THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 12, Issue of March 19, pp. 7803–7815, 1999

Printed in U.S.A.

Transcriptional Repression of the Cystic Fibrosis Transmembrane Conductance Regulator Gene, Mediated by CCAAT Displacement Protein/*cut* Homolog, Is Associated with Histone Deacetylation*

(Received for publication, August 12, 1998, and in revised form, December 14, 1998)

SiDe Li‡, Libia Moy‡, Nanci Pittman‡, Gongliang Shue‡, Barbara Aufiero§¶, Ellis J. Neufeld‖, Neal S. LeLeiko‡, and Martin J. Walsh‡‡‡**

*From the ‡Department of Pediatrics, Division of Pediatric Gastroenterology and Liver Diseases, the **Program in Molecular, Cellular, Biochemical, and Developmental Sciences, and the §Department of Medicine, Division of Molecular Medicine, Mount Sinai School of Medicine, New York, New York 10029 and the ‖Medical Division of Hematology-Oncology, Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115*

Human cystic fibrosis transmembrane conductance regulator gene (*CFTR*) transcription is tightly regulated by nucleotide sequences upstream of the initiator sequences. Our studies of human *CFTR* transcription focus on identifying transcription factors bound to an inverted CCAAT consensus or "Y-box element." The human homeodomain CCAAT displacement protein/*cut* homolog (CDP/*cut*) can bind to the Y-box element through a *cut* repeat and homeobox. Analysis of stably transfected cell lines with wild-type and mutant human *CFTR*-directed reporter genes demonstrates that human histone acetyltransferase GCN5 and transcription factor ATF-1 can potentiate *CFTR* transcription through the Y-box element. We have found 1) that human CDP/*cut* acts as a repressor of *CFTR* transcription through the Y-box element by competing for the sites of transactivators hGCN5 and ATF-1; 2) that the ability of CDP/*cut* to repress activities of hGCN5 and ATF-1 activity is contingent on the amount of CDP/*cut* expression; 3) that histone acetylation may have a role in the regulation of gene transcription by altering the accessibility of the *CFTR* Y-box for sequence-specific transcription factors; 4) that trichostatin A, an inhibitor of histone deacetylase activity, activates transcription of *CFTR* through the Y-box element; 5) that the inhibition of histone deacetylase activity leads to an alteration of local chromatin structure requiring an intact Y-box sequence in *CFTR*; 6) that immunocomplexes of CDP/*cut* possess an associated histone deacetylase activity; 7) that the carboxyl region of CDP/*cut*, responsible for the transcriptional repressor function, interacts with the histone deacetylase, HDAC1. We propose that *CFTR* transcription may be regulated through interactions with factors directing the modification of chromatin and requires the conservation of the inverted CCAAT (Y-box) element of the *CFTR* promoter.

The gene responsible for cystic fibrosis encodes the *c*ystic *f*ibrosis *t*ransmembrane conductance *r*egulator (CFTR)[1] protein (1, 2). The expression of *CFTR* is confined primarily to specific epithelial cell types and is ordinarily expressed in low levels. The low levels and cell type-specific expression of *CFTR* appear to be dictated primarily by sequences upstream of the transcriptional start sites of *CFTR* which correspond to functional promoter sequences (3, 4). The elements required for active, cell type-specific *CFTR* transcription lie in a narrow band of nucleotide sequences, proximal to the multiple transcript initiation (5). The levels of *CFTR* transcripts in individual cell types appear to correspond to the ability of specific epithelia to modulate expression of *CFTR* variably in response to 1) levels of cAMP (6), 2) the stimulation of protein kinase A and C activities (7–9), and 3) phorbol esters (10) likely through signaling pathways or mechanisms that converge ultimately on gene transcription (for review, see Ref. 11).

The tight transcriptional control of *CFTR* requires a conserved inverted CCAAT (Y-box) element (12). Despite the absence of a TATA signal, the human *CFTR* gene can initiate RNA transcription through multiple, discrete start sites requiring conserved residues within the Y-box. Thus, an intact CCAAT consensus on the opposing strand of the human *CFTR* appears to be a requirement for accurate transcript initiation (12). Both basal and cAMP-mediated regulation of *CFTR* transcription have implicated the Y-box (12) in tandem with a weak CRE nucleotide consensus downstream (13). *CFTR* transcription appears to be influenced by additional *cis*-acting elements, including a site located in the first intron of the human *CFTR* (14). The activation of *CFTR* transcription by cAMP correlates with increasing sensitivity to DNase I overlapping the Y-box element (12). This may reflect the role of *trans*-acting factors in modifying local chromatin structure. Although it has been shown previously that individual members of bZIP families of transcription factors have the capacity to bind and activate *CFTR* transcription (12, 13), little is known about the parameters and proteins involved in directing transcription of the *CFTR in vivo*.

* This work was supported by awards (to M. J. W.) from the Cystic Fibrosis Association of Greater New York, the Cystic Fibrosis Foundation, and in part from a Public Health Service Award from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Present address: WRAIR, Dept. of Immunology, 14th and Dahlia St. NW, Washington, D. C. 20307.

‡‡ To whom correspondence should be addressed: Dept of Pediatrics, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029. E-mail: walsh@msvax.mssm.edu.

[1] The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; CRE, cyclic AMP-responsive element; P/CAF, p300/CBP-associated factor; CREB, CRE-binding protein; CBP/p300, CREB-binding protein/p300; wt, wild-type; HDAC1/2, histone deacetylase 1/2; bp, base pair(s); CBF·NF-Y, CCAAT-binding factor/nuclear factor Y; CDP/*cut*, CCAAT displacement protein/*cut* homolog; ATF-1, activating transcription factor 1; hGH, human growth hormone; CMV, cytomegalovirus; TK, thymidine kinase; GST, glutathione *S*-transferase; PCR, polymerase chain reaction; Br-cAMP, bromo cyclic AMP; TSA, trichostatin; LW, lysis washing; EMSA, electrophoretic mobility shift assay; C/EBP, CAAT enhancer-binding protein.

Histone modification plays an important role in regulating gene transcription (15, 16). The level of acetylation of conserved residues on the core histones H3 and H4 appears to plays a critical role in the regulation of gene transcription (17), and the presence of histone acetyltransferase activity correlates with the activation of other genes (18, 19). Conversely, it is believed that gene repression is associated with the increased activity of histone deacetylases (17, 20). Although it is accepted that histone acetyltransferases and deacetylases are tightly associated with transcriptional machinery through several well characterized transcriptional cofactors or adaptor proteins (21–23), little is known about how such activities are directed to a specific gene. Several genes encoding histone acetyltransferase and deacetylase activities have been identified in human. Interestingly, many of these genes encode both transcriptional coactivators and corepressors, such as hGCN5, P/CAF, p300/CBP, TAF250, and HDAC1/2 (for review see Ref. 18). Recent observations have suggested that transcriptional cofactors such as p300/CBP and HDAC1/2 encode proteins for the enzymatic histone acetyltransferase and deacetylase activities, respectively (22, 24). These proteins can form complexes with sequence-specific nuclear hormone receptors and transcription factors (25–28). Although these cofactors do not possess sequence-specific DNA binding activities, a transcriptional cofactor can target a specific gene when tethered to a gene promoter through a heterologous DNA binding domain as shown previously (29). Therefore, transcriptional cofactors may regulate the level of histone acetylation of a specific gene through interactions with sequence-specific transcription factors.

Our present objective was to identify proteins bound to the human *CFTR* promoter through the Y-box nucleotide consensus of the *CFTR*. We have now identified two such proteins: CDP/*cut*, a homeodomain repressor, and the transcription factor complex CBF·NF-Y. CBF·NF-Y is a highly conserved complex of heterotrimeric subunits shown to interact with CCAAT sequence elements common to many eukaryotic promoters (30, 31). Conserved residues within subunits of the CBF·NF-Y complex, which include a region homologous to the histone-fold motif found in histones H2A and H2B, are necessary for subunit interaction to form the heterotrimeric complex (32). Domains of CBF·NF-Y, located in two of the subunits (CBF-c and CBF-b) are necessary for the transactivation by the CBF·NF-Y complex (33). The CBF·NF-Y-bound complex can form tight interactions with known histone acetyltransferases hGCN5 and P/CAF to transactivate transcription (27). Mammalian *C*CAAT *d*isplacement *p*rotein/*cut* (CDP/*cut*) repressor is a 180–190-kDa polypeptide (34–36) closely related to the *cut* protein of *Drosophila* (37), which determines cell fate in *Drosophila* (38, 39). CDP was first described as a CCAAT-box-binding protein in the sea urchin sperm histone promoter (40). It was defined subsequently as a gene-specific transcriptional repressor associated with the differential regulation of the gp91^{phox} gene in myeloid cell differentiation (41). Human CDP/*cut* also binds to promoters of the c-*myc*, *N*-cam, thymidine kinase, c-*mos*, and histone genes in a variety of cell types (42–46) and is implicated in the regulation of gene transcription through sequence elements associated with nuclear matrix attachment (47). The competing activities between CBF·NF-Y and CDP/*cut* have been postulated as a mechanism to regulate several genes through the CCAAT motif (44, 48, 49).

The objective of this study was to determine further whether transcription of *CFTR* is regulated by nuclear factors associated with histone acetylation. Because the regulation *CFTR* transcription is potentiated through cAMP and the CREB/ATF activators (12, 13), we postulate that mechanisms directing the recruitment of histone acetyltransferase coactivators could, conversely, direct a histone deacetylase complex through a separate set of CCAAT-binding factors within the context of the human *CFTR* promoter Y-box element. In this report, we present evidence that the Y-box element of the *CFTR* promoter is a site that intersects histone acetylation and deacetylation processes with mechanisms to modulate transcription of *CFTR*.

EXPERIMENTAL PROCEDURES

Cell Culture—Pancreatic carcinoma (PANC1) and histiocytic lymphoma (U937) cells were obtained from the American Type Culture Collection (Rockville, MD). PANC1 cells stably transfected with human *CFTR* promoter-directed human growth hormone transgenes pCF-197wt/hGH and pCF-197ΔCCAAT/hGH and the pØGH promoterless vector have been described (12). All PANC1 cell cultures were maintained in Dulbecco's modified Eagle's medium in glucose (4.5 g/liter) (Life Technologies, Inc.). U937 cell were maintained in RPMI. Medium was supplemented with 10% fetal calf serum (Hyclone, Logan, UT) and 1 × gentamycin (Life Technologies, Inc.). Cell cultures were maintained in 5% CO_2 incubator at 37 °C.

Expression Plasmids—Plasmids for hGCN5 (50) (Dr. Yoshihiro Nakatani, NIH, Bethesda, MD) and ATF-1 (51) (Dr. Michael Green, University of Massachusetts, Amherst) cDNAs were generously provided as gifts. Fragments containing cDNAs were amplified by polymerase chain reaction (PCR) using primers carrying *Bam*HI adaptors and subcloned into a mammalian expression plasmid pAsc.CMV-SV*neo*VA.[2] The expression of ATF-1 and hGCN5 cDNAs was directed by a cytomegalovirus immediate-early promoter in plasmid pAsc.CMV-SV*neo*Va. The expression vector for full-length human CDP/*cut*, pMT2-CDP, was described previously (34) as well as the bacterial expression plasmids for pGEX*cut3*-HD, pGEX*cut3*-term, and pGEX*cut3*+HD-term (35). Expression and purification of CDP/*cut* fragments by subcloning into the pGEX2TK vector (Amersham Pharmacia Biotech) and expression in *Escherichia coli* bacterial strain BL-21 as glutathione *S*-transferase (GST) fusion proteins were as described previously (12).

Cell Transfections and Reporter Gene Analysis—Cells were plated at a density of 5 × 10^5 cells/35-mm plate (approximately 75% confluent) 18 h prior to transient transfections. Transfections were carried out as described previously (12). Briefly, plasmid expression reporter and 100 ng of internal control pRSV/CAT were diluted to a final volume of 50 μl in 20 mM Hepes buffer, pH 7.6. The DNA mixture was transferred to a tube containing 30 μl of LipofectAMINE™ (Life Technologies, Inc.) diluted in 1 ml of Opti-MEM (Life Technologies, Inc.). The mixture was incubated for 15 min at room temperature. This medium mixture was used to replace the existing medium in the cell cultures and rocked gently, briefly, for even distribution of the mixture. Transfected cells were incubated for 6 h at 37 °C in a 5% CO_2 atmosphere. At this time, medium was replaced with fresh culture medium and cultured for an additional 48 h before assaying the cultures and culture medium for chloramphenicol acetyltransferase activity and human growth hormone (hGH) (12). The levels of hGH secreted into the medium from transfected cells were assayed by hGH enzyme-linked immunosorbent assay (Boehringer Mannheim) according to the manufacturer's instructions. hGH expression was normalized to the level of chloramphenicol acetyltransferase expression directed by the Rous sarcoma virus long terminal repeat sequences in each experiment.

