Molecular Cloning of the Human Leukotriene C_4 Synthase Gene and Assignment to Chromosome 5q35[1]

Timothy D. Bigby,*†‡ Craig R. Hodulik,*† Karen C. Arden,*§ and Lixia Fu*†

*Department of Medicine, †Division of Pulmonary and Critical Care Medicine, and §Ludwig Institute for Cancer Research, University of California, San Diego, California, U.S.A.

‡Department of Veterans Affairs Medical Center, San Diego, California, U.S.A.

ABSTRACT

Background: Cysteinyl leukotrienes (LT) are mediators involved in inflammatory and allergic disorders. LTC_4 synthase catalyzes the first committed step in the synthesis of these inflammatory mediators, and its cellular distribution appears to be unique.

Materials and Methods: A human genomic library was screened by polymerase chain reaction (PCR) with primers that were designed based on the reported cDNA sequence for the LTC_4 synthase gene. The gene was identified in one clone by Southern blotting of restriction enzyme digests, subcloning of fragments containing regions of interest, and DNA sequencing of these subclones. The transcription initiation site was determined by primer extension analysis. Chromosome location was determined by fluorescent in situ hybridization and screening of somatic cell hybrids by PCR.

Results: The LTC_4 synthase gene is ~2.5 kb in length, consisting of five exons (136, 100, 71, 82, and 257 bp, respectively) and four introns (1,447, 102, 84, and 230 bp, respectively). Transcription initiation occurs at a single site 78 bp upstream of the coding region. The 5'-flanking region contains neither a TATA nor a CAAT box. The first 1 kb of the 5'-flanking region, however, contains putative DNA binding motifs for SP-1, AP-1, AP-2, *ets* factors, and CREB/ATF. A STAT binding motif is present in the first intron. The LTC_4 synthase gene is located in the distal region of the long arm of chromosome 5 in 5q35.

Conclusions: The LTC_4 synthase gene does not contain elements of a typical regulated gene and may therefore contain novel regulatory elements. This gene is also located in a region on chromosome 5 that appears to play a role in allergic and inflammatory disorders, such as asthma.

INTRODUCTION

Leukotrienes are oxygenated metabolites derived from the 5-lipoxygenase pathway of arachidonic acid metabolism. They are formed via the unstable epoxide intermediate 5,6-*trans*-oxido-7,9-trans-11,14-*cis*-eicosatetraenoic acid or leukotriene (LT) A_4 (1). The first two committed steps in this pathway are catabolized by the enzyme 5-lipoxygenase (2), and the activity of this enzyme within the cell requires the presence of the 5-lipoxygenase–activating protein (FLAP) (3). The resulting LTA_4 can then be further metabolized either to 5(S),12(R)-dihydroxy-6,14-*cis*,8,10-*trans*-eicosatetraenoic acid (LTB_4) by the enzyme LTA_4 hydrolase (4) or to 5(S)-hydroxy-6(R)-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (LTC_4) by the enzyme LTC_4 synthase (5). LTC_4 and its metabolites, LTD_4 and LTE_4, are the components of slow-reacting substance of anaphylaxis (6) but are more properly termed cysteinyl leukotrienes. LTC_4 synthase converts LTA_4 to LTC_4 by conjugating a glutathione residue at

Address correspondence and reprint requests to: Timothy D. Bigby, 111J, Department of Veterans Affairs Medical Center, 3350 La Jolla Village Drive, San Diego, CA 92161, U.S.A.

[1]The nucleotide sequence reported in this paper has been deposited in the GenBank database (accession no. U62025).

Copyright © 1996, *Molecular Medicine*, 1076-1551/96/$10.50/0

C_6 of LTA_4. The cysteinyl leukotrienes are known to have potent pro-inflammatory effects including sustained smooth muscle contraction, increased vascular permeability, and hypersecretion of mucus (7). These compounds are thought to play a significant role in disorders such as asthma, allergic diseases, pulmonary hypertension, acute lung injury, and acute coronary syndromes (7).

LTC_4 synthase, an integral membrane protein, has been purified to homogeneity and partial N-terminal sequence obtained (5). This enzyme is a homodimeric protein with an estimated monomeric molecular size of 18 kD. Two independent groups have cloned the cDNA for LTC_4 synthase (8,9). These two groups reported cDNA sequences for LTC_4 synthase that differed minimally (694 versus 679 bp) and which had identical open reading frames of 450 bp, predicting a protein of 150 amino acids and a M_r of 16,568. The deduced amino acid sequence consists primarily of hydrophobic amino acids and has no structural similarity to other glutathione-*S*-transferase enzymes. However, LTC_4 synthase does have 31% sequence identity to FLAP, and it can be inhibited by the FLAP antagonist MK886 (8,9). A number of reports indicate that LTC_4 synthase is a phospho-regulated protein and is a target of protein kinase C (10–12). These data suggest that phosphorylation of this protein by protein kinase C inhibits enzymatic activity.

