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Expression of the cystic fibrosis transmembrane conductance regulator gene in cells of non-epithelial origin

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ABSTRACT

Consistent with the fact that the clinical disorder cystic fibrosis (CF) is manifested on epithelial surfaces, active transcription of the CF transmembrane conductance regulator (CFTR) gene and CFTR mRNA transcripts are detectable in a variety of epithelial cells, suggesting CFTR gene expression might be epithelial cell-specific. However, analysis of the CFTR gene promoter suggests it is a housekeeping gene, implying more widespread expression than only in epithelial cells. To evaluate the latter hypothesis, various human cells of non-epithelial origin, including lung fibroblasts, U-937 histiocytic lymphoma cells, K-562 erythroleukemia cells, HL-60 promyelocytic leukemia cells as well as freshly isolated blood lymphocytes, neutrophils, monocytes, and alveolar macrophages were examined for CFTR gene expression. Although Northern analysis failed to show CFTR mRNA transcripts in these cells, amplification of mRNA (after conversion to cDNA) by polymerase chain reaction combined with Southern analysis demonstrated the presence of CFTR mRNA transcripts at low levels in all cells evaluated except HL-60 cells. Comparative quantitative analysis showed fibroblasts contained 200 – 400 fold less CFTR mRNA transcripts than the T84 and HT-29 colon carcinoma epithelial cell lines, but had similar levels of CFTR transcripts to those of other epithelial cell lines. Nuclear transcription run-on analyses demonstrated very low level CFTR gene transcription in fibroblasts and U-937 cells, similar to that of other epithelial cells, but lower than the T84 and HT-29 colon carcinoma cell lines. Interestingly, while chromatin DNA of fibroblasts had no DNase I hypersensitivity sites in the 5′ flanking region of the CFTR gene, HT-29 chromatin DNA exhibited four DNase I accessible sites in the same region, suggesting that these sites may be related to more active transcription of the CFTR gene in the intestinal epithelial cells than in fibroblasts.

INTRODUCTION

Cystic fibrosis (CF), one of the most common fatal hereditary disorders of Caucasians, is a disease manifested on epithelial surfaces, particularly in the lung, pancreas and intestine (1). The disease is caused by mutations of the 'cystic fibrosis transmembrane conductance regulator' (CFTR) gene, a 27 exon, 250 kb gene on chromosome 7 at q31, whose predicted primary translation product is a 1480 amino acid protein (2,3). Although all of the functions of this CFTR protein are not clear, available evidence from a variety of sources suggests it may be a regulated anion channel which secretes Cl^- across the apical membrane of epithelial cells in response to activation of specific pathways regulated by increased intracellular cyclic AMP (cAMP) or protein kinases (4–12). Consistent with this concept, CFTR mRNA transcripts have been observed in organs with epithelia and in epithelial cell lines derived from colon and pancreas carcinomas, as well as transformed airway epithelial cells, cultured sweat gland cells, and fresh bronchial epithelial cells (3,13–18). Further, nuclear transcription run-on analyses have shown epithelial cells actively transcribe the CFTR gene, and transfection studies have demonstrated that 5′ flanking sequences of the CFTR gene are capable of promoting reporter gene expression in epithelial cells (13).

Although this evidence suggests the expression of the CFTR gene might be epithelial cell-specific, there is also evidence that the expression of the gene may be more widespread. In this regard, CFTR mRNA transcripts in lymphocytes have been used to identify mutations in the CFTR gene (19), and fibroblasts and lymphocytes derived from individuals with CF have defective Cl^- secretory responses to secretagogues which increase intracellular cAMP levels (20–22). Further, the promoter of the CFTR gene has characteristics of a housekeeping gene in that it has no TATA box, a high G + C content, multiple transcription start sites, several putative binding sites for the transcription factor Sp1, and supports only low level transcription (13,23), implying that the expression of the CFTR gene may not be limited to epithelial cells.

With this as background, the present study has been directed toward evaluating the expression of the CFTR gene in human cells of non-epithelial origin. Interestingly, we have found that although the levels of expression vary, the CFTR gene is expressed in many of these cells.

MATERIALS AND METHODS

Source of cells and cell culture

Non-epithelial cells included both cell lines and freshly isolated cells, all of human origin. The non-epithelial culture cell lines evaluated were HFL1 human diploid lung fibroblasts [American