Preparation of Nuclear Extracts, Electrophoretic Mobility Shift Assays, and DNase I Protection Assay—Nuclear extracts from human PANC1 cells were prepared essentially as described (52) with modifications to the preparation of nuclear extracts described previously (12). The extracts were frozen under liquid N_2 and stored at −80 °C until further use. The concentrations of the nuclear extracts were determined by colorimetric quantitation assays according to the manufacturer's directions (Bio-Rad). The double-stranded oligonucleotide corresponding to sequences of the *CFTR* inverted CCAAT element 5′-tgggggaatggaagccaaatgacatcac-3′ was synthesized and labeled at the 5′-end by incubating with [γ-^{32}P]ATP and T4 polynucleotide kinase. The binding reactions were carried out at 4 °C for 30 min with 0.1 ng of the ^{32}P-labeled oligonucleotide, 0.5 μg of nonspecific competitor DNA (poly(dI·dC)), and either 2 or 10 μg of PANC1 nuclear extract in the following buffer conditions: 20 mM Hepes, pH 7.9, 60 mM KCl, 0.1 mM EDTA, 5% (v/v) glycerol. For competition experiments, unlabeled competitor oligonucleotides corresponding to nucleotide sequences of the *CFTR* gene promoter bearing a mutant inverted CCAAT element (5′-

[2] Y. Ioannou, unpublished data.

tgggggggaagaagccaaatgacatcac-3′) and mutant (5′-tgggggggaattggaagccaaataacatgac-3′) were added in 100 × molar excess to the binding reactions prior to the introduction of labeled oligonucleotides. All DNA-protein complexes were resolved by electrophoresis on 5% polyacrylamide:bisacrylamide (80:1) in 0.25 Tris acetate and EDTA at 10 volts/cm at 4 °C. Antibody supershift assays were performed similarly, with the exception that polyclonal antiserum (1 μg) to CBF-c (33), CDP/*cut*, or preimmune serum was added along with bovine serum albumin (5 μg) directly to the DNA-nuclear extract mixture and allowed to incubate at 4 °C for an additional 30 min. DNA-protein and antibody complexes were fractionated on 5% polyacrylamide:bisacrylamide (80:1) in 0.25 Tris acetate and EDTA at 10 volts/cm at 4 °C.

Deoxyribonuclease I (DNase I) protection assay was performed using nuclear extracts prepared from untreated and 8-Br-cAMP-treated cells on the 127-bp fragment from the region of the *CFTR* promoter between −198 and −71 upstream of the translation start site. A DNA fragment was generated with the primers 5′-gggcagtgaaggcgggggaaagagc-3′ and 5′-ctgggtgcctgccgctcaaccctt-3′. The 5′-oligonucleotide 5′-gggcagtgaaggcgggggaaaga-3′ was labeled by using [γ-^{32}P]ATP and T4 polynucleotide kinase. Addition of the [γ-^{32}P]ATP-labeled oligonucleotide (~ 5 pmol) to the PCR was used to generate a ^{32}P-labeled DNA fragment. The DNA fragment was excised and purified from a 5% polyacrylamide gel. DNase protection experiments were carried out as described previously (12). To confirm the location of protection from DNase hydrolysis, corresponding Maxam-Gilbert [G +A] reactions were conducted on the ^{32}P-labeled DNA fragment.

Endonuclease Sensitivity Assays—Nuclei from trichostatin A (TSA)-treated, 8-Br-cAMP/TSA-treated, and untreated PANC1 cells were digested with *Bsp*MI (500 units, New England Biolabs, Beverly, MA) at 37 °C for 2 h in digestion buffer (10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 60 mM KCl, 0.1 mM EDTA, 5 mM $MgCl_2$, 5% (v/v) glycerol, and 0.5 mM dithiothreitol). After digestion with *Bsp*MI *in vivo*, DNA was extracted with a phenol/chloroform/isoamyl alcohol (24:24:1) mixture and recovered by ethanol precipitation. Extracted DNA was digested further with the restriction enzymes *Hin*dIII and *Eae*1, fractionated on 1.8% agarose gels by electrophoresis, and transferred onto nylon membrane filters. Filters were hybridized to a ^{32}P-labeled DNA probe as indicated. Filters were washed three times using high stringency washing conditions with a final wash (0.1 × SSC at 68 °C) for 15 min. Filters were exposed to x-ray film or evaluated with a Storm 860 PhosphoImager using ImageQuant™ software (Molecular Dynamics, Sunnyvale, CA).

Genomic DNase I Hypersensitivity Assays—Cells were treated with 100 ng/ml TSA (Sigma) for 30 min at 37 °C prior to assays. Nuclei (20 μg of DNA equivalent) from stably transfected PANC1 cells (12) were treated with the addition of the RNase-free DNase I (Promega, Madison, WI) with increasing concentrations from 5 to 50 units in a buffer. Nuclease reactions were terminated by the extraction of genomic DNA with phenol/chloroform, and DNA was then precipitated with ethanol at −20 °C from 2 h. DNase-treated treated DNA was digested with *Sac*II (420 units) and *Sac*I (340 units, New England Biolabs) at 37 °C for 18 h in a 1 °C digestion buffer. Digested genomic DNA was fractionated by agarose gel electrophoresis, blotted onto membranes, and hybridized to a human genomic *CFTR* ^{32}P-labeled *Sac*II-*Sac*I probe, indicated in the experiment shown, washed, and autoradiographed.

Immunoprecipitations and GST Pull-down Assay—U937 cells were metabolically labeled with [^{35}S]methionine/cysteine (NEN Life Science Products). Nuclear lysates of ^{35}S-labeled U937 cells were precleared with rabbit preimmune serum and immunoprecipitated with protein A-Sepharose (Amersham Pharmacia Biotech). The remaining supernatant was used for immunoprecipitation experiments. Immunoprecipitation of HDAC1 was performed using nuclear lysates from metabolically ^{35}S-labeled U937 cells pretreated with GST to eliminate background and immunoprecipitated with HDAC1 antiserum (Santa Cruz Biotechnology, Santa Cruz, CA) as described (53). The GST and the GST-CDP fusion proteins were expressed as described above and conjugated to glutathione-Sepharose beads (Amersham Pharmacia Biotech) after low stringency washing procedure (54). The GST and GST-CDP/*cut* fusion proteins, bound to conjugated glutathione-Sepharose beads, were equilibrated in a lysis washing (LW) buffer (10 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.5 mM EDTA, 10 mM Na_3 (PO_4), 10 mM NaF, 2.5 mM leupeptin, 10 mM phenylmethylsulfonyl fluoride, 100 μg of aprotinin), then incubated with nuclear extracts of ^{35}S-labeled U937 cells (2×10^5 dpm) in a final volume of 250 μl for 3 h at 4 °C. Then the beads were centrifuged and washed three times in LW buffer and treated with HDAC1 antiserum. The antiserum was passed over a GST column to preclear anti-GST antibodies. After elution of GST fusion protein from the beads with glutathione, immunoprecipitations with HDAC1 antiserum were performed as described below. Mixtures were washed twice with LW buffer containing 0.5% deoxycholate and immunoprecipitated with protein A-Sepharose beads. The immunoprecipitates were washed three times with LW buffer containing 0.5% deoxycholate, resuspended in a 2 × Laemmli gel loading buffer, and loaded onto a 12% SDS-polyacrylamide gel. Rabbit polyclonal antibodies for CDP/*cut* were generated against the GST fusion containing the human CDP/*cut* protein encoding the homeodomain through the carboxyl (COOH)-terminal region using standard protocols (54).

In Vitro Protein-Protein Binding Assays—The GST and the GST-CDP/*cut* fusion proteins were expressed as described above and immobilized on glutathione-conjugated beads, washed, and equilibrated in HEKG binding buffer (40 mM Hepes, pH 7.9, 50 mM KCl, 0.2 mM EDTA, 2.5 mM leupeptin, 10 mM phenylmethylsulfonyl fluoride, 100 μg of aprotinin, 1.0 mM dithiothreitol, and 0.5 mg of bovine serum albumin). Transcription template for HDAC1 was created by the reverse transcription PCR method with 0.5 μg of human HeLa cell mRNA using a primer pair set corresponding to the translational open reading frame (5′-cgagcaagatggcgcagacgcag-3′) and terminus (5′-cgtgagggactcagcaggaagcc-3′) of human HDAC1. The correct PCR product was obtained, verified by gel electrophoresis and nucleotide sequence analysis, and then cloned into the vector pCR®-Blunt according to the manufacturer's instructions (Invitrogen) to generate the *in vitro* transcription template pHDAC1-T7. The ^{35}S-labeled HDAC1 protein was synthesized *in vitro* from the linearized plasmid template by the use of the coupled transcription-translation system (TNT® system, Promega). The ^{35}S-labeled HDAC1 protein was incubated with a 50% slurry of the corresponding GST-CDP/*cut* fusion protein in 400 μl of HEKG binding buffer for 1 h at 4 °C with gentle rocking. The Sepharose beads were then washed four times with 1 ml each time of HEKG binding buffer (lacking bovine serum albumin, leupeptin, aprotinin, and phenylmethylsulfonyl fluoride). Bound proteins were eluted in 25 μl of 50 mM Tris-HCl, pH 6.9, 0.1 mM EDTA, containing 100 mM glutathione (Sigma). Eluted proteins were resuspended in a 2 × Laemmli gel loading buffer and loaded onto a 12% SDS-polyacrylamide gel and resolved by electrophoresis.

In Vitro Histone Deacetylase Assays—Histone deacetylase activity was assayed essentially as described (23) with 50 μl of crude cell extract, then immunoprecipitated with rabbit polyclonal antiserum against recombinant human CDP/*cut* (34) for 2.5 h at 37 °C. Immunoprecipitates of human CDP/*cut* were washed three times at 4 °C in a low stringent wash containing 0.1% Nonidet P-40 in phosphate-buffered saline. Pretreatment of immunoprecipitates with 100 ng/ml TSA occurred for 30 min at 4 °C before addition of the peptide substrate. The synthetic peptide substrate was purchased commercially (Accurate Chemical, Westbury, NY) and corresponds to the first 24 residues of the amino-terminal domain of bovine histone H4 (23). Immunoprecipitates were then incubated with 2.5 mg (60,000 dpm) of acid-soluble peptide for 30 min at 37 °C. The reaction was terminated with acetic acid and HCl. Released [^{3}H]acetic acid was obtained by extraction with ethyl acetate and quantitated by liquid scintillation counting. Samples were were assayed triplicate (in four trials), and the nonenzymatic release of label was subtracted to obtain the final value.

