A Naturally Occurring Sequence Variation That Creates a YY1 Element Is Associated with Increased Cystic Fibrosis Transmembrane Conductance Regulator Gene Expression*

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We have identified previously a novel complex mutant allele in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in a patient affected with cystic fibrosis (CF). This allele contained a mutation in CFTR exon 11 known to cause CF (S549R(T>G)), associated with the first alteration described so far in the minimal CFTR promoter region (−102T>A). Studies on genotype-phenotype correlations revealed striking differences between patients carrying mutation (S549R(T>G)) alone, who had a severe disease, and patients carrying the complex allele (−102(T>A)+S549R(T>G)), who exhibited milder forms of CF. We thus postulated that the sequence change (−102T>A) may attenuate the effects of the severe (S549R(T>G)) mutation through regulation of CFTR expression. Analysis of transiently transfected cell lines with wild-type and −102A variant human CFTR-directed luciferase reporter genes demonstrates that constructs containing the −102A variant (which creates a Yin Yang 1 (YY1) core element) increases CFTR expression significantly. Electrophoretic mobility shift assays indicate that the −102 site is located in a region of multiple DNA-protein interactions and that the −102A allele recruits specifically an additional nuclear protein related to YY1. The finding that the YY1-binding allele causes a significant increase in CFTR expression in vitro may allow a better understanding of the milder phenotype observed in patients who carry a severe CF mutation within the same gene.

Cystic fibrosis (CF), 1 the most common lethal inherited autosomal recessive disease in the worldwide Caucasian population, is characterized by severely altered functions in the adsorbing and secreting epithelia of pulmonary, digestive, and reproductive systems (1). CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), which functions as a cAMP- and ATP-regulated Cl− channel at the apical cell surface (2–4).

Expression of the CFTR gene (5, 6) occurs in a subset of specialized cells of epithelial origin and is regulated tightly both temporally and spatially (7–10). The minimal CFTR promoter lies within about 226 base pairs upstream of the transcription start site (11) and appears to have structural features of a “housekeeping” gene promoter (11–14). Analysis of the DNA sequence of this promoter revealed potential regulatory elements. Both basal and cAMP-mediated regulation of CFTR transcription involved a consensus inverted CCAAT (Y-box) element (15) in tandem with a weak CRE nucleotide consensus downstream (16).

However, no sequences that might account for tissue specificity have yet been found, and regulatory elements conferring tissue-specific and temporal regulation are thought to lie mainly outside the promoter region. Analyses of the CFTR gene chromatin structure have identified in all cell types a number of DNase I-hypersensitive sites at −20.5 kb and −79.5 kb relative to the translational start codon (17). An additional cis-acting element has been characterized by DNase I analyses in the first intron of the human CFTR gene at 181–10 kb (18). In vitro analysis by electrophoresis mobility shift, DNase I footprinting, and transient expression assays showed that this putative regulatory element in the first intron has a positive effect on the activity of the CFTR promoter in cells that express endogenous CFTR.

A recent study demonstrated that transcription factor ATF-1 and human histone acetyltransferase GCN5, directing the structural modification of chromatin, stimulated CFTR transcription through the intact inverted CCAAT (Y-box) element (19). In addition, the authors showed that inhibition of histone deacetylase activity by trichostatin A potentiated CFTR transcription through the Y-box element. It was suggested that activities competing for the association with the Y-box element regulate the level of histone acetylation in chromatin surrounding the transcription start sites, leading to either activation or repression of gene transcription. However, although significant progress has been made in understanding the mechanisms that underlie the strict cellular control of CFTR expression, the genetic elements and transcription factors that regulate it, including those controlling tissue specificity, are not yet well defined.