Type Culture Collection (ATCC) CCL 153], WI-26VA4 transformed lung fibroblasts (ATCC CCL 95.1), U-937 histiocytic lymphoma cells (ATCC CRL 1593), K-562 erythroleukemia cells (ATCC CCL 243) and HL-60 promyelocytic leukemia cells (ATCC CCL 240). Fresh T-lymphocytes, neutrophils and monocytes were isolated from blood of normal individuals (24). Alveolar macrophages were obtained by bronchoalveolar lavage from normal nonsmoking volunteers (25). As epithelial cell controls, the colon adenocarcinoma cell lines T84 (ATCC CCL 248) and HT-29 (ATCC HTB 38) [cells known to express the CFTR gene (3,13,14)], as well as the HS-24 human bronchial squamous carcinoma cell line (provided by W. Ebert, Thoraxklinikum, Heidelberg-Rohrbach, West Germany) (26) and the HeLa cervical carcinoma cell line (ATCC CCL 2) were evaluated. All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM, Whittaker Bioproducts) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 50 units/ml penicillin and 50 μg/ml streptomycin (all from Biofluids, Inc.), except T84 cells which were maintained in DMEM with 5% FBS, 2 mM glutamine, 50 units/ml penicillin and 50 μg/ml streptomycin, and HS-24 cells in RPMI 1640 with 25 mM N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), pH 7.4, 2 mM glutamine, 100 μg/ml gentamicin (all from GIBCO/BRL). All experiments with adherent cell lines were carried out when the cells were 80−90% confluent. Suspension cell lines were utilized during exponential growth ($5-10\times10^5$/ml). All cell populations were >95% viable by trypan blue exclusion.

Evaluation of CFTR mRNA transcript levels

CFTR mRNA transcript levels were evaluated by two separate methods: Northern analysis (27) and amplification of mRNA (after conversion to cDNA) using the polymerase chain reaction (PCR) followed by Southern hybridization (28,29). Total cellular RNA was isolated from each cell type by the guanidine thiocyanate-cesium chloride gradient method (30). Northern analysis was carried out using 10 μg of total RNA from each cell type. For mRNA evaluation by PCR, the equal amount of total RNA (5 μg) from each cell type was first incubated (50 μl total volume) with Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL) and either oligo-dT primer (for subsequent amplification of the region spanning exons 21−24 only), or random hexanucleotide primers (for subsequent amplification of the regions spanning exons 1−5, exons 17b−19, and exons 21−24) (both primers from Promega) to convert mRNA to cDNA (31). To quantify CFTR gene mRNA transcripts, PCR amplification was performed for the region spanning exons 21−24 (603 bp) using 5 μl of each reverse transcription product as a template, *Taq* DNA polymerase (Perkin-Elmer Cetus), and CFTR gene specific primers in exon 21 (HCF-12: 5'-AGTGGAGTGATCAAGAAATATGG-3') and exon 24 (HCF-6: 5'-TCCACGAGCTCCAATTCCATGAGG-3'), primers chosen to cross exon-intron boundaries to preclude amplification of potentially contaminating genomic DNA (3,13,16,28). The relative CFTR mRNA transcript levels among various cell types were evaluated by PCR amplification (94°C−2 min×1 cycle; 94°C−1 min, 58°C−2 min, 72°C−3 min, ×25 cycles; 72°C−5 min×1 cycle) (DNA Thermal Cycler, Perkin-Elmer Cetus) of serial dilutions of each cDNA (13,16). To demonstrate that cDNA could be quantitatively evaluated, standard curves were established using 5 μl of serially diluted reverse transcription products (1, 10, 10^2 and 10^3-fold) as templates. To confirm the quantification of CFTR mRNA transcripts in non-epithelial cells, two other regions of CFTR mRNA transcripts were also amplified: exons 1−5 (686 bp; with HCF-33, 5'-AGTAGTAGGTCTTTGGCATTAGG-3' and HCF-60, 5'-CATCAAATTTGTTCAGGTTGTTGG-3'), and exons 17b−19 (538 bp; with HCF-47, 5'-TCATCTTGTTACAAGCTTAAAAGG-3' and HCF-48, 5'-AGAAGGAAATGTTCTCTAATATGG-3'). As a control, γ-actin mRNA transcripts were evaluated by the similar techniques except the primers were specific for γ-actin transcripts (HAG-3, 5'-ATGAAGATCAAGATCATCGCACCC-3'; and HAG-4, 5'-CACCAAGCCACCGACTTGTCTTCC-3'), and amplification was carried out for only 18 cycles (16,32). Following amplification, the cDNA was subjected to agarose gel electrophoresis, transferred to nylon membranes (Nytran, Schleicher and Schuell), cross-linked by ultraviolet (UV) irradiation (Stratalinker, Stratagene), and hybridized with ^{32}P-labeled probes [prepared by the random priming method (33)]. For evaluation of CFTR exon 21−24 amplified product, a nested cDNA probe was used; for the comparative analysis of amplification of 3 different CFTR transcript regions, a 4.5 kb cDNA probe was used; and for evaluation of the γ-actin gene, a nested cDNA probe was used (13,16,32). Hybridization was carried out in 0.5 M sodium phosphate buffer, pH 7.0, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% (w/v) bovine serum albumin (BSA), and 7% (w/v) sodium dodecyl sulfate (SDS) at 65°C for 36 h (34). Membranes were then washed twice in 2×SSC (1×SSC: 150 mM sodium chloride and 15 mM sodium citrate), 0.1% SDS at 23°C for 30 min, and twice in 0.1×SSC, 0.1% SDS at 65°C for 30 min, and evaluated by autoradiography. To quantify the relative CFTR gene mRNA transcript levels, the resulting autoradiograms were scanned by a laser densitometer (Ultroscan Laser Densitometer, Pharmacia-LKB).

