A dominant negative inhibitor of the cAMP-dependent protein kinase has been shown to inhibit the basal expression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in the human colon carcinoma cell line, T84. A functional cAMP response element (CRE) was localized at −48 in the CFTR promoter, and we have analyzed the interactions of this regulatory region with transcription factors. An adjacent inverted CCAAT element (Y box) at position −60 was also investigated. Mutation of the CRE or the Y box decreases the activity of the promoter in transient transfections of T84 or JEG-3 cells. Electrophoretic mobility shift assays demonstrate that CRE-binding protein (CREB) binds to the CFTR CRE with high affinity and independently of the adjacent Y box and that the CFTR CRE binds CREB and activating transcription factor-1 in nuclear extracts of T84 and CaLu-3 cells. In transient transfections of JEG-3 cells, activation of the CFTR promoter is blocked by a dominant negative CREB mutant. The CFTR CRE will also drive cAMP-mediated expression when placed upstream of a heterologous basal promoter. These results demonstrate that CFTR is a bona fide CRE-dependent gene, and we suggest that CFTR expression levels in vivo may be responsive to hormones or drugs that activate the cAMP-dependent protein kinase system.

Many genes are induced by cAMP, including those coding for hormones, metabolic enzymes, structural proteins, and transcription factors. Although most of these genes contain a CRE sequence, their promoter, other regulatory sequences, such as the AP-1 (1), AP-2 (2), Sp1 (3), Pit-1 (4), Y box (inverted CCAAT element) (5), and estrogen response element (6), may also mediate a cAMP-dependent gene activation. The CRE is bound by the cAMP response element-binding protein (CREB)/activating transcription factor (ATF) family of proteins, which can be activated by phosphorylation by the cAMP-dependent protein kinase (PKA) to stimulate gene transcription (7). A group of indirectly activated CRE-responsive genes consists of those that respond to changes in the levels of the transcription factors induced by cAMP, and this group probably accounts for the broad changes in cellular function characteristic of physiological responses to prolonged cAMP activation.

The consensus CRE, TGACGTCA, provides the strongest and most frequently observed cAMP mediation of various promoters. Nonconsensus CREs that mediate PKA responsiveness frequently exhibit changes in the middle two bases. Examples of the variant CRE, TGACaTCA or TGaTGTCA, include the CREs found in the nonprimate glycoprotein hormone α subunit (8), neurotensin/neuromedin M (9), tissue-type plasminogen activator (t-PA) (10), and retinoic acid receptor β2 (11) genes. Other variations of the xonense CRE site have also been shown to be functional. The variant CREs are often weaker than the consensus palindromic sequence at conferring cAMP responsiveness and may require cooperation with other sites to mediate this regulation. Occasionally, the variant CREs mediate responsiveness from other signaling stimuli, such as the phorbol ester responsiveness of the t-PA CRE (10) and the retinoic acid-mediated responsiveness of the retinoic acid receptor β2 site (11).

A variant CRE sequence (TGACaTCA) is present in the promoter for the cystic fibrosis transmembrane conductance regulator (CFTR) gene, and this element has recently been implicated in basal and PKA-mediated responsiveness of the promoter. We have determined that the basal activity of the CFTR gene is regulated by cAMP in the human colon carcinoma cell line, T84, and that inhibition of PKA eliminates CFTR-mediated halide efflux (12) and gene expression (13) in these cells. This regulation is mimicked by a reporter construct, CFTR(wt)-luc, T84 cells, and JEG-3 cells (13), a human chorioncarcinoma cell line frequently utilized to study PKA-mediated promoter regulation. Mutation of the variant CRE sequence in the CFTR promoter decreases both basal and PKA-stimulated CFTR-luc activity in transient transfections of T84 cells (13), suggesting that this element may function as a CRE to confer cAMP regulation to CFTR gene expression.