Enzymes of the 5-lipoxygenase pathway appear to have cell-specific expression, with the possible exception of LTA_4 hydrolase. The range of cell types in which expression is detected is not, however, generalized for all enzymes of this cascade. For example, 5-lipoxygenase expression is limited primarily to cells of myeloid origin (1) and appears to be regulated by cytokines (13) and growth factors (14). By contrast, the expression of LTC_4 synthase in humans appears be limited to eosinophils, mast cells, platelets, endothelial cells, and perhaps, to some extent, mononuclear phagocytes (7,15,16). Although a report suggests that interleukin 3 may play a role in the regulation of expression of LTC_4 synthase in mouse mast cells (17), the regulation of expression of LTC_4 synthase in humans is unknown.

The purpose of this investigation was to characterize the structure of the human gene encoding for LTC_4 synthase because of the substantial body of evidence that suggests that its product may play an important role in inflammatory and allergic disease. We also wished to determine the sequence of its promoter as the first step in understanding the regulation of this gene, and to define the chromosome location of the LTC_4 synthase gene to begin to examine the possibility that abnormalities in the regulation of this gene may be associated with inflammatory disorders.

MATERIALS AND METHODS

Cell Preparation

THP-1 cells, a monocyte-like cell line derived from a patient with acute monocytic leukemia (18), were obtained from American Tissue Culture Collection (Rockville, MD, U.S.A.) and were cultured in RPMI 1640 with 10% fetal calf serum, 50 μM 2-mercapto ethanol, 100 U/ml of penicillin, and 100 μg/ml of streptomycin at 37°C in 5% CO_2.

Total Cellular RNA

Total RNA was extracted from THP-1 cells by using the single-step, guanidium thiocyanate procedure (19).

cDNA Cloning of LTC_4 Synthase

The cDNA encoding for LTC_4 synthase was obtained by polymerase chain reaction (PCR) cloning techniques. In brief, RNA was reverse transcribed as previously reported (20). Primers were prepared corresponding to the 5′ (5′-AGCGTTCCCCAGCTCGC) and 3′ (5′-GGGT CACTAGAACTGA) ends of the reported cDNA sequence (8). PCR reactions were carried out in a 50-μl reaction containing 1 μg of DNA template, 1 mM deoxynucleotide triphosphates, 20 pmol of each oligonucleotide primer, and 2.5 U of Taq polymerase, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9), 5% DMSO, and 5% glycerol. Thirty cycles of PCR were performed, with each cycle consisting of denaturation at 94°C for 60 sec, annealing at 60°C for 30 sec, and extension at 72°C for 60 sec. Amplified products were electrophoresed through 1% agarose gels and visualized by ethidium bromide staining. These primers were used to perform PCR that generated a faint band at the appropriate size of ~620 bp on a 1% agarose gel. This band was excised and used for a second amplification step. The product of this reaction was again excised from agarose and was ligated into the PCR cloning vector, pGEM T (Promega, Madison, WI, U.S.A.). Dideoxy chain

termination sequencing was performed yielding the exact anticipated sequence that corresponded fully to the published sequence (8).

Genomic Cloning

A variety of pairs of PCR primers were designed based on the reported cDNA sequence for LTC_4 synthase (8,9) and were tested to determine if they would prime products from both LTC_4 synthase cDNA and genomic DNA. PCR reactions were carried out in a 50-μl reaction containing 1 μg of DNA template, 400 μM deoxynucleotide triphosphates, 20 pmol of each oligonucleotide primer, and 2.5 U of Taq polymerase, 1.5 mM $MgCl_2$, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 5% DMSO, and 5% glycerol. Thirty cycles of PCR were performed with each cycle consisting of denaturation at 94°C for 60 sec, annealing at 60°C for 30 sec, and extension at 72°C for 45 sec. PCR was preceded by a 3-min incubation at 94°C and followed by a 72°C final soak for 10 min. Amplified products were electrophoresed through 2% agarose gels and visualized by ethidium bromide staining. The forward primer 5′-CCAGCTCGCCTTCACACACAG and the reverse primer 5′-TTGCAGCAGGACTCCCAGGAG primed a product of 103 bp from both cDNA and genomic DNA that corresponded to bp 9 to 111 of the cDNA. This primer set was utilized to screen a human P1 library (Genome Systems, St. Louis, MO, U.S.A.) by PCR techniques. A single positive clone was subjected to restriction digestion and fragments were separated on 1% agarose gels. The gels were then transferred to nylon membranes (Zeta Probe; Biorad, Hercules, CA, U.S.A.) for Southern blot analysis. Blots were probed with the full-length LTC_4 synthase cDNA and hybridization was performed as per the manufacturers' instructions (Zeta Probe; Biorad). Fragments from a *Bam*HI digest that hybridized to the LTC_4 cDNA were excised from agarose and extracted. These purified bands were then ligated into pBluescript KS^- (Stratagene, La Jolla, CA, U.S.A.). Sequences of the cloned inserts were determined from double-stranded templates by the dideoxy chain termination method using sequence specific primers. Some sequences were also determined using the original genomic clone by sequencing toward the 5′ end of the gene using successive sequencing primers. Sequence data were collected from a minimum of two overlapping runs derived from each DNA strand and analyzed by the MacVector sequence analysis software (Eastman Kodak Co., Rochester, NY, U.S.A.).