RESULTS

CBF·NF-Y Is Associated with the CFTR Gene Proximal Y-box Element—Because basal and cAMP-mediated *CFTR* transcription requires the conservation of the inverted CCAAT motif in the promoter of *CFTR* (12, 13) and is a potential consensus to bind CBF·NF-Y (55), we reasoned that CBF·NF-Y or similar CCAAT-binding factors could have a role in directing *CFTR* transcription. To identify the nuclear proteins associated with basal and inducible activation of *CFTR* transcription, we tested whether the transcription factor CBF·NF-Y complex could bind to the proximal Y-box consensus site of the human *CFTR* promoter. Fig. 1*A* demonstrates the binding of nuclear protein prepared from confluent monolayers of PANC1 cells in competitive EMSA in the presence of unlabeled competing oligonucleotides corresponding to wild-type human *CFTR* sequences or mutant *CFTR* oligonucleotide sequences. The unlabeled homologous *CFTR* Y-box element sequence depletes the visible complex in a competition assay. Addition of an oligonucleotide corresponding to the same sequence with the exception of a three-nucleotide deletion directed at the ATTGG to A—G failed to compete for binding. Moreover, a mutation introduced into a CRE consensus 11 bp downstream of the ATTGG sequence

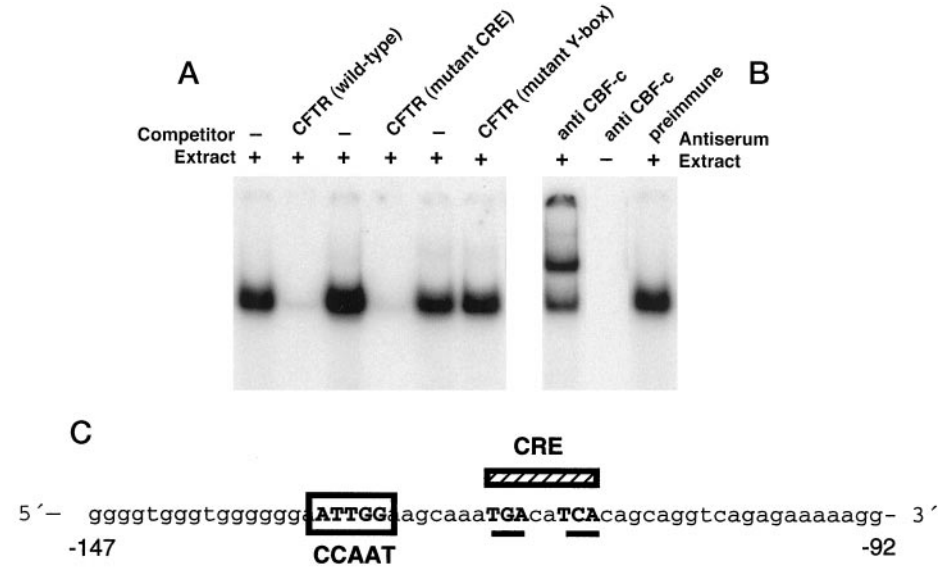

FIG. 1. **EMSA/supershift demonstrates that CBF·NF-Y binds to the proximal Y-box of the human CFTR promoter.** *Panel A*, competitive EMSA was performed with nuclear extracts prepared from PANC1 cells after confluent growth. Using 2 μg of nuclear extract and 1×10^5 cpm of a ^{32}P-labeled oligonucleotide of the wild-type *CFTR* inverted CCAAT element, binding was competed with unlabeled oligonucleotides containing sequences of *CFTR* bearing mutant CCAAT and CRE elements. *Panel B*, supershifted complexes containing CBF·NF-Y bound to the radiolabeled wild-type *CFTR* oligonucleotide are recognized by the antiserum against the CBF-c subunit of CBF·NF-Y as shown in the EMSA. *Panel C*, nucleotide sequence of the human *CFTR* promoter between −147 and −92, relative to the first open reading frame. The sequence corresponding to the inverted CCAAT element of *CFTR* is *outlined* in the *open box*. *Underlined* sequences corresponding to the nucleotide consensus of the CRE are shown and are indicated as the *hatched* box above the sequence.

competed equally as well for the binding of nuclear protein from PANC1 cells (Fig. 1). Shown in *panel B* are supershift assays (EMSA) performed with rabbit antibodies directed against the (c) subunit of CBF·NF-Y; they indicate a supershifted band of the CBF·NF-Y complex bound to the Y-box motif (*first lane*). The CBF·NF-Y supershift was compared with nuclear extracts treated with preimmune serum and shows no altered mobility of the DNA-protein complex in the *last lane* (Fig. 1*B*). This result confirms that CBF·NF-Y can bind the Y-box sequence of human *CFTR* and correlates with the relative consensus necessary for binding CBF·NF-Y (55). It is important to note that previous studies by this laboratory have also documented the ability of this site to bind C/EBP (12). Antibodies directed against the C/EBPβ and C/EBPδ proteins alter complex formation in these experiments (data not shown) as well as in other experiments (12) but failed to immunoprecipitate any histone acetyltransferase activity.[3] However, the question that still remains is whether cell type specificity plays a role in the ability of either CBF·NF-Y or C/EBP to bind the *CFTR* Y-box element to activate transcription *in vivo*. Here we addressed the role of CBF·NF-Y to bind the *CFTR* promoter because of the purported role of CBF·NF-Y in histone acetylation (27, 28). Therefore, we wanted to expand our studies to include other relevant DNA-binding proteins to act through the inverted CCAAT element.

Activation of CFTR Transcription by the Transcription Factor ATF-1 and the Histone Acetyltransferase hGCN5—The correlation between the DNA-binding proteins of the Y-box element with the local structural changes in chromatin (12) has prompted speculation that the modification of core histones, which overlap the Y-box, may reflect a change to chromatin structure favorable for the activation of transcription (27, 28). As a way of determining the role of transcription factors and chromatin accessory factors associated with the proximal Y-box (inverted CCAAT) element to influence the transcription of *CFTR*, transactivation assays were performed on PANC1 cells carrying stably transfected *CFTR* promoter-directed transgenes. Clonal populations of stably transfected cells bearing wild-type (pCF-197wt/hGH-PANC1) and mutated Y-box (pCF-197ΔCCAAT/hGH-PANC1) transgenes were used to assess the ability of cotransfected effector genes to alter expression of the integrated reporter gene. Six individual stably transfected PANC1 clonal populations were isolated and tested for each of the two transgene constructs (pCF-197wt/hGH-PANC1 and pCF-197ΔCCAAT/hGH-PANC1) to confirm that repression is not the result of integration artifacts of the two stably transfected transgenes (Fig. 2). The background expression of promoter function was determined by including stably transfected PANC1 cells bearing the parental hGH reporter gene construct lacking a promoter (Fig. 2). The cDNAs tested include the cyclic AMP-responsive transcription factor ATF-1 and the human histone acetyltransferase hGCN5 and activate transcription of *CFTR* in pancreatic carcinoma cells (Fig. 2). Analysis of reporter transgene expression (Fig. 2) indicates that both ATF-1 and hGCN5 direct the activation of a *CFTR*-directed reporter gene. The observed 4–5-fold activation was dependent on an intact Y-box element consensus site, suggesting that both ATF-1 and hGCN5 functionally converge on the Y-box element to stimulate *CFTR* transcription. Although the histone acetyltransferase P/CAF (56), a close homolog of hGCN5, was not tested here, similar results have been obtained from other studies (28), suggesting the involvement of histone acetyltransferase in the activation of *CFTR*. A mutated *CFTR* Y-box element failed to confer activation by either ATF-1 or hGCN5, suggesting that the activity by each of the effectors tested requires the intact Y-box sequence. These data support the role of histone acetyltransferase in the activation of *CFTR* and the requirement for an intact CCAAT consensus in response to the transient expression of histone acetyltransferase activity in the PANC1 cell type.

The Human Transcriptional Repressor CDP/cut Binds to the Y-box Region of the CFTR Promoter—We had previously suggested a strong correlation between the Y-box (inverted CCAAT) element of the *CFTR* promoter and repression of *CFTR* transcription (12). Previous experiments had demonstrated the absence of protein binding with the induction of *CFTR* transcription by cAMP (12). Although repression of *CFTR* transcription could not be confirmed by site-directed mutagenesis because of the intrinsic nature of the inverted CCAAT in basal transcription (12), other reports suggest that the Y-box element of *CFTR* could be a target for CDP/*cut* (44, 48). To determine the functional role of CDP/*cut* in *CFTR* transcription, PANC1 cell clones (pCF-197wt/hGH-PANC1 and pCF-197ΔCCAAT/hGH-PANC1) were cotransfected with an ex-

[3] M. J. Walsh, unpublished data.

pression vector encoding the full-length CDP/*cut* cDNA, pMT2-CDP (Fig. 3). Because of the lack of CDP/*cut* protein in PANC1 cells compared with U937 cells by Western blot analysis (data not shown), PANC1 cells provided the opportunity to introduce the ectopic expression of CDP/*cut* in a relatively naïve model. Results (Fig. 3) demonstrate that the function of CDP/*cut* was dependent on the Y-box to repress activation, mediated by either ATF-1 or the human histone acetyltransferase hGCN5. Although repression of a constitutively expressed *CFTR*-directed transgene was relative, a striking reduction (20-fold) in hGH expression was apparent when either ATF-1 or hGCN5 was used as an activator in the cotransfection experiments (Fig. 3). It is apparent that overexpression of CDP/*cut* can directly antagonize either ATF-1 or hGCN5, and repression of the constitutive expression of *CFTR* was consistent with the antagonism of ATF-1 and hGCN5 transactivation, as similarly reported with heterologous promoters (57). To determine if the behavior of CDP/*cut* to repress the activity by either ATF-1 or hGCN5 is stoichiometric, we titered the amount of the plasmid expression vector for CDP/*cut*, pMT2/CDP/*cut* used in each cotransfection experiment (Fig. 4). In these experiments the cell line pCF-197wt/hGH-PANC1, bearing the wild-type Y-box motif of *CFTR*, was used to determine the ability of CDP/*cut* to compete with the transactivation potential ATF-1 and hGCN5. Results shown in Fig. 4 demonstrate that the transfected CDP/*cut* expression vector competes in a dose-dependent manner with hGCN5 and to a lesser degree ATF-1.

To confirm whether CDP/*cut* does bind the *CFTR* promoter, studies were conducted using nuclear extracts from U937 cells and recombinant CDP/*cut* to determine the binding pattern of CDP/*cut* to the Y-box consensus site of *CFTR*. Results in Fig. 5*A* show an EMSA antibody supershift experiment performed using an oligonucleotide corresponding to a *CFTR* inverted CCAAT sequence (Y-box) and antibodies generated against the CDP/*cut* protein and a recombinant GST fusion protein of CDP/*cut* expressed in *E. coli* (see "Experimental Procedures"). Results shown in Fig. 5*A* indicate that CDP/*cut* is a component

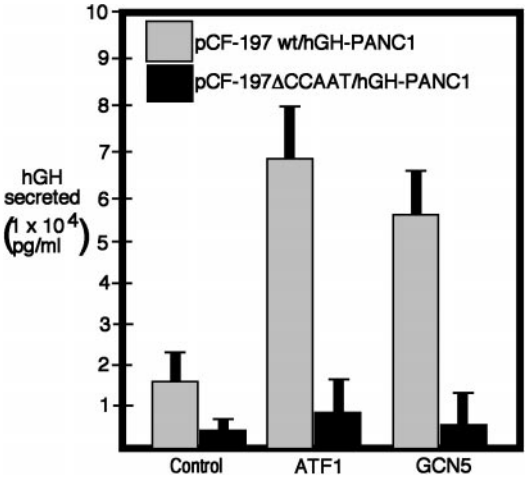

FIG. 2. **Transactivation of CFTR gene transcription by human histone acetyltransferase hGCN5 and transcription factor ATF-1.** The two cell lines bearing wild-type or mutant *CFTR* promoters were cotransfected with the effector plasmid expressing the transcription factors ATF-1 or human hGCN5 with an internal control pCMVβ (β-galactosidase expression). Each transfection was normalized to levels of β-galactosidase expressed to correct for transfection efficiency. Human GH production was expressed as pg of hGH produced in 1 ml of culture medium. Values represent the mean ± S.E. ($n \geq 5$).