The CFTR promoter was sequenced and initially characterized by two separate groups (14, 15). These investigators identified several potential regulatory elements by sequence homology, including the variant CRE. One of these studies demonstrated that the CFTR promoter was down-regulated by phorbol ester (15), which is consistent with the effects of phorbol ester on CFTR mRNA (16). This finding was supported by a later study of the CFTR promoter, which also determined that the chromatin context and methylation of the promoter, as well as unidentified regulatory elements close to the transcription start sites, contribute to cell type-specific expression (17).

Although none has yet been identified, mutations of the CFTR promoter, including mutations in the variant CRE, might lead to the lack of CFTR expression that causes cystic fibrosis. This lack of CFTR at the plasma membrane prevents the activation of chloride efflux and inhibition of sodium influx that is necessary for proper exocrine fluid homeostasis, resulting in inspissated secretions that lead to organ failure and...
opportunist infection. The potential for cAMP-mediated regulation of CFTR gene expression may offer therapeutic benefit for patients with milder forms of cystic fibrosis in which the function of CFTR may be boosted by increased expression.

In this report we characterize the variant CRE in the CFTR promoter and its ability to bind members of the CREB/ATF transcription factor family. Based on mutational analysis and electrophoretic mobility shift assays, we conclude that this site is indeed a bona fide CRE. Examination of other potential cAMP regulatory sites in the CFTR promoter suggests that there may be sites between −305 and −70 and that the Y box at −60 recently identified by Pittman et al. (18) as a potential cAMP regulatory element in pancreatic cells also functions modestly as such in colonic cells. Furthermore, we identify the CRE-binding proteins in two CFTR-expressing cell lines as CREB and ATP-1, and we determine that CREB will stimulate CFTR expression through the CRE. We have also established that human lung-derived epithelial cells can regulate CFTR gene expression in response to PKA.

MATERIALS AND METHODS

Cell Culture—CaLu-3 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 1 mM sodium pyruvate. HTE-1 cells were generously provided by Christine Hall (American Cancer Society), and were obtained from tracheal biopsy of a 50-year-old male. Cells were transformed with SV40 large T antigen and passed on fibronectin-coated plates in supplemented KGM medium (Clonetics). T84 and JEG-3 cells were grown as described previously (13).

Solution Hybridizations—CaLu-3 or HTE-1 cells were treated with 30 µM N-(12-p-bromosaminylaminoethyl)-S-isouquinolinesulfonamide (H-89) for 24 h, 30 µM forskolin for the next morning for 6 h. At this time, all cells were harvested, using 200 µl of lysis buffer/well (200 µM potassium phosphate, pH 7.8, 6 mM MgSO4, 4 mM ATP, 0.1% Triton X-100, 1 mM dihtiothreitol) and assaying 60 µl for luciferase activity as described elsewhere for the JEG-3 cells (20). Luciferase activity was normalized for the protein amount (Bradford), and for those figures in which the mean is more than one experiment is presented, the basal activity of the wild-type CFTR-luc was set at 100%.

The JEG-3 cells were transfected using a calcium phosphate procedure for 24-well dishes described previously (21). In these experiments, we used 100 ng/well for wild-type or mutant CFTR-luc reporter. For the experiments examining the effect of transfected PKA on CFTR-luc expression, we cotransfected the cells with 1 ng/well of an expression vector for the C-terminal CREB (22). For the experiments examining the effect of transfected CREB on CFTR-luc expression, we transfected 5 ng of RSV-CREB or 5 ng of RSV-KCREB. RSV-KCREB drives the expression of a mutant CREB that does not bind DNA but will bind wild-type CRE, preventing the association of CREB with the CRE (23). In all JEG-3 transfection experiments, cells were transfected with 50 ng of pCMV- lacZ with K3 (+) bringing the total amount of DNA to 250 ng/well. After a 24-h transfection, the medium was changed to Dulbecco’s modified Eagle’s medium and 10% fetal bovine serum, except for the experiments examining C-α expression, in which cells were treated with 40 µM ZnSO4 in 2.5% fetal bovine serum for 18–24 h prior to harvesting to induce expression of the CREB plasmid in the CEV-transfected cells. After harvesting, cells were assayed for luciferase and β-galactosidase activity as described before (20).

