The unfolded protein response (UPR) aids cellular recovery by increasing the capacity and decreasing the protein load of the endoplasmic reticulum (ER). Although the main pathways of the UPR are known, the mechanisms of UPR-associated transcriptional repression have not been explored in mammalian cells. Previous studies indicate that endogenous cystic fibrosis transmembrane conductance regulator (CFTR) mRNA levels and protein maturation efficiency decrease when the UPR is activated. In the present study, we demonstrate that inhibition of CFTR expression under ER stress leads to reduced cAMP-activated chloride secretion in epithelial monolayers, an indication of diminished CFTR function. Moreover, ER stress and the UPR obliterate endogenous ΔF508 CFTR mRNA expression in CFPAC-1 cells without affecting recombinant ΔF508 CFTR mRNA levels or mRNA half-life. These results emphasize that transcriptional repression of CFTR under ER stress, in concert with decreased CFTR maturation efficiency, leads to diminished function. Using human CFTR promoter reporter constructs, we confined the ER stress-associated CFTR transcriptional repression to the minimal promoter. Chromatin immunoprecipitation assays established the binding of the UPR-activated ATF6 transcription factor to this region under ER stress, which links the repression to the UPR. Methylation-specific PCR (MSP) revealed hypermethylation of Cpg sites inside and in the vicinity of the MAZ transcription factor binding region of CFTR, demonstrating methylation-dependent repression. Using pharmacological inhibitors, we show that both DNA methylation and histone deacetylation contribute to CFTR transcriptional inhibition. These studies provide novel insight into the mechanism of gene repression during the mammalian UPR.

In eukaryotic cells, the endoplasmic reticulum (ER) is the site of protein folding and assembly. The unfolded protein response (UPR) can result from ER stress brought on by any number of insults (1–3), including depletion of ER Ca²⁺ stores (1), proteasome blockade (4), increase in the concentration of reactive oxygen species (5, 6), inflammation (7), overexpression of secretory proteins (2, 8), or altered glycosylation (9). In addition to increasing the capacity of the ER by enhancing the synthesis of membrane components and chaperones (10), the UPR also decreases the ER protein load by enhancing ERAD (11) and to some extent by inhibiting transcription and translation (10, 12). Although the principal mechanisms of the UPR have been studied extensively, only limited information is available regarding the extent and specificity of transcriptional repression during the UPR. In yeast, the limited number of genes that are transcriptionally repressed by the UPR encode secreted or cell surface proteins (6). Importantly, neither the extent nor the mechanisms of UPR-associated transcriptional repression have been investigated in mammalian cells.

The cystic fibrosis transmembrane conductance regulator (CFTR), an integral membrane glycoprotein expressed in the apical membrane of epithelial cells, plays a central role in ion transport and multiple regulatory processes (13–16). Epithelial cells are often exposed to environmental insults and inflammation that could activate the UPR (7), and we have previously reported that the UPR inhibits endogenous wild-type (WT) CFTR biogenesis by decreasing mRNA levels, translation, and maturation efficiency. Decreased endogenous CFTR mRNA levels were not the result of enhanced cytosolic RNase activity, and no decreases in transferrin receptor (TR) or glyceraldehyde-3-phosphate dehydrogenase mRNA levels were detected. Importantly, activation of the UPR did not affect levels of recombinant CFTR mRNA, suggesting that the transcriptional repression targeted the endogenous CFTR promoter (17). Based on this background, the present study was designed to understand the functional consequences of decreased CFTR expression and to explore the molecular mechanism of CFTR transcriptional repression by the UPR.

The major regulatory sequences for the human CFTR gene have been identified (for review, see Ref. 18), and it is accumu-
edged that CFTR expression is strictly regulated during development (16, 18). In contrast, we lack data concerning epigenetic regulation of CFTR expression, especially under pathological conditions. Furthermore, based on recent developments regarding the molecular pathogenesis of other human disorders, it is evident that understanding cellular stress responses in general, and ER stress responses in particular, is necessary for progress against the myriad of human diseases for which cellular stress is a component. These disorders include a growing number of genetic diseases such as cystic fibrosis (CF), as well as many neurodegenerative and metabolic disorders (1, 19).

