A)$^+$ RNA was used for each lane.

![Figure 6](https://example.com/fig6.png)

**Figure 6.** Effect of the methylation at CpG sites on the promoter activity. The insert of p(–123/+91) was incubated with or without SssI methylase, ligated into pGL3 basic vector, and transfected into HeLa cells. The relative luciferase activities are shown as mean ± SD from three independent experiments performed in triplicate.
The cDNA cloning of an LTB₄ receptor, BLT₁, showed that BLT₁ is a GPCR with seven transmembrane domains with few similarities to known receptors, including those for chemoattractants and prostanoids (10). We and others have successfully cloned cDNA and genomic DNAs of BLT₁ from other species, including mouse (11, 12), guinea pig (14, 15), and rat (13), and have shown that the primary structures of BLT₁ are conserved among species especially in the TM domains. BLT₁ isolated from various animals showed subnanomolar Kᵩ values for [³H]-LTB₄ in transfection studies, and the binding was inhibited by a number of specific BLT antagonists. Therefore, BLT₁ is a high-affinity receptor for LTB₄. The distribution of BLT₁ is restricted, with expression observed almost exclusively in peripheral leukocytes (10), (14), activated macrophages (13), and eosinophils (11).

In this manuscript, genomic structure, characterization of the promoter region, and mechanism of cell-specific transcription of the BLT₁ gene are reported. The BLT₁ gene consists of three exons, and the ORF is intronless like other receptors for chemoattractants such as FM LP (31), IL-8 (32), and platelet-activating factor (PAF [33]). Owman et al. (9) have shown that the human BLT₁ gene is localized in 1q11.2-q12. Three transcription initiation sites were detected by 5' RACE analysis when the primer specific to the 5' UTR of HL-5 clone was used, and these sites were scattered over 120 bp. There are no TATA or CAAT elements around these initiation sites, but one of initiation sites is highly homologous to the initiator sequence. The initiator sequence has been reported to play a role in transcription initiation of various genes (19, 33). No transcription initiation site was detected using the primer specific to the 5' UTR of HL-1 clone. Two human BLT₁ cDNAs, HL-1 and HL-5, were isolated, and were shown to share the same ORF, but different 5' UTRs (34). In Northern blotting of HL-60 and U937 cells, the probe containing the 5' UTR specific to HL-5 clone gave positive signals of ~1.6 kb and 3.0 kb (data not shown), which were similar to the results from the probe of the entire ORF (Fig. 5 D; reference 10). In contrast, the probe containing the 5' UTR specific to HL-1 clone gave no signals on the same membrane (data not shown), suggesting that HL-5 and not HL-1 is the major transcript of BLT₁, at least in HL-60 and U937 cells.

We next examined the BLT₁ promoter activity using the 5' flanking region--luciferase fusion plasmids. The results from various truncated mutants showed that the region between −76 and the initiator sequence is required for the basal transcription of the human BLT₁ gene. EMSA analysis revealed that Sp₁ binds to −52/−47 of the BLT₁ promoter. By introducing the mutation at this Sp₁ binding site in p(−123/+91), the promoter activity was decreased to ~25% of the wild-type. These results clearly indicate that Sp₁ is important for the basal transcription of the human BLT₁ gene. In the promoter of several myeloid-specific genes such as CD11b (35), CD18 (36), and hematopoietic cell kinase genes (37), Sp₁ was reported to act as a major activator. Although Sp₁ is a ubiquitous transcription factor, it is preferentially expressed in hematopoietic cells (38). It is still undetermined how Sp₁ mediates the tissue-specific transcription. In our study, significant basal promoter activity was observed in HeLa cells, which do not express BLT₁ intrinsically. EMSA showed that Sp₁ binds to the BLT₁ promoter using nuclear extracts from HeLa cells, and mutagenesis in this Sp₁ binding site abolished the promoter activity in HeLa cells, as observed in the case of THP-1 cells. Therefore, Sp₁ binding to the BLT₁ promoter clearly can not explain the tissue- and cell-specific expression of BLT₁. The promoter analysis of l-histidine decarboxylase (39) and leukosialin (CD43 [reference 40]) genes revealed that Sp₁ is a major activator for their transcription. The methylation of their promoter regions at CpG sites plays an important role in cell- and tissue-specific transcription. As the promoter region of BLT₁ gene has high CpG contents with Sp₁ as a major activator, we studied the methylation state of the region surrounding the BLT₁ promoter in BLT₁-expressing and nonexpressing cells.