Recombinant Protein and Nuclear Extract Preparation—Recombinant CREB (24) was provided by Dr. Richard Goodman (Vollum Institute), while the recombinant ATF-2 fragment (25) was provided by Dr. James Hoefler (In vitrogen). Nuclear extracts were prepared using an approach similar to that used by Kim et al. (26). Cells were trypsinized off the plates, the trypsin was neutralized with medium containing fetal bovine serum, and the cells were pelleted. Cells were resuspended in five packed cell volumes of 3 × TGE (150 mM Tris, pH 7.4, 22.5 mM EDTA, 30% glycerol) with protease inhibitors (3 µg/ml aprotinin, 1 mM 4-(2-aminoethyl)-benzenesulfonic acid, 0.2% mg/ml soybean trypsin inhibitor, and 2 µg/ml leupeptin) and broken in a Dounce homogenizer. Nuclei were collected by a quick spin at 10,000 rpm for 10 s, the supernatant was removed, and the nuclear pellet was resuspended in two-thirds of the original packed cell volume of 3 × TGE, 500 mM KCl, protease inhibitors. After 20 min at 4 °C, the extracted nuclei were spun at 46,000 rpm for 23 min in an SW41 rotor, at which point the nuclear pellet was removed and the protein content of the nuclear extract was determined. No dialysis was performed, so the final concentration of KCl in the electrophoretic mobility shift assay (EMSA) reflected the diluted KCl from the extraction step; the final concentration ranged from 50 to 75 mM KCl.

Electrophoretic Mobility Shift Assays—Oligonucleotide probes were end-labeled using T4 kinase and γ32P-ATP. The probes and other oligonucleotides in these experiments were stored in 50 mM NaCl and prior to use were heated to 95 °C and cooled slowly to 45 °C. In all experiments, we used 0.1 ng of labeled probe, the specific activity of which was approximately 100,000 cpm/ng.

For the experiments using the double-stranded 27-bp oligonucleotide probe, cfr(CRE), the reaction consisted of the following: 0.1 ng of labeled probe; 200 ng of poly(dIdC); 6 µg of bovine serum albumin; a 30 × 100, or 300 × excess of unlabeled competitor (see below) or buffer control; 60 ng of CREB or 4 ng of ATF-2 fragment; 12% glycerol; 12 mM HEPES, pH 7.9; 60 mM KCl; 1 mM EDTA, and 1 mM dihtiothreitol in a final volume of 20 µl. The unlabeled competitors were in 50 mM NaCl, and the sequences of all double-stranded oligonucleotides were as follows: scf(CRE), AGAGATTGGCTGATGACACAG; cfr(CRE), TGGAGGCTATGCATGACAGCT; cfr(CRE), TGGAGACATGTCAGAGCAG; cfr(CRE), TGGAGACATAATGTAGTACAGCAGGCAGT. The binding reactions were performed at 30 °C for 30 min, and 10 µl were loaded onto a nondenaturing minigel. The gel consisted of 4% acrylamide, 0.1% bisacrylamide, 2.5% glycerol, 50 mM Tris-HCl, 380 mM glycine, 2 mM EDTA at 1 h at 100 V on ice. The gel was dried and autoradiographed at −80 °C.

For the experiments using the 37-bp oligonucleotide probes and nuclear extracts, the conditions varied somewhat from those described above. The cfr(Ybox/CRE) oligonucleotide was labeled as the 27-mer above. These binding reactions consisted of the following: 0.1 ng of C-α expression, in which cells were treated with 40 µM ZnSO4 in 2.5% fetal bovine serum for 18–24 h prior to harvesting to induce expression of the CREB plasmid in the CEV-transfected cells. After harvesting, cells were assayed for luciferase and β-galactosidase activity as described before (20).
Regulation of CFTR Gene Expression by PKA