Primer Extension Analysis

Total RNA was isolated as noted above, and 44 μg were used per primer extension reaction. The primer for the extension reactions was 25 bp in length, with the sequence 5′-AGTAGAGCTAC CTCGTCCTTCATGG, and corresponded to bp 100 to 76 of the genomic sequence. This primer was ^{32}P-labeled using T4 polynucleotide kinase. Total RNA was denatured at 85°C for 10 min and was then annealed with RNA at 62°C for 20 min. The samples were then cooled to 42°C and were reverse transcribed with 60 units per reaction of Superscript II reverse transcriptase (Gibco/BRL Life Sciences, Gaithersburg, MD, U.S.A.) by previously reported techniques (19). The location of transcription initiation was determined by comparing the sizes of the primer extension bands with a radiolabeled standards. The exact size the primer extension product was confirmed by performing a dideoxy sequencing reaction using the above primer and running this reaction in parallel with primer extension products on 8% acrylamide gels.

Chromosome Analysis

FLUORESCENT IN SITU HYBRIDIZATION. Purified DNA from the P1 clone containing the LTC_4 synthase gene was labeled with digoxigenin dUTP by nick translation. Labeled probe was combined with sheared human DNA and hybridized to metaphase spreads of chromosomes, derived from phytohemagglutinin-stimulated normal human peripheral blood lymphocytes, in a solution containing 50% formamide, 10% dextran sulfate, and 2 × SSC. Specific hybridization signals were detected by incubating the hybridized slides in fluoresceinated anti-digoxigenin antibodies followed by counterstaining with propidium iodide (21).

PCR ANALYSIS OF SOMATIC CELL HYBRIDOMAS. The human × hamster cell hybrids were obtained from the Human Genetic Cell Repository (Camden, NJ, U.S.A.). The human chromosome content of each somatic cell hybrid was established by the Human Genetic Mutant Cell Repository using standard cytogenetic as well as molecular cytogenetic techniques. The hybrids used for detection of LTC_4 sequences are the same cell lines

which appear in the NIGMS human/rodent somatic cell hybrid mapping panel #2 - version 2 (1994/1995 Catalog of Cell Lines, NIGMS Human Genetic Mutant Cell Repository, NIH Publication No. 94-2011) with two exceptions; GM10478 containing human chromosomes 4 and 20 was substituted for GM10115, containing only human chromosome 4, GM11441 containing human chromosomes 5 and 20 was substituted for GM13140 containing only chromosome 20. Hamster DNA was isolated from the E36 cell line and mouse DNA was isolated from a C57BL/6J animal. All cell lines were maintained under the recommended selective conditions. DNA was isolated using the Puregene DNA Isolation Kit by Gentra (Research Triangle Park, NC, U.S.A.). PCR reactions were carried out in a 50-μl reaction containing 1 μg of DNA template with the primers used to screen the P1 library, as noted above.

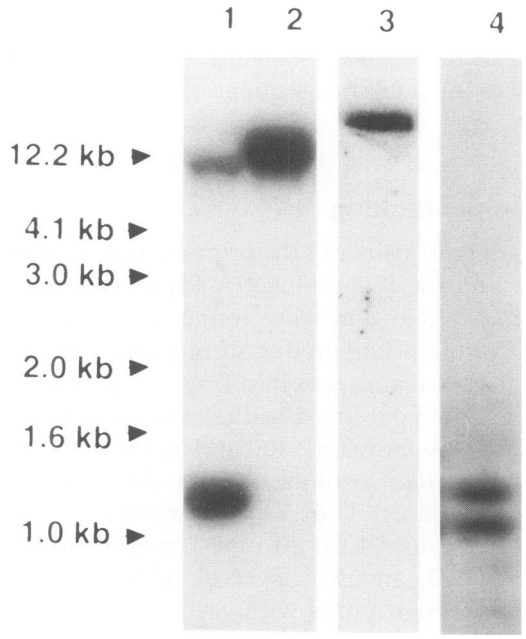

FIG. 1. Southern blot analysis of P1 plasmid containing the LTC_4 synthase gene

P1 plasmid DNA was digested with the restriction enzymes *Bam*HI (Lane 1), *Eco*RI (Lane 2), *Hin*dIII (Lane 3), and *Apa*I (Lane 4). These digests were subjected to electrophoresis on a 1% agarose gel, transferred to nylon membranes, and probed with the complete cDNA for LTC_4 synthase. These digests resulted in 2 (1.0 and 8.5 kb), 1 (~14 kb), 1 (~18 kb), and 2 (1.0 and 1.1 kb) bands.

RESULTS

Identification and Initial Analysis of Clones

Three positive clones in the P1 library were identified by PCR screening with primers designed based on the reported cDNA sequence for LTC_4 synthase. One of these was chosen for complete evaluation. The genomic DNA insert in this plasmid clone was ~77 kb in size. Southern blots probed with the complete LTC_4 synthase cDNA of *Bam*HI, *Eco*RI, *Hin*dIII, and *Apa*I digests of this P1 plasmid revealed 2 (1.0 and 8.5 kb), 1 (~14 kb), 1 (~18 kb), and 2 (1.0 and 1.1 kb) bands, respectively (Fig. 1). Gel purified fragments from the *Bam*HI digest were subcloned into pBluescript II KS^-.