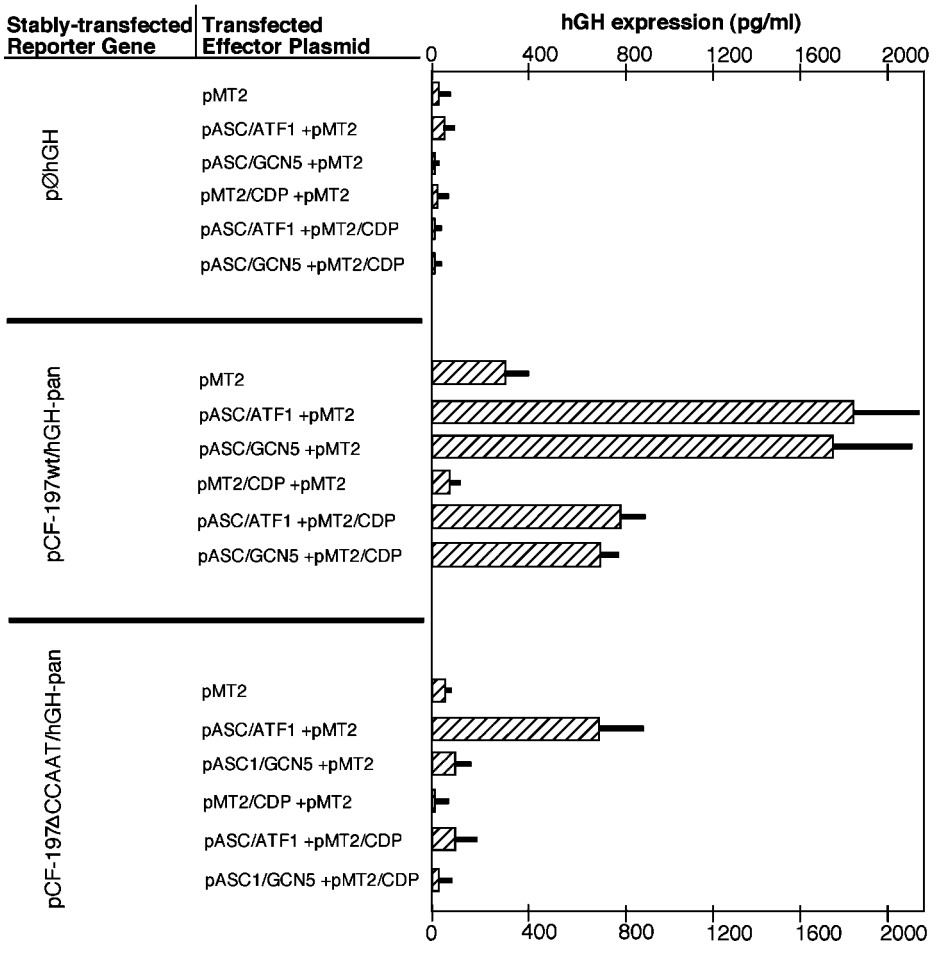

FIG. 3. **CDP/*cut* represses gene expression mediated through the Y-box element of a CFTR-directed transgene.** Cotransfection experiments were performed using LipofectAMINE reagent (Life Technologies, Inc.) of stably transfected cell lines each containing one of the three independent human *CFTR*-directed transgenes under the selection of 400 μg/ml G418 (12). Secretion of the reporter gene product hGH was monitored from medium of stably transfected cells lines after cotransfections performed with the parental control vector (pMT2), the expression vectors for CDP/*cut* (pMT2/CDP/*cut*), ATF-1 (pASC/ATF1), or the histone acetyltransferase hGCN5(pASC1/hGCN5). Each transfection was normalized with an internal control plasmid vector (pCMVβ) expressing β-galactosidase to correct for transfection efficiency. Values ($n \geq 8$) represent the mean ± the S.D. expressed as pg of hGH secreted in 1 ml of medium.

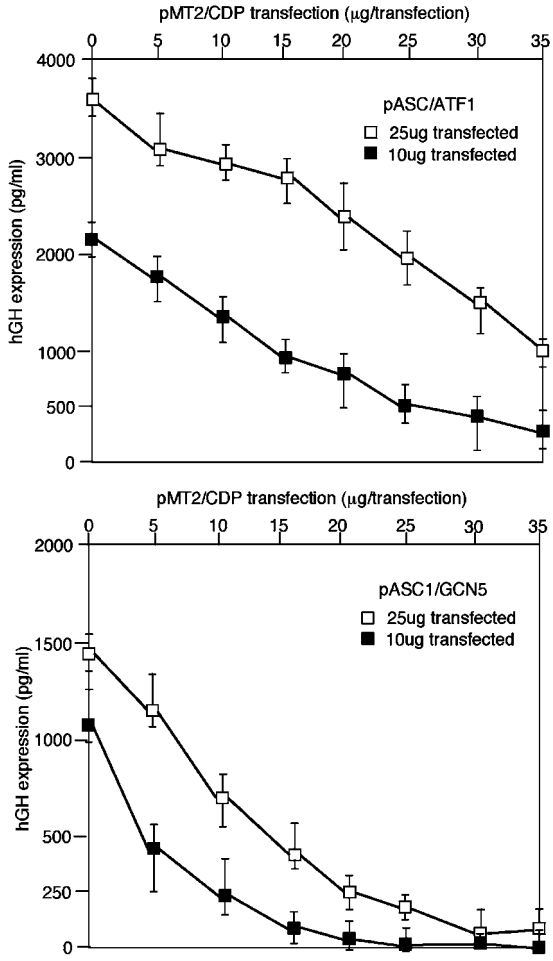

FIG. 4. **Dose-dependent expression of human CDP/*cut* represses ATF-1 and human hGCN5 transactivation of CFTR transcription.** Cotransfection with pMT2/CDP/*cut* and pASC/ATF1 or pASC1/hGCN5 (*bottom panel*) into the stably transfected cell lines pCF-197wt/hGH-PANC1 and pCF-197ΔCCAAT/hGH-PANC1 was performed and assayed for hGH expression. Varible but linear amounts of pMT2/CDP/*cut* plasmid were cotransfected with 10 and 25 μg of either pASC/ATF1 or pASC1/GCN5. Each cotransfection was normalized with an internal control plasmid (pCMVβ) to correct for the efficiency of each transfection with the expression of β-galactosidase activity. Human GH was expressed as pg/ml medium. *Bars* above and below *boxes* represent the S.E. ($n \geq 5$).

of the complex bound to the Y-box inverted CCAAT sequence of the *CFTR*. A subsequent DNase I footprinting experiment was performed to establish the location of recombinant CDP/*cut* binding within the human *CFTR* 5′-flanking region. Protection from hydrolysis by DNase I in the presence of recombinant CDP/*cut* reveals a pattern of protection overlapping the inverted CCAAT (Y-box) motif as shown in Fig. 5*B*. The figure indicates the position relative to the open reading frame of CFTR. A GST-CDP/*cut* fusion protein carrying the third *cut* repeat through the termination of human CDP/*cut*, which includes the homeodomain, was expressed in *E. coli* as a GST fusion protein and used in DNase I footprint analysis (Fig. 5*B*). The GST-CDP/*cut* fusion protein was able to confer protection of the same region overlapping the Y-box motif of the *CFTR* promoter (Fig. 5*B*).

Inhibition of Histone Deacetylase Activity by TSA Corresponds with Activation of CFTR Transcription Mediated through the Proximal Y-box Element—The recent findings that coactivators and transcription factors, such as p300/CBP, C/EBP, and CBF·NF-Y, encode or are tethered to enzymatic activity associated with histone acetylation provide rationale for investigating whether local chromatin, overlapping the Y-box, may be a direct target for histone acetylation. To test this hypothesis, we demonstrate a relationship between histone acetylation and transcription of *CFTR*. TSA is a specific inhibitor of histone deacetylase activity and has been associated with potentiating gene transcription in cell cultures (58, 59). We determined the relationship of TSA treatment in multiple populations of stably transfected cells to the stimulation of reporter gene expression when directed by the human wild-type *CFTR* promoter. Results of our preliminary studies indicate that administration of TSA potentiates the expression of the hGH reporter gene and that potentiation of a *CFTR*-directed transgene specifically requires the Y-box consensus (Fig. 6). To investigate whether transcription, potentiated by TSA, is mediated through the Y-box motif, we then tested a stably transfected transgene in which the Y-box was mutated and no longer bound nuclear protein (12). In the transgene constructs shown in Fig. 6, the Y-box was either left intact in the wild-type *CFTR* promoter or mutated by the deletion of four nucleotides in the Y-box element. The control reporter transgene with the intact Y-box responded positively to both cAMP and TSA induction. As anticipated the reporter with the mutated Y-box sequence was not activated by either cAMP or TSA. These results indicate that TSA enhances transcription through the Y-box. Therefore, the inhibition of histone deacetylase activity is directed through interactions with the Y-box sequence of *CFTR*.

Inhibition of Histone Deacetylase Activity Is Associated with Chromatin Accessibility of CFTR in Vivo—To establish a correlation between the inhibition of histone deacetylation with increased accessibility in the *CFTR* promoter *in vivo*, we examined whether TSA treatment alters the sensitivity of nuclei to hydrolysis with a restriction endonuclease. The experimental strategy was based on the ability to detect the sensitivity of the *CFTR* promoter in nuclei to endonuclease digestion after treatment with the histone deacetylase inhibitor, TSA. *Bsp*MI was chosen (Fig. 7*B*) because it recognizes a nucleotide sequence immediately adjacent to the Y-box of the *CFTR* promoter. PANC1 cells, treated with TSA, were used to prepare nuclei and digested with *Bsp*MI *in vivo*, DNA was purified, restricted with *Hin*dIII and *Eae*1, and then digestion products were detected by Southern blot hybridization using a ^{32}P-labeled probe corresponding to the *Hin*dIII/*Eae*1 fragment of the *CFTR* promoter. Fig. 7*A* shows that both TSA-treated and cAMP+TSA-treated cells display a specific pattern that corresponds with the digestion by *Bsp*MI endonuclease. This pattern demonstrates the presence of the 673-bp and 180-bp fragments shown (Fig. 7*A*). Decreasing the amount of TSA, added to PANC1 cell cultures, reflects the loss of specific *Bsp*MI cleavage. The treatment of PANC1 cells with both 8-Br-cAMP and TSA (100 ng/ml) complemented the sensitivity to *Bsp*MI cleavage. Thus, TSA by itself and in combination with 8-Br-cAMP directs *Bsp*MI cleavage of nucleotide sequences adjacent to the Y-box of *CFTR*, suggesting that histone acetylation contributes to an alteration of chromatin structure proximal to the Y-box element of the *CFTR* promoter.

DNase I Hypersensitivity in the CFTR Promoter Requires an Intact Proximal Y-box Element—Because the status of histone acetylation may determine the structure of chromatin, we examined whether increased sensitivity to DNase I hydrolysis gives a pattern consistent with the stimulation of *CFTR* transcription. The experiment shown in Fig. 8 examined whether mutation to the intact Y-box element alters sensitivity to nuclease hydrolysis surrounding the *CFTR* promoter/hGH gene junction from a stably transfected transgene. Nuclei from multiple clonal populations of stably transfected pancreatic carci-

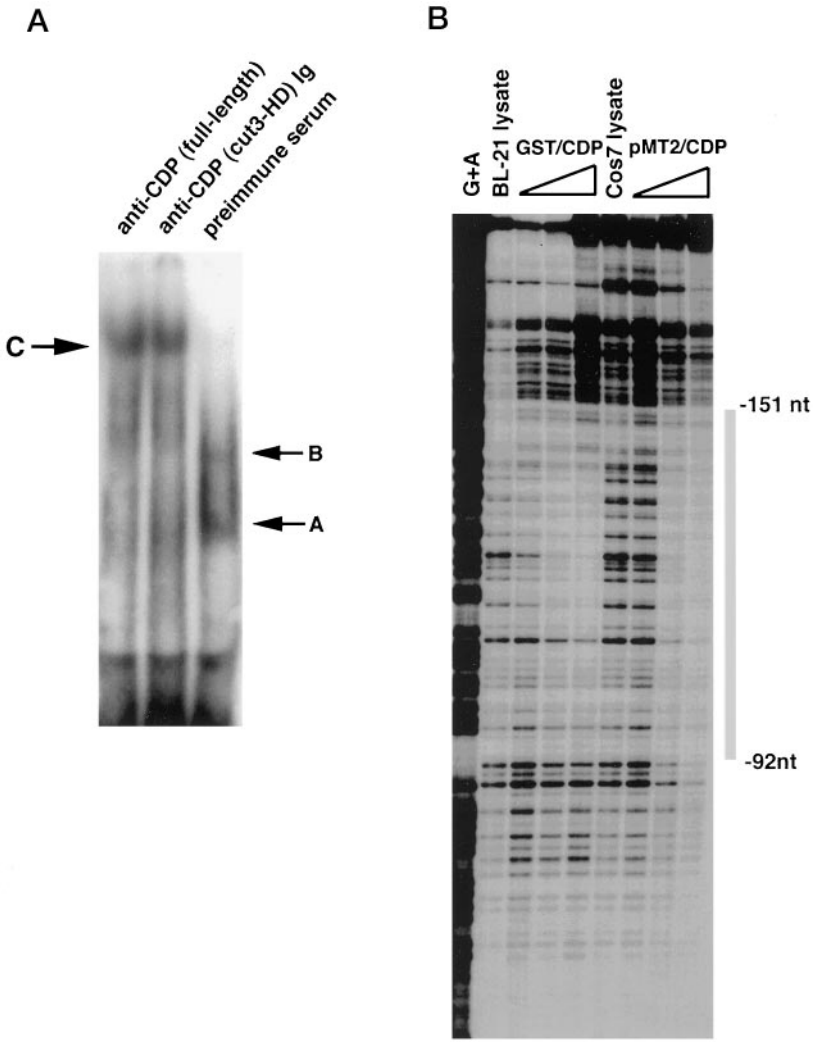

FIG. 5. **Human CDP/*cut* binds the human CFTR gene.** *Panel A*, supershift analysis by EMSA of CDP/*cut* bound to the *CFTR* Y-box element. Using extracts from U937 cells, gel shift analysis of the Y-box sequence representative of −92 to −151 nucleotides of the human *CFTR* promoter was radiolabeled with [^{32}P]ATP. EMSA, using two different polyclonal antisera directed at CDP/*cut*, was performed to identify DNA-protein mobility, shown as complex C. *Panel B*, protection from hydrolysis by DNase I between the nucleotides located −92 and −151 relative to the 5′-upstream region of the first AUG codon overlapping the Y-box nucleotide consensus of the human *CFTR* gene promoter is shown. Extracts from COS-7 cells transfected with the CDP/*cut* expression vector pMT2/CDP/*cut* and the GST fusion protein of CDP/*cut* were used in a DNase I protection experiment and compared with control nuclear extracts from the identical cells. Corresponding Maxam-Gilbert reactions carried out to identify the boundaries of protection rendered by the extracts are *outlined* by the *hatched bar* adjacent to the photograph.