We have previously determined that mutation of the variant CRE at position −48 diminishes the activity of the CFTR promoter in transient transfections of T84 cells (13). Recently, others have reported that an inverted CCAAT element (Y box) at position −60 mediates basal and PKA-stimulated activity of the CFTR promoter in a pancreatic cell line (18), with the contribution of the variant CRE being unclear in this cell line. To determine the relative importance of the variant CRE and Y box element, as well as to further characterize the contribution of upstream sequences, we have produced the constructs depicted in Fig. 2A, containing various CFTR promoter regions upstream of a luciferase reporter. We constructed a series of truncations, (−650)CFTR-luc, (−305)-CFTR-luc, (−151)CFTR-luc, (−70)CFTR-luc, and (−55)CFTR-luc. The (−70)CFTR-luc contains both the Y box and the CRE, while (−55)CFTR-luc contains only the CRE. In addition, we have produced several promoter-reporter constructs in which there are one or more mutations in the CFTR promoter. The CFTR(CRE)-luc contains a mutation that changes the variant CRE to a consensus CRE, while the CFTR(mCRE)-luc contains an unrelated sequence in place of the variant CRE. The CFTR(mYbox)-luc contains a mutation in the Y box that destroys the core sequence, mutating the same bases that were deleted by Pittman et al. (18) resulting in less activity of the promoter. The CFTR(mYbox/mCRE)-luc construct contains mutations in both the Y box and the variant CRE.

Transient Transfections of CFTR Promoter Truncations—Fig. 2B depicts the results from transient transfections of T84 cells, which demonstrates that the (−55)CFTR-luc is active, although only −30% as active as (−2150)CFTR-luc, and is capable of approximately the same -fold stimulation by cAMP as (−2150)CFTR-luc (−1.8 ×). The (−70)CFTR-luc is clearly less active (−50%) than the (−2150)CFTR-luc promoter, and even the (−650)CFTR-luc is less active (−70%) than (−2150)CFTR-luc.

Fig. 2C depicts the results from transient transfections of JEG-3 cells, demonstrating that promoter truncations as small as −55 bp are active and responsive to CAMP-mediated stimulation. Moreover, these results suggest that the Y box element is not required for basal activity or PKA-mediated stimulation in JEG-3 cells. We have used the JEG-3 cells previously to study the CFTR promoter, because they exhibit a stronger cAMP-mediated regulation of gene expression than the T84 cells (13) and can be transfected more reliably than the Calu-3 cells.

Progressive truncation from the (−151) position to the (−70) position produces an increase in promoter activity. This may reflect an increase in PKA-independent activity, since, as seen in Fig. 2B, H-89 treatment does not have much of an effect on the activity of (−70) and (−55) in T84 cells. The gain of basal activity in the shorter promoters is not unusual in promoter truncation studies. One interpretation of these results is that there are negative regulatory elements in the truncated region, although the difference in activity might also be related to the proximity of plasmid vector sequences.
The results presented in Fig. 2 demonstrate that in T84 cells there are several elements mediating promoter activity, although the CRE and downstream sequences are sufficient for the PKA-stimulatable activity. In contrast, in JEG-3 cells the smallest truncation is as active as the (-2150)CFTR-luc. Perhaps the longer CFTR promoter constructs are actually more active in T84 cells as a result of upstream regulatory sequences that are inactive in JEG-3 cells. In both cell lines, however, we observe promoter activity in the truncations containing only the CRE and downstream sequences, suggesting that the CRE is sufficient for activity in the context of a minimal CFTR promoter.

**Mutation of the Variant CRE and Y Box in the CFTR Promoter**—To further examine the PKA-mediated regulation of the CFTR promoter, we performed transient transfections using (-2150)CFTR-luc constructs containing mutations in the variant CRE or in the Y box, changing these elements to unrelated sequences. We also examined the effect of mutating the variant CRE to a consensus CRE, to determine whether this would enhance cAMP-mediated stimulation of the CFTR promoter. In Fig. 3, we demonstrate that in T84 cells, the CFTR(cCRE)-luc construct has essentially the same activity as the wild-type construct. When the CRE is mutated to an unrelated sequence (CFTR(mCRE)-luc), both basal and forskolin-induced activity are reduced by 75%. Mutation of the Y box in the CFTR promoter also reduces activity by approximately 75%, as depicted in Fig. 3. The double mutant CFTR(mYbox/mCRE) has less activity than either of the single mutants in T84 cells, suggesting that regulation through these two elements occurs separately. The double mutant retains a measurable amount of PKA-dependent basal activity, however, as do the single mutants.