Characterization of LTC_4 Synthase Genomic Clone

Sequencing and sequence analysis revealed that the LTC_4 synthase gene is ~2.5 kb in size and consists of 5 exons (136, 100, 71, 82, and 257 bp, respectively) (Fig. 2). Four introns measured 1447, 102, 84, and 257 bp, respectively. All introns began with GC and ended with AG, as expected for exon-intron boundaries. Sequence analysis included 1.0 kb of the 5′-flanking region (Fig. 3). Computer assisted analysis of the 5′-flanking region for possible binding sites for known transcription factors revealed putative DNA binding motifs for CREB/ATF (−918 bp), AP-1 (−825 bp), AP-2 (−329 bp), *ets* factors (−178 bp), and SP-1 (−42 bp). The first intron also contained binding motifs for STAT (266 bp), AP-2 (624, 995, and 1363 bp), and SP-1 (1445 and 1446 bp). Primer extension analysis revealed a single product of 100 bp indicating that the

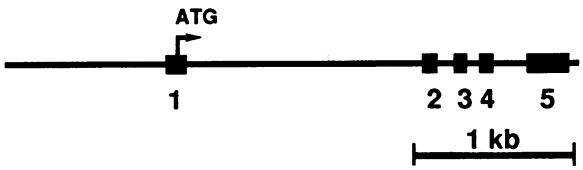

FIG. 2. Schematic representation of the inserts isolated from human P1 genomic clone containing the LTC_4 synthase gene

The ATG codon is located at nucleotide 78 in exon 1 (arrow). The open reading frame is ~2.5 kb and the portion of 5′-flanking region sequenced is ~1.0 kb. Five exons (136, 100, 71, 82, and 257 bp) are represented by the filled rectangles interspersed by four introns (1,447, 102, 84, and 230 bp).