Genomic Southern blotting was performed to investigate the methylation state in various cell lines. BLT₁-expressing cells showed the shorter positive bands by digestion with HpaII, which can cleave only unmethylated CCGG sequences. In contrast, HeLa and HepG2 cells, which do not express BLT₁, exhibited the longer digested bands by HpaII. This implies the significant correlation between the expression of BLT₁ and the methylation state. Next, we methylated the BLT₁ promoter construct p(−123/+91) by SssI methylase in vitro, and examined the effect of this methylation on the promoter activity in HeLa cells. The methylated construct showed only 15% promoter activity of the unmethylated construct. These results show that the cell-specific expression of BLT₁ is primarily dependent on the methylation of the promoter region. However, EMSA showed that Sp₁ binds similarly to methylated and unmethylated DNA probes (−76/−33) in THP-1 and HeLa cells (data not shown). Sp₁ is known to bind to the GC box in a methylation-insensitive manner (41). The methyl-CpG binding proteins MecP₁ (42) and MecP₂ (43), have been reported to interact specifically with methylated DNA and repress the transcription. These MecPs might play a role as a repressor in the BLT₁ transcription.

Furthermore, we found another ORF overlapping the promoter and 5' UTR of the BLT₁ gene. This gene has high homology with BLT₁ and encodes a novel GPCR. We have named this GPCR as BLT₂, and have reported the role as a low-affinity receptor for LTB₄ (16). In prokaryotes, the overlapping of the promoter and ORF has been reported (44). For example, in E. coli, the promoter of the b-lactamase gene (ampC) is located in the last structural gene of the fumarate reductase (frd) operon, and the ampC attenuator served as the terminator for transcription of the frd operon (45). In our case, the expression pattern of BLT₁ and BLT₂ is partially overlapped at tissue level, as observed in Northern blotting (10, 16). However, the biological significance of the overlapping of the promoter and ORF was not clarified. Further study should be
needed to demonstrate the significance of gene organization of two related receptors, BLT1 and BLT2. To our knowledge, this is the first example in mammals that the ORF localizes within the promoter region of another gene, so called “promoter in ORF.”

In conclusion, we have determined the genomic structure of the human BLT1 gene, and have also identified the transcription initiation sites and the promoter region of this gene. Sp1 binding to the promoter region was required for the basal transcription of the BLT1 gene. Although the precise mechanism of the tissue-specific transcription of the BLT1 gene is still not clear, we found that the CpG island of the BLT1 promoter is methylated in the cells that do not express BLT1, and also that this methylation inhibits BLT1 transcription in vitro. Our findings enhance the understanding of how genes are transcribed in a cell- and tissue-specific manner, and assist the understanding of the pathogenesis of inflammatory diseases in which LTB4 and LTB4 receptors are involved. In addition, the presence of “promoter in ORF” in the human genome promises to provide insights into how mammalian genes show flexibility and complexity in the transcriptional regulation.

We thank Drs. S. Hoshiko, F. Osawa, and Y. Akanatsu at Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd. for encouragement, and Drs. M. Taniguchi, N. Uozumi, S. Ishii, D.A. Wong, K. Takeyama, and S. Kato at the University of Tokyo for their discussions.

This work was supported in part by grants-in-aid from the Ministry of Education, Science, Sports, and Culture and the Human Science Foundation, and by grants from the Yamanouchi Foundation for Metabolic Disorders, the Uehara Memorial Foundation, and the Cell Science Research Foundation.

Submitted: 27 March 2000
Revised: 3 May 2000
Accepted: 18 April 2000

References

1. Ford-Hutchinson, A., M.V. Doig, M.E. Shipley, and M.J. Smith. 1980. Leukotriene B4, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature. 286:264–265.

2. Samuelsson, B., S.E. Dahlén, J.A. Lindgren, C.A. Rouzer, and C.N. Serhan. 1987. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. Science. 237:1171–1176.

3. Griffiths, R.J., E.R. Pettipher, K. Koch, C.A. Farrell, R. Breslow, M.J. Conklyn, M.A. Smith, B.C. Hackman, D.J. Wimberly, A.J. Milici, et al. 1995. Leukotriene B4 plays a critical role in the progression of collagen-induced arthritis. Proc. Natl. Acad. Sci. USA. 92:517–521.

4. Turner, C.R., R. Breslow, M.J. Conklyn, C.J. Andresen, D.K. Patterson, A.A. Lopez, B. Owens, P. Lee, J.W. Watson, and H.J. Showell. 1996. In vitro and in vivo effects of leukotriene B4 antagonism in a primate model of asthma. J. Clin. Invest. 97:381–387.