Transcription of the CFTR gene

The CFTR gene transcription rates in various cell lines were evaluated by nuclear transcription run-on analysis (13,35,36). Nuclei isolated from 5×10^7 cells of each cell line were incubated (37°C, 30 min) with 5 mM ATP, 2 mM CTP, 2 mM UTP, 250 μCi [α-^{32}P]GTP (>400 Ci/mmol, Amersham) in the presence of 2 units/μl RNase inhibitor (RNasin, Promega) to label actively transcribed RNA. RNA was then recovered by the acid guanidinium thiocyanate-phenol-chloroform method (37) using RNAzol B (Cinna/Biotex Laboratories International), and resuspended in 10 mM HEPES, pH 7.4, 5 mM EDTA, 0.1% SDS. ^{32}P-labeled nascent RNA was purified by Sephadex G-50 column chromatography (5 prime → 3 Prime), denatured (65°C, 10 min), and hybridized to filter-bound DNA targets in 1 ml of hybridization buffer at 65°C for 36 h as described above. Plasmids containing the individual DNA targets (5 μg each) were denatured in 200 mM NaOH at 95°C for 5 min, neutralized with 300 mM HEPES (free acid), blotted on Nytran membranes using a slot blot apparatus (Minifold II, Schleicher & Schuell), and immobilized by UV cross-linking. DNA targets included a human CFTR cDNA pTG4964 (13), genomic clones for the human proto-oncogenes c-*fos* and c-*myc* containing no repetitive sequences (both from Lofstrand Labs), a human β-actin cDNA pHFβA-1 (32), and as a negative control, the plasmid pUC19. Except for 0.5% BSA, hybridization was carried out under the same conditions as described for Southern analysis above. After hybridization, the membranes were washed 3 times in 2×SSC at 23°C for 10 min, exposed to 5 μg/ml RNase A and 5 units/ml RNase T1 in 2×SSC, 10 mM Tris-HCl, pH 7.4, at 37°C for

30 min, followed by incubation with 50 mg/ml proteinase K (all from Boehringer Mannheim Biochemicals) in 2×SSC, 10 mM Tris-HCl, pH 7.4, 0.5% SDS at 37°C for 45 min, washed in 2×SSC, air dried and evaluated by autoradiography (13,35). To determine the relative transcription rate of the CFTR gene compared to the β-actin gene (defined as 100%), the autoradiograms were quantified by laser densitometric scanning, and after subtracting background hybridization to the plasmid pUC19, the values normalized to the relative length of the RNA coding sequences within DNA targets (CFTR 4.5 kb; β-actin 2.0 kb, respectively).

DNase I hypersensitivity site mapping of CFTR gene 5′ flanking region

The 4.4 kb *Eco*RI segment of the CFTR gene containing the 5′ flanking sequences was evaluated for DNase I hypersensitivity sites in chromatin DNA of freshly isolated nuclei from HT-29 epithelial cells and HFL1 fibroblasts (38,39). Briefly, cells were detached from culture plates with trypsin, washed with phosphate buffered saline, pH 7.4, and resuspended in nuclear isolation buffer [15 mM Tris-HCl, pH 7.4, 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 0.1 mM ethylene glycol-bis(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA), 0.5 mM dithiothreitol] containing 0.3 M sucrose and 0.2% (v/v) Nonidet P-40. Nuclei were isolated using sucrose gradient centrifugation at 25,000×g, 18 min, resuspended in 10 mM Tris-HCl, pH 7.4, 10 mM KCl, 3 mM $MgCl_2$, and exposed to bovine pancreatic DNase I (10 ng/μg DNA, Boehringer Mannheim Biochemicals) for 0, 1, 2, or 4 min. The chromatin DNA was extracted and purified. DNA (20 μg) from each DNase I exposure was completely digested with *Eco*RI (New England Biolabs, Inc.), subjected to agarose gel electrophoresis, and evaluated by Southern hybridization analysis as described above. The ^{32}P-labeled probe used was a 450 bp *Eco*RI-*Acc*I fragment located at the 5′ end of the 4.4 kb *Eco*RI segment of the CFTR gene (prepared by the random priming method) (13,33).