Decreased CFTR function may also be associated with other chronic airway diseases besides CF, including chronic bronchitis, chronic obstructive pulmonary disease, and asthma (20, 21). The molecular pathology is not fully understood for any of these disorders, but several approaches that rescue the folding-deficient ΔF508 CFTR mutant from ERAD have been reported to deliver functional CFTR protein to the cell surface. Such approaches include proteasome inhibition, ER Ca\(^{2+}\) transport blockers, and depletion of ER Ca\(^{2+}\) (22–25). Interestingly, all of these interventions cause ER stress and activate the UPR (4, 9, 26). Consequently, and given the emergence of ΔF508 CFTR correction as a therapeutic approach for CF, studies concerning how cellular stress responses might regulate CFTR expression, both at the ER and in post-ER compartments, are of significant interest.

In the present study, we tested the functional consequences of decreased endogenous CFTR expression under ER stress. Moreover, we explored the molecular mechanism by which the UPR suppresses CFTR transcription. In addition to enhancing our knowledge of cellular mechanisms behind CF and many other pulmonary diseases, the results presented herein provide novel information regarding UPR-associated transcriptional regulation of membrane protein expression in mammalian cells.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture Conditions**—Calu-3, CFPAC-1 (expressing endogenous ΔF508 CFTR) (27), and HeLa cells were obtained from the ATCC. CFB410ΔF (expressing recombinant ΔF508 CFTR) was produced as previously described (29). Cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) with 10% fetal bovine serum in a humidified incubator at 37 °C in 5% CO\(_2\). For Ussing chamber studies, Calu-3 cells were grown on permeable supports (6.5-mm diameter Transwell filters, Costar).

**Antibodies**—Monoclonal CFTR C-terminal antibody (24-1) was purified from hybridoma supernatant at the University of Alabama Hybridoma Core Facility (ATCC # HB-11947).

**Induction of ER Stress and Activation of the UPR**—Pharmacological induction of ER stress and activation of the UPR was performed according to previously described methods (9, 17, 29). Briefly, cells were treated with 50 μM ALLN (Sigma) (9) or 5 μg/ml tunicamycin (TM, Sigma) (29) for the time intervals specified in each experiment.

**Functional Studies (Ussing Chamber)**—cAMP-activated chloride currents were measured through Calu-3 cell monolayers mounted in Ussing chambers as previously described (28, 30–32). Briefly, following stabilization of the basal current, the solution in the apical chamber was replaced with low chloride Ringer’s solution (6 mM sodium gluconate substitution), short-circuit currents (I\(_sc\)) were measured with an epithelial voltage clamp (VCC-600, Physiologic Instruments, San Diego, CA). A 10-mV pulse of 1-s duration was imposed every 10 s to monitor the transepithelial resistance (R\(_t\)), which was calculated using Ohm’s law. I\(_sc\) was activated by addition of 20 μM forskolin and blocked by 200 μM glybenclamide. Results were plotted as ΔI\(_sc\) (μA/cm\(^2\)), representing the fraction of current blocked by glybenclamide and normalized to the area of insert. Additionally CFTR inhibitor-172 (20 μM, Calbiochem) was also used to inhibit currents (33).

**Measurement of CFTR Protein Levels**—CFTR was immuno-precipitated from 250 μg of total protein, in vitro phosphorylated with \([γ\text{-}^{32}P]ATP\) (PerkinElmer Life Sciences) and cAMP-dependent protein kinase A (Promega, Madison, WI), separated on 6% SDS-PAGE gels, and analyzed using phosphorimaging, as described previously (17, 31).

**Relative Quantification of CFTR mRNA Using Real-time RT-PCR**—CFTR mRNA levels were quantified using real-time PCR, as previously described (17).

**Quantification of UPR Reporter and Control mRNA Levels**—HSPAS/Bip ( assay ID: Hs00607129_gh) and XBPI ( assay ID: Hs00231936_m1) mRNA levels were measured to test for UPR activity. Transferrin receptor (TR, assay ID: Hs99999911_m1) was amplified and measured as an additional control (17).

**CFTR mRNA Half-life Measurements**—CFTR mRNA half-lives were measured as described (34), with modifications explained below. Cells were grown on 10-cm plastic dishes to ~80% confluency. Parallel experiments were performed in ER-stressed and control cells. ER stress was induced by 50 μM ALLN or 5 μg/ml TM for 2 h. Actinomycin D (5 μg/ml, Sigma) was added concurrently to stop transcription, after which the RNA was isolated at the indicated time intervals using Qiagen mRNaseasy. Actinomycin D and ALLN or TM (in ER-stressed samples) were maintained throughout every experiment (34).

