The Cystic Fibrosis Gene Has a "Housekeeping"-type Promoter and Is Expressed at Low Levels in Cells of Epithelial Origin*

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Evaluation of the expression of the cystic fibrosis (CF) gene in human epithelial cell lines demonstrated active, but low level, transcription of the gene. Analysis of 3.8 kilobases of genomic sequences 5' to exon 1 of the CF gene demonstrated no TATA promoter element, but a high G + C content, multiple transcription start sites, and several potential Sp1 binding sites. Fragments of 5'-flanking sequences from 2.2 kilobases to as small as 102 base pairs 5' to the major transcription start site supported constitutive reporter gene expression in epithelial cells, but at low levels, and independent of the length of the 5' fragment. CF gene transcription was down-regulated by phorbol myristate acetate. Importantly, evaluation of freshly isolated normal human bronchial cells also demonstrated CF gene transcription at a relatively low rate. Together, these observations suggest that although the normal CF gene promoter has characteristics of a "housekeeping"-type gene, and the gene is expressed at low levels in cells of organs that manifest the clinical disorder "cystic fibrosis," its expression can be modulated transcriptionally, implying a possible therapeutic approach for the disease.

Cystic fibrosis (CF), a fatal recessive hereditary disorder characterized by abnormalities of electrolyte transport in organs with exocrine glands, results from mutations of a 27-exon 250-kb gene on chromosome 7 at q31 (1-7). The major clinical manifestations of CF are in the lung, with mucus impaction, bacterial colonization, chronic infection, and consequent derangements of airways and lung parenchyma. In the gastrointestinal tract, pancreatic insufficiency and large bowel water and electrolyte abnormalities can cause diarrhea or intestinal obstruction (1). In the skin, sweat gland secretions have a high content of Cl-, a feature that forms the basis of the classic laboratory diagnosis of the disease (1).

The predicted primary translation product of the CF gene is a 1480-amino acid protein termed the "cystic fibrosis transmembrane conductance regulator (CFTR)" (3). Although the function of this putative protein is unknown, evidence from a variety of sources suggests it modulates Cl-, and possibly Na+, transport across the apical surface of epithelial cells (8-14). In this regard, compared with normal cells, cultured airway and pancreatic epithelial cells derived from individuals with CF secrete less Cl- in response to secretagogues that increase intracellular CAMP or activate protein kinase C (10-14). Importantly, transfer of a normal CF cDNA reverses these abnormalities in Cl- secretion (15-17). In addition to modulating the electrolyte milieu on epithelial apical surfaces, it has been hypothesized that CFTR may function to regulate electrolyte transport of intracellular vesicles (18).

The purpose of the present study is to evaluate the level of CF gene transcription in epithelial cells and to characterize the promoter of the gene. Interestingly, the structure of the promoter and its function in cells of epithelial origin show that it has the characteristics of a promoter of a "housekeeping"-type gene, but is capable of being modulated at the transcriptional level, an observation with important implications for strategies designed to reverse the CF phenotype in vivo.

MATERIALS AND METHODS

Cell Cultures—Evaluation of CF gene expression was carried out in the colon adenocarcinoma cell lines HT-29 (American Type Culture Collection ATCC HTB 38) and T84 (ATCC CCL 248). HT-29 cells were cultured in Dulbecco's modified Eagle's medium (Whittaker Bioproducts) supplemented with 10% fetal bovine serum (Biofluids) and T84 cells in Dulbecco's modified Eagle's medium with 5% fetal bovine serum using standard techniques.

CF mRNA Transcripts—CF mRNA transcripts were evaluated by Northern analysis (19). Total RNA was isolated from HT-29 or T84 cells by the guanidinium thiocyanate-CsCl gradient method. RNA (10 μg) was subjected to formaldehyde-agarose gel electrophoresis, transferred to a nylon membrane (Nytran, Schleicher and Schuell), hybridized with a 32P-labeled 1.6-kb CF cDNA probe (PouII-Acl fragment spanning exons 2-12 (ATCC 61136)) generated by random priming method, and evaluated by autoradiography.

