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Molecular cloning of cDNA and a chromosomal gene encoding GPE1-BP, a nuclear protein which binds to granulocyte colony-stimulating factor promoter element 1

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Granulocyte colony-stimulating factor (G-CSF) is produced from macrophages in response to lipopolysaccharide (LPS). GPE1, a *cis*-acting element of the G-CSF gene promoter, functioned as an LPS-inducible element. We isolated cDNA and a chromosomal gene encoding the mouse GPE1-binding protein (GPE1-BP). A 150-amino acid protein deduced from the cDNA has a basic domain and a leucine zipper motif, and seems to be identical to that of recently isolated Ig/EBP. The nuclear extract from COS cells transfected with the cDNA showed GPE1-binding activity. Transcripts were ubiquitously detected, and may be spliced from two exons of a single gene.

Granulocyte colony-stimulating factor; Macrophage; Lipopolysaccharide; Leucine zipper; C/EBP; DNA-binding protein

1. INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein which regulates proliferation and differentiation of the progenitor cells of neutrophilic granulocytes [1,2]. G-CSF is induced in macrophages in response to bacterial endotoxin i.e. lipopolysaccharide (LPS) [3]. The promoter region of human and mouse G-CSF genes is highly homologous, up to 300 base pairs (bp) from the transcription initiation site [4]. *E. coli* chloramphenicol acetyltransferase (CAT) assay of various mutants in the 300-bp promoter of the mouse G-CSF gene, suggested that at least three *cis*-controlling elements, namely G-CSF promoter elements (GPE) 1 through 3, were required for G-CSF gene expression [3,5]. Analysis of mutants with reiterated GPE indicated that GPE1 functioned as an LPS-inducible regulatory element in macrophages [3].

In this report, we isolated cDNAs encoding mouse GPE1-binding protein (GPE1-BP) and its chromosomal gene. The deduced amino acid sequence revealed that it is probably identical to the Ig/EBP which was recently isolated as a protein binding to the enhancer element of immunoglobulin genes [6].

2. MATERIALS AND METHODS

2.1. *Isolation of cDNA and genomic clones*

cDNA libraries were constructed in the λgt11 vector with mRNA from mouse macrophage BAM3 [7] or monocyte-macrophage PU5-1.8 cells (ATCC TIB61). The BAM3 cDNA library was screened by the Southwestern method [8] with a slight modification. As a probe, ^{32}P-labeled GPE1 DNA was prepared by polymerase chain reaction. The plasmid pGPE1-4, containing four tandem GPE1 fragments (5′ AGAGATTCCCCGATTTCACAAAAACTTTCGCAAACAG-CTTTT 3′) in the pUC-SX vector [3], was used as the template. The DNA fragment was amplified with the universal M4 and RV primers (Takara Shuzo Co.) as described [9] except for the substitution of bromodeoxyuridine triphosphate for TTP. After the binding of labeled DNA to expressed proteins on nitrocellulose filters (Schleicher & Schuell), they were exposed to 254-nm ultraviolet light using a UV Stratalinker (Stratagene), and washed as described [8]. Full-length cDNA clones and genomic clones were identified by plaque hybridization [10].

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3. RESULTS AND DISCUSSION

To isolate cDNA encoding GPE1-binding protein (GPE1-BP), a λgt11 expression library of mouse macrophage BAM3 cells was screened by a modified Southwestern method [8]. One clone was positive among 7.5×10^5 clones, but the cDNA insert (950 bp) did not contain the initiation methionine codon. cDNA libraries from BAM3 and PU5-1.8 cells were screened by plaque hybridization [10], and three cDNAs with different 3′-encoding regions were isolated. Northern blot analysis of RNA prepared from various cells [11] with the coding region of this cDNA resulted in one major band (about 5 kilobases (kb) in length) and minor bands (Fig. 1). These mRNAs were detected not only in G-CSF-producing macrophages (BAM3 and PU5-1.8), but also in the G-CSF-nonproducing macrophage line P388D$_1$, the pre-B lymphocyte line 70Z/3, and fibroblast L929 cells, regardless of LPS-treatment.