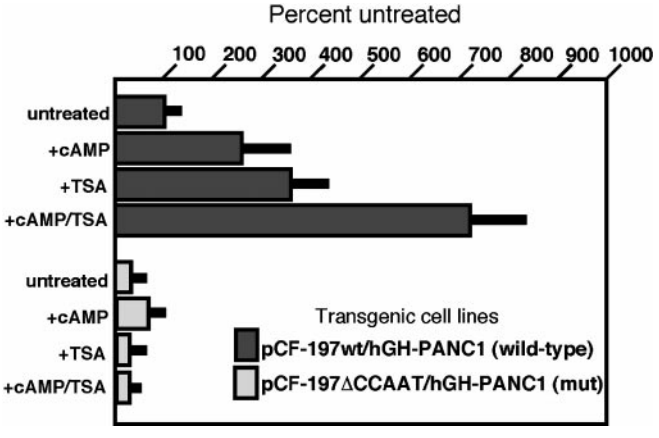

FIG. 6. **Requirement of the CFTR gene Y-box element for the induction of gene transcription by TSA.** Experiments were performed using multiple clonal populations of stably transfected cell lines carrying wild-type and mutant *CFTR*-directed hGH transgenes. Each of the two transgene constructs bearing either the wild-type or mutant *CFTR*-directed reporter gene received either TSA, cAMP, or TSA+cAMP. The production of hGH was monitored 48 h after each transfection. Values reflect the percent of hGH production relative to the untreated PANC1 cells stably transfected with the wild-type transgene ± the S.D. (n = 6).

noma PANC1 cell lines were pooled and used to determine whether a mutation directed at the Y-box sequence alters the pattern of nuclease sensitivity consistent with the treatment with TSA. Shown is an autoradiograph of a Southern blot hybridization (Fig. 8) depicting the relationship of an intact Y-box to DNase I hydrolysis of nuclei after treatment with TSA and cAMP. The *top panels* demonstrate a stably transfected PANC1 cell line, pCF-197wt/hGH-PANC1, bearing the intact wild-type Y-box sequence of the human *CFTR*-directed transgene (Fig. 8). In the comparative experiment, mutation directed at the Y-box nucleotide consensus in cell line pCF-197ΔCCAAT/hGH-PANC1 failed to confer the same sensitivity to DNase I hydrolysis after treatments with either cAMP or TSA. Experiments were also performed in the absence of both TSA and cAMP in each of the transgenic constructs and demonstrate a pattern corresponding to the lack of DNase I hypersensitivity within the *CFTR* promoter (not shown). Although this is consistent with the activation of *CFTR* transcription, the comparison that is made in this experiment was planned only to reflect differences between wild-type and mutant sequences of the inverted CCAAT element. Therefore, results shown in Fig. 8 suggest that increased sensitivity to DNase I by cAMP or TSA is dependent on the intact Y-box sequence. Our results suggest that the intact inverted CCAAT sequence regulates the accessibility of chromatin after treatment with TSA and cAMP. This observation is consistent with the induction of the activation of gene transcription (shown in Fig. 6) and accessibilty of chromatin to *Bsp*MI cleavage (shown in Fig. 7). We support the notion that the acetylation of histones, mediated by the CCAAT sequence element, contributes to the activation of *CFTR*-directed transcription. However, we do not know the precise

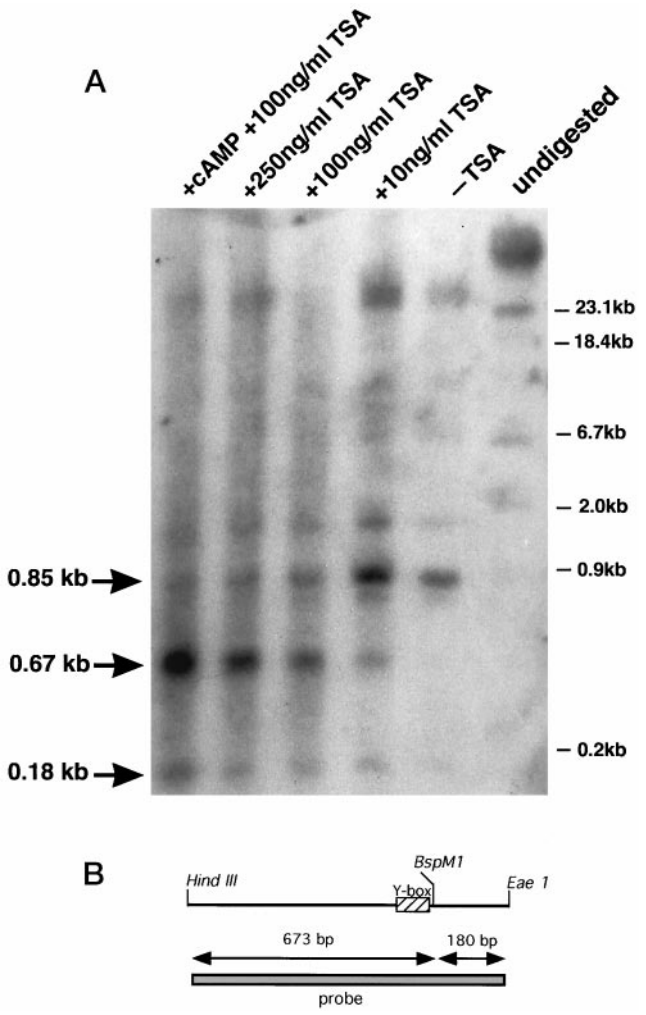

FIG. 7. **Endonuclease sensitivity of the CFTR promoter by TSA.** *Panel A*, PANC1 cells were treated with increasing amounts of TSA (10, 100, and 250 ng/ml) or TSA (100 ng/ml) with 500 μM 8-Br-cAMP. Nuclei were digested with *Bsp*MI, genomic DNA was then extracted followed by digestion with *Hin*dIII and *Eae*1. Digested DNA was fractionated by electrophoresis, transferred to filters, and hybridized to the indicated probe. The *upper most band* and two *lower bands* represent undigested or *Bsp*MI-digested bands, respectively. In the *last lane*, TSA-treated nuclei were digested with *Bsp*MI, and genomic DNA was extracted and loaded on to the gel in the absence of *Hin*dIII and *Eae1* digestion. Plasmid DNA, corresponding to the identical genomic region, was digested *in vitro* (naked DNA) and used as a control for comparison (not shown). Molecular weight DNA markers were used to confirm the length of the fragments. The lengths of undigested and digested fragments are shown to the *left*. *kb*, kilobases. *Panel B*, diagram of *CFTR* and the corresponding endonuclease digestion. The inverted CCAAT (Y-box) element is indicated as the *hatched box*. *Double arrows* indicate the anticipated fragments after digestion with *Bsp*MI. The *shaded bar* indicates the length of the ^{32}P-labeled probe used corresponding to the restriction fragment of *CFTR*.

mechanism for this result, nor do we suggest that CBF·NF-Y (or other CCAAT-binding factor) is responsible for the targeted recruitment of histone acetyltransferase activity to *CFTR*. We observed that DNase I hypersensitivity may correlate closely with the accessibility of the sequences in chromatin by potential *trans*-acting factors. Access to the CCAAT element in chromatin may be stimulated further by histone acetylation, and this, in turn, potentiates further remodeling of chromatin through the association of histone acetyltransferases consistent with other findings (27, 28).

HDAC1 Is Associated with the COOH-terminal Region of CDP/cut—Recent observations have shown that repressors of transcription alter chromatin structure through the association of histone deacetylase activities (60, 61). Studies also have shown the association of a novel mammalian transcriptional corepressor, mSin3A, with the histone deacetylases HDAC1 and HDAC2 (23, 60). Interactions are mediated through a paired amphipathic helix domain conserved within several proteins and associated with protein-protein interactions (60–62). We tested the hypothesis to determine if human CDP/*cut*, which binds to the Y-box of the *CFTR*, may recruit the interactions of a histone deacetylase through a domain similar to the paired amphipathic helix motif of mSin3A (57, 60). To determine if histone deacetylase activity was associated with CDP/*cut in vivo* histone deacetylase activity was tested on immunoprecipitates of CDP/*cut* as shown in Fig. 9*A*. CDP/*cut* immunoprecipitates possess histone deacetylase activity when using a synthetic peptide substrate corresponding to the first 24 amino-terminal residues of histone H4 (23). However, only background levels of histone deacetylase activity were detected in the immunoprecipitates when the antiserum was blocked with the specific immunogen, recombinant CDP/*cut*. To determine if the deacetylase activity is present, as described in Figs. 6 and 7, we tested whether TSA would inhibit the activity associated with the immunoprecipitates of CDP/*cut*. Results show that treatment of the immunoprecipitates of CDP/*cut* with TSA reduces the acetylation by approximately 60–70% (Fig. 9*A*). This suggests that the associated histone deacetylase activity in the CDP/*cut* immunoprecipitates is sensitive to TSA treatment. Using a truncated human CDP/*cut* corresponding to the carboxyl-terminal region of human CDP/*cut*, we generated three GST fusion proteins representative of the carboxyl terminus of CDP/*cut* (shown in Fig. 9*B*). Previous studies have shown that the repressor function of CDP/*cut* was confined to the COOH-terminal region of the CDP/*cut* gene product (57). In the experiment shown in Fig. 9*C*, GST fusion proteins were expressed and purified. Proteins shown in Fig. 9*C* correspond to the regions of human CDP/*cut* which encode fusion proteins overlapping the homeodomain through the end of CDP/*cut*, the *cut3* domain to the COOH-terminal end of CDP/*cut*, and the *cut3* through the homeodomain shown as GST-CDP/*cut* (*HD-term*), GST-CDP/*cut* (*cut3-term*), and GST-CDP/*cut* (*cut3-HD*), respectively. Each of these GST-CDP/*cut* fusion proteins was used as a target to attract interactions to the COOH-terminal third of CDP/*cut* of extracts from U937 cells metabolically labeled with [^{35}S]methionine/cysteine, followed by the GST pull-down assay with glutathione-agarose beads. The ^{35}S-labeled protein complexes, bound to the GST-CDP/*cut* fusion proteins, were precipitated with glutathione-conjugated agarose and then characterized by immunoprecipitation with a rabbit polyclonal antibody directed against HDAC1 fusion protein (Fig. 9*B*). The polyclonal antibody and ^{35}S-labeled protein extracts were preabsorbed with recombinant GST protein prior to the immunoprecipitation. Results of the immunoprecipitation experiment (Fig. 9*D*) show the association of the recombinant CDP/*cut* fusion protein with HDAC1 (55 kDa) (60, 61) as the candidate protein indicated in Fig. 9*D*. Immunoprecipitation of ^{35}S-labeled extracts is shown in the *first lane* and indicates the immunoprecipitation of HDAC1 from protein extracts of U937 cells as well as two consistent bands corresponding to molecular masses of 150 and 50 kDa.