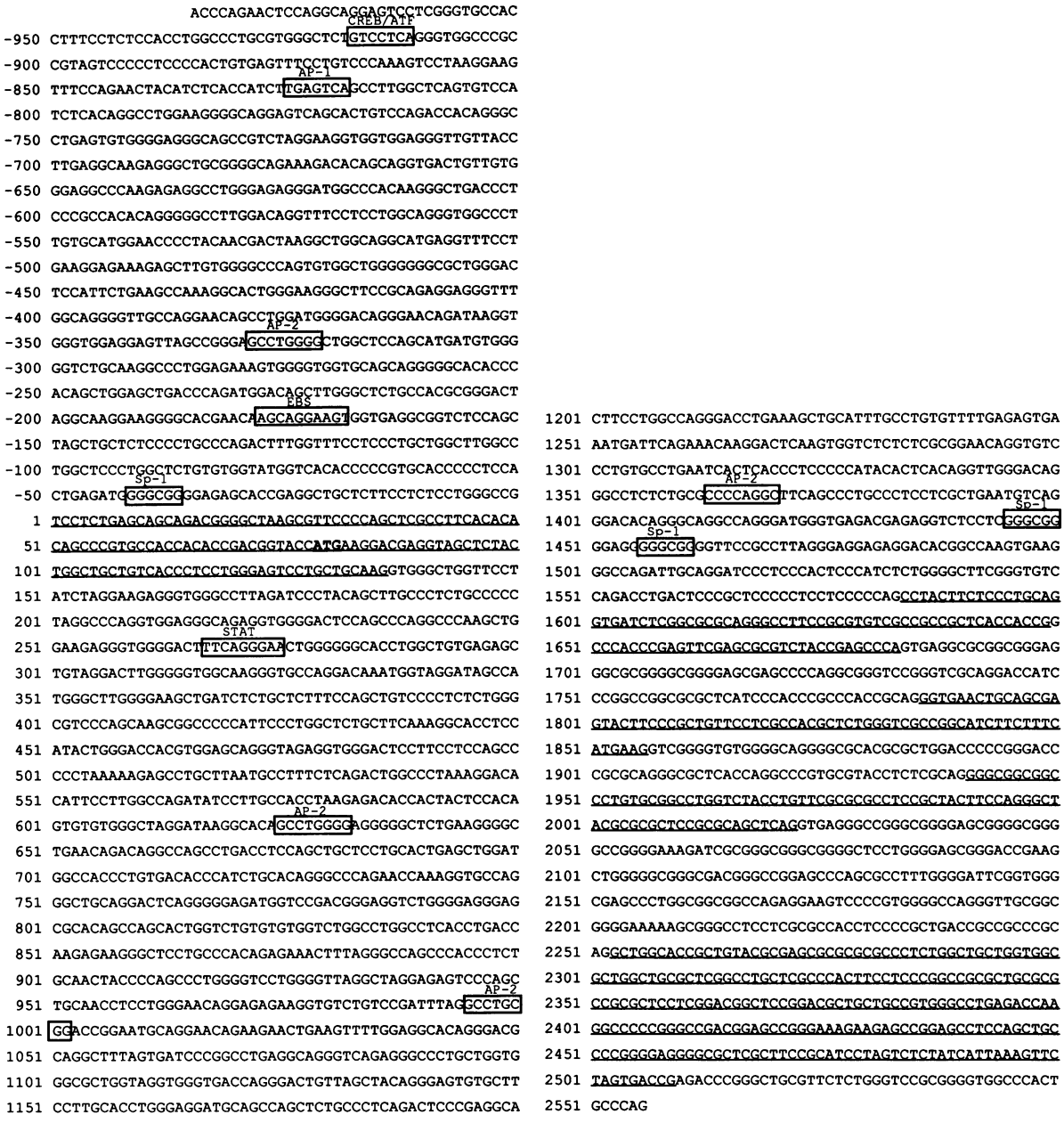

FIG. 3. Nucleotide sequence of the LTC_4 synthase gene

Putative DNA binding motifs of known transcription factors in the 5′-untranslated region and in the first intron are denoted by a box. The open reading frame is denoted by underlining. The ATG codon at the start of the coding region is denoted in bold.

transcription initiation site was 78 bp upstream of the initiator ATG codon (Fig. 4).

When our genomic sequence was compared with the two published cDNA sequences for LTC_4 synthase (8,9), the entire sequence reported by Lam and colleagues (8) was accounted for in our genomic sequence. However, 14 bp at the 5′ end of the Welsch sequence (9) were not present near the transcription initiation site of our clone. To exclude the possibility that another exon existed in the 5′-flanking region of the gene, we initially scanned the complete sequence of the LTC_4 synthase gene and these 14 bp were not not found. In addition, an oligonucleotide corresponding to these 14 bp was synthesized and ^{32}P-labeled using T4 polynucleotide kinase. This

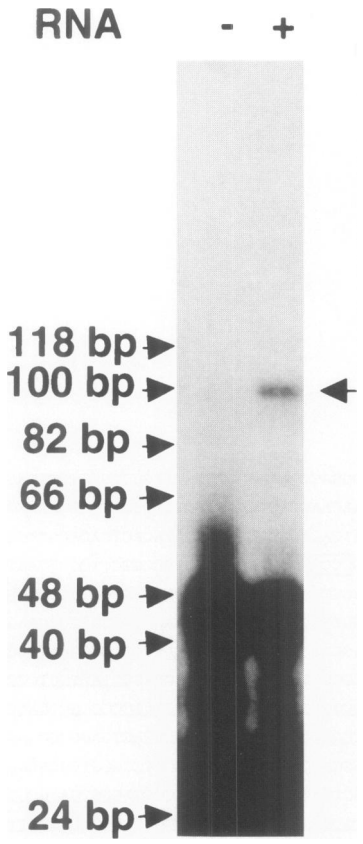

FIG. 4. Determination of transcription initiation site

Total RNA was isolated from THP-1 cells and was annealed with a 25-bp reverse primer corresponding to nucleotides 76 to 100 of the genomic sequence and labeled with T4 polynucleotide kinase with [γ-^{32}P] ATP. The samples were then reverse transcribed using Superscript II. Electrophoresis of these samples was performed in 8% denaturing polyacrylamide and the gel was subjected to autoradiography. The left lane (−) lacks RNA (negative control). The right lane (+) is the same reaction conducted with 44 μg of total RNA. The primer extension product was found to be 100 bp. This size was confirmed by repeat gels run in parallel with a sequencing reaction using the P1 plasmid and the same primer. By comparison to labeled markers, the transcription initiation site mapped to a single site 78 bp upstream of the ATG initiator codon.

this labeled oligonucleotide did not detect any specific bands in Southern blots of LTC_4 synthase gene DNA digested with *Bam*HI (data not shown).

Chromosome Localization of LTC_4 Synthase

FLUORESCENT IN SITU HYBRIDIZATION. A total of 80 metaphase cells were analyzed and 70 exhibited specific labeling with the LTC_4 synthase gene. Initial fluorescent in situ hybridization analysis revealed specific labeling of the long arm of a group B chromosome. In additional experiments a control probe that has been previously mapped to 5q21 (this was confirmed by co-hybridization with a probe from the Cri du Chat locus on chromosome 5q15) was co-hybridized with the P1 plasmid containing the LTC_4 synthase gene. The results showed specific labeling of the mid (control probe) and distal (experimental probe) long arm of chromosome 5 (Fig. 5). Measurements of 10 specifically labeled chromosomes demonstrated that the LTC_4 synthase gene is located at 99% of the distance from the centromere to the telomere of chromosome arm 5q, a region corresponding to band 5q35.