The data shown in Fig. 3 demonstrate that mutation of the variant CRE to an unrelated sequence reduces the activity of
CFTR mutations are within the context of the one band (lane 2–6) migrating more slowly. Interestingly, both of these bands depend on the CRE, since the shifted bands persist in the T84 nuclear extracts (Fig. 4). Preincubation with the polyclonal anti-ATF-2 antiserum totally eliminates the upper band (lane 10), which is consistent with the effect of this antiserum on the recombinant protein fragment (lane 14). The combination of anti-CREB and anti-ATF-1 or anti-ATF-2 most clearly demonstrates that the lower band is CREB, while the upper band is either ATF-1 or -2, although closely related proteins are also possibilities.

To identify the proteins binding to the CRE in other cell types, we performed EMSAs similar to those in Fig. 4 on nuclear extracts from Calu-3 cells. As demonstrated in Fig. 1A, Calu-3 cells display a more robust PKA-modulated stimulation of gene expression than the T84 cells, so we examined whether the identity of the CRE-binding proteins differed in these cells. Calu-3 nuclear extracts produce two bands (Fig. 4C) similar to those observed with T84 nuclear extracts in Fig. 4B, although the intensity of the upper band a appears generally diminished with respect to the analogous band in the T84 nuclear extracts (Fig. 4 and data not shown). The CaLu-3 shifted bands show the same specificity as those from the T84 extract (Fig. 4B) when incubated with the same cold competitor oligonucleotides (data not shown). Fig. 4C depicts the CaLu-3 EMSAs after preincubation with the same antibodies used in Fig. 4B. As with the T84 nuclear extracts, bands a and b from the Calu-3 nuclear extracts are recognized by anti-ATF and anti-CREB antibodies, respectively. Interestingly, the anti-CREB supershifting in Calu-3 cells is essentially complete, while in T84 cells we are unable to shift the entire CREB band under the same conditions. The results depicted in Fig. 4 demonstrate that the CRE CRE binds recombinant CREB as well as CREB and ATF-1 or -2 in nuclear extracts from two CFTR-expressing cell types. This binding is totally dependent on the CRE, and the Y box does not appear to bind a nuclear extract protein under our EMSA conditions.

Electrophoretic Mobility Shift Assays Using Recombinant CREB/ATF Proteins—To further characterize the ability of the CRE in the CFTR to bind members of the CREB/ATF family in vitro, we performed EMSAs with recombinant CREB/ATF family members and serial dilutions of competitor oligonucleotide. These results provide an estimate of the relative affinity of the CRE for CREB/ATF family members, as demonstrated in Fig. 5. Fig. 5A depicts an EMSA in which we have used a 27-bp oligonucleotide consisting of the CFTR variant CRE and surrounding sequence from –58 to –32, cftr(CRE), as the labeled probe. Fig. 5 demonstrates that recombinant CREB produced a shifted band, as expected from the results of Fig. 4, and competition with the unlabeled consensus CRE from the somatostatin promoter, ss(CRE), competes away this binding. Additionally, competition with unlabeled wild-type CFTR sequence (cftr(CRE)) or with an oligonucleotide in which the CFTR variant CRE is mutated to the consensus core sequence (cftr(cCRE)), eliminates the binding of the CFTR CRE probe to CREB in a dose-dependent manner. CREB binds equally well to either the cftr(cCRE) oligonucleotide or the cftr(CRE) oligonucleotide, as determined by comparing the dose response of the competition. Competition with an unlabeled oligonucleotide containing the mutant CRE does not compete away CREB binding, similar to the results using the cftr(Ybox/mCRE) oligonucleotide in Fig. 4.

