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Leukotriene C_4 synthase promoter driven expression of GFP reveals cell specificity

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Abstract

Leukotriene C_4 synthase is a key enzyme in leukotriene biosynthesis. Its gene has been cloned and mapped to mouse chromosome 11. Expression occurs in cells of myeloid origin and also in the choroid plexus, the hypothalamus and the medial eminence of mouse brain. In this study a vector that expresses enhanced green fluorescent protein (eGFP) under the control of the mouse leukotriene C_4 synthase promoter was constructed and used to study promoter activity in different cell lines. Specific eGFP expression was observed in human monocytic leukemia (THP-1) and rat basophilic leukemia (RBL-1) myeloid cells which both express leukotriene C_4 synthase, but not in human embryonic kidney (HEK293/T) epithelial cells which do not express this enzyme. In the myeloid cells, but not in the epithelial cells, we observed that the leukotriene C_4 synthase promoter activity was stimulated by 12-*O*-tetradecanoylphorbol-13-acetate and all-*trans*-retinoic acid. In contrast dimethyl sulfoxide did not affect promoter activity.

Keywords: Leukotriene; Leukotriene C_4 synthase; Expression; GFP; TPA; Promoter; Retinoic acid; DMSO

Cysteinyl leukotrienes are powerful mediators of inflammatory responses and immediate hypersensitivity reactions [1,2]. They are formed from arachidonic acid in activated myeloid cells by sequential reactions catalyzed by 5-lipoxygenase and leukotriene C_4 synthase. The 2.01 kb gene encoding leukotriene C_4 synthase on mouse chromosome 11 comprises five exons with intron/exon boundaries identical to those of the human gene. Promoter-enhancer elements, including binding sites for AP-2 and C/EBP, were recognized in a 1.2 kb 5′-flanking region of the gene [3]. Tissue distribution studies have shown leukotriene C_4 synthase mRNA or protein expression in mouse choroid plexus [4], hypothalamus and brain medial eminence [5].

In this study we report the construction of a plasmid expressing enhanced green fluorescent protein (eGFP) under control of the leukotriene C_4 synthase promoter. Transient transfection of this plasmid into human monocytic leukemia (THP-1), rat basophilic leukemia (RBL-1), and human embryonic kidney (HEK293/T) epithelial cells showed that eGFP was expressed by cells which express leukotriene C_4 synthase (RBL-1 and THP-1) but not by the leukotriene C_4 synthase negative HEK293/T cells. The recombinant vector constructed should be useful for investigations concerning the control of leukotriene C_4 synthase expression *in vitro* and *in vivo*.

Materials and methods

Materials. Restriction enzymes, T4 DNA polymerase, T4 DNA ligase, pcDNA3, competent *E. coli* DH5α, antibiotics, Dulbecco's modified Eagle's medium, and fetal calf serum were obtained from Invitrogen (Paisley Scotland). DE81 anionic exchange paper was purchased from

Abbreviations: AP-2, activating protein 2; C/EBP, CCAAT/enhancer binding protein; eGFP, enhanced green fluorescent protein; HEK293/T, human embryonic kidney 293T cell line; HL-60, human promyelocytic leukemia cell line; LTC_4S, leukotriene C_4 synthase; $pLTC_4S$, leukotriene C_4 synthase promoter; PBS, phosphate buffered saline; RBL-1, rat basophilic leukemia cell line-1; Retinoic acid, all-*trans*-retinoic acid; Sp1, specificity protein 1; Sp3, specificity protein 3; THP-1, human monocytic leukemia cell line; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

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Whatman International Ltd (Maidstone, England). A plasmid encoding enhanced green fluorescent protein (p-eGFP-C1) was obtained from Clontech (Mountain View, CA). Plasmid midi purification kit was purchased from Savéen (Malmö, Sweden). Mouse clone RP23-319B15 containing the leukotriene C_4 synthase gene was obtained from BACPAC Resources (Oakland, CA). All other chemicals were from Sigma-Aldrich (St Louis, MO).

Recombinant plasmid. A 1764 bp fragment of the 5′-flanking region of the leukotriene C_4 synthase gene was amplified by PCR from clone BAC RP23-319B15 (see Table 1), and ligated into the EcoRV and XhoI restriction sites of pcDNA3. Another fragment containing intron 2 of the leukotriene C_4 synthase gene was similarly amplified by PCR (Table 1), and ligated into the same plasmid at NheI and XhoI restriction sites. The pLTC$_4$S-intron 2 cassette was verified by sequencing using a MegaBACE 500. eGFP cDNA was amplified by PCR from p-eGFP-C1 (Table 1), and ligated into the XbaI and ApaI sites of an empty pcDNA3 vector. The resulting vector was cleaved by BglII and BamHI to remove its cytomegalovirus promoter and then religated. The insert containing the promoter and intron 2 regions of the leukotriene C_4 synthase gene was excised with EcoRI and XhoI from the pcDNA3 vector and religated into the cut pcDNA3-eGFP vector to generate a recombinant eGFP plasmid driven by the leukotriene C_4 synthase gene promoter (Fig. 1A). The pLTC$_4$S-intron 2-eGFP cassette is readily removable by EcoRI and ApaI digestion.