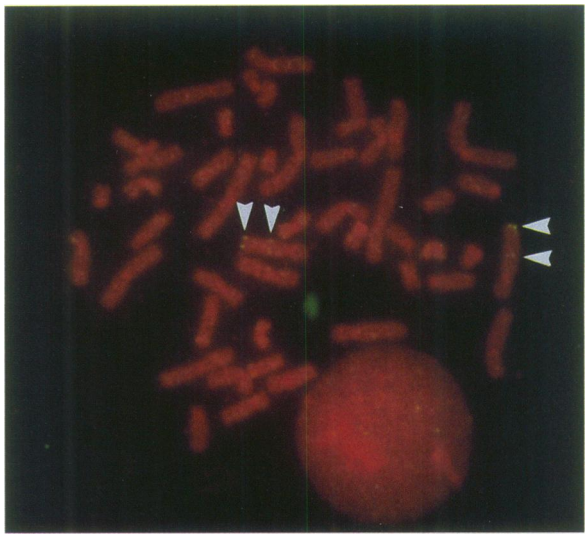

FIG. 5. Fluorescent in situ hybridization

Metaphase chromosomes were hybridized with purified DNA from the P1 clone labeled with digoxigenin dUTP by nick translation. Specific hybridization signals were detected by incubating with a fluoresceinated anti-digoxigenin antibodies. Doublet positive signals are observed on the far distal end of the long arm (q arm) of a group B chromosome (distal arrow). Measurements of 10 chromosomes revealed that the signal was localized 99% of the distance from centromere to the telomere. Metaphase chromosomes were also hybridized with a control probe that is known to localize to 5q21 (proximal arrow). These data taken together indicate that LTC_4 synthase localizes to 5q in an area corresponding to band 35 (5q35).

PCR ANALYSIS OF SOMATIC CELL HYBRIDOMAS. To confirm the chromosomal localization obtained by fluorescence in situ hybridization, PCR analysis

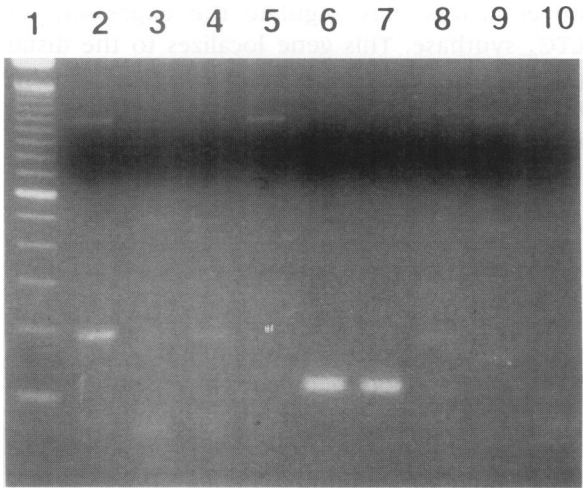

FIG. 6. PCR of the LTC_4-specific 103-bp fragment from genomic DNA of human somatic cell hybrids cell lines

(Lane 1) 100 bp ladder (Gibco). (Lane 2) GM13139 containing human chromosome 1. (Lane 3) GM 10826 containing human chromosome 2. (Lane 4) GM10253 containing human chromosome 3. (Lane 5) GM11441 containing human chromosomes 4 and 20. (Lane 6) GM10114 containing human chromosome 5. (Lane 7) Human genomic DNA. (Lane 8) Hamster genomic DNA. (Lane 9) Mouse genomic DNA. (Lane 10) No DNA template. Only the presence or absence of human chromosome 5 was concordant with the presence or absence of the LTC_4-specific PCR product (~100 bp; Lanes 6 and 7). Faint bands (~200 bp) were present in hybridomas prepared from rodent somatic cells (Lanes 2, 4, and 8) but did not correspond to the anticipated product for LTC_4 synthase.

of DNA from a panel of hamster or mouse × human somatic cell hybrids was performed using primers corresponding to the first exon of the LTC_4 gene. The chromosomal localization was determined by scoring the presence or absence of the LTC_4-specific band in each lane of the agarose gel, which had amplified DNA from hybrids containing different human chromosomes (Fig. 6). In this panel of hybrids, only the presence of chromosome 5 in the hybrid was concordant with presence of the LTC_4 PCR product. Fainter bands were sometimes detected in the absence of chromosome 5 and may be a result of the presence of homologous genes in rodent genomic DNA.

DISCUSSION

This study reports the initial characterization of the LTC_4 synthase gene. We have demonstrated that the LTC_4 synthase gene is relatively small (2.5 kb), consisting of five exons and four introns. This is in contrast to the related protein, FLAP, whose cDNA has 31% sequence identity with the LTC_4 synthase gene but whose gene is 35 kb in length (22). The initiation of transcription has been confirmed to be at a single site 78 bp upstream of the initiator methionine. The chromosome location of this gene was mapped (by using two distinct techniques) to the long arm of chromosome 5, probably to region 5q35.

The coding region of the LTC_4 synthase gene corresponds to the two reported cDNA sequences (8,9). The portion of the sequence upstream to the ATG start codon reported by Lam and colleagues (8) is fully contained in our sequence (bp 25–78). The portion of the sequence upstream to the ATG start codon reported by Welsch (9) is, however, only partially found in our genomic sequence (bp 7–78) since the most proximal 14 bp of the Welsch sequence are not found in ours. Every attempt has been made to exclude another exon containing these 14 bp. Scanning of the sequence of the 5′-flanking region of the gene did not reveal these 14 bp. Furthermore, probing Southern blots of the P1 clone containing the LTC_4 synthase gene with an oligonucleotide probe corresponding to these 14 bp did not reveal another exon. We thus conclude that the 14 bp reported by Welsch and colleagues are not present in the LTC_4 synthase gene and might represent a cloning artifact.