Fig. 5B demonstrates that the CFTR CRE probe will also bind a fragment of recombinant ATF-2, another member of the CREB/ATF family. Although ATF-2 is not phosphorylated or activated by PKA, this protein does bind the CRE and probably mediates stimulation through the Jun kinase signaling cascade (29, 30). The strength of binding of ATF-2 appears very similar to the profile observed with CREB in Fig. 5A. These results
demonstrate that the CFTR CRE will specifically bind two CREB/ATF family members with an affinity approximately equal to the affinity of these proteins for the canonical CRE. Furthermore, disruption of the CRE in the CFTR oligonucleotides eliminates the binding to CREB/ATF family members, which correlates with the decrease in basal activity of the CFTR(mCRE)-luc construct observed in Fig. 3.

Regulation of the CFTR Promoter by CREB in Transient Transfections—To determine whether CREB can activate CFTR expression in vivo, we performed transient transfections of JEG-3 cells and examined the ability of CREB and PKA to activate the wild-type and mutant CFTR promoters. As shown in Fig. 6, cotransfection with an expression vector encoding the catalytic subunit of PKA causes a stimulation of wild-type CFTR promoter, and this stimulation is enhanced by cotransfection with a CREB expression vector. Mutation of the CRE abolishes at least 80% of the basal and PKA-mediated transcriptional response, as demonstrated by the middle panel.
of Fig. 6. Since our binding studies (Figs. 4 and 5) indicate that the mutant CRE has no in vitro affinity for CREB, we suggest that the residual PKA response of the CFTR(mCRE)-luc is mediated by other transcription factors that bind to the CFTR promoter. The mutant Y box-containing CFTR promoter is stimulated both by PKA and by CREB, although to a lesser extent than the wild-type promoter. These results suggest that PKA and CREB stimulate wild-type CFTR promoter activity predominantly through the CRE. Mutation of the adjacent Y box does, however, cause approximately a 2-fold attenuation of the response of the CFTR promoter to PKA, although the response of this construct to CREB alone remains similar to that of the wild-type construct.

**DISCUSSION**

The CFTR promoter contains a variant CRE (TGACaTCA) at position −48 that we have shown to have all of the properties expected of a functional CRE. The CFTR CRE binds CREB and ATF family members with high affinity, and nuclear extracts from CFTR-expressing cell lines contain CREB/ATF family members that bind this sequence in electrophoretic mobility shift assays. Mutation of the variant CRE within the context of the 2.2-kilobase pair CFTR promoter reduces basal activity when assayed in T84 cells and prevents much of the PKA and CREB-mediated induction when assayed in JEG-3 cells. The CFTR CRE can be transferred to a heterologous promoter, where it confers robust cAMP-induced promoter activity, which is lost if the CRE is mutated.

Clearly, other elements within the CFTR promoter are also important for transcriptional activity and probably act cooperatively with the CRE in governing basal and cAMP-mediated CFTR expression in vivo. The persistent basal and cAMP-inducible activity of the CFTR(mCRE) promoter suggests that some of these additional regulatory regions may in fact be bound by transcription factors that are regulated by cAMP. These regions include putative AP-1 sites at −2,1058, −2,976, −2,745, and −2,283, putative Sp1 sites at positions −3,335 and −3,256, and a putative AP-2 site at −1,1108 (17). Although the AP-1 site generally confers Fos/Jun-mediated activation and the Sp1 site generally modulates basal activity, both the AP-1 and Sp1 sites have been shown to mediate PKA responsiveness in certain promoters. AP-2 sites mediate basal and phorbol ester-stimulated activity and have also been
shown to mediate cAMP responsiveness, alone or in concert with other cAMP-responsive elements (2, 10, 34, 35).