RESULTS

CFTR mRNA transcript levels

Although Northern analyses readily demonstrated 6.5 kb CFTR mRNA transcripts in the intestinal epithelial cell lines T84 and HT-29 as previously described (3,13,14), no CFTR mRNA transcripts were observed in any non-epithelial cell line under identical conditions with the equal amount of total RNA and the same ^{32}P-labeled 4.5 kb CFTR cDNA probe (data not shown). However, using the more sensitive technique of PCR amplification after reverse transcription followed by Southern hybridization, it was apparent that almost all non-epithelial cells contained CFTR mRNA transcripts, albeit in reduced quantity (Figure 1).

First, comparative analysis using quantitative PCR (in which the amplification of CFTR mRNA transcripts in the region of exons 21−24 was in linear relationship to the input template cDNA amount) showed that diploid HFL1 fibroblasts contained CFTR mRNA transcripts, although at levels 200- to 400-fold less than T84 or HT-29 cells (Figure 1A). However, the differences between non-epithelial and epithelial cells were less striking when the fibroblasts were compared to the HS-24 lung epithelial cell line, in which the level of CFTR transcripts was only 1.5 to 2-fold greater. In contrast, as control, γ-actin mRNA transcript levels evaluated by the identical methodology were quite similar among these four cell lines.

Second, evaluation of a variety of human cells of non-epithelial origin demonstrated that the presence of CFTR mRNA transcripts

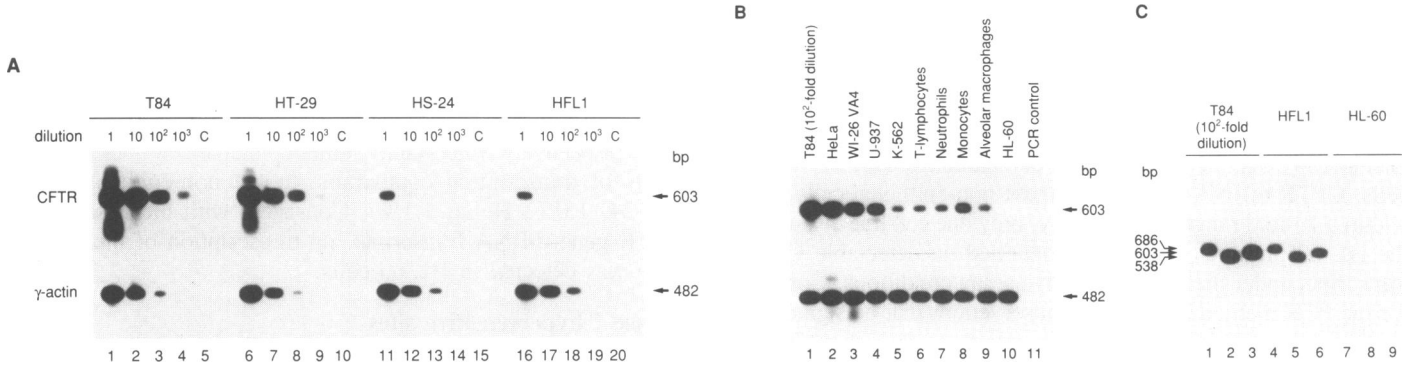

Figure 1. Expression of the CFTR gene in human non-epithelial cells compared to that in epithelial cells. (A) Quantitative analysis of the levels of CFTR gene mRNA transcripts (*top*), and as a control, γ-actin mRNA transcripts (*bottom*). mRNA transcripts were evaluated by polymerase chain reaction (PCR) amplification of cDNA (converted from mRNA by reverse transcriptase) and Southern hybridization. Data shown from left to right are: T84 colon carcinoma cells (lanes 1−4), HT-29 colon carcinoma cells (lanes 6−9), HS-24 bronchial squamous carcinoma cells (lanes 11−14), and HFL1 lung fibroblasts (lanes 16−19). Serially diluted (from 1 to 10^3-fold) reverse transcription products were used as the templates for PCR amplification for each cell line. PCR control lane for each cell type contained no DNA as a template (labeled 'C': lanes 5,10,15,20). The sizes of the amplified mRNA transcripts (cDNA) are indicated (CFTR 603 bp, γ-actin 482 bp). (B) CFTR gene mRNA transcripts (*top*), and γ-actin mRNA transcripts (*bottom*) in various human cell types. Human cells included: T84 colon carcinoma cells (lane 1), HeLa cervical carcinoma cells (lane 2), WI-26VA4 transformed lung fibroblasts (lane 3), U-937 histiocytic lymphoma cells (lane 4), K-562 erythroleukemia cells (lane 5), blood T-lymphocytes (lane 6), neutrophils (lane 7), monocytes (lane 8), alveolar macrophages (lane 9) and HL-60 promyelocytic leukemia cells (lane 10). The reverse transcription products of T84 cells (diluted 10^2-fold) served as a positive control for the CFTR transcripts (lane 1) and no DNA template served as a negative PCR control (lane 11). For γ-actin mRNA amplification in T84 cells, reverse transcription products used as a template for the PCR were undiluted. The sizes of the amplified mRNA transcripts are the same as those in Figure 1A (as noted above). (C) Amplification of three different regions of CFTR gene mRNA transcripts. Lane 1—T84 cells (with 10^2-fold diluted PCR template) evaluated for exon 1−5 containing transcripts; lane 2—same as lane 1 but for transcripts containing exons 17b−19; lane 3; same as lane 1 but for exons 21−24; lane 4—HFL1 fibroblasts for exons 1−5; lane 5—same as lane 4 but for exons 17b—19; lane 6—same as lane 4 but for exons 21−24; lane 7—HL-60 cells for exons 1−5; lane 8—same as lane 7 but for exons 17b−19; lane 9—same as lane 7 but for exons 21−24. The expected sizes of the amplified products are shown (exons 1−5, 686 bp; exons 17b−19, 538 bp; exons 21−24, 603 bp).