Another means of obtaining insights into the mechanisms governing the expression of the CFTR gene is to identify naturally occurring disease-causing mutations or sequence variations affecting CFTR regulation. So far, only three laboratories have screened the 5′-upstream region of the CFTR gene in affected patients for sequence alterations, mostly by using de-naturing gradient gel electrophoresis/sequencing assays. Bien-
ven et al. (20) have investigated a segment extending from nucleotides −531 to −1000 relative to the Cap site and identified two polymorphisms (−895T>G and −816C>T) and one putative disease-associated mutation (−741T>G). More recently, Verlingue et al. (21) analyzed a region that spans more than 3.9 kb of sequences upstream of the first CFTR exon, including the CFTR promoter, down to 1.3 kb within the first intron, as these sequences had been shown previously to contain potential regulatory elements highly conserved throughout evolution from rodents to primates (12, 22, 23). Their analysis resulted in the detection of one polymorphism only (−966T>G). We have identified a novel complex allele (102T>A)+S549R(T>G)) containing a previously described CF-causing mutation (S549R(T>G)) in exon 11 and a sequence alteration in the minimal CFTR promoter region (−102T>A) (24). S549R(T>G) was initially considered as a “severe” mutation on the basis of clinical features presented by patients who carry it, particularly pancreatic insufficiency. We discovered recently, through a genotype-phenotype correlation analysis, that this mutation, when occurring in cis with the sequence change (−102T>A), is associated with milder forms of CF than (S549R(T>G)) alone (25). This finding suggested that the (−102T>A) naturally occurring nucleotide variation may, at least partially, compensate for the severity of the (S549R(T>G)) mutation by CFTR overexpression.

The objective of this study was to determine further whether the (−102T>A) sequence alteration is a relevant regulatory mutation. We have investigated its effects on transcription using reporter gene assays and conducted analysis of cis-acting elements by in vitro DNA-protein interactions. In this report, we demonstrate that this first naturally occurring sequence variation in the minimal CFTR promoter creates a YY1 transcription factor binding site and has a substantial impact on the basal rate of transcription of the CFTR gene in vitro.

MATERIALS AND METHODS

**Reporter Gene Constructs**—A 362-base pair fragment spanning the minimal CFTR promoter region and extending from positions −227 to +135 (102T>A) was inserted into the pGL3 control vector (Promega Corp.). This vector contains a modified firefly luciferase coding sequence upstream of the SV40 late poly(A) signal and no eukaryotic protein binding in the assay, 10 µM MgCl2, 0.2 mM EDTA with freshly added protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol). The cell pellet was homogenized in 400 µl of annealing buffer (67 mM Tris-HCl (pH 7.5), 13 mM MgCl2, 1.3 mM EDTA, 1.3 mM spermidine, and 6.7 mM dithiothreitol) and cooling in 4 °C. The samples were then dried and autoradiographed with an intensifying screen. The intensity of firefly luciferase activity was measured in a LumiGene 5000 luminometer (EG & G Berthold, Wilbad, Germany) with a 2-s premeasurement delay and a 10-s measurement period. Firefly luciferase activity was normalized to Renilla luciferase activity.

**Electrophoretic Mobility Shift Assays**—Oligonucleotides for radiolabeled probes and competitor DNAs were synthesized on an Applied Biosystems 373 automated sequencer. Nonzymatic and nonantisense strand pairs were annealed by heating at 85 °C for 2 min in 40 µl of annealing buffer (10 mM Tris-HCl (pH 7.5), 13 mM MgCl2, 1.3 mM EDTA, 1.3 mM spermidine, and 6.7 mM dithiothreitol) and cooling in two steps at 65 °C for 15 min then 37 °C for 1 h. Double-stranded oligomeric probes were end labeled with [γ-32P]ATP (5000 Ci/mmol) using T4 polynucleotide kinase (Roche Diagnostic) according to the manufacturer’s instructions and separated from free nucleotides by QIAquick Nucleotide Removal kit (Qiagen). Data were deconvoluted using the Instat Program (Graph Pad Software).