Our data suggest that one additional element important for the activity of the CFTR promoter is the Y box at position −60. This element was originally identified as a modulator of basal and PKA-stimulated activity of the CFTR promoter in PANC1 cells (18), and our results in JEG-3 and T84 cells support these findings. We have demonstrated that the CRE and Y box are independently regulated in T84 cells, although we have not observed binding of nuclear extract proteins to the Y box. This discrepancy may result from a low level of Y box-binding proteins in our nuclear extracts that is below the threshold for detection in the in vitro EMSA, or it may indicate that the Y box-binding proteins require additional sequences outside of the sequence of our probes for optimal binding. Our experiments have demonstrated, however, that there is no contribution by the Y box to CREB/ATF binding to the CFTR CRE and that the CREB-mediated stimulation of the promoter occurs almost exclusively through the CRE.

The regulation of the CFTR gene by PKA has been established now in several cell lines, including the T84 (13), HT-29 (27), CaLu-3, and HTE-1 cells. The level of inducibility of the endogenous CFTR gene by cAMP is dependent on cell type. We have observed only modest induction in T84 cells, although the high basal activity of the promoter in these cells is almost completely dependent on PKA activity. In contrast, the human tracheal epithelial cell line, HTE-1, expresses CFTR basally at very low levels, while the gene is induced 5–10-fold by cAMP. The CaLu-3 cells fall between the T84 and HTE-1 cells, with a moderately high degree of basal expression and a cAMP inducibility of ~3-fold. This range of regulation may reflect different “set points” for basal PKA activity, or it may be due to different compositions of cAMP-regulated transcription factors in the various cell lines.

The relative ratio of CREB to ATF proteins may govern the range of inducibility of the CFTR promoter in various cell lines. In general, we have observed a greater proportion of ATF with respect to CREB in the less cAMP-regulatable T84 cells in our EMSAs. Regardless of whether the ATF protein is ATF-1 or ATF-2, these proteins have been shown previously to be less responsive to cAMP than CREB. ATF-2 is not activated by PKA, and it forms heterodimers with Fos or Jun but not with CREB or ATF-1 (36), suggesting that ATF-2 bound to the CFTR CRE may mediate signaling through pathways other than the cAMP pathway. CREB and ATF-1 form heterodimers (36), and in cells with a high ratio of ATF-1 to CREB the magnitude of cAMP-mediated gene expression is attenuated (37). Clearly, however, there are several other possible mechanisms that could lead to the range of cAMP inducibility between cell lines, including differences in the amount and types of PKA present, and the final profile of regulation probably depends on multiple factors.

The potential for cAMP to regulate both the transcriptional activity of the CFTR gene and the functional activity of the CFTR protein provides a dual mechanism of control. Under normal physiological conditions, the CFTR protein is phosphorylated directly by PKA and thus regulated by hormones that signal stress. A greater augmentation of CFTR activity might occur when the signal is sufficient for an increase in CFTR gene expression. The clinical significance of the transcriptional effect is as yet unknown, but patients are often treated with cAMP-modulating drugs for a variety of conditions. For example, long term treatment of asthmatic patients with β2-adrenergic agonists might cause a chronic stimulation of the PKA pathway and an increase in CFTR gene expression. Chronic treatment of cystic fibrosis patients with cAMP-modulating drugs might be beneficial if there is an increase in the level of CFTR at the plasma membrane, especially for those patients with milder disease that have mutations that produce lower levels or moderately functional CFTR. A recent report that overexpression of the ΔF508 CFTR mRNA leads to functional expression (38), is interesting in this regard, since ΔF508
is the most common mutation in cystic fibrosis patients. Clinical studies examining the effects of PKA-stimulating bronchodilators, such as the β2-adrenergic agonist albuterol, on cystic fibrosis patients suggest that there may indeed be some therapeutic benefit with these drugs. Hordvik et al. (39) observed that long term albuterol treatment significantly improved the respiratory function of cystic fibrosis patients in a manner that was unrelated to the patients’ asthma. Interestingly, the response required 2 weeks of treatment before most patients’ conditions improved, and after the drug was discontinued many patients rebounded to conditions worse than before the onset (39). These changes are consistent with changes in gene expression. Our examination of the regulation of CFTR gene expression by PKA suggests that the potential mechanism by which drugs such as the β-adrenergic agonists may be beneficial could involve regulation through the CRE in the CFTR promoter.

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