Total CFTR mRNA levels at each time point were measured as described previously (35). Briefly, mRNA levels were obtained by RT-PCR using a TaqMan-based assay (Applied Biosystems # hs_00357011_m1) and normalized to endogenous 18 S rRNA levels (amplified using a standard primer set, Applied Biosystems # hs_99999901_m1). CFTR mRNA values for each time point were calculated from four individual samples generated in at least two independent experiments. Relative CFTR mRNA levels at the time points indicated were plotted as percent differences from CFTR mRNA levels at the initial time point (t = 0). The mRNA half-lives were calculated from the exponential decay using the trend line equation C/C\(_0\) = e\(^{-kt}\) (where C and C\(_0\) are mRNA amounts at time t and the t\(_0\), respectively, and k\(_d\) is the mRNA decay constant), as previously described (36).

**CFTR Gene Promoter Constructs**—A human CFTR promoter-driven firefly luciferase reporter construct (pCFTR-pLuc) was purchased from Panomics (Cat. #: LR1020, Panomics Inc., Fremont, CA). This construct contains a 1000-bp fragment of
the human CFTR 5′-UTR upstream of firefly luciferase (“1-kb promoter”). Using the 1-kb promoter construct as a template, we created additional reporter vectors containing various fragments of the human CFTR 5′ UTR. To create the “no promoter” construct, our negative control, the 1-kb promoter was excised from the pCFTR-pLuc construct using the flanking Nhel and BglII sites. To create the “minimal promoter” construct, the 1-kb promoter region was replaced with a 372-bp segment of the human CFTR regulatory region. This segment, known to be the minimal promoter region of human CFTR (37), was amplified by PCR and inserted using the Nhel and BglII sites in original pCFTR-pLuc, as before.

CFTR Gene Promoter Reporter Assay—To test the transcriptional activity of the human CFTR promoter regions, HeLa cells were transfected with the constructs described above or with control plasmids provided by Panomics. Twenty-four hours before experiments, cells were seeded on 6-well tissue culture plates at ~40% confluency and transfected using FuGENE6 (Roche Applied Science). Each well received 2 μg of total plasmid DNA, 1 μg of vector of interest plus 1 μg of Renilla luciferase as an internal control. At 12 h post-transfection, cells were given normal medium containing 5 μM forskolin, 5 μg/ml TM, or a vehicle control for an additional 12 h. At the time points indicated, cells were lysed using luciferase assay lysis buffer (Promega) and firefly/Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay (Promega), according to the manufacturer’s protocol. Results in treated cells were plotted as percent decrease in arbitrary light units compared with control cells.

Chromatin Immunoprecipitation Assay—ChIP assays were performed as described (38, 39). Briefly, control and ER-stressed cells were fixed using formaldehyde that cross-links and preserves protein-DNA interactions. Cell nuclei were then isolated, suspended in 10 mM Tris (pH 7.5) and 1 mM EDTA, and sonicated. The soluble chromatin was placed into radioimmune precipitation assay buffer (0.1% SDS, 1% Triton X-100, 0.1% sodium deoxycholate 140 mM NaCl) and pre-cleared. Immunoprecipitation was performed with 2–5 μg of appropriate antibody (anti-ATF6α (H-280) and anti-XBP1 (M-186), rabbit polyclonal antibodies, Santa Cruz Biotechnology Inc.), and the immune complexes were absorbed to protein A/G beads (Pierce) and blocked with a mixture of bovine serum albumin and salmon sperm DNA. Protein-DNA complexes were resolved by reverse cross-linking, and proteins were removed by digestion. After standardizing DNA content, the sequence of interest was amplified by PCR using specific primers. The PCR products were tested in 1.5% agarose gels in 40 mM Tris acetate (pH 8.3) and 1.0 mM EDTA and stained with ethidium bromide. The ERSE region was amplified using 5′-GTTGACACT-TAGAAGCGAGAGAAGAGAACC-C3′ and 5′-GTTGACCTCAGGCTG-CCTA-3′ primers. The CFTR minimal promoter region was amplified using 5′-CTCCTGAAAAGGGCACCAGGTT-3′ and 5′-GCATTTTTTAAAACCTGCT-3′ primers.

Inhibition of ER Stress-induced ATF6 Cleavage by the Serine Protease Inhibitor AEBSF—AEBSF is a water-soluble, irreversible serine protease inhibitor that prevents the ER stress-induced cleavage of the Golgi membrane-bound ATF6 (40). Calu-3 cells were treated with 300 μM AEBSF (Calbiochem) for 6 h to inhibit ATF6 cleavage. ER stress was induced with TM (5 μg/ml). When AEBSF and TM were applied together, AESBF was added 1 h prior to TM (29).