**Preparation of Nuclear Extracts**—Crude nuclear extracts from HeLa, Calu-3, and CF-PAC1 cell lines were prepared as described previously (26) with minor modifications. Briefly, actively growing cells were collected by scraping in 10 ml of phosphate-buffered saline, pelleted by centrifugation, and washed once in hypotonic buffer (10 mM HEPES (pH 7.9), 1.5 mM MgCl2, 10 mM KCl, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol). The cell pellet was homogenized in Nonidet P-40 buffer (0.1% Nonidet P-40 in the same buffer above) with approximately 100 strokes in a Dounce homogenizer with a B-type pestle. The homogenate was centrifuged at 14,000 rpm for 30 min in a Beckman P2402 rotor (4 °C) and the nuclear fraction extracted with 20 mg/ml HEPES (pH 7.9), 25% glycerol, 0.42 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA with freshly added protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 2 μg/ml aprotinin, and 1 μg/ml pepstatin). Extracts were clarified by centrifugation at 14,000 rpm for 30 min at 4 °C (GS-15 centrifuge, Beckman) before overnight dialysis at 4 °C against 100 volumes buffer containing 20 mM glycerol and 0.14 mM NaCl by using DispoDialyzer systems, Spectra/ Por quality (Spectrum). The nuclear extracts were flash frozen in aliquots in liquid nitrogen and stored at −80 °C. Protein concentrations were determined by Bradford assay (Bio-Rad).

**Statistical Analysis**—Comparisons were made with analysis of variance, followed by the Welch’s alternate t test, a modification of the unpaired t test. A value of p < 0.05 was considered statistically significant. Data were presented using the Instat Program (Graph Pad Software).

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**Electrophoretic Mobility Shift Assays**—Oligonucleotides for radiolabeled probes and competitor DNAs were synthesized on an Oligo 1000 DNA synthesizer (Beckman). The sequences of the sense strand of each of the probes used in this study are shown in Table I. Appropriate sense and antisense strand pairs were annealed by heating at 85 °C for 2 min in 40 µl of annealing buffer (67 mM Tris-HCl (pH 7.5), 13 mM MgCl2, 1.3 mM EDTA, 1.3 mM spermidine, and 6.7 mM dithiothreitol) and cooling in two steps at 65 °C for 15 min then 37 °C for 1 h. Double-stranded oligomeric probes were end labeled with [γ-32P]ATP (5000 Ci/mmol) using T4 polynucleotide kinase (Roche Diagnostic) according to the manufacturer’s instructions and separated from free nucleotides by QIAquick Nucleotide Removal kit (Qiagen). Data were deconvoluted using the Instat Program (Graph Pad Software).

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The −102A CFTR Allele Recruits a YY1-related Protein

TABLE I

| Oligonucleotide | Sequence (5’ to 3’; sense strand) |
|-----------------|--------------------------------|
| WT              | −121GAA AGC CCC TAG AGC AAA TTT GGC GCC GGA CCA GCC AGC−83 |
| 102A            | −121GAA AGC CCC TAG AGC AAA TTT GGC GCC GGA CCA GCC AGC−83 |
| CArG           | −121GAA AGC CCC TAG AGC AAA TTT GGC GCC GGA CCA GCC AGC−83 |
| iCCAAT         | −121GAA AGC CCC TAG AGC AAA TTT GGC GCC GGA CCA GCC AGC−83 |
| YY1sa         | −121GAA AGC CCC TAG AGC AAA TTT GGC GCC GGA CCA GCC AGC−83 |
| nYY1          | −121GAA AGC CCC TAG AGC AAA TTT GGC GCC GGA CCA GCC AGC−83 |
| c-fos         | −121GAA AGC CCC TAG AGC AAA TTT GGC GCC GGA CCA GCC AGC−83 |

This is the human minimal CFTR promoter region, sequence from −121 to −83 relative to A of the translation codon at +1 (11).

Consensus CArG box, CC(A/T-rich)GG.

Consensus inverted CCAAT box with consensus flanking sequences.