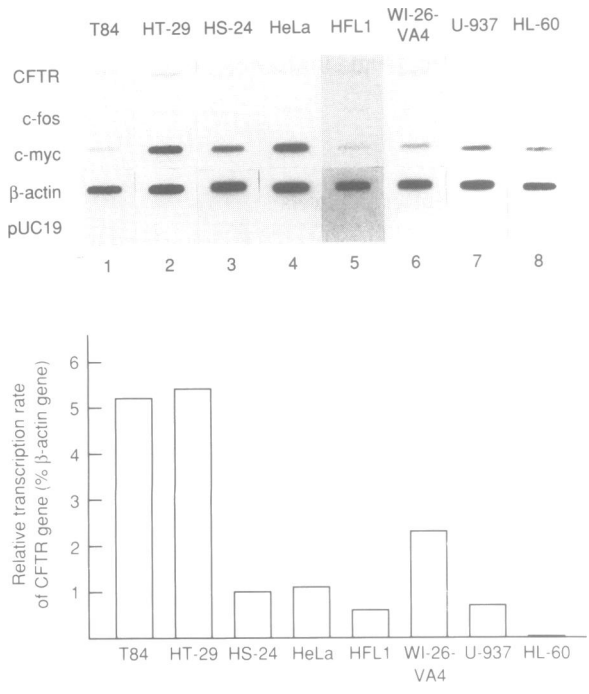

Figure 2. Comparison of the transcription rate of the CFTR gene to control genes in epithelial cells and in non-epithelial cells. (*Top*) Shown is transcription run-on analysis for: T84 (lane 1), HT-29 (lane 2), HS-24 (lane 3), HeLa (lane 4), HFL1 (lane 5), WI-26VA4 (lane 6), U-937 (lane 7) and HL-60 (lane 8). For further comparison to the data for the CFTR, also shown are c-*fos*, c-*myc* and β-actin gene transcription run-on data. The plasmid pUC19 is a negative control. (*Bottom*) Relative transcription rate of the CFTR gene compared to that of the β-actin gene defined as 100% (see Materials and Methods).

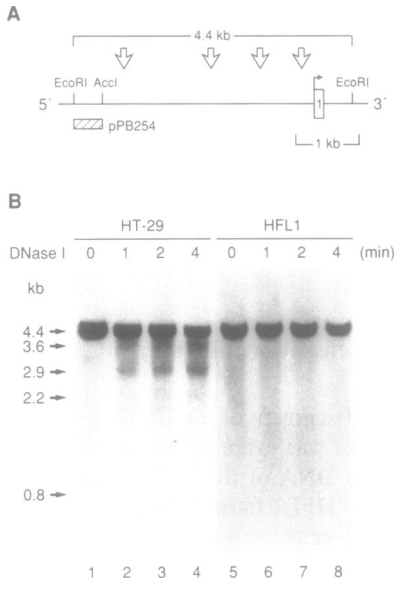

Figure 3. Evaluation of chromatin DNA for the presence of DNase I hypersensitivity sites in the 5′ flanking and the intervening sequences of the exon 1 of the CFTR gene in epithelial cells (HT-29 colon carcinoma) compared to non-epithelial cells (HFL1 fibroblasts). (A) Partial map of the CFTR gene and 5′ flanking region showing the *Eco*RI restriction fragment used for evaluation of DNase I hypersensitivity sites. The location of a ^{32}P-labeled probe encompassing a 5′ *Eco*RI-*Acc*I fragment (pPB254) is indicated (hatched box). Arrows indicate the approximate locations of the DNase I hypersensitivity sites observed in HT-29 cell chromatin DNA. (B) Comparison of DNase I hypersensitivity sites in chromatin DNA from HT-29 cells and HFL1 lung fibroblasts. Nuclei isolated from HT-29 and HFL1 cells were treated with DNase I for 0 to 4 min, DNA was extracted, digested with *Eco*RI, and evaluated by Southern analysis with the 5′ *Eco*RI-*Acc*I probe as describe above. Lanes 1–4—chromatin DNA from HT-29 cells. The appearance of 3.6, 2.9, 2.2 and 0.8 kb bands is indicated. Lanes 5–8—chromatin DNA from HFL1 fibroblasts. No hypersensitivity sites emerge over the period of DNase I treatment.