MSP—methylSEQR™ (Applied Biosystems) was used according to the manufacturer’s protocol. Briefly, unmethylated cytosines were converted to uracils using an Applied Biosystems methylSEQR™ bisulfite conversion kit, according to the manufacturer’s protocol. Following PCR using primers specific for methylated versus unmethylated DNA, the 5′-UTR region of CFTR was analyzed with Methyl Primer Express® v1.0. The primers used in the final assays were as follows: Fw-5′-CGTTAGAGTAAATTGTGGGTC-3′ and Rw-5′-ATCTCTC-GAACGCTAAAAATC-3′. The resulting 246-bp PCR fragments were purified and cloned into the TopoTA vector (Invitrogen), followed by sequencing and analysis. For each condition, at least 20 clones were selected and sequenced. Results were analyzed and presented as previously described (41).

DNA MTase and HDAC Inhibitor Studies—Cells were treated with trichostatin A (a DNA MTase inhibitor, 50 ng/ml), 5′-azacytidine (an HDAC inhibitor; 1 μM), or both compounds as specified. RNA was isolated, and CFTR mRNA levels were measured in real-time RT-PCR experiments, as previously described (17).

Statistical Analysis—Results were expressed as means ± S.D. Statistical significance among means was determined using the Student’s t test (paired and unpaired samples).

RESULTS

ER Stress Decreases cAMP-activated Chloride Secretion in Epithelial Monolayers—Using two different human epithelial cell lines that endogenously express WT CFTR (Calu-3 and T84), we had previously demonstrated that pharmacological induction of ER stress and activation of the UPR decrease both the mRNA levels and the protein maturation efficiency of endogenous WT CFTR (17). Herein, we used these two cell lines to determine the functional consequences of this decreased CFTR expression in the context of the UPR. To conduct these studies, Calu-3 and T84 cells were treated with TM (5 μg/ml, 6–14 h) to induce ER stress (9, 17, 29). Activation of the UPR was monitored by measuring the increase in spliced XBP1 (sXBP1) mRNA, a well accepted indicator of UPR activation (17). XBP1 is a transcription factor synthesized upon frameshift splicing of XBP1 mRNA by IRE1 RNase, one of the three ER-localized sensors of the mammalian UPR (42). The XBP1 mRNA encodes a basic leucine zipper transcription factor that mediates ER stress responses by entering the nucleus and binding directly to the UPR element of UPR target genes (42, 43). As an additional indicator of UPR activation, we monitored mRNA levels of BiP, the central regulator of ER stress that controls the activity of all three ER resident sensors: IRE1, PERK, and ATF6 (44). In Calu-3 cells, both sXBP1 and BiP mRNA levels were elevated following TM treatment, but CFTR mRNA levels decreased to <30%
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Activation of the UPR in Calu-3 cells decreases CFTR mRNA levels and function. A, ER stress induction is followed by activation of the UPR and decreased CFTR mRNA levels. Following induction of ER stress in Calu-3 cells using TM (5 μg/ml), UPR activity was monitored by measuring sXBP1 and BIP mRNA levels. CFTR mRNA levels were measured in real-time RT-PCR experiments. Results are plotted relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels; n = 6, p < 0.001. B, activation of the UPR decreased CFTR protein levels. CFTR protein was measured after 6 h (left panel) or 14 h (right panel) of ER stress induction by TM. CFTR (arrows) was immunoprecipitated from 250 μg of total cellular protein using anti-CFTR (24-1) antibody, in vitro phosphorylated, subjected to SDS-PAGE, and detected by phosphorimaging analysis. Two individual samples were tested at each time point; n = 2 per condition. C, ER stress decreased cAMP-mediated chloride currents (Isc) in Calu-3 and T84 cell monolayers. Polarized Calu-3 and T84 monolayers were mounted into Ussing chambers for analysis. Transepithelial chloride currents were recorded following activation with 20 μM forskolin and blockade with 200 μM glybenclamide or CFTR inhibitor-172. Results are plotted as ΔIsc (μA/cm²), representing currents blocked by glybenclamide or CFTR inhibitor-172, and normalized to 1 cm² (upper panels). Representative tracings from control and ER-stressed Calu-3 and T84 monolayers are also shown (lower panels); n > 6 per condition.