Mutated YY1 box.

c-fos consensus SRE (28).

ble-stranded oligonucleotide (Promega Corp.) was included as a nonspecific DNA probe.

The proteins that bind the same probe from different cell lines were compared by limited proteolytic cleavage using a modified proteolytic clipping band shift assay (27). Briefly, a predetermined amount of a specific protease (trypsin) was added to the relevant binding reaction tube and the same amount of water to the non-protease control tube. The binding reaction was incubated at room temperature for a further 10 min.

To identify nuclear factors that bind to DNA probes, 1 µg of specific antibody (rabbit polyclonal anti YY1, sc-281; mouse polyclonal anti YY1, sc-7341; or rabbit polyclonal anti-SRF, sc-335; Santa Cruz Biotechnology) or antibody (rabbit polyclonal anti YY1, sc-281; mouse polyclonal anti YY1, sc-7341; or rabbit polyclonal anti-SRF, sc-335; Santa Cruz Biotechnology) was incubated with nuclear extracts 20 min prior to in vitro DNA binding. Rabbit polyclonal anti-mouse IgG,b (kindly provided by Dr. Devaux, Laboratoire Infections rétroviraux et signalisation cellulaire, Montpellier, France) was included as a negative control.

RESULTS

The −102A Allele Affects CFTR Expression in Vitro—To explore specifically the effects of the −102T>A sequence variation on CFTR expression, we generated two luciferase reporter constructs driven by the minimal CFTR promoter, which differed only by the presence of either a thymine or an adenine at the −102 position (Fig. 1 left panel). Multiple plasmid preparations were made from each reporter construct and transiently transfected into the Calu-3 and CF-PAC1 cells, which express endogenous CFTR, and into HeLa cells, which do not express endogenous CFTR. All experiments included parallel transfections of a promotorless negative control (pGL3-Basic). The relative luciferase activity of the mutant construct (102A-pGL3) was increased by −60% (p < 0.005), −66% (p = 0.0033), or −45% (p = 0.0001) in Calu-3, CF-PAC1, or HeLa cells, respectively, compared with the activity of the wild-type construct (Fig. 1, right panel). These results suggest that the −102 nucleotide change occurred in an activator element.

Electrophoretic Mobility Shift Assays Demonstrate That the −102A Allele Creates a YY1 Binding Site—To determine which nuclear proteins are bound to the DNA sequence at the −102 site, we performed in vitro gel band shift experiments. Interestingly, a previous sequence comparison with known transcription factor binding sites indicated that this promoter substitution occurred in a region highly conserved throughout many species. As shown in Fig. 2A, this region contains two overlapping binding sites: a CArG-like motif, similar to the consensus CC(A/T-rich)GG (CARG element) and an inverted CCAAT-like motif. Remarkably, the sequence alteration (−102T>A) creates a core element for the multifunctional transcription factor YY1 in reverse orientation. Various oligonucleotides harboring one of the cis-acting consensus elements (Table I) were assayed for binding activity with nuclear extracts from Calu-3, CF-PAC1, and HeLa cells. Nucleoprotein complexes with identical electrophoretic mobility were detected with WT and CArG probes in all cell types examined (Fig. 2B, lanes 3, 4, 8, 9, 13, and 14). As shown in Fig. 2B (lanes 5, 10, and 15), the complex formed with the inverted CCAAT probe resulted in a pattern slightly different from those observed with the WT and CArG probes in all cell lines. These results suggest that the minimal CFTR promoter encompassing the −102 site might interact with a SRF-like protein.

In the presence of the −102A probe, an additional band, indicated by arrowheads, was generated in all cell types (Fig. 2B, lanes 2, 7, and 12). This finding, together with DNA sequence data, led us to postulate that this additional allele-specific binding protein might be YY1. However, the band shift patterns observed with different nuclear extracts indicate that another trans-acting factor can bind to this site, depending on the cell type.