CF Gene Expression Rate—CF gene transcription rate was examined by nuclear transcription run-on analysis (20). Nuclei isolated from 5 x 10⁶ cells of each cell line were incubated with 5 mM ATP, 2 mM CTP, 2 mM UTP, 250 μCi of [α-32P]GTP (Amersham Corp., >400 Ci/mmol), and RNase inhibitor (RNasin, Promega) to label actively transcribed RNA. The RNA was recovered by the acid guanidinium thiocyanate-phenol-chloroform method, purified by Sephadex G-50 column chromatography, and hybridized to excess amounts (6 μg) of DNA targets (see below) immobilized on Nitran. The membranes were then washed, exposed to Rnase A and Rnase T1, followed by proteinase K (all from Boehringer Mannheim), and evaluated by autoradiography. The DNA targets included a human CF cDNA (pTG4964; a 4.5-kb cDNA encompassing the entire coding sequence, constructed by standard methods from polymerase chain...
reaction (PCR) amplified fragments of cDNA derived from human lung poly(A) RNA, genomic clones for c-fos and c-myc (both from Lofstrand Labs), human β-actin cDNA (pHFA-1) (21), and, as a negative control, the plasmid pUC19. To determine the relative transcription rate of the CF gene compared with the β-actin gene (designated 100), we quantified by densitometric scanning and the values normalized to the relative length of the mRNA coding sequences within DNA targets (CF, 4.5 kb; β-actin, 2.0 kb, respectively). To evaluate the effect of phorbol 12-myristate 13-acetate (PMA) on CF gene transcription, HT-29 and T84 cells were incubated in the absence or presence of PMA (HT-29, 100 nM; T84, 40 nM) for 24 h. Nuclear run-on analyses were carried out as described above, and the changes in the transcription level of the CF gene as well as the control β-actin gene were assessed by the laser densitometry.

Sequence of the 5′-Flanking Region of the CF Gene—A 4.3-kb EcoRI DNA fragment containing CF gene exon 1 and 5′-flanking region was isolated from a human chromosome 7-specific library (ATCC 57722), subcloned into pUC19 (pPB236), and sequenced by the dyeoxy chain termination method (22). An homology search between the human CF gene 5′-flanking region sequence and sequences in GenBank was performed using DNATIS (Hitachi America) and FASTA (23).

Identification of Transcription Start Sites—Three methods were used to identify the CF gene transcription start sites in HT-29 and T84 cells: primer extension analysis, S1 nuclease mapping, and RNase protection analysis. Primer extension analysis was performed using two different antisense primers located in exon 1 (CFEXI-2 (5′-GTTTTGAGGACACCTGGTTTTTC-3′) and HCF-13 (5′-CTG-AAGGTTCGAGACACCGC-3′)) 5′ end-labeled with [32P]ATP (Amersham Corp., >5000 Ci/mmol). Briefly, 100 µg of total RNA was hybridized with 5 × 10^6 cpm of CFEXI-2 or HCF-13, incubated with Moloney murine leukemia virus reverse transcriptase (GIBCO/Bethesda Research Laboratories) and unlabeled nucleotide triphosphates. Primer extension products were then fractionated on denaturing polyacrylamide gels and evaluated by autoradiography. S1 nuclease mapping was carried out using standard techniques with a 32P-end-labeled 800-bp HindIII-AcoI fragment of pPB236. RNase protection analysis was done using a 1.3-kb HindIII-EcoRI fragment of pPB236 after subcloning into the transcription vector plBluescript II SK+ (Stratagene). A β-actin antiserum cDNA was synthesized by T3 RNA polymerase, hybridized with cellular RNA, and evaluated by nuclear transcription run-on analysis as described above.

RESULTS AND DISCUSSION

Consistent with the concept that the disease “cystic fibrosis” is manifest in epithelial tissues, and the observation of CF mRNA transcripts in the pancreatic adenocarcinoma cell line CFPAC-1 (26) and cells derived from skin exocrine sweat gland ducts (3), analysis of total cellular RNA by Northern hybridization demonstrated 6.5-kb CF mRNA transcripts in the human colon epithelial cell lines HT-29 and T84 (Fig. 1A). Furthermore, consistent with the presence of CF mRNA transcripts, HT-29 and T84 cells actively transcribed the CF gene (Fig. 1B). However, the rate of transcription of the CF gene was relatively low, representing approximately 5% of that of a control β-actin gene in both HT-29 and T84 cells (data based on densitometric scanning of the autoradiograms in Fig. 1B).

Surprisingly, but consistent with the relatively low rate of transcription of the CF gene, sequence analysis of 3.8 kb 5′ to exon 1 of the CF gene demonstrated structural features typical of a housekeeping gene (27) (Fig. 2). First, there was no TATA promoter element within 500 bp upstream of the major transcription start site defined as +1 by primer extension analysis (Fig. 2B). Two sequences with homology to a CCAAT box and one inverted CCAAT box motif flanking exon 1 were observed, but they did not occur at positions typical for functional CCAAT sites in most regulated genes (28). Second, the G + C content was high; for example, it averaged 65% in the 500 bp immediately 5′ to exon 1, compared with 40% for the entire human genome. Third, multiple transcription start sites were observed in cells expressing the CF gene. This was observed among mRNA transcripts in both