Southern blot analysis of mouse genomic DNA suggested that there is a single gene per haploid genome

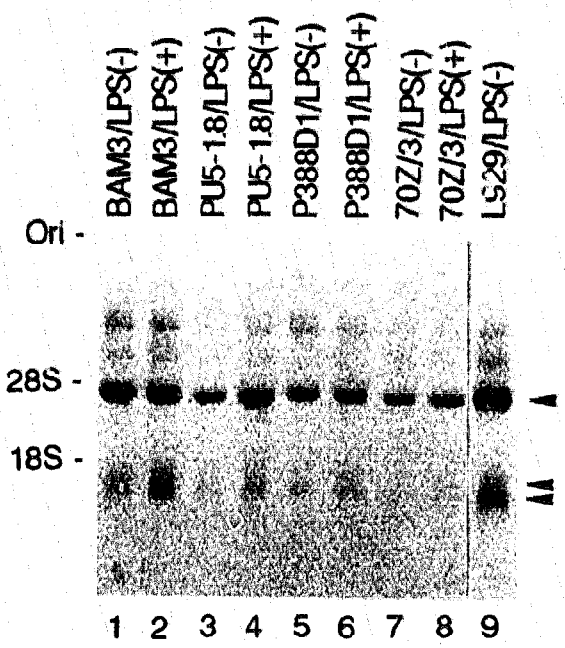

Fig. 1. Expression of GPE1-BP mRNA. Northern blot analysis was performed with total RNA (10 μg) prepared from cells treated with or without LPS.

(data not shown). Sequences of genomic clones isolated from a mouse spleen library [4], compared with that of cDNA, revealed that this gene consists of two exons (Fig. 2); exon 1 codes for the 5'-noncoding region and exon 2 for the coding and 3'-noncoding regions, the latter having at least three polyadenylation signals and a B1 repetitive sequence [12] located between the first and second signals. Primer extension analysis and S1 nuclease mapping indicated that transcription of this gene starts just downstream of an Sp1 binding site [13] (data not shown). Furthermore, only the 5 kb mRNA hybridized with a DNA probe containing the sequence downstream of the second poly(A) signal (data not shown). These results suggested that mRNAs detected in Northern blot analysis (Fig. 1) are spliced in the same way but are terminated at different polyadenylation sites in exon 2: two minor short transcripts are terminated at the first and the second polyadenylation sites and the major transcript (about 5 kb) at a third site 4 kb downstream of the second.

The protein sequence deduced from this cDNA consists of 150 amino acid residues (M_r = 16 399) (Fig. 2), and has a basic domain (residues 53–89) and a leucine zipper motif (residues 90–128). These regions exhibit

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TCTAGAGGCGAGCGCGTCGTTCTCCAAGCAGCGCTTCTATTGGTCACGGCGGCGGCGGCAGCGCGCGGACCCGAAGAGGCGGGGAAAACCGGTGGCCGCGGTGGC

                                  Exon 1
GGAACGGGCGGAGGTTGCCGGTTTCGTAACCGTCGCTCCTCCTCGCCGACTCGCGGGCTGCGAGGCCTGGGTCGGGTCGGGTCGGGCCGCGCCGCGCGGGGCCGC
      Sp1                         *
                                                  Intron
TCGGAGTGGAGGCCGTCTGGGGGCGGGCGGGCCGGCCGGAGGCGCAG GTAAGGAGGCCGCGGGTTGTAGCTCGGAGGGGTT----------------------