We next examined the specificity of the DNA-protein interactions by competitive gel band shift assays. As seen in Fig. 3A, the −102A allele-specific binding protein was inhibited by unlabeled 102A oligonucleotide, whereas the complex formation was not altered by the addition of unlabeled nonspecific oligonucleotide (Sp1). Similarly, because the −102A allele is located in the potential composite element (Fig. 2A) and generates a YY1 consensus element (ANATGNN/C), competition experiments were performed with various oligonucleotides containing either consensus or mutated motifs (sequences listed in Table I). As shown in Fig. 3B (lanes 9 and 10), effective competition was obtained with either the YY1sa oligonucleotide, which contains a skeletal α-actin muscle regulatory element-like motif, or the c-fos SRE oligonucleotide (28). The latter contains a core YY1 element in direct orientation (CCAT) and differs from other oligonucleotides both in the AT-rich core element and flanking sequences outside the conserved CC and GG nucleotides. The c-fos SRE oligonucleotide, which does not contain any sequence similar to CFTR, was chosen to evaluate the fine specificity of the interaction and contribution of surrounding nucleotides. In contrast, oligonucleotides not containing the −102T>A substitution (WT, CArG, inverted CCAAT) or comprising a mutated YY1 motif (mYY1) did not compete for the −102A allele-specific binding protein under the same conditions (Fig. 3B, lanes 3–6). However, a simultaneous addition of unlabeled CArG and YY1sa oligonucleotides resulted in total inhibition of DNA-protein complex formation (data not shown). These observations suggest that a SRF-like protein and YY1 factor could simultaneously occupy the region containing the (−102T>A) sequence variation.

To examine further the nature of factors specifically binding the −102A allele in different nuclear extracts from cells that either do or do not express endogenous CFTR (indicated by arrowheads in Fig. 2B), we performed band shift assays after

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2 S. Vuillaumier and E. Denamur, personal communication.
The mobility shift assays using Calu-3, CF-PAC1, or HeLa nuclear extracts indicated on motif. Panel B binding site. Stippled solid box boxes highlight CArG-like element. Thick solid box bars respectively, expressing endogenous CFTR. Open bars show the results of the dual luciferase assay with the constructs described above. Results were normalized to the WT-pGL3, and results from pGL3-basic shows the inverted CCAAT element characterized previously (15, 19).

Human nuclear proteins binding the −102T>A site. Panel A, graphical representation of the CFTR promoter region containing the −102T>A naturally occurring sequence variation. Broken boxes highlight CArG-like element. Thick solid box highlights the YY1 binding site. Stippled solid box highlights the inverted CCAAT-like motif. Panel B, the WT, 102A, CArG, and inverted CCAAT oligomers indicated on top were radiolabeled and used as probes in electrophoretic mobility shift assays using Calu-3, CF-PAC1, or HeLa nuclear extracts. The arrowhead on the left of each autoradiogram indicates the complex selectively forming with the mutant probe (102A).

Proteolytic clipping of DNA-protein complexes. Proteolytic clipping was carried out with defined amounts of trypsin (determined by serial dilution of the protease; data not shown). When the 102A probe was used, the resulting clipping pattern was very similar to the pattern obtained with the YY1sa probe (Fig. 3C, lanes 4 and 8). Moreover, a similar pattern of partial complexes, indicated by arrowheads (Fig. 3C, lanes 4, 6, 8, and 10), was observed with cells that express endogenous CFTR (CF-PAC1) and with those that do not (HeLa). These findings suggest that the nuclear factor that specifically binds to the −102A allele in the minimal CFTR promoter is very similar to the ubiquitous YY1 transcription factor.