was a common phenomenon (Figure 1B). Comparative analysis using the identical amplification conditions (see above) demonstrated that the non-epithelial cells WI-26VA4, U-937, K-562, T-lymphocytes, neutrophils, monocytes and alveolar macrophages all contained CFTR transcripts. Further, while CFTR mRNA levels in these cells were several hundred-fold less than in T84 cells, the amounts were comparable to HeLa epithelial cells. CFTR mRNA levels in all these non-epithelial cells were within a 10-fold range. Interestingly, only one cell line evaluated, the HL-60 promyelocytic line, contained no detectable CFTR transcripts under these very sensitive assay conditions. Controls for the PCR method included the observations that no signal was present in samples containing no template, and that similar amounts of γ-actin mRNA transcript levels were observed in all cells analyzed.

Finally, not only were CFTR transcripts evident in non-epithelial cells such as HFL1 fibroblasts, but the transcripts contained sequences spanning the range of the entire CFTR coding exons, including exons 1–5, 17b-19, and 21–24 (Figure 1C). A similar observation was made in T84 cells, but not in the HL-60 cells where no CFTR transcripts were observed.

CFTR gene transcription in cells of non-epithelial origin

Comparative nuclear transcription run-on analyses demonstrated that, like epithelial cells, non-epithelial cells such as fibroblasts and U-937 cells also actively transcribed the CFTR gene, although the rate of transcription was generally lower in non-epithelial cells (Figure 2). The relative transcription rate of the CFTR gene in HFL1, WI-26VA4 and U-937 cells compared to that of the β-actin gene (defined as 100%) was 0.6, 2.3 and 0.7%, respectively, while the rate was 5.2 and 5.4% in T84 and HT-29 cells, respectively. Importantly, other epithelial cell lines showed levels of transcription comparable to the non-epithelial cells (HS-24, 1.0%; HeLa, 1.1%). Consistent with the absence of CFTR gene mRNA transcripts, no transcription of the CFTR gene was detected in HL-60 cells.

DNase I hypersensitive sites

DNase I hypersensitivity site mapping studies of HT-29 cell chromatin showed four hypersensitivity sites in the 5′ flanking region of the CFTR gene at approximately −3000, −1600, −900 and −200 bp relative to the major transcription start site (Figure 3; see reference 13 for details regarding the major transcription start site and the 5′ flanking region). In contrast, no DNase I hypersensitivity sites were observed in HFL1 fibroblast chromatin DNA.

DISCUSSION

The pathological consequences of cystic fibrosis unfold almost entirely on epithelial surfaces, including the lung, pancreas and gastrointestinal tract (1). Consistent with this concept, the CFTR gene is actively transcribed by epithelial cells freshly isolated from

normal human bronchi, and by the T84 and HT-29 carcinoma cell lines derived from colonic epithelia (13). Further, CFTR mRNA transcripts are found in these and other (Caco-2) colonic epithelial cell lines (18,40), as well as other human epithelial cells, including normal and CF bronchial epithelial cells (13,16,17), normal sweat glands (3), and various cell lines such as CFPA-C-1 pancreatic adenocarcinoma cells (15), bronchial carcinoma cells (HS-24) and cervical carcinoma cells (HeLa) as shown in the present study. While the relative levels of CFTR transcripts vary among these epithelial cells, the observation that all epithelial cells evaluated express the CFTR gene suggests that the CFTR gene may be expressed universally in human epithelial cells.

Analysis of the 3.8 kb segment of the 5′ flanking region to exon 1 of the CFTR gene, however, reveals structural and functional features typical of a 'housekeeping' gene, implying broader expression of the CFTR gene than only in epithelial cells (13). The present study clearly shows that this is the case, in that a broad variety of cells of non-epithelial origin contains CFTR gene mRNA transcripts. Further, it appears that the entirety of CFTR gene coding sequence is transcribed in non-epithelial cells, as evidenced by the presence of transcripts in fibroblasts containing sequences encoded by exons 1−5, exons 17b−19, and exons 21−24. In addition, cells such as fibroblasts transcribe the CFTR gene.