                                                                                  Exon 2
------- about 5 kb -----------ACTTGATCCTGTCATTTCAGATATTTTAATGCGACTTATTTTCTAACTAG GTACATGTGAAGATTTTTTGGCAG

                                                                                M S K L S Q P A T T P   11
TTGAGTGTGGCCTCCTCGGATCACATTGCTCTGATTTCTACCTATTCTGTGTTGGCAAAGGAACGTGCCCAAATGAGCAAGCTGTCGCAGCCAGCCACTACTCCA

G V N G I S V I H T Q A H A S G L Q Q V P Q L V P A G P G G G G K A V   46
GGAGTGAATGGAATAAGTGTCATTCATACTCAGGCACATGCCAGCGGCTTACAGCAGGTTCCTCAGCTGGTGCCCGCTGGGCCTGGGGGAGGGGGCAAGGCTGTG

P P S K Q S K K S S P M D R N S D E Y R Q R R E R N N M A V K K S R L   81
CCTCCAAGCAAGCAAAGCAAAAAGAGCTCACCCATGGATCGGAATAGTGACGAATACCGCCAGCGCAGAGAGCGGAACAATATGGCGGTGAAAAAAAGCCGGTTA

K S K Q K A Q D T L Q R V N Q L K E E N E R L E A K I K L L T K E L S  116
AAAAGCAAGCAGAAAGCTCAAGATACACTGCAAAGAGTAAACCAGCTCAAGGAAGAGAATGAACGGTTGGAAGCCAAAATTAAGTTGCTGACAAAGGAATTAAGT

V L K D L F L E H A H S L A D N V Q P I S T E T T A T N S D N P G Q *  150
GTACTGAAAGATTTGTTTCTTGAGCATGCGCACAGCCTCGCAGACAACGTGCAGCCCATCAGCACGGAAACTACAGCGACAAATTCTGATAACCCAGGGCAGTAG

ATCTCCTTCCAGGCCCAGGGCTTGTGACTTGAACATGAGAGGTGTGACCACCCTGCACCCCTTGCTTCAGTGGCTGAACTCGGTCTCCTTCCATTGGAGGTTGTT

TTCTAGGCTCAACACTGAACAGCTGATTAGCCATGTAATTTATCTGGTCTTAAATGATAATGGATTTTTGAAGCACTAAAAGGATTTAGGGTTTATCGTTAAAAC

AAAATTCCTGGACTTTAATATTCTTAATAAATCCTCACTTCCCCAGAAATGGCTCTTTTGTAGGAATGGGACAATGGAAGGTCATGTAGAAGGTACTGGGTTCTA
                          1st
                                    <---   B1 Sequence
ATGAGAGAATACCTTGGATTAAGAAAAGCTGAATTCTAGCCGGGCGGTGGTGGCACACGCCTTTAATCCCAGCACTTAGGAGACAGAGGCAGGCGGATTTCTGAG

                                                                  --->
TTCGTTGCCAGCCTGGTCTACAAAGTGAGTCCTAGGTCAGCCAGGGCTATACAGAAAAACCCTGTCTCGAAAAACAAAAACAAATAAACAAAAAAAACAAAACAA
                                                                      2nd

ACAAACAAAAAGAAAAGCTGAATTCCATCTGGGTTCCTCAGTTTGTGATTTTATTCTAAATTGTGTATGGTAAATTCTGGGGCTACAAAAACCAGTCCTAATAAT
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Fig. 2. Nucleotide sequence of the mouse GPE1-BP gene. DNA sequencing was performed by the chain termination method using T7 DNA polymerase (Pharmacia) and [α-^{35}S]dATP (Amersham). The coding sequence is translated and numbered from the initiation methionine. An Sp1 binding site is underlined, the putative initiation site for transcription is indicated by an asterisk, and polyadenylation signals are underlined. The B1 repetitive sequence is delineated by arrows. The G residue deleted in Ig/EBP cDNA [6] is indicated by an arrowhead (▲).