To characterize nuclear factors in the protein-DNA complexes, we performed an extensive immunological analysis with specific antibodies against transcription factors having the potential to bind YY1 and CArG sites in the composite element shown in Fig. 2A. As expected, preincubation with the anti-YY1 antibodies specifically prevented the formation of the −102A allele-specific complex. Upper and lower bands were completely inhibited with the rabbit polyclonal anti-YY1 (Fig. 4A, lane 5, and Fig. 4B, lane 4), whereas only the upper band formation was abolished with mouse polyclonal anti-YY1 (Fig. 4A, lane 4). A nonspecific antibody failed to inhibit or supershift the nucleoprotein complex (Fig. 4A, lane 8). As positive controls, we incubated the anti-YY1 antibody with the complexes obtained with YY1sa and c-fos probes, which contain a core YY1 element in reverse (ATGG) and direct orientation (CCAT), respectively (Fig. 4B, lanes 6 and 8). For negative supershift experiments, we incubated the same antibody with WT and mYY1 probes, which do not contain a YY1 motif in either orientation (Fig. 4B, lanes 2 and 10). To verify further whether the DNA-protein complexes obtained with WT and 102A probes contained SRF transcription factor, we incubated the band shift reaction mixtures with a rabbit polyclonal anti-SRF. Binding of this antibody was not detected, which could be due to the naturally occurring transversion at −108 (GC instead of CC in consensus CArG box), limitations of the antibody, steric hindrance within the complexes preventing antibody access to the epitope, and/or because the SRF transcription factor does not bind (data not shown). However, limitation of the antibody appears unlikely because we observed a supershifted band with the c-fos SRE probe (data not shown). These results suggest that our standard mobility shift protocol was unable to detect a ternary complex containing SRF protein. Previous studies reported that in the absence of both covalent and noncovalent modifications, SRF interaction with SRE was extremely slow (29–32). In addition, it has been shown that proteins such as Phox1 or YY1 could enhance the rate of SRF binding through an effect on either the conformation of SRF or the structure of DNA (33, 34). Alternatively, Gualberto et al. (28) showed that YY1 could be a more potent...
inhibitor of SRF binding to the α-actin muscle regulatory element than to the c-fos SRE.

Taken together, these data demonstrate that both WT and −102A alleles bind specifically a common factor, possibly related to the SRE-binding protein family, which is not yet completely characterized, and that the −102A allele recruits an additional factor immunologically related to YY1.

DISCUSSION

In this study, we examined the functional relevance of the −102T>A sequence variation on the CFTR transcriptional activity. Transient expression experiments showed, in all transfected cell types (Calu-3, CF-PAC1 cell lines expressing endogenous CFTR, or HeLa cells, which do not), that the −102A allele caused a significant increase in basal CFTR expression.

DNA-protein interaction assays demonstrated the binding of one or more nuclear protein(s) to the sequence surrounding the −102 site. By competition and antibody cross-reactivity studies, we demonstrated that (i) although the identity of the protein involved in the formation of the −102 site-specific complexes is uncharacterized, this protein shares DNA binding sequence specificity with the SRE-binding protein family, and (ii) the −102A allele recruited specifically an additional nuclear protein related to YY1.

Although numerous studies have reported that mutations in promoter regions of genes modify DNA-protein binding, recent reports have demonstrated the regulatory effect of naturally occurring gene promoter polymorphisms. For example, an additional guanine at −1607 base pairs in the matrix metalloproteinase 1 promoter has been shown to create an Ets binding
site and increase transcription, providing one molecular mechanism for enhanced extracellular matrix degradation (35). Knight et al. (36) have shown that the tumor necrosis factor C–376A allele characterized in the tumor necrosis factor promoter region, was a genetic determinant of susceptibility to cerebral malaria. They demonstrated that the tumor necrosis factor C–376 polymorphism recruited an OCT-1 binding protein and increased modestly but significantly TNF expression in human monocytes.

In the CFTR gene, only one putative deleterious mutation (−741T>G) within the transcriptional regulatory region has been functionally characterized by electrophoretic mobility shift assay. However, the nuclear protein bound to this polymorphic site remained uncharacterized (20). We identified a novel complex allele (−102T>A+S549R>T>G) containing a putative regulatory sequence alteration in the minimal CFTR promoter region (24). In a recent report, we showed that the CF patients carrying this complex allele were diagnosed at a much later age, had pancreatic functions relatively preserved, and had a milder lung disease and better overall clinical course compared with patients carrying the (S549R>T>G) mutation alone (25). We postulated that the −102T>A nucleotide change in the proximal CFTR promoter region, might, at least partially, compensate for the severity of the (S549R>T>G) mutation by CFTR overexpression.