Based upon the observations made in the present study, it is possible to categorize the various cells into four groups according to the origin of cells and the level of CFTR gene expression: (1) colon carcinoma cells derived from intestinal epithelium such as T84 and HT-29 cells which show the highest levels of transcription and mRNA transcripts of the CFTR gene of all known cells; (2) other epithelial cells, including carcinoma cell lines such as HeLa cervical carcinoma cells and HS-24 bronchial carcinoma cells (this study), as well as transformed cell lines such as BET-1A bronchial cells and 293 kidney epithelial cells (unpublished data), which demonstrate lower levels of transcription and mRNA transcripts; (3) non-epithelial cells, including HFL1 diploid lung fibroblasts, WI-26VA4 transformed lung fibroblasts, hematopoietic tumor cells such as U-937 histiocytic lymphoma cells, K-562 erythroleukemia cells as well as freshly isolated blood cells (T-lymphocytes, neutrophils and monocytes) and alveolar macrophages, which show very low levels of CFTR gene transcription and mRNA transcripts, but similar to those of the cells in group 2; and (4) non-epithelial HL-60 promyelocytic leukemia cells, the only cell known that shows no detectable transcription or mRNA transcripts of the CFTR gene.

Although there is some variation in the levels of CFTR gene expression among non-epithelial cells categorized in group 3, in general, the degree of expression is quite similar. In this regard, the level of CFTR transcripts in HFL1 and WI-26VA4 fibroblasts, U-937 and K-562 cells derived from bone marrow precursors, and primary cells such as blood T-lymphocytes, neutrophils and monocytes, and alveolar macrophages are all within a 10-fold range. Interestingly, CFTR mRNA transcript levels in non-epithelial cells are within the same range as that in some epithelial cell lines categorized in group 2 such as HS-24 and HeLa. Similarly, we have observed that transformed epithelial cell lines from normal and individuals with CF express the CFTR gene at transcript levels below detection by Northern analysis (unpublished data). Consistent with the similarities of CFTR gene mRNA transcript levels in group 3 non-epithelial cells and group 2 epithelial cells, the rate of CFTR gene transcription analyzed by the nuclear transcription run-on assay is also quite similar. In this regard, while T84 and HT-29 cells (which contain the highest levels of CFTR transcripts known) have much more active transcription of the CFTR gene than all other cells examined, HS-24 and HeLa cells show rates of transcription similar to the non-epithelial cells including HFL1, WI-26VA4 and U-937.

Quantitative analyses of the level of CFTR mRNA transcripts demonstrated that T84 and HT-29 cells contain, in general, several hundred-fold more CFTR transcripts than non-epithelial cells in group 3. The number of CFTR transcript copies in such colon carcinoma cells are approximately 20 per cell (unpublished data). Thus, we can estimate CFTR mRNA transcript numbers in individual groups as follows: group 1, 20 copies/cell; group 2, an average of 0.2 copies/cell; group 3, 0.02 to 0.2 copies/cell; and group 4, no copies/cell.

Similar quantitative analysis of CFTR gene expression in normal human airway epithelium reveals CFTR mRNA levels are actually very low, averaging 1−2 copies/cell in nasal, tracheal and bronchial epithelia (16). Although the direct comparison in CFTR mRNA transcript level between these primary bronchial epithelial cells and other cell types are difficult because the numbers of available bronchial epithelial cells recovered by brushing through a fiberoptic bronchoscope are very limited, the relative abundance of CFTR transcripts in primary airway epithelia are likely within the range of group 2 transformed or tumor epithelial cells.

Although the estimated level of CFTR gene expression in non-epithelial cells is less than one mRNA transcript per cell, there are several reasons to believe such a low level of CFTR transcripts might be physiologically relevant. First, there are examples of other genes whose physiologic function can be achieved in the condition that the cellular mRNA copy number is very much less than 1 transcript per cell. For example, in mature blood neutrophils, elastase is very abundant and plays a critical role in health and disease. However, neutrophils do not contain detectable levels of elastase mRNA nor transcribe the gene (41,42). In this case, the neutrophil elastase gene is expressed only in promyelocytes within the bone marrow, but not in mature blood neutrophils. Second, if only some of the cells in the population are expressing the CFTR gene at any time and others are not, for a gene that is normally expressed at very low levels (like CFTR), the average copy number could be less than 1 per cell, or even far less than 1 per cell. For example, expression of CFTR may be intermittent, perhaps related to the cell cycle (22). Third, CFTR gene expression may be related to the state of differentiation of the cell, even among a population of cells with an apparent similar phenotype. Fourth, the CFTR gene might be 'turned off', requiring a stimulus for its expression, and its expression in any given cell in a population might have to be induced. Finally, the potential physiologic relevance of CFTR gene expression in non-epithelial cells is supported by the observations that the physiologic function of CFTR (cAMP regulated Cl^- channel activity) has been demonstrated in fibroblasts (20,43), T- and B-lymphocytes (21,21).