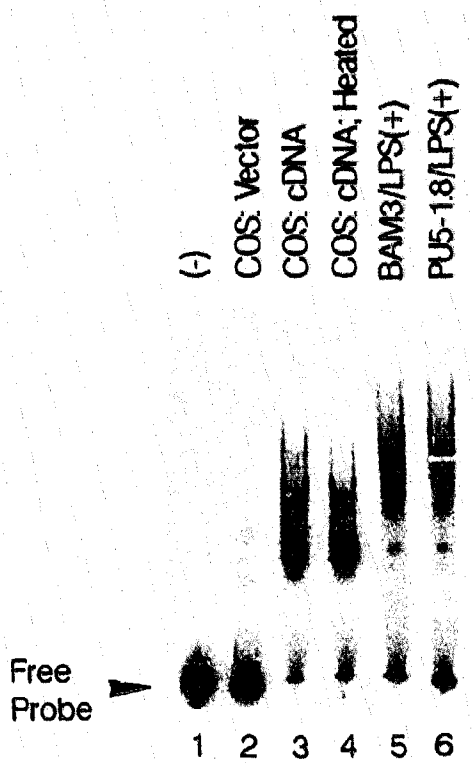

Fig. 3. Binding of GPE1-BP to GPE1. Gel retardation assay [3] was performed with the end-labeled GPE1 monomer as the probe (lane 1), and 1 μg of nuclear extract from COS-7 cells transfected with pEF-BOS (lane 2) of pEF-BOS carrying the cDNA (lanes 3 and 4), or 2 μg of extract from LPS-treated BAM3 (lane 5) and PU5-1.8 (lane 6) cells. In lane 4, the nuclear extract was heated (90°C, 10 min), centrifuged, and the supernatant was assayed.

high homology with the basic domain and the leucine zipper motif of a nuclear factor C/EBP [14] (45% identity) and NF-IL6 [15] (46% identity), although the N-terminal 52 residues share little homology to them. To confirm the DNA-binding activity, the cDNA was placed downstream of the elongation factor 1α promoter of the mammalian expression vector pEF-BOS [16] and introduced into COS-7 cells by the DEAE-dextran method [5]. Shifted bands were observed with the nuclear extracts from cells transfected with the cDNA-expression plasmid in a gel retardation assay (Fig. 3, lanes 2 and 3). As found in C/EBP [14] and NF-IL-6 [15], the nuclear protein binding to the GPE1 was heat-stable (Fig. 3, lane 4). From these results, we concluded that the encoded protein is a GPE1-binding protein related to C/EBP. However, unlike other members of C/EBP family [14,15], the chromosomal gene for GPE1-BP contained an intron.

Recently, cDNA for a nuclear protein Ig/EBP which binds to the immunoglobulin enhancer has been cloned [6]. Nucleotide sequence comparison revealed that most of this cDNA was identical with the GPE1-BP cDNA except the deletion of the G residue in the 5'-noncoding region and the divergence in the 3'-noncoding region of the Ig/EBP cDNA. These differences seem to be cloning artifacts in Ig/EBP, since the nucleotide sequence of the chromosomal gene of the GPE1-BP was completely identical to that of cDNAs (Fig. 2). Since Roman et al. could detect only mRNA of about 5 kb, and the open reading frame continued through the 5'-noncoding region, they postulated that their cDNA is partial, and the actual Ig/EBP is larger than the protein coded by Ig/EBP cDNA [6]. However, our current analyses indicate that the GPE1-BP (Ig/EBP) codes for a protein of 150 amino acids.

In any event, it is interesting that the same nuclear factor (GPE1-BP, Ig/EBP) binds to the enhancer of the G-CSF gene in macrophage or to the enhancer of immunoglobulin genes in B cells. How the GPE1-BP is involved in transcription of the G-CSF gene remains to be studied. It is possible that the GPE1-BP polypeptides may associate with each other as a homodimer or with other leucine zipper proteins such as C/EBP and NF-IL6 as a heterodimer. Further analysis of the role of the GPE1-BP may lead to an understanding of the general mechanism of LPS-inducible gene expression.

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