5. Iversen, L., K. Krågskår, and V.A. Ziboh. 1997. Significance of leukotriene-A4 hydrolase in the pathogenesis of psoriasis. Skin Pharmacol. 10:169–177.

6. Cole, A.T., B.J. Pilikington, J. McLaughlan, C. Smith, M. Balstis, and C.J. Hawkey. 1996. Mucoal factors inducing neutrophil movement in ulcerative colitis: the role of interleukin 8 and leukotriene B4. Gut. 39:248–254.

7. Zimmerman, B.J., D.J. Guillery, M.B. Grisham, T.S. Gaginella, and D.N. Granger. 1990. Role of leukotriene B4 in granulocyte infiltration into the postischemic feline intestine. Gastroenterology. 99:1358–1363.

8. Nöri, E., T. Yokomizo, A. Nakao, T. Izumi, T. Fujita, S. Kimmura, and T. Shimizu. 2000. An in vivo model showing the chemotactic activity of leukotriene B4 in acute renal ischemic-reperfusion injury. Proc. Natl. Acad. Sci. USA. 97:823–828.

9. O’Neill, C., C. Nilsson, and S.J. Lølø. 1996. Cloning of cDNA encoding a putative chemotactic receptor. Genomics. 37:187–194.

10. Yokomizo, T., T. Izumi, K. Chang, Y. Takuwa, and T. Shimizu. 1997. A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. Nature. 387:620–624.

11. Huang, W.W., E.A. Garcia-Zepeda, A. Sauty, H.C. Oettgen, M.E. Rothenberg, and A.D. Luster. 1998. Molecular and biological characterization of the murine leukotriene B4 receptor expressed on eosinophils. J. Exp. Med. 188:1063–1074.

12. Martin, V., P. Ronde, D. Unette, A. Wong, T.L. Hoffman, A.L. Edinger, R.W. Doms, and C.D. Funk. 1999. Leukotriene binding, signaling, and analysis of HIV coreceptor function in mouse and human leukotriene B4 receptor-transfected cells. J. Biol. Chem. 274:8597–8603.

13. Toda, A., T. Yokomizo, K. Maeda, A. Nakao, T. Izumi, and T. Shimizu. 1999. Cloning and characterization of rat leukotriene B4 receptor. Biochem. Biophys. Res. Commun. 262:806–812.

14. Maeda, K., T. Yokomizo, T. Izumi, and T. Shimizu. 1999. cDNA cloning and characterization of guinea-pig leukotriene B4 receptor. Biochem. J. 342:79–85.

15. Boie, Y., R. Stocco, N. Sawyer, M.G. Greig, S. Kargman, M.D. Slipetz, P.G. O’Neill, T. Shimizu, T. Yokomizo, M.K. Metters, and M. Abramovitz. 1999. Characterization of the cloned guinea pig leukotriene B4 receptor: comparison to its human orthologue. Eur. J. Pharmacol. 380:203–213.

16. Yokomizo, T., K. Kato, K. Terawaki, T. Izumi, and T. Shimizu. 2000. A second leukotriene B4 receptor, BLT2: a new therapeutic target in inflammation and immunological disorders. J. Exp. Med. 192:421–431.

17. Dignam, J.D., P.L. Martin, V. Ronde, D. Unette, A. Wong, T.L. Hoffman, A.L. Edinger, R.W. Doms, and C.D. Funk. 1999. Leukotriene binding, signaling, and analysis of HIV coreceptor function in mouse and human leukotriene B4 receptor-transfected cells. J. Biol. Chem. 274:8597–8603.

18. Gilmore, T.D. 1990. NF-κB, KBF1, dorsal, and related components. Genes Dev. 4:1730–1740.

19. Smale, S.T., and D. Baltimore. 1989. The “initiator” as a transcription control element. Proc. Natl. Acad. Sci. USA. 86:823–828.

20. Mermod, N., E.A. O’Neill, T.J. Kelly, and R. Tjian. 1989.
The proline-rich transcriptional activator of CTF/NF-I is distinct from the replication and DNA binding domain. C. el. 58:741–753.

24. Schmidt, M.C., Q. Zhou, and A.J. Berk. 1989. Sp1 activates transcription without enhancing DNA-binding activity of the TATA box factor. Mol. Cell. Biol. 9:3299–3307.

25. Klinker, J.F., K. Wenzen-Seifert, and R. Seifert. 1996. G-protein-coupled receptors in HL-60 human leukemia cells. Gen. Pharmacol. 27:33–54.