Interaction of GST-CDP with HDAC1 in Vitro—Studies described above suggest that ^{35}S-labeled HDAC1, from metabolically radiolabeled U937 cells, is somehow tethered to CDP (Fig. 9). To confirm whether the interaction between GST-CDP and HDAC1 is a result of a direct interaction or mediated by other factors (*i.e.* mSin3A or mSin3B) we examined the nature of the interaction *in vitro*. We tested the ability of ^{35}S-labeled HDAC1, synthesized and radiolabeled *in vitro* using a coupled

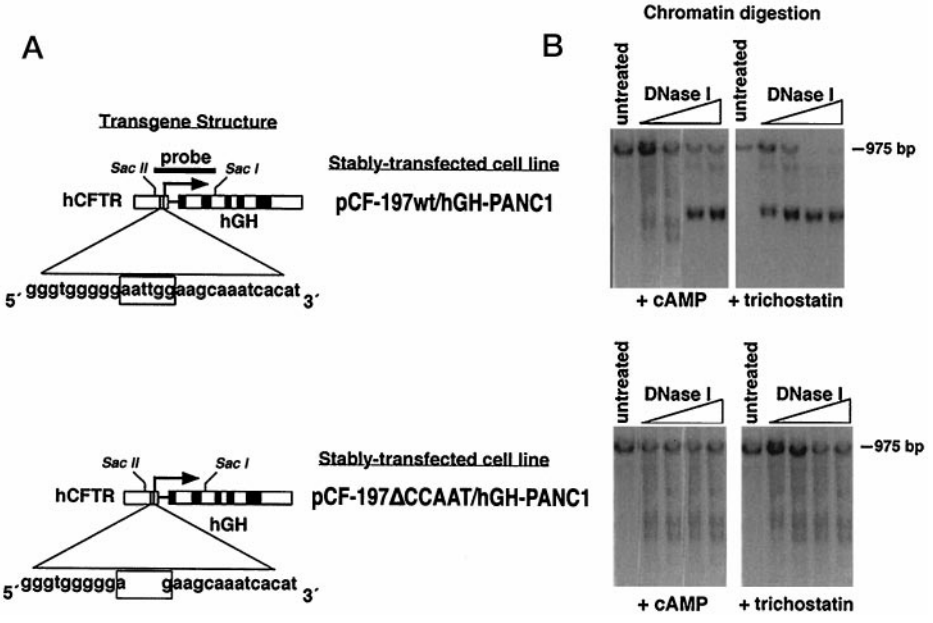

FIG. 8. **DNase I hypersensitivity of the Y-box in the human CFTR gene promoter by trichostatin A.** *Panel A*, schematic diagram depicting the transgenic constructs used in the stably transfected cell lines. The diagram indicates the structural relationship of the human *CFTR* gene promoter to sequences fused with the hGH gene. The *sequences* beneath the diagram are representative of the wild-type and mutant *CFTR* promoters used to construct the minigenes in stably transfected cell lines. The probe used for the Southern hybridization analysis is indicated as the *Sac*II-*Sac*I fragment of the transgene. *Panel B*, stably transfected cells were treated with TSA at 100 ng/ml for 24 h. Nuclei were prepared and digested with increasing amounts of DNase I. Genomic DNA was extracted by organic phase extraction, digested with restriction enzymes *Sac*II and *Sac*I, fractionated by gel electrophoresis, transferred on to nitrocellulose filters, followed by hybridization with a ^{32}P-labeled probe as indicated.

transcription-translation system, to bind to different domains of CDP/*cut*, expressed as GST fusion proteins and immobilized on glutathione-agarose beads. The structure of full-length CDP/*cut*, depicted in Fig. 10*A*, is comprised of a single coiled-coil domain, three *cut* repeats, and one homeodomain in addition to the transcriptional repressor domain at the most COOH-terminal region (57). We suggest that the repressor domain of CDP/*cut* recruits the interaction with HDAC1, and we sought to localize the region of CDP/*cut* to interact with HDAC1. Consistent with our hypothesis (Fig. 10*B*) the COOH-terminal end of CDP/*cut*, expressed as GST-CDP (*cut3*-term), is competent to interact with HDAC1 by this *in vitro* binding assay, whereas HDAC1 exhibited no binding to the GST protein control. Furthermore, truncation of the COOH-terminal region, shown as the GST-CDP (*cut3*-HD) protein (Fig. 10*B*), impaired the ability of the fusion protein to interact with HDAC1. The GST-CDP (*HD-term*) construct containing the homeodomain and COOH-terminal domain maintained the interaction with HDAC1 (Fig. 10*B*).

DISCUSSION

The architecture of chromatin plays a fundamental role in the regulation of genes. The underlying behavior of gene transcription *in vivo* may be determined more by the overall structure of chromatin as a result of the cumulative function transcription factors have in regulating local chromatin structure. The relationship between the remodeling of chromatin by the modification of histones and gene-specific transcription factors recently has become more apparent (15, 20). Since the observation of transcriptional regulatory proteins encoding intrinsic activity for the acetylation or deacetylation of histones, this mechanism for altering chromatin structure has gained prominence in the study of gene regulation (19). Although it is believed that acetylation of histones may neutralize charged lysine residues to allow for the movement or displacement of nucleosomes along a DNA template, making local chromatin more accessible and active for gene transcription, a precise mechanism for this model has yet to be elucidated.

In this report, we provide evidence that the *CFTR* gene Y-box element is a potential target of the CBF·NF-Y activation complex. This was demonstrated by competitive EMSA and supershift analysis (Fig. 1). CBF·NF-Y has been implicated previously in the assembly and remodeling of chromatin structure surrounding its cognate sequence (63). The trimeric subunit complex of CBF·NF-Y has been shown previously to possess histone acetyltransferase activity through the tethering of hGCN5 and/or P/CAF to CBF·NF-Y (27, 28). Previously, we have also demonstrated the binding of the *CFTR* inverted CCAAT (Y-box) sequence by the transcription factor C/EBP (12). Although our study here has not included the role of C/EBP on *CFTR* transcription, it is important to note that C/EBP can interact with CBP/p300 *in vivo* (64) and is the focus of other studies. We believe that C/EBP may play a particularly intriguing role in *CFTR* transcription. The absence of C/EBP in cell lines derived from transgenic "knockout" mice, when compared with wild-type littermates, show the lack of p300-containing complexes associated with the CFTR Y-box. However, this observation is in contrast to results showing CBP associated with the same Y-box element in both knockout and wild-type cell lines.[4] Interestingly, the rate of *CFTR* transcription *in vivo* in these same "knockout" cell lines is precisely half of that compared with the wild-type cell lines. It is plausible to consider that the composition of individual coactivator complexes could reflect a preferential stoichiometric arrangement or dosage between CBP and p300 (65) and may demonstrate some distinction and/or differential regulation between the two coactivators.

We have demonstrated that transcription of *CFTR* is mediated by the transcription factor ATF-1 and the human histone acetyltransferase hGCN5 (Fig. 2). Transfection studies have demonstrated the activating potential of the transcription factor ATF-1 and the human histone acetyltransferase hGCN5 to stimulate *CFTR* transcription. Evidence that hGCN5 potentiates transcription of *CFTR* indicates a role for histone acetylation in the regulation of *CFTR* transcription. We also show that CDP/*cut* has a functional role in repressing transcription of *CFTR*. This result suggested to us that CDP/*cut* effectively competes with transcriptional activators hGCN5 and ATF-1 to antagonize directly the activation of *CFTR* transcription (Figs. 3 and 4). The binding of CDP/*cut* (shown in Fig. 5, *A* and *B*) overlaps the Y-box of *CFTR*; therefore, we speculate that transcription factors bound to the Y-box may, differentially, alter the pattern of histone acetylation in chromatin proximal to transcript initiation to regulate transcription. In our studies, we have used the CDP/*cut* protein, from nuclear extracts,

[4] M. J. Walsh, S.-D. Li, and G. Shue, manuscript in preparation.

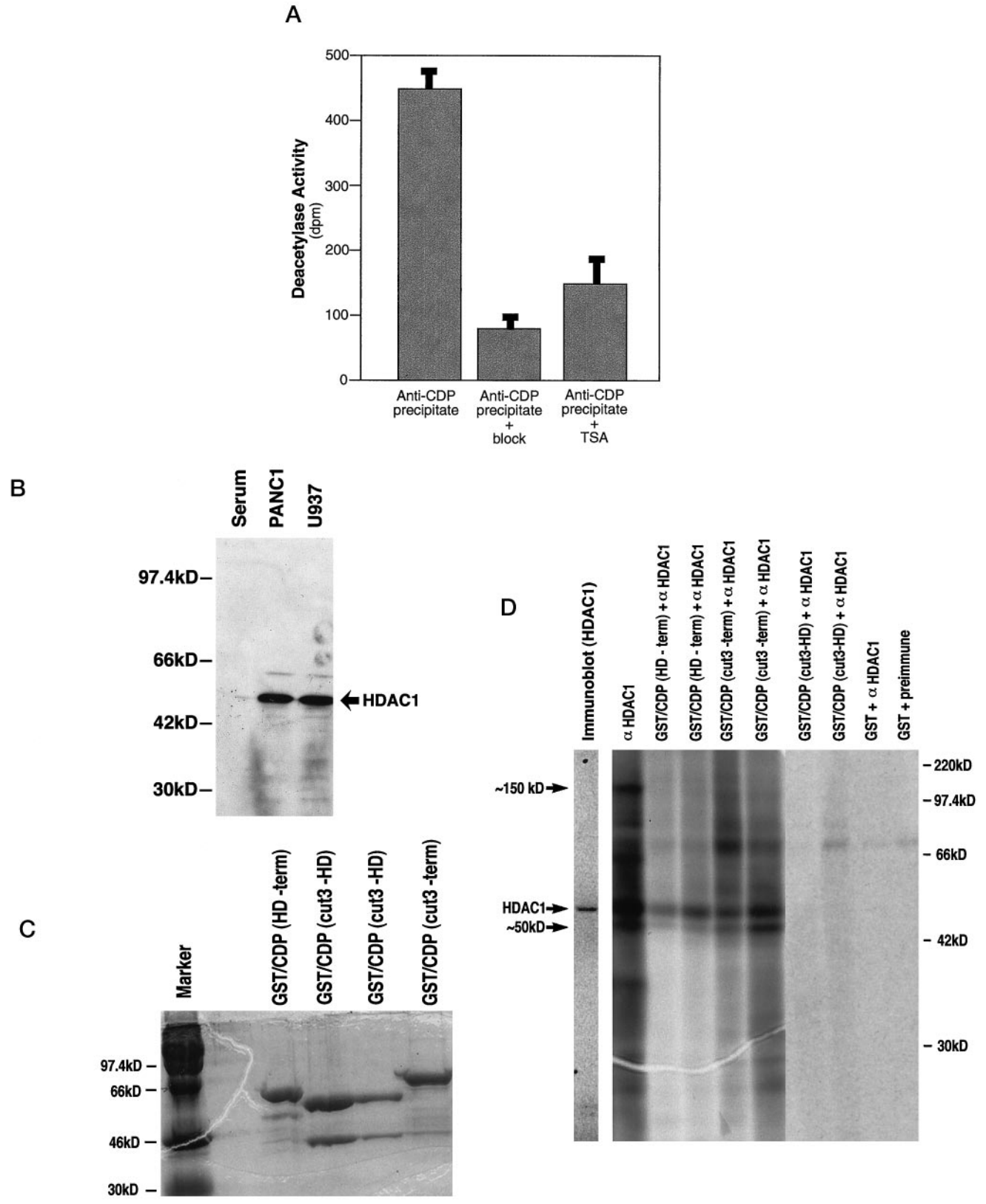

FIG. 9. **Association of CDP/*cut* with HDAC1 activity.** *Panel* A, *in vitro* histone deacetylase activity of anti-CDP/*cut* immunoprecipitates are shown from human U937 cell extracts. Cell extracts (approximately 10 mg) were immunoprecipitated using anti-human CDP/*cut* polyclonal antibodies. +*block* indicates that the anti-CDP/*cut* antibodies were preabsorbed with recombinant CDP/*cut* fusion proteins. +*TSA* indicates that the anti-CDP/*cut* immunoprecipitates were pretreated with 100 ng/ml TSA for 30 min at 4 °C before being assayed for histone deacetylase activity. *Panel B*, truncated COOH-terminal constructs of CDP/*cut* in-frame with the GST protein were used to purify ^{35}S-labeled proteins bound to the carboxyl-terminal domain of CDP/*cut*. Several fusion proteins encoding a fusion of GST in-frame with the following corresponding COOH-terminal truncations of the human CDP/*cut* protein are: GST-CDP/*cut* (HD-term), GST-CDP/*cut* (*cut3*-HD), and GST-CDP/*cut* (*cut3*-term). Proteins were characterized by SDS-polyacrylamide gel electrophoresis and shown with a corresponding molecular mass marker. The proteins were expressed in *E. coli* strain BL21 after induction with 0.1 mM isopropyl 1-thio-β-D-galactopyranoside for 1 h at 37 °C. Bacteria lysates expressing GST fusion proteins were prepared and purified with agarose beads conjugated with glutathione, eluted, and electrophoresed through a 9.5% SDS-