FIGURE 1. Activation of the UPR in Calu-3 cells decreases CFTR mRNA levels and function. A, ER stress induction is followed by activation of the UPR and decreased CFTR mRNA levels. Following induction of ER stress in Calu-3 cells using TM (5 μg/ml), UPR activity was monitored by measuring sXBP1 and BIP mRNA levels. CFTR mRNA levels were measured in real-time RT-PCR experiments. Results are plotted relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels; n = 6, p < 0.001. B, activation of the UPR decreased CFTR protein levels. CFTR protein was measured after 6 h (left panel) or 14 h (right panel) of ER stress induction by TM. CFTR (arrows) was immunoprecipitated from 250 μg of total cellular protein using anti-CFTR (24-1) antibody, in vitro phosphorylated, subjected to SDS-PAGE, and detected by phosphorimaging analysis. Two individual samples were tested at each time point; n = 2 per condition. C, ER stress decreased cAMP-mediated chloride currents (Isc) in Calu-3 and T84 cell monolayers. Polarized Calu-3 and T84 monolayers were mounted into Ussing chambers for analysis. Transepithelial chloride currents were recorded following activation with 20 μM forskolin and blockade with 200 μM glybenclamide or CFTR inhibitor-172. Results are plotted as ΔIsc (μA/cm²), representing currents blocked by glybenclamide or CFTR inhibitor-172, and normalized to 1 cm² (upper panels). Representative tracings from control and ER-stressed Calu-3 and T84 monolayers are also shown (lower panels); n > 6 per condition.

(Fig. 1A). Similar results were obtained in T84 cells (data not shown). Transferrin receptor (TR) and glyceraldehyde-3-phosphate dehydrogenase mRNA levels did not change under ER stress in either cell line (data not shown), suggesting that the effect was specific for CFTR (17).

To evaluate the consequences of diminished CFTR mRNA levels under ER stress, CFTR protein levels were analyzed by immunoprecipitation and in vitro phosphorylation in control and TM-treated cell lysates. Although CFTR levels remained unchanged after 6 h of ER stress (Fig. 1B, upper panel), a 14-h exposure to TM significantly decreased CFTR levels (Fig. 1B, lower panel).

It is clear from these experiments that induction of ER stress significantly reduced both CFTR mRNA and protein levels. To determine whether there were any functional consequences of this decrease, transepithelial short-circuit currents (∆Isc) were compared in control and ER-stressed Calu-3 and T84 monolayers mounted in Ussing chambers. After 14 h of ER stress, when CFTR protein levels were markedly lower than control, ΔIsc were measured following forskolin activation (increased intracellular cAMP levels) and CFTR inhibition (using glybenclamide or CFTR inhibitor-172) (33). To maximize chloride secretion, Isc measurements were performed under specific conditions when the bathing fluid in the apical compartment was replaced with low chloride Ringer’s solution (see “Experimental Procedures”).

The Isc measured in intact epithelial monolayers results from a transepithelial current and represents cumulative movement of solutes across the cells (basolateral and apical membranes). To assess CFTr-associated ΔIsc, we measured to what extent the ΔIsc was inhibited by glybenclamide or CFTR inhibitor-172. In agreement with previous results (45, 46), no significant difference in Isc inhibition was observed when using glybenclamide versus CFTR inhibitor-172, suggesting that the majority of ΔIsc observed in these experiments was CFTR-driven. After 14 h of induced ER stress, the cAMP-stimulated ΔIsc decreased to <40% in both Calu-3 and T84 monolayers (Fig. 1C), which is consistent with the decrease in CFTR mRNA and protein levels observed during ER stress.

ER Stress Eliminates Endogenous ∆F508 CFTR mRNA in CFPAC-1 Cells—It has been reported that ∆F508 CFTR mRNA levels were markedly lower than WT levels in native human tissues (47). CF tissues are affected by chronic inflammation, which inflicts diverse insults on CFTR-expressing cells (see Ref. 48 for a review). We therefore sought to test whether the UPR contributes to decreased ∆F508 CFTR mRNA levels. In these studies, we induced ER stress in CFPAC-1 cells, which express low levels of endogenous ∆F508 CFTR. Two different pharma-
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FIGURE 2. ER stress decreases endogenous, but not recombinant, CFTR mRNA levels. A, activation of the UPR eliminates CFTR mRNA from CFPAC-1 cells expressing low levels of endogenous ΔF508 CFTR. Following induction of ER stress in CFPAC-1 cells using proteasome inhibition or TM, UPR activity was monitored by measuring sXBP1 mRNA levels. Endogenous ΔF508 CFTR mRNA levels decreased to undetectable levels (see error bars) following activation of the UPR. Results are plotted relative to control (untreated) cells. B, no decrease in recombinant ΔF508 CFTR and endogenous TR mRNA levels following induction of ER stress in CFBE41o-ΔF cells. ER stress was induced in CFBE41o-ΔF as in CFPAC-1 cells. Measurements of sXBP1 mRNA levels indicate induction of the UPR, but no changes in recombinant CFTR or endogenous TR mRNA levels were detected.