Cell-specific expression of LTC_4 synthase has been demonstrated in a limited number of cell types. Aside from this, little information is available regarding the regulation of expression of this enzyme. Initial studies demonstrated that LTC_4 synthase activity increased in thioglycolate-elicited rat peritoneal macrophages four to six times above the levels in resting macrophages (23). Studies in human erythroleukemia cells also indicate that LTC_4 synthase activity is induced by phorbol esters (consistent with the presence of AP-1 sites in the 5′-flanking region), 1α,25-dihydroxy vitamin D_3, and DMSO (24). However, molecular probes to examine the level of protein or rate of RNA transcription were not available at the time these studies were performed. More recent work in U937 cells suggests that conditioning with DMSO induces an increase in LTC_4 synthase activity, and this appears to be correlated with an increase in LTC_4 synthase protein (25). Another study, by Murakami and colleagues, demonstrates that mouse bone marrow–derived mast cells differentiated with c-*kit* ligand and IL-10, and then conditioned with

IL-3 express greater quantities of LTC_4 synthase protein and steady-state mRNA (17). This latter report suggests the possibility that LTC_4 synthase expression may, in part, be regulated by IL-3. Although these authors did not directly examine transcriptional rates of LTC_4 synthase, their studies suggest that IL-3 may act by increasing transcription. IL-3 regulation could potentially be mediated by the STAT recognition motif identified in intron 1.

In the 5′-flanking region of the human LTC_4 synthase gene, we have not found putative DNA binding motifs that are typical of regulated genes with cell-specific expression (i.e., a TATA or CAAT box). Sequence analysis has, however, demonstrated putative DNA binding motifs for CREB/ATF, AP-1, AP-2, *ets*, SP-1, and STAT transcription factors. The functional significance of these sites remains to be determined. However, we conclude that novel regulatory elements may play an important role in the regulation of this gene and that functional promoter analysis will be necessary to gain further insight into its regulation.

We have demonstrated that the human LTC_4 synthase gene is located on the most distal portion of the q arm of chromosome 5 (5q35). This finding is particularly intriguing in light of recent reports demonstrating a cytokine gene cluster, including IL-3, -4, -5, -9, and -13, on the q arm of chromosome 5 (5q31.1–q33) (26,27). In addition, linkage analysis studies suggest that familial elevations of IgE levels in Amish patients is linked to a locus on the distal q arm of chromosome 5 (27). Another study has examined polymorphic markers in this same region of chromosome 5 and found that both elevated levels of IgE and bronchial hyperresponsiveness localize to this region (28). These reports, along with our findings, suggest the possibility that the distal arm of chromosome 5 is a locus for pro-inflammatory genes. More importantly, LTC_4 synthase may represent another candidate gene that may be dysregulated in patients with inflammatory or allergic diseases, such as asthma. This regulation could be due to enhanced expression of the LTC_4 synthase via increased expression of cytokine genes such as IL-3 or via structural mutations of the regulatory elements of LTC_4 synthase.

In summary, we have demonstrated that LTC_4 is a small gene consisting of five exons and four introns. The 5′-flanking region does not contain typical regulatory elements of a gene with cell-specific expression, thus suggesting that novel factors may regulate the expression of LTC_4 synthase. This gene localizes to the distal portion of the q arm of chromosome 5 (5q35), raising the possibility that it is involved in inherited inflammatory or allergic diseases.

ACKNOWLEDGMENTS

The authors would like to acknowledge helpful discussions with Dr. Christopher K. Glass. This work was supported in part by a grant-in-aid from the American Heart Association of California, a grant from the Merit Review Board of the Department of Veterans Affairs, and a Career Investigator Award from the American Lung Association (TDB).

ADDENDUM

At the time of submission of this manuscript, the authors noted the recent publication by Penrose and colleagues reporting similar findings (Penrose JF, Spector J, Baldasaro M, et al. [1996] Molecular cloning of the gene for human leukotriene C_4 synthase. Organization, nucleotide sequence, and chromosomal localization to 5q35. *J. Biol. Chem.* **271:** 11356–11361).

REFERENCES

1. Samuelsson B. (1983) Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. *Science* **220:** 568–575.
2. Rouzer CA, Matsumoto T, Samuelsson B. (1986) Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A_4 synthase activities. *Proc. Natl. Acad. Sci. U.S.A.* **83:** 857–861.
3. Dixon RA, Diehl RE, Opas E, et al. (1990) Requirement of a 5-lipoxygenase–activating protein for leukotriene synthesis. *Nature* **343:** 282–284.
4. Rädmark O, Shimizu T, Jornvall H, Samuelsson B. (1984) Leukotriene A_4 hydrolase in human leukocytes. Purification and properties. *J. Biol. Chem.* **259:** 12339–12345.
5. Nicholson DW, Ali A, Vaillancourt JP, et al. (1993) Purification to homogeneity and the N-terminal sequence of human leukotriene C_4 synthase: A homodimeric glutathione *S*-

transferase composed of 18-kDa subunits. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 2015–2019.