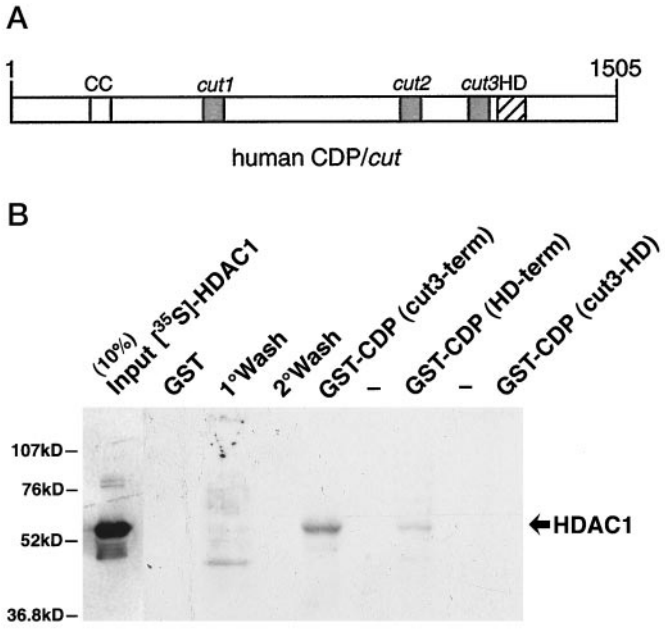

FIG. 10. **Localization of HDAC1 binding to CDP/cut.** *Panel A*, schematic representation of the human CDP/*cut* protein with conserved domains of the protein highlighted as *open*, *shaded*, or *hatched boxes* (*CC*, coiled coil; *cut1*, *cut* repeat 1; *cut2*, *cut* repeat 2; *cut3*, *cut* repeat 3; *HD*, homeodomain). *Panel B*, the full-length HDAC1 protein was synthesized and radiolabeled by *in vitro* transcription-translation system. ^{35}S-Labeled HDAC1 was incubated with the various COOH-terminal constructs of CDP/*cut*, expressed as GST fusion proteins, and immobilized on glutathione-Sepharose beads. The domains of CDP/*cut* included in the GST fusion protein and used in the protein affinity-binding assay are indicated above the *lane*. The nonrecombinant form of GST was employed as a negative control. The ^{35}S-labeled HDAC1 protein, bound by the GST-CDP/*cut* constructs, was eluted by soluble glutathione, resolved by SDS-PAGE, and visualized by PhosphoImager analysis (Molecular Dynamics, Inc). Sampled primary (*1° Wash*) and secondary (*2° Wash*) washes of ^{35}S-labeled HDAC1 were taken before the elution of the bound GST-CDP (cut3-term) fusion protein and are shown in the corresponding the *third* and *fourth lanes*. 10% of total input of ^{35}S-labeled HDAC1 is shown in the *first lane*. Molecular weight markers (Bio-Rad) were used to confirm the size of the bands.

transfected COS-7 cells, and from protein expressed in bacteria to show binding to the *CFTR* Y-box (Fig. 5*B*). How does the Y-box element function to regulate *CFTR* transcription *in vivo*? Our studies demonstrate that inhibition of histone deacetylase activity with TSA corresponds to the stimulation of *CFTR* transcription mediated through an inverted and intact CCAAT sequence. Evidence in this report implies that activities competing for the association with the Y-box element regulate the level of histone acetylation in chromatin surrounding the transcript start sites, leading to either activation or repression of gene transcription. We have shown that inhibition of histone deacetylase activity by TSA potentiates the transcription of a *CFTR*-directed transgene through the Y-box element. Thus, the activation of *CFTR* transcription could be linked to histone acetyltransferase activity through the inverted CCAAT nucleotide consensus (Y-box) sequence. Examination of the *CFTR* promoter *in vivo* using a *Bsp*MI endonuclease sensitivity assay suggests that TSA will promote cleavage of nucleotide sequences proximal to the *CFTR* Y-box element and CRE (Fig. 7). Furthermore, DNase I hypersensitivity assays shown in Fig. 8 demonstrate that an intact 5′-ATTGG-3′ sequence of human *CFTR* is required for DNase I hypersensitivity of nuclei. DNase I hypersensitivity of two *CFTR*-directed transgenic constructs reveals that, in fact, the ATTGG consensus is an essential nucleotide requirement for the nuclease hypersensitivity of the *CFTR* promoter in chromatin as a result of the inhibition of histone deacetylase activity *in vivo*. This has been demonstrated in several randomly selected stably transfected clones (data not shown). Despite the inhibition of histone deacetylase activity by TSA to stimulate *CFTR* transcription through the putative alteration of chromatin structure, overlapping the Y-box element, there is no direct evidence to show the modification of histones within the context of the *CFTR* promoter to undergo changes in acetylation patterns as a result of TSA treatment. However, our observations suggest that the specialized sensitivity of local chromatin to nuclease hydrolysis is linked to the inhibition of histone deacetylase activity. We have yet to establish whether *trans*-acting factors bound to the *CFTR* Y-box element (such as CDP/*cut*, C/EBP, or CBF·NF-Y) can target the enzymatic modification of local histones *in vivo* (66). Although the correlation between histone acetylation and gene activation is established, further studies to determine the extent of local chromatin to undergo histone modification will have to be performed.

Our observations suggest the association of CDP/*cut* with histone deacetylase activity. We propose that transcriptional repression by CDP/*cut* may recruit histone deacetylases to *CFTR* and that certain factors tethered to the DNA-binding proteins (such as CDP/*cut*) can target the deacetylation (or acetylation) of specific lysine residues of core histones such as those established through similar models (67, 68). Because *CFTR* transcription is potentiated through TSA we tested whether CDP/*cut* may function to interact with any known histone deacetylase. Immunoprecipitation of CDP/*cut* demonstrates that along with CDP/*cut*, histone deacetylase activity is coimmunoprecipitated and is inhibited by TSA. Furthermore, studies using fusion proteins of CDP/*cut* indicate that CDP/*cut* may recruit interactions with histone deacetylase, HDAC1, through the COOH-terminal domain known to contain the repression domain of CDP/*cut* (57). It is only implied here that CDP/*cut* may repress gene transcription of *CFTR* through a mechanism incorporating the histone deacetylation of chromatin in proximity to the *CFTR* promoter. Although the molecular basis for the repression mediated by CDP/*cut in vivo* is not known, it could conceptually involve competition between enzymes involved in histone acetylation associated with sequence-specific transcription factors. Although this simple model could explain the role CDP/*cut* may have in transcriptional repression of *CFTR*, still little is known about the precise mechanism directing specific alterations in local chromatin domains *in vivo*.

The acetylation state of local chromatin may represent a

polyacrylamide gel before staining with Commassie Blue dye. *Panel C*, immunoblot analysis of HDAC1 from nuclear extracts isolated from PANC1 and U937 cells. *Panel D*, immunoblot analysis and immunoprecipitation of HDAC1 from a GST-CDP pull-down assay. From *left*, *first lane*, unlabeled U937 cell extract immunoprecipitated with HDAC1 antiserum was separated from the remainder of the gel as an individual lane, transferred onto a filter, and immunoblotted with HDAC1 antiserum. *Second lane*, immunoprecipitation of HDAC1 from ^{35}S-labeled nuclear extracts of U937 cells. *Third* through *eighth lanes*, immunoprecipitation of HDAC1 from ^{35}S-labeled nuclear extracts of U937 cells after GST pull-down assay with either the GST-CDP/*cut* (HD-term), GST-CDP/*cut* (*cut3*-term), GST-CDP/*cut* (*cut3*-HD), and GST fusion protein. The washing of the ^{35}S-labeled proteins bound to the GST-CDP/*cut* fusion protein and affixed to glutathione beads was performed three times with LW buffer. The GST proteins were eluted with glutathione, immunoprecipitated with HDAC1 antiserum, and washed twice with LW buffer containing 0.5% deoxycholate followed by precipitation with protein A-Sepharose. Autoradiographs of SDS-polyacrylamide gels are shown with the corresponding molecular mass determination.

stoichiometric relationship between competing acetylation and deacetylation processes. Thus, the requirement for the Y-box in hGCN5-mediated transactivation of *CFTR* suggests that the Y-box element likely binds proteins necessary for the conformational changes in chromatin structure. There is evidence to suggest that certain transcription factors have higher affinity for their binding sites when the nucleotide sequences are embedded in chromatin assembled from hyperacetylated histones (69). It is plausible that competing activities directed by CDP/*cut* or CBF·NF-Y, as an example, recruit activities favorable for either the repression or activation of *CFTR* transcription, respectively. Although we suggest that CDP/*cut* recruits histone deacetylase activity, it is unclear precisely how such a ternary complex of CDP/*cut* is assembled in the context of the *CFTR* promoter Y-box element and whether such events do take place *in vivo*. Future studies will have to take into account the enzymatic modification of histones associated with the site of transcriptional repression (and activation) and will likely provide a more detailed model for the regulation of *CFTR* transcription. Investigations to determine the extent of acetylation directed at specific lysine residues within core histones H4 and H3 of local chromatin will provide important information about how such activity is targeted to the *CFTR* promoter. However, our observations are consistent with the role of both CBF·NF-Y and CDP/*cut* to regulate genes through the recruitment of accessory enzymatic function to enhance the effect of these factors either to activate or repress specific genes, respectively (27, 28, 57). The recent demonstration that HDAC1 and the mSin3A transcriptional corepressor are present as a complex *in vivo* and physically interact (60, 61), along with results presented here, suggests that histone deacetylase activity is targeted to the *CFTR* inverted CCAAT sequence by the association with the transcriptional repressor CDP/*cut*. A consistent pattern in the GST pull-down assay with GST-CDP/*cut* fusion protein (Fig. 9*C*) indicates bands of ~150 and 50 kDa, respectively; these bands closely correlate with the molecular mass of mSin3A or *N*-CoR and HDAC2, respectively (70). In a model proposed for the repression of Pit-1-mediated transcription it was shown that the transcriptional repressors RPX and *Msx-1*, both homeodomain proteins, are tethered to mSin3A and HDAC1 through the mSin3A corepressor homolog *N*-CoR to repress transcription (71). It is likely that CDP/*cut*, like CBF·NF-Y or CREB/ATF, recruits transcriptional adaptor proteins that are comprised of either coactivator or corepressor ternary complexes dependent on the factor bound to the Y-box element. For transcription factors bound to a chromatin template in both activated and repressive states of transcription this would determine the coactivator or corepressor composition. For example, the activation of *CFTR* transcription by ATF-1 and hGCN5 may account for the presence of a transcriptional coactivator p300/CBP-bound complex to displace further nucleosome-mediated repression of *CFTR* through histone acetyltransferase activity (20). Conversely, CDP/*cut* bound to the Y-box of *CFTR* may displace the p300/CBP coactivator ternary complex in the activated state of transcription through the association with histone deacetylase activity. Therefore, the amount of CDP/*cut* expressed in the cell (Fig. 4) may reflect a conversion of the exposed Y-box to a less accessible element in chromatin as a result of increased recruitment of histone deacetylase activity. Thus, the absence (or displacement) of CDP/*cut* correlates with the ability of activating mechanisms of transcription associated with histone acetyltransferase activities to promote transcription. Although we do not preclude the role of other *cis*-acting regulatory elements of *CFTR* to promote the induction of transcription, here we indicate a correlation between the accessibility of chromatin in *CFTR* transcription with the intact ATTGG sequence of *CFTR*. Even though several mechanisms control transcription *in vivo*, the regulation of chromatin architecture by opposing enzymatic activities is implied here as central to this process. Therefore, the regulation of enzymatic function by histone acetylation factors could conceivably benefit those afflicted with cystic fibrosis through the enhancement of *CFTR* expression in cells that bear mutations conferring a mild cystic fibrosis phenotype.