Cologal methods of activating the UPR (TM and proteasome inhibition by ALLN), both reduced endogenous ΔF508 CFTR mRNA levels to very low, nearly undetectable levels (Fig. 2A). Interestingly, when testing CFBE41o-ΔF cells expressing recombinant ΔF508 CFTR (28), we detected no change in recombinant CFTR mRNA levels after induction of ER stress conditions, even though the change in sXBP1 mRNA levels was comparable to that in CFPAC-1 cells (Fig. 2B). No changes in TR mRNA levels were detected in CFPAC-1 cells under the same ER stress conditions (Fig. 2C). Taken together, these results indicate that the negative effect of the UPR on endogenous CFTR mRNA is detectable, pronounced, and specific to endogenous CFTR, even in cells with very low CFTR mRNA expression.

ER Stress and the UPR Have No Effect on CFTR mRNA Stability—Next, we sought to reveal the mechanism behind the decrease in endogenous WT and ΔF508 CFTR mRNA levels, because it could result from transcriptional repression, enhanced mRNA decay, or both. Our previous studies on cytosolic RNase activity in CFTR-expressing cells revealed no difference between control and ER stress conditions (17). To supplement these results, we determined the half-life of CFTR mRNA under control and ER stress conditions. In these experiments, ER stress was induced by 50 μM ALLN or 5 μg/ml TM for 2 h, a time period after which UPR activation was apparent based on an increase in UPR reporter mRNA levels (sXBP1 and BiP) (17). At 5.8 h and 5.5 h, respectively, the half-lives of CFTR mRNA in control and ER-stressed Calu-3 cells were not significantly different, indicating that ER stress and the UPR did not affect CFTR mRNA half-life (Fig. 3). This result supports the hypothesis that decreased CFTR mRNA levels under ER stress are the result of transcriptional inhibition.

Repression of the Human CFTR Minimal Promoter under ER Stress—To further investigate the mechanism behind how ER stress and the UPR transcriptionally repress CFTR, we designed experiments to elucidate the specific region in the human CFTR 5′-UTR that is targeted for repression during ER stress. For these experiments, we utilized firefly luciferase reporters driven by different fragments of the human CFTR 5′-UTR (Fig.
ER Stress, the UPR, and CFTR Expression Regulation

FIGURE 4. ER stress decreased CFTR promoter activity. A, schematic drawings of the human CFTR reporter vectors. B, luciferase expression from the 1-kb promoter vector in control and ER-stressed HeLa cells. Luciferase expression in control cells was set to 100%, and results for ER-stressed cells are plotted relative to control cells (n = 12). As a positive control, forskolin (30 μM for 12 h) was used in some samples to induce luciferase expression through the CRE and variant CRE elements. Luciferase expression decreased to ~40% of control expression levels following ER stress induction. A ~40% increase in luciferase expression was measured in response to forskolin (n = 12; p < 0.005). C, luciferase expression in control and ER-stressed cells following transfection with no promoter (negative control), minimal promoter, 1-kb promoter, and 2.3-kb promoter vectors. Results are plotted as relative light units (n = 12, p = <0.001 for all constructs).

To establish a baseline by which to design the experiments, we tested a commercially available firefly luciferase construct, in which luciferase expression was driven by a 1-kb segment of the human CFTR promoter (pCFTR-pLuc). This 1-kb segment contained two cAMP-responsive elements, CRE and variant CRE, that have been shown to enhance CFTR expression by ~30% in response to cAMP (Fig. 4B). This observation demonstrated that our 1-kb promoter responded to stimuli known to affect transcription of CFTR in vivo.

For all experiments, transfected cells not subjected to ER stress were used as negative controls. Additionally, in some experiments, a plasmid construct expressing Renilla luciferase (phRL-CMV, Promega) was also transfected into HeLa cells as an internal transfection control. Renilla luciferase activity did not change under any of our experimental conditions (data not shown).