Cell cultures. Cell cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere. RBL-1 and THP-1 cells were cultured in RPMI-1640 medium supplemented with 10% (v/v) fetal calf serum, penicillin 100 units/ml, and streptomycin 100 μg/ml. Cell cultures were split 1:10 every third day. HEK293/T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, penicillin 100 units/ml, and streptomycin 100 μg/ml. These cultures were split 1:10 at confluence.

Transfection and other treatments of cells. One hour prior to transfection cells cultured in 6-well plates were washed and 3 ml of serum free medium per well was added. A mixture containing 5 μg recombinant plasmid construct or p-eGFP-C1 control vector, 2 μl 0.1 M polyethyleneimide, and 5 μl 20% (w/v) glucose in a total volume of 22 μl was incubated for 20 min at room temperature and then added to the serum-starved cells. Fetal calf serum was added 3 h after transfection to a final concentration of 10% (v/v) and the cells were incubated for 24 h. At this time final concentrations of 100 nM 12-*O*-tetradecanoylphorbol-13-acetate, 50 μM retinoic acid or 1.5% (v/v) dimethyl sulfoxide were added for another 24 h prior to mounting the cells for microscopic analyses.

Fluorescence microscopy. Cells were washed twice in PBS and fixed with 4% w/v paraformaldehyde. Upon illumination with a 488 nm argon laser eGFP fluorescence was imaged using a Nikon C1 confocal microscope.

Quantitation of fluorescence. The fluorescence in transfected cells was analyzed from images taken on the Nikon microscope, using Volocity 4.1 software (Improvision, Coventry, England). Average fluorescence in individual cells (F) was determined and corrected for background fluorescence in non-transfected cells (B). The corrected fluorescence was

Table 1
Oligonucleotide primers for PCR

Gene sequence	Forward primer	Reverse primer	Product size and position
LTC$_4$S promotor	5′-GCAGATACTGTGCTCGAGATGCG-3′	5′-GGGCAAGCTTGAACAAAGAGACCG-3′	1764 bp (−1230 − +534)
LTC$_4$S intron 2	5′-CTCGAGGAAGAAGATGCCG-3′	5′-CGCTAGCGGTGATCTCTGCAC-3′	237 bp (1068–1304)
eGFP ORF	5′-GCTCTAGAGGTCGCCACCATG-3′	5′-AGTCCGGACTTGTAGGGCCCGTCC-3′	743 bp

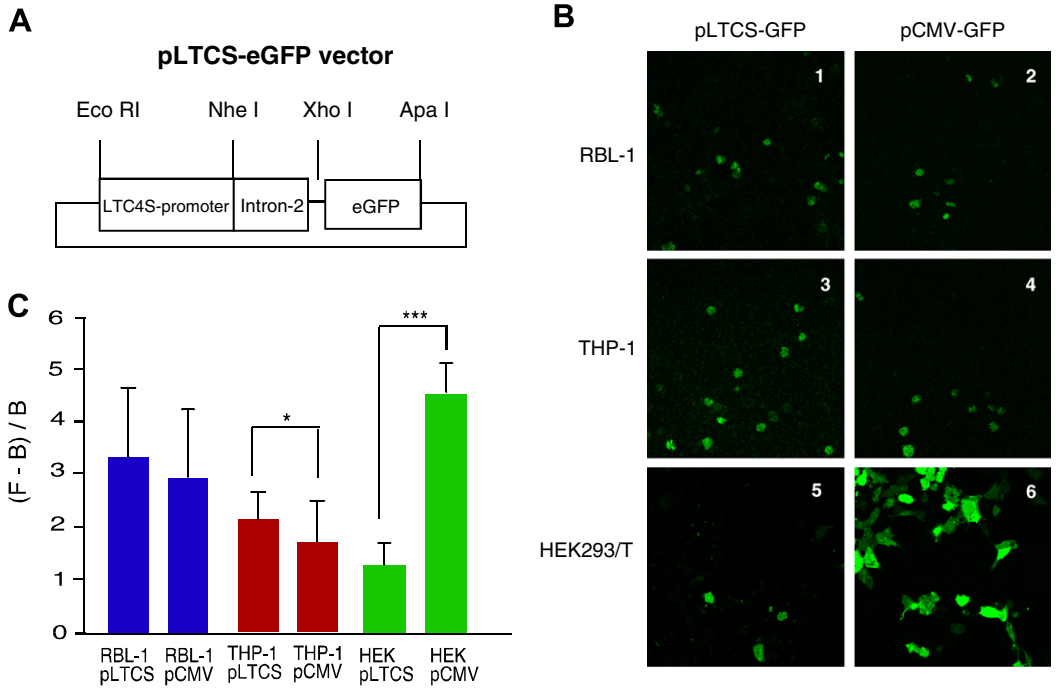

Fig. 1. (A) A vector in which eGFP expression is driven by the promoter and intron 2 of the LTC$_4$ synthase gene was constructed using pcDNA3 as backbone. (B) Fluorescence microscopy of RBL-1 (1 and 2), THP-1 (3 and 4), and HEK293/T (5 and 6) cells transfected with pLTC$_4$S-eGFP or with p-eGFP-C1, a cytomegalovirus promoter (pCMV) driven control plasmid. (C) Quantitative fluorescence measurements on the cells shown in (B). F, average cell fluorescence; B, background fluorescence. The bars show mean values ± SD. Statistical significance is indicated by $^{*}p < 0.05$ or $^{***}p < 0.001$.