Acknowledgments—We thank the laboratories of Drs. Michael Green, Yoshihiro Nakatani, Yiannis Ioannou, and Sankar Maity for plasmid reagents of ATF-1, hGCN5, the expression vector pASC-*8neoVA*, and antiserum to CBF·NF-Y, respectively. We are also grateful for advice received from Drs. Shelley Berger, Yoshihiro Nakatani, Checco Ramirez, and from David Zappulla.

REFERENCES

1. Rommens, J. M., Iannuzzi, M. C., Kerem, B.-S, Drumm, M. L., Melmer, G., Dean, M., Rozmahel, R., Cole, J., Kennedy, D., Hidaka, N., Zsiga, M., Buchwald, Riordan, J. R., Tsui, L.-C., and Collins, F. S. (1989) *Science* **245,** 1059–1065
2. Riordan, J. R., Rommens, J. M., Kerem, B.-S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M.-L., Iannuzzi, M. C., Collins, F. S., and Tsui, L.-C. (1989) *Science* **245,** 1066–1073
3. Yoshimura, K., Nakamura, H., Trapnell, B. C., Dalemans, W., Pavirani, A., Lecocq, J.-P., and Crystal, R. G. (1991) *J. Biol. Chem.* **266,** 9140–9144
4. Chou, J.-L., Rozmahel, R., and Tsui, L.-C. (1991) *J. Biol. Chem.* **266,** 24471–24476
5. Koh, J., Sferra, T. J., and Collins, F. S. (1993) *J. Biol. Chem.* **268,** 15912–15921
6. Breuer, W., Kartner, N., Riordan, J. R., and Cabantchik, Z. I. (1992) *J. Biol. Chem.* **267,** 10465–10469
7. Bargon, J., Trapnell, B. C., Chu, C.-S., Rosenthal, E. R., Yoshimura, K., Guggino, W. B., Dalemans, W., Pavirani, A., Lecocq, J. P., and Crystal, R. G. (1992) *Mol. Cell. Biol.* **12,** 1872–1878
8. Bargon, J., Trapnell, B. C., Yoshimura, K., Dalemans, W., Pavirani, A., Lecocq, J.-P., and Crystal, R. G. (1992) *J. Biol. Chem.* **267,** 16056–16060
9. Breuer, W., Glickstein, H., Kartner, N., Riordan, J. R., Ausiello, D. A., and Cabantchik, I. Z. (1993) *J. Biol. Chem.* **268,** 13935–13939
10. Trapnell, B. C., Zeitlin, P. L., Chu, C. S., Yoshimura, K., Nakamura, H., Guggino, W. B., Bargon, J., Banks, T. C., Dalemans, W., Pavirani, A., Lecocq, J.-P., and Crystal, R. G. (1991) *J. Biol. Chem.* **266,** 10319–10323
11. Hunter, T., and Karin, M. (1992) *Cell* **70,** 375–387
12. Pittman, N., Shue, G., LeLeiko, N. S., and Walsh, M. J. (1995) *J. Biol. Chem.* **270,** 28848–28857
13. Matthews, R., P., and McKnight, G. S. (1996) *J. Biol. Chem.* **271,** 31869–31877
14. Smith, A., Barth, M. L., McDowell, T. L., Moulin, D. S., Nuthall, H. N., Hollingsworth, M. A., and Harris, A. (1996) *J. Biol. Chem.* **271,** 9947–9954
15. Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996) *Cell* **84,** 843–851
16. Brownell, J. E., and Allis, C. D. (1996) *Curr. Opin. Genet. Dev.* **6,** 176–184
17. Wolffe, A. P. (1996) *Science* **272,** 371–372
18. Wade, P. A., and Wolffe, A. P. (1997) *Curr. Biol.* **7,** 82–84
19. Wade, P. A., Pruss, D., and Wolffe, A. P. (1997) *Trends Biochem. Sci.* **22,** 128–132
20. Wolffe, A. P., and Pruss, D. (1996) *Cell* **84,** 817–819
21. Bannister, A. J., and Kouzarides, T. (1996) *Nature* **384,** 641–643
22. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., and Nakatani, Y. (1996) *Cell* **87,** 953–959
23. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) *Science* **272,** 408–411
24. Rundlett, S. E., Carmen, A. A., Kobayashi, R., Bavykin, S., Turner, B. M., and Grunstein, M. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 14503–14508
25. Nagy, L., Kao, H. Y., Chakravarti, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L., and Evans, R. M. (1997) *Cell* **89,** 373–380
26. Minucci, S., Horn, V., Bhattacharyya, N., Russanova, V., Ogryzko, V. V., Gabriele, L., and Howard, B. H. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 11295–11300
27. Currie, R. A. (1998) *J. Biol. Chem.* **273,** 1430–1434
28. Jin, S., and Scotto, K. W. (1998) *Mol. Cell. Biol.* **18,** 4377–4384
29. Wang, H., and Stillman, D. J. (1993) *Mol. Cell. Biol.* **13,** 1805–1814
30. Maity, S. N., Golumbek, P. T., Karsenty, G., and de Crombrugghe, B. (1988) *Science* **241,** 582–585
31. Maity, S. N., Sinha, S., Ruteshouser, E. C., and de Crombrugghe, B. (1992) *J. Biol. Chem.* **267,** 16574–16580
32. Sinha, S., Kim, I.-S., Sohn, K.-Y., de Crombrugghe, B., and Maity, S. N. (1996) *Mol. Cell. Biol.* **16,** 328–337
33. Coustry, F., Maity, S. N., Sinha, S., and de Crombrugghe B. (1996) *J. Biol. Chem.* **271,** 14485–14491
34. Neufeld, E. J., Skalnik, D. G., Lievens, P. M., and Orkin, S. H. (1992) *Nat. Genet.* **1,** 50–55
35. Aufiero, B., Neufeld, E. J., and Orkin, S. H. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 7757–7761
36. Andres, V., Nadal-Ginard, B., and Mahdavi, V. (1992) *Development* **116,** 321–334
37. Blochlinger, K., Bodmer, R., Jack, J., Jan, L. Y., and Jan, Y. N. (1988) *Nature* **333,** 629–635
38. Jack, J., Dorsett, D., Delotto, Y., and Liu, S. (1991) *Development* **113,** 735–747
39. Blochlinger, K., Jan, L. Y., and Jan, Y. N. (1993) *Development* **117,** 441–450
40. Barberis, A., Superti-Furga, G., and Busslinger, M. (1987) *Cell* **50,** 347–359

41. Skalnik, D. G., Strauss, E. C., and Orkin, S. H. (1991) *J. Biol. Chem.* **266,** 16736–16744
42. Dufort, D., and Nepveu, A. (1994) *Mol. Cell. Biol.* **14,** 4251–4257
43. Valarche, I., Tissier-Seta, J. P., Hirsh, M. R., Martinez, S., Goridis, C., and Brunet, J. F. (1993) *Development* **119,** 881–896
44. Kim, E. C., Lau, J. S., Rawlings, S., and Lee, A. S. (1997) *Cell Growth Differ.* **8,** 1329–1338
45. Higgy, N. A., Tarnasky, H. A., Valarche, I., Nepveu, A., and van der Hoorn, F. A. (1997) *Biochim. Biophys. Acta* **1351,** 313–324
46. el-Hodiri, H. M., and Perry, M. (1995) *Mol. Cell. Biol.* **15,** 3587–3596
47. Banan, M., Rojas, I. C., Lee, W.-H., King, H. L., Harriss, J. V., Kobayashi, R., Webb, C., and Gottlieb, P. D. (1997) *J. Biol. Chem.* **272,** 18440–18452
48. Luo, W., and Skalnik, D. G. (1996) *J. Biol. Chem.* **271,** 18203–18210
49. Mantovani, R. (1998) *Nucleic Acids Res.* **26,** 1135–1143
50. Candau, R., Moore, P. A., Wang, L., Barlev, N., Ying, C. Y., Rosen, C. A., and Berger, S. L. (1996) *Mol. Cell. Biol.* **16,** 593–602
51. Hai, T., Liu, F., Coukos, W. J., and Green, M. R. (1989) *Genes Dev.* **3,** 2083–2090
52. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489
53. Harlow, E., and Lane, D. (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
54. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., and Smith, A. E. (eds) (1992) *Short Protocols in Molecular Biology*, pp. 16.28–16.31, J. Wiley & Sons, Inc., New York
55. Bi, W., Wu, L., Coustry, F., de Crombrugghe, B., and Maity, S. N. (1997) *J. Biol. Chem.* **272,** 26562–26572
56. Yang, X.-J., Orgyzko, V. V., Nishiikawa, J., Howard, B. H., and Nakatani, Y. (1996) *Cell* **87,** 953–959
57. Mailly, F., Bérubé, G., Harada, R., Mao, P.-L., Phillips, S., and Nepveu, A. (1996) *Mol. Cell. Biol.* **16,** 5346–5357
58. Kijima, M., Yoshida, M., Sugita, K., Horinouchi, S., and Beppu, T. (1993) *J. Biol. Chem.* **268,** 22429–22431
59. Yoshida, M., Horinouchi, S., and Beppu, T. (1995) *Bioassays* **17,** 423–430
60. Laherty, C. D., Yang, W.-M., Sun, J.-M., Davie, J.- R., Seto, E., and Eisenman, R. N. (1997) *Cell* **89,** 349–356
61. Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L., and Ayer, D. E. (1997) *Cell* **89,** 341–347
62. Wang, H., Clark, I., Nicholon, P. R., Herskowitz, I., and Stillman, D. J. (1990) *Mol. Cell. Biol.* **10,** 5927–5936
63. Linhoff, M. W., Wright, K. L., and Ting, J. P.-Y. (1997) *Mol. Cell. Biol.* **17,** 4589–4596
64. Mink, S., Haenig, B., and Klempnauer, K. H. (1997) *Mol. Cell. Biol.* **17,** 6609–6617
65. Yao, T. P., Oh, S. P., Fuchs, M., Zhou, N. D., Ch'ng, L.-E., Newsome, D., Bronson, R. T., Li, E., Livingstone, D. M., and Eckner, R. (1998) *Cell* **93,** 361–372
66. Kadosh, D., and Struhl, K. (1998) *Mol. Cell. Biol.* **18,** 5121–5127
67. Yang, W. M., Inouye, C., Zeng, Y., Bearss, D., and Seto, E. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 12845–12850
68. Ayer, D. E., Kretzner, L., and Eisenman, R. N. (1993) *Cell* **72,** 211–222
69. Vettese-Dadey, M., Grant, P. A., Hebbes, T. R., Crane-Robinson, C., Allis, C. D., and Workman, J. L. (1996) *EMBO J.* **15,** 2508–2518
70. Laherty, C. D., Billin, A. N., Lavinsky, R. M., Yochum, G. S., Bush, A. C., Sun, J.-M., Mullen, T. M., Davie, J. R., Rose, D. W., Glass, C. K., Rosenfeld, M. G., Ayer, D. E., and Eisenman, R. N. (1998) *Mol. Cell* **2,** 33–42
71. Xu, L., Lavinsky, R. M., Dasen, J. S., Flynn, S. E., McInerney, E. M., Mullen, T.-M., Heinzel, T., Szeto, D., Korzus, E., Kurokawa, R., Aggarwal, A. K., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1998) *Nature* **395,** 301–306

Transcriptional Repression of the Cystic Fibrosis Transmembrane Conductance Regulator Gene, Mediated by CCAAT Displacement Protein/ *cut* Homolog, Is Associated with Histone Deacetylation

SiDe Li, Libia Moy, Nanci Pittman, Gongliang Shue, Barbara Aufiero, Ellis J. Neufeld, Neal S. LeLeiko and Martin J. Walsh

J. Biol. Chem. 1999, 274:7803-7815.
doi: 10.1074/jbc.274.12.7803