Using the 1-kb promoter construct as a template, we further refined the segment of the human CFTR 5′ regulatory region that is repressed under ER stress by constructing additional firefly luciferase vectors containing various segments of the CFTR promoter and enhancer regions (Fig. 4A). The human CFTR minimal promoter corresponds to a 372-bp segment of the 5′-UTR that contains the variant CRE element, but not the CRE element (50). This segment has been demonstrated as the minimum sequence to drive CFTR expression (38). The 2.3-kb reporter construct contains the 1-kb construct, plus an additional segment of the CFTR 5′-UTR. The “no promoter” construct, which was the pCFTR-pLuc construct with the 1-kb promoter deleted, was used as a negative control.

Utilizing an experimental design similar to that described above for the 1-kb promoter vector, we measured the effects of ER stress and activation of the UPR on the luciferase expression driven by these promoter constructs. The results indicate that luciferase expression following induction of ER stress decreased from all constructs except the negative control (Fig. 4C). Because the decrease in luciferase expression affected all constructs, including the minimal promoter, and because the decrease corresponded to the relative endogenous CFTR mRNA decrease measured in Calu-3 cells under identical conditions, these results suggested that the region repressed by ER stress conditions was within the minimal promoter region (372-bp segment of the 5′-UTR).

Binding of a UPR-activated Transcription Factor (ATF6) to the Human CFTR Minimal Promoter under ER Stress—To link ER stress-associated transcriptional repression of CFTR to the UPR, we analyzed whether the minimal promoter region contained putative binding sites for ATF6 and for a number of previously

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**Figure 5. ATF6 interacts with the minimal CFTR promoter under ER stress conditions.** A. Schematic drawing of transcription regulatory factors binding to the minimal promoter of human CFTR. Binding sites for ATF6, Sp1, NF-Y/CDP-cut, and SRF/YY1 are shown. The arrow represents the target DNA fragment, amplified by PCR using the primer pair specified in the method section. TSS: transcription start site. ER stress conditions increase the binding of XBP1 and ATF6 to the ERSE element of BiP/GRP78. ChIP assays were performed in control and ER-stressed Calu-3 cells to validate the assay. DNA input controls were tested in all experiments to assure that the assay is quantitative (data not shown). The arrow shows PCR products of the sizes predicted for XBP1 and ATF6 binding. Enhanced binding of ATF6 and XBP1 to the ERSE element under ER stress are reflected in the higher density bands under ER stress conditions. Non-immune IgG was also included to validate the assay. Antibodies were anti-ATF6 or anti-XBP1, respectively. M: molecular weight marker. C. ATF6 binds to the minimal promoter of CFTR under ER stress. ChIP assays were performed using anti-ATF6α antibody. The resulting sequence, a putative binding site in the overlapping sections of the minimal promoter region of the CFTR gene, was amplified by PCR. The arrow shows the location of a PCR product of predicted size. No bands of predicted size were detected in control samples immunoprecipitated with anti-ATF6 antibody or in controls not subjected to ER stress but immunoprecipitated with anti-ATF6 antibody (far left lane = bp standards). D. Inhibition of ATF6 cleavage eliminated transcriptional repression of CFTR under ER stress. Calu-3 cells were treated with AEBSF (300 μM) for 6 h. CRP stress was induced with TM (5 μg/ml). BiP and CFTR mRNA levels were measured in real-time RT-PCR. Results are plotted as relative BiP and CFTR mRNA compared with untreated control (Calu-3, n = 6).

Identified regulatory factors, including SRF/YY1 and CRE (37). ATF6 is a transcription factor that is activated by the UPR and helps facilitate cell recovery by regulating the transcription of ER chaperones and folding enzymes (12, 19). Although ATF6 is most commonly known to function as a transcriptional activator, it has been shown to repress transcription by recruiting HDAC1 to the ATF6-SREBP2 complex (51). We therefore hypothesized that binding of ATF6 to the CFTR minimal promoter caused the UPR-associated transcriptional repression of CFTR, either by directly inhibiting the basal transcriptional complex of RNA polymerase and its associated factors (52), or indirectly by interfering with positively acting influences, such as cAMP through CRE (53).