26. Bonventre, J.V., Z. Huang, M.R. Taheri, E. O'Leary, E. Li, M.A. Moskowitz, and A. Sapirstein. 1997. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. Nature. 390:622–625.

27. Uozumi, N., K. Kume, T. Nagase, N. Nakatani, S. Ishii, F. Tashiro, Y. Komagata, K. Maki, K. Ikuta, Y. Ouchi, et al. 1997. Role of cytosolic phospholipase A2 in allergic response and parturition. Nature. 390:618–622.

28. Serhan, C.N., J.Z. Haeggstrom, and C.C. Leslie. 1996. Lipid mediator networks in cell signaling: update and impact of cytokines. FASEB J. 10:1147–1158.

29. Alexander, S.P.H., and J.A. Peters. 1999. Receptor and ion channel nomenclature supplement. Trends Pharmacol. Sci. Suppl.:53–54.

30. Chiang, N., K. Gronert, C.B. Clish, J.A. O'Brien, M.W. Freeman, and C.N. Serhan. 1999. Leukotriene B4 receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. J. Clin. Invest. 104:309–316.

31. Perez, H.D., R. Holmes, E. Kelly, J. McClary, Q. Chou, and W.H. Andrews. 1992. Cloning of the gene coding for a human receptor for formyl peptides. Characterization of a promoter region and evidence for polymorphic expression. Biochemistry. 31:11595–11599.

32. Ahuja, S.K., A. Shetty, H.L. Tiffany, and P.M. Murphy. 1994. Comparison of the genomic organization and promoter function for human interleukin-8 receptors A and B. J. Biol. Chem. 269:26381–26389.

33. Mutoh, H., H. Bito, M. Minami, M. Nakamura, Z. Honda, T. Izumi, R. Nakata, Y. Kurachi, A. Terano, and T. Shimizu. 1993. Two different promoters direct expression of two distinct forms of mRNAs of human platelet-activating factor receptor. FEBS Lett. 322:129–134.

34. Yokomizo, T., K. Mase, K. Kato, A. Toda, T. Izumi, and T. Shimizu. 2000. Leukotriene B4 receptor. Cloning and intracellular signaling. A m. J. Respir. Crit. Care Med. 161:S51–S55.

35. Chen, H.M., H.L. Pahl, R.J. Schelbe, D.E. Zhang, and D.G. Tenen. 1993. The Sp1 transcription factor binds the CD11b promoter specifically in myeloid cells in vivo and is essential for myeloid-specific promoter activity. J. Biol. Chem. 268:8230–8239.

36. Rosmarin, A.G., M. Luo, D.G. Caprio, J. Shang, and C.P. Simkevich. 1998. Sp1 cooperates with the ets transcription factor, GABP, to activate the CD18 (beta2 leukocyte integrin) promoter. J. Biol. Chem. 273:13097–13103.

37. Hauses, M., R.R. Tonjes, and M. Grez. 1998. The transcription factor Sp1 regulates the myeloid-specific expression of the human hematopoietic cell kinase (HCK) gene through binding to two adjacent GC boxes within the HCK promoter-proximal region. J. Biol. Chem. 273:31844–31852.

38. Saffer, J.D., S.P. Jackson, and M.B. Annarella. 1991. Developmental expression of Sp1 in the mouse. Mol. Cell. Biol. 11:2189–2199.

39. Kuramasu, A., H. Saito, S. Suzuki, T. Watanabe, and H. Ohtsu. 1998. Mast cell-/basophil-specific transcriptional regulation of human l-histidine decarboxylase gene by CpG methylation in the promoter region. J. Biol. Chem. 273:31607–31614.

40. Kudo, S., and M. Fukuda. 1995. Tissue-specific transcriptional regulation of human leukosialin (CD43) gene is achieved by DNA methylation. J. Biol. Chem. 270:13298–13302.

41. Tate, P.H., and A.P. Bird. 1993. Effects of DNA methylation on DNA-binding proteins and gene expression. Curr. Opin. Genet. Dev. 3:226–231.

42. Boyes, J., and A. Bird. 1991. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. Cell 64:1123–1134.

43. Nan, X., F.J. Campoy, and A. Bird. 1997. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell. 88:471–481.

44. Normark, S., S. Bergstrom, T. Edlund, T. Grundstrom, B. Jaurin, F.P. Lindberg, and O. Olsson. 1983. Overlapping genes. Nature. 306:471–481.

45. Grundstrom, T., and B. Jaurin. 1982. Overlapping between ampC and frd operons on the Escherichia coli chromosome. Proc. Natl. Acad. Sci. USA. 79:1111–1115.