![Fig. 1. Expression of the CF gene in human epithelial cell lines. A, CF gene mRNA transcripts. Shown are Northern analyses of total cellular RNA. Lane 1, HT-29 colon carcinoma cells (ATCC HTB 29); lane 2, T84 colon carcinoma cells (ATCC CCL 240). The position of the 6.5-kb CF mRNA transcripts is indicated. B, CF gene transcription. The rate of transcription was evaluated in HT-29 (lane 3) and T84 (lane 4) cell lines by nuclear transcription run-on analysis (20). In comparison to the data for the CF gene, data for the genes c-fos, c-myc, and β-actin are shown. The plasmid pUC19 is a negative control.](https://example.com/fig1.png)
HT-29 and T84 cells, where multiple minor transcription start sites were observed in addition to the major start site -72 bp 5' to the putative translation start codon in exon 1. The same observations were made using three separate analytical methods, primer extension analysis (Fig. 2B), S1 nuclease mapping, and RNase protection analysis (data not shown). Finally, there were three potential binding sites for the transcription factor Spl (GC boxes at -950, -334, and -255). A 38-bp segment from -168 to -141 has an 89% homology with the promoter sequence of human α(1) collagen gene (dashed underline). B, determination of the transcription start sites. Shown is the primer extension analysis of CF mRNA from HT-29 and T84 cell lines utilizing the antisense primer CFX1-2. The results are shown diagrammatically at the top with representative autoradiograms at the bottom. Multiple transcription start sites were observed ranging over 110 bp in both HT-29 and T84 cells, with one prominent band (defined as +1) with microheterogeneity. Minor bands were observed at -61, -60, -1, +2, +4, +35, +44, +52, and +53.

**Fig. 2. Structural organization of the 5'-flanking region of the CF gene.** A, sequence of 5'-flanking region of exon 1. Of the 3.8 kb sequenced 5' to exon 1, the nucleotide sequence from -1000 to +99 is shown; the sequence from -3825 to +574 has been submitted to GenBank. Numbers are relative to the major transcription start site (indicated by the arrow) as defined below in B. The putative start codon in exon 1 is located at position +72, and the deduced amino acid sequence is shown below (single-letter code). There are two CCAAT-like sequences (-168 and -52, underline) and one inverted CCAAT motif (-60, overline). Three putative binding sites for the nuclear transcription factor Spl are indicated by the boxed GGGCGG core sequences at -950, -334, and -255. A 38-bp segment from -168 to -141 has an 89% homology with the promoter sequence of human α(1) collagen gene (dashed underline). B, determination of the transcription start sites. Shown is the primer extension analysis of CF mRNA from HT-29 and T84 cell lines utilizing the antisense primer CFX1-2. The results are shown diagrammatically at the top with representative autoradiograms at the bottom. Multiple transcription start sites were observed ranging over 110 bp in both HT-29 and T84 cells, with one prominent band (defined as +1) with microheterogeneity. Minor bands were observed at -61, -60, -1, +2, +4, +35, +44, +52, and +53.

**Table I**

| Gene | Experiment | HT-29 | T84 |
|------|------------|-------|-----|
|      | Resting    | +PMA  | Resting | +PMA  |
| CF   | 1          | 2,119 | 1,104 | 7,101 | 2,492 |
|      | 2          | 5,938 | 3,581 | 3,004 | 1,542 |
| β-Actin | 1        | 14,490 | 14,220 | 23,460 | 33,360 |
|      | 2          | 32,210 | 37,060 | 8,372 | 33,290 |

**Cystic Fibrosis Gene Promoter**

in intracellular cAMP is one mechanism by which the CF gene product may modulate Cl− secretion (8–14, 18, 26). There were also a number of potential glucocorticoid response elements (positions -1466, -1226, -1140, -941) as well as AP-2 binding sites (-1107, -344) and one C/EBP binding site (-196) (30). Comparison of the sequence of 9.9 kb 5' to exon 1 of the CF gene to that of other promoters demonstrated no striking similarities except for a 28-bp sequence at -168 that had an 89% homology with a segment of the promoter of the human α(1) collagen gene, a sequence important in the regulation of that gene (32). In addition, CF gene sequences

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2 Trappell, B. C., Zeitlin, P. L., Chu, C.-S., Yoshimura, K., Nakamura, H., Guggino, W. B., Bargan, J., Banks, T. C., Dalemans, W., Pavirani, A., Lecocq, J.-P., and Crystal, R. G. (1991) *J. Biol. Chem.*, in press.
Fig. 3. Promoter activity of the CF gene 5'-flanking region. Levels of luciferase expression by fusion gene constructs of CF 5'-flanking region and a luciferase reporter gene are shown relative to the expression of the positive control pRSVL. pLucO, a promoterless luciferase plasmid, served as a negative control. Each data point is the average of three independent transfection experiments.