In recent years, the highly conserved YY1 transcription factor has been the subject of extensive study. YY1 (also known as Δ, UCRRB, and NF-E1), a zin finger DNA-binding protein of the C2H2 type, has been shown to interact with regulatory sequences for a number of mammalian genes (37). YY1 is characterized as a multifunctional transcription factor that can affect gene expression through many different mechanisms. Indeed, depending on its binding sequence, YY1 can act as a transcriptional repressor or activator and, when binding at the initiator element, it becomes a component of the basal transcription complex (37–40). It has been proposed that the YY1 dual activity implies the presence of two distinct functional domains in the protein, one for repression and one for activation (38, 41, 42). Another attempt to explain these opposing actions of YY1 has been based on DNA binding and sequence orientation (34, 43, 44). Moreover, YY1 activation or repression might be mediated by protein-protein interactions. Lee et al. (41) recently characterized a specific interaction between YY1 and the transcriptional modulator p300 and suggested the formation of a three-protein complex composed of YY1, p300, and E1A in mediating transcription. Various chromatin-remodeling proteins including histone deacetylases, histone acetyltransferases, corepressors, and coactivators have been shown to interact with YY1 (45, 46). YY1 has also been reported to be associated with the nuclear matrix, which can affect chromosomal topology (47). Recently, YY1 has been reported as essential in enhancing the transcriptional level of the human dopamine β-hydroxylase gene after 12-O-tetradecanoylphorbol-13-acetate stimulation, through interaction with two proteins human hDTRE (dopamine 12-O-tetradecanoylphorbol-13-acetate-binding factor) and CREB (CRE-binding protein (48)). YY1 is also known to be a partner protein of poly(ADP) ribosyltransferase, which is involved in DNA repair (49). Many studies reported composite sites that bind YY1 and another factor in a mutually exclusive fashion. Indeed, YY1 can act as a repressor by decreasing transcription through a repressor domain in the COOH terminus (50), by displacing an activator from the site, or both. A few reports have described the role of YY1 as a transcription activator at composite sites (51, 52).

Here we have presented the first evidence for a compensatory mutation in the CFTR promoter that up-regulates its expression and implicates a protein that could be YY1. The mechanism by which the YY1 protein can exert its action on CFTR expression remains to be determined. YY1 may induce DNA binding and thus create a promoter architecture that facilitates or impairs transcription factor activity. CRE-binding protein and histone acetyltransferases are involved in CFTR regulation and represent components of the basal transcription machinery. As such, they may be targets for such chromatin remodeling. YY1 could also interact competitively with proteins that recognize the YY1 overlapping site or facilitate the binding of other proteins to this region of the promoter. Furthermore, because the YY1-binding element created in the −102A allele corresponds closely to the YY1 consensus sequence (37, 50, 53) and occurs within a CArG-like box, we can imagine that YY1 could enhance occupancy of the SRE-like element by a SRE-binding protein such as SRF.

Further characterization of the proteins that bind to the CFTR promoter containing the −102A allele will be required to explain how YY1 activates the CFTR promoter. Future analysis of the regulation of CFTR transcription in vivo will allow detailed characterization of cell type and temporal expression control by this new cis-acting sequence. These studies may shed light on the role of YY1 in the complex series of events involved in the regulation of CFTR transcription. Hence, the potential for YY1-involved regulation of the CFTR gene expression may offer benefit for CF patients, as a mutant CFTR protein that retains some function could be partially compensated by increased expression.

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A Naturally Occurring Sequence Variation That Creates a YY1 Element Is Associated with Increased Cystic Fibrosis Transmembrane Conductance Regulator Gene Expression

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