In the context of above discussion, it is reasonable to accept the potential physiologic relevance of less than 1 CFTR mRNA copy per cell. It is important, however, to prove that the detection of CFTR transcripts in epithelial cells other than colon carcinoma cells and non-epithelial cells is not an artifact of the sensitivity of the methodology employed in the present study. In this regard, the observed PCR amplification products did not originate from

contaminating DNA (such as genomic DNA or plasmid containing CFTR cDNA), but from CFTR mRNA transcripts (after conversion to cDNA) because the negative PCR controls did not show amplification signal [all PCR reagents (except template cDNA) were pretreated by short wavelength UV irradiation to exclude amplification of any possible DNA contamination (16,44)]. Further, under the conditions of PCR amplification utilized in this study, 'illegitimate transcription' (transcription of any gene in any cell type), described by Chelly et al. (45), was not observed. This was demonstrated using HL-60 promyelocytic leukemia cells which did not show CFTR mRNA transcripts despite efforts to do so including amplification of 3 separate regions of CFTR transcripts.

These observations of widespread distribution of CFTR gene expression have several important implications. First, the available evidence suggests the CFTR protein may be a regulatable Cl^- channel (11,12). Consistent with the structural and functional characteristics of the promoter of the CFTR gene as a housekeeping gene (13,23), it is conceivable that the CFTR protein may function in most human cells to maintain cellular anion secretory properties. However, since the pathophysiology of cystic fibrosis is largely confined to epithelia, the CFTR protein is probably not an absolute prerequisite for the well-being of individual cells, but may be for the overall function of specific organs. In this regard, although mutations of the CFTR gene have profound consequences for organs such as the lung or pancreas, expression of the CFTR gene in epithelial cells is not required to sustain life, or even to maintain the viability of the cells that express the mutated gene (2–10,46–52). Consistent with this concept, it is known that epithelial cells expressing the CFTR gene have multiple mechanisms regulating Cl^- secretion (5–10,53), suggesting the CFTR gene product may be only part of the mechanism for maintaining the intracellular, or extracellular electrolyte milieu, and/or the electrolyte milieu within intracellular organelles such as those involved in mucus secretion (54–56).

Second, there is clear evidence that the transferred CFTR gene product can function in cells of non-epithelial origin with the same anion transport features and regulatory properties as in epithelial cells (11,12,57). In this context, transfer of the CFTR gene to NIH/3T3 mouse fibroblasts (a cell line with no detectable cAMP-regulated chloride channels) provides the 3T3 cells with cAMP-regulatable Cl^- channels (11), a characteristic of epithelial cells expressing CFTR (6–10). Further, concordant with the observations that non-epithelial cells such as lymphocytes and fibroblasts contain CFTR mRNA transcripts, normal T-lymphocytes, and B- and T-lymphocyte cell lines exhibit cell cycle-dependent expression (mostly during the G_1 phase) of cAMP-regulatable Cl^- permeability as discussed above, while lymphocytes from CF individuals lack this cell cycle-dependent Cl^- permeability and second messenger regulation (21,22). Moreover, phosphorylation-activated Cl^- channels (a characteristic of a normal CFTR gene product) have also been found in normal human fibroblasts, but are defective in fibroblasts derived from individuals with CF (20,43), further implying that the CFTR gene product may play a physiologic role in such non-epithelial cells as well as in epithelial cells.

Third, although a variety of non-epithelial cells such as fibroblasts and U-937 cells transcribe the CFTR gene and contain its mRNA transcripts, the activity of gene transcription is lower than that of colon carcinoma cells. Consistent with the difference in CFTR gene transcription level between these two cell groups, HT-29 cells demonstrated several DNase I hypersensitivity sites in the 5′ flanking region of the CFTR gene while HFL1 fibroblasts did not, suggesting that these sites may be related to more active transcription of the gene in these colon carcinoma cells than in fibroblasts, and also potentially different regulation of CFTR gene expression in these two cell types.

Fourth, blood T-lymphocytes from individuals with CF may be suitable non-transformed human target cells to test *in vitro* the feasibility of direct gene therapy for CF. Because of their easy accessibility and ability to be maintained *in vitro* without need for modification, T-lymphocytes provide an opportunity to evaluate the expression of the transferred normal CFTR gene and its protein product while maintaining expression of the endogenous defective CFTR gene at levels similar to that *in vivo*.

Finally, the fact that the CFTR gene is widely expressed in cells of non-epithelial origin, and the CFTR gene product can function in such cells as a regulatable Cl^- channel, will have to be considered in developing strategies for gene therapy for cystic fibrosis. Since the lethal manifestations of CF originate primarily on the respiratory epithelial surface, the respiratory epithelium should be the primary target for gene therapy of CF, i.e., the recombinant normal CFTR gene should be transferred to airway epithelial cells. However, in the context that other normal lung cells express the CFTR gene, including fibroblasts, T-lymphocytes and alveolar macrophages, inadvertent transfer of the CFTR gene to those cells will have to be monitored for consequences that conceivably would alter the function of the cells, or the overall electrolyte milieu of the organ.

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