6. Murphy RC, Hammarstrom S, Samuelsson B. (1979) Leukotriene C: A slow-reacting substance from murine mastocytoma cells. *Proc. Natl. Acad. Sci. U.S.A.* **76:** 4275–4279.
7. Lewis RA, Austen KF, Soberman RJ. (1990) Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *N. Engl. J. Med.* **323:** 645–655.
8. Lam BK, Penrose JF, Freeman GJ, Austen KF. (1994) Expression cloning of a cDNA for human leukotriene C_4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A_4. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 7663–7667.
9. Welsch DJ, Creely DP, Hauser SD, Mathis KJ, Krivi GG, Isakson PC. (1994) Molecular cloning and expression of human leukotriene C_4 synthase. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 9745–9749.
10. Ali A, Ford-Hutchinson AW, Nicholson DW. (1994) Activation of protein kinase C downregulates leukotriene C_4 synthase activity and attenuates cysteinyl leukotriene production in an eosinophil substrin of HL-60 cells. *J. Immunol.* **153:** 776–788.
11. Kargman S, Ali A, Vaillancourt JP, Evans JF, Nicholson DW. (1994) Protein kinase C-dependent regulation of sulfidopeptide leukotriene biosynthesis and leukotriene C_4 synthase in neutrophilic HL-60 cells. *Mol. Pharmacol.* **45:** 1043–1049.
12. Sjölinder M, Tornhamre S, Werga P, Edenius C, Lindgren JA. (1995) Phorpbol ester–induced suppression of leukotriene C_4 synthase activity in human granulocytes. *FEBS Lett.* **377:** 87–91.
13. Ring WL, Riddick CA, Baker JR, Munafo DA, Bigby TD. (1996) Lymphocytes stimulate expression of 5-lipoxygenase and its activating protein in monocytes in vitro via granulocyte macrophage colony-stimulating factor and interleukin-3. *J. Clin. Invest.* **97:** 1293–1301.
14. Steinhilber D, Radmark O, Samuelsson B. (1993) Transforming growth factor β upregulates 5-lipoxygenase activity during myeloid cell maturation. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 5984–5988.
15. Bigby TD, Meslier N. (1989) Transcellular lipoxygenase metabolism between monocytes and platelets. *J. Immunol.* **143:** 1948–1954.
16. Penrose JF, Spector J, Lam BK, et al. (1995) Purification of human lung leukotriene C_4 synthase and preparation of a polyclonal antibody. *Am. J. Respir. Crit. Care Med.* **152:** 283–289.
17. Murakami M, Austen KF, Bingham CO, Friend DS, Penrose JF, Arm JP. (1995) Interleukin-3 regulates development of the 5-lipoxygenase/leukotriene C_4 synthase pathway in mouse mast cells. *J. Biol. Chem.* **270:** 22653–22656.
18. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. (1980) Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int. J. Cancer* **26:** 171–176.
19. Ausbel FM, Brent R, Kingston RE, et al. (1992) *Current Protocols in Molecular Biology.* Greene Publishing Association and John Wiley and Sons, Brooklyn, NY.
20. Barrett KE, Yen A, Bigby TD, Montisano D, Gigli I. (1994) Inhibition of human peripheral blood lymphocyte function by protoporphyrin and longwave ultraviolet light. *J. Immunol.* **153:** 3286–3294.
21. Stokke T, Collins C, Kuo W-L, et al. (1995) A physical map of chromosome 20 established using fluorescence *in situ* hybridization and digital image analysis. *Genomics* **26:** 134–137.
22. Kennedy BP, Diehl RE, Boie Y, Adam M, Dixon RAF. (1991) Gene characterization and promoter analysis of the human 5-lipoxygenase-activating protein (FLAP). *J. Biol. Chem.* **266:** 8511–8516.
23. Abe M, Hara N, Muranishi H, Ikeda T, Nagata N, Shigematsu N. (1990) Enhanced leukotriene C_4 synthase activity in thioglycollate-elicited peritoneal macrophages. *Biochem. Biophys. Res. Commun.* **171:** 1344–1352.
24. Söderström M, Bolling A, Hammarström S. (1992) Induction of leukotriene C_4 synthase activity in differentiating human erythroleukemia cells. *Biochem. Biophys. Res. Commun.* **189:** 1043–1049.
25. Nicholson DW, Ali A, Klemba MW, Munday NA, Zamboni RJ, Ford-Hutchinson AW. (1992) Human leukotriene C_4 synthase expression in dimethyl sulfoxide-differentiated U937 cells. *J. Biol. Chem.* **267:** 17849–17857.
26. van Leeuwen BH, Martinson ME, Webb GC,

Young IG. (1989) Molecular organization of the cytokine gene cluster, involving the human IL-3, IL-4, IL-5, and GM-CSF genes, on human chromosome 5. *Blood* **73:** 1142–1148.
27. Marsh DG, Neely JD, Breazeale DR, et al. (1994) Linkage analysis of IL-4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* **264:** 1152–1156.
28. Postma DS, Bleecker ER, Amelung PJ, et al. (1995) Genetic susceptibility to asthma—Bronchial hyperresponsiveness coinherited with a major gene for atopy. *N. Engl. J. Med.* **333:** 894–900.

Contributed by K. S. Warren on June 13, 1996.