A. PCR

Gel

Southern

bp

603

1

2

CF

c-fos

-1575

-718

-1036

pUC19

B. Relative activity (% RSV control)

CF 5'

+1

exon I

Major start

+24

Luciferase

RSV LTR

pCF2244L

Luciferase

pCF1575L

Luciferase

pCF1036L

Luciferase

pCF718L

Luciferase

pCF391L

Luciferase

pCF187L

Luciferase

pCF102L

Luciferase

pLucO

Relative activity (% RSV control)

0 5 10 100

Fig. 4. CF gene expression in normal human bronchial epithelial cells. A, CF gene mRNA transcripts. The size of the expected amplified CF cDNA fragment is indicated. Shown are an ethidium bromide-stained agarose gel (lane 1) and Southern hybridization (lane 2). B, transcription of the CF gene. Shown in comparison to the CF gene are the genes for c-fos, c-myc, and β-actin. The plasmid pUC19 served as a negative control.

At positions −680 to −670, −497 to −478, −286 to −274, and −105 to −90 showed approximately 80% homology with sequences of the promoter of the secretory leukoprotease inhibitor gene, a gene expressed only in epithelial cells such as the bronchial epithelium.7

Transfection of fusion genes composed of CF gene 5'-flanking sequences and a luciferase reporter gene into HT-29 cells proved that these CF gene sequences had promoter function in cells that normally transcribed the endogenous CF gene (Fig. 3). Interestingly, the region including only 102 bp immediately 5' of the major transcription start was capable of promoting luciferase expression. Consistent with the housekeeping gene concept (27), the relative amount of reporter gene expression did not change appreciably by progressively including additional 5' sequences up to −2244 from the transcription start. Importantly, and consistent with the relatively low level expression of the CF gene in epithelial cells is not necessary to sustain life or even to maintain the viability of the cells that express the gene (2-14, 26). Consistent with this concept, there is evidence that epithelial cell genes code for proteins that are required for general cellular metabolic functions, the CF gene product probably is not absolutely required for function of the organ, rather than for the well-being of the cell. In this context, although mutations of the CF gene have profound consequences to organs such as the lung or pancreas, expression of the CF gene in epithelial cells is not necessary to support life or even to maintain the viability of the cells that express the gene (2-14, 26). Consistent with this concept, there is evidence that epithelial cells expressing the CF gene may be in a subgroup of genes with structural characteristics of housekeeping genes, but that have tissue-specific functions such as the human nerve growth factor receptor gene (37) and the Pim-1 proto-oncogene (33) and/or that exhibit regulation in gene expression by specific agents.

Irrespective of the classification of the promoter of the CF gene, the observation that the CF gene is transcribed in gene transcription in a fashion as close to the in vivo milieu as possible, normal human bronchial epithelial cells were evaluated as quickly as possible after removal, i.e. obviating potential modification by in vitro maintenance in culture. This evaluation is particularly relevant to CF, as the bronchial epithelium is the major site of the clinical manifestations of the disease. Not only were CF mRNA transcripts observed in freshly isolated bronchial cells (Fig. 4A), but nuclei isolated from normal bronchial epithelium demonstrated active transcription of the CF gene (Fig. 4B). Importantly, as observed in HT-29 and T84 cells, the level of transcription was quite low, representing only approximately 6% of that of the β-actin gene.

Together, the structural features of the CF promoter and the low level expression of the CF gene in epithelial cells suggest that it should be classified as a housekeeping gene, together with other genes such as dihydrofolate reductase, hypoxanthine phosphoribosyl transferase, adenosine deaminase, and phosphoglycerate kinase genes (27). However, whereas most housekeeping genes code for proteins that are required for general cellular metabolic functions, the CF gene product probably is not absolutely required for function of the cell itself, i.e. the housekeeping function of the CF gene product may be more relevant to the overall function of the organ, rather than for the well-being of the cell. In this context, although mutations of the CF gene have profound consequences to organs such as the lung or pancreas, expression of the CF gene in epithelial cells is not necessary to maintain the extracellular milieu and/or within specialized vesicles such as those of bronchial mucus-producing cells (18, 35, 36). Alternatively, the electrolyte-related functions of CFTR may be only one part of its function, with other specialized functions linked to the disease process of cystic fibrosis. Thus, the CF gene may be in a subgroup of genes with structural characteristics of housekeeping genes, but that have tissue-specific functions such as the human nerve growth factor receptor gene (37) and the Pim-1 proto-oncogene (33) and/or that exhibit regulation in gene expression by specific agents.
normal bronchial tissues at a low rate, together with the knowledge that individuals homozygous for the \( \Delta \text{Phe}^{508} \) mutation have CF mRNA transcript levels in respiratory epithelium comparable with normals (31). It is important to note that the low level expression of the two endogenous CF genes sufficient to convert the respiratory epithelium to the status of at least that of a CF heterozygote. Furthermore, the observation of the down-regulation of CF gene transcription by PMA leads to the possibility that the normal CF gene can be corrected by developing agents that would mimic this effect.

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