The human CFTR minimal promoter contains an inverted CCAAT consensus, also known as a “Y-box” element. The human homeodomain CCAAT displacement protein/cut homolog (CDP/cut) can cause transcriptional repression by inhibiting a transcriptional activating complex consisting of SRF, NF, and the Y box element (54). According to the sequence data, ATF6 could bind adjacent to this region or to pre-formed complexes, either of which could result in transcriptional repression (Fig. 5A).

Based on these hypotheses, we utilized ChIP assays to test whether ATF6 could bind to the CFTR minimal promoter, and whether ATF6 binding was different under control and ER stress conditions. ATF6 binding assays to the minimal promoter were performed following optimization of ChIP assays, as described previously (39). In the initial control experiments, we compared the binding of ATF6 and XBP1 to the BiP/GRP78 region under normal and ER stress conditions (55). This region, called the ERSE region, contains known binding sequences that these factors utilize during ER stress conditions. The results indicate a significant increase in a PCR product of predicted size (210 bp) during ER stress for both XBP1 and ATF6, which corresponds to an enhanced binding of these factors to the ERSE region of BiP/GRP78 under ER stress (51, 56). No PCR product was detected in samples where anti-XBP1 or anti-ATF6 antibodies were replaced with non-immune rabbit IgG (Fig. 5B).

Next, we tested whether ATF6 interaction with the CFTR minimal promoter increased under ER stress conditions. Sheared DNA-protein samples were immunoprecipitated with anti-ATF6α (H-280) antibodies. Unstressed cells immunoprecipitated with anti-ATF6α and ER-stressed samples immunoprecipitated with nonspecific (anti-XBP1 antibodies) were tested as controls. Following reverse cross-linking, protease digestion, and standardization of DNA content, the region of interest was amplified by PCR (Fig. 5C). A PCR product of the predicted size (indicated by the arrow) was present in samples tested following induction of ER stress. No PCR products of the predicted size were detected in control samples immunoprecipitated with anti-XBP1 antibody (used as control) or, more importantly, in unstressed samples immunoprecipitated with anti-ATF6. These results confirm that induction of ER stress enhances the binding of ATF6 to the predicted region of the human CFTR 5’-UTR. Importantly, this finding provides the first direct link between CFTR transcriptional repression and the UPR.

Inhibition of ATF6 Cleavage Abolishes Transcriptional Repression of CFTR under ER Stress—To confirm the role of ATF6 in ER stress-associated CFTR transcriptional repression, we performed experiments in which we inhibited the release of
Transcriptional repression under ER stress.

To further investigate this possibility, we designed experiments to compare the methylation status of the CFTR minimal promoter under normal and ER stress conditions. To begin, we analyzed the human CFTR minimal promoter and located all of the CpG sites (Fig. 6). Although the human CFTR CRE sequence does not contain CpG dinucleotide, the two other regulatory sequences, the Sp1 and MAZ binding sites, do contain CpGs. It is notable that the PCR product obtained in the ChIP assays for ATF6 binding overlaps this region, suggesting that ATF6 may bind this regulatory site to mediate its effects.

To determine whether the methylation status of any of these regions changes during ER stress, we isolated genomic DNA from control and ER-stressed cells and performed MSP. This assay requires very small quantities of DNA and has been shown to detect 0.1% changes in allele methylation within a given CpG island (70). Six different primer pairs were tested to optimize the assay. The results indicate that the methylation pattern in the majority of CpGs was not different under control or ER stress conditions (Table 1). Interestingly, some CpGs were methylated even under basal, non-stress conditions (Fig. 6, underlined). More importantly, however, specific CpG dinucleotides inside and in the vicinity of the MAZ and Sp1 binding sites were hypermethylated under ER stress conditions (Fig. 6, overlined). These results suggest that a methylation-dependent mechanism involving this region might mediate transcriptional repression of CFTR during ER stress.

**DNA Methylation and Histone Deacetylation in Concert**

**Contribute to Transcriptional Repression of CFTR during ER Stress—**

Promoter methylation may result in transcriptional repression through a number of different mechanisms (63–66, 71, 72). For example, methylation has been shown to recruit HDACs, which ultimately results in histone and/or inhibitory transcription factor deacetylation and gene silencing (71). To determine whether this mechanism is behind CFTR transcriptional repression during ER stress, we tested whether inhibition of DNA MTase activity and/or HDAC activity alleviated the decrease in CFTR mRNA levels we observed during ER stress.

In these studies, we used pharmacological inhibitors to block DNA MTase and HDAC activity (5’-azacytidine for 96 h and trichostatin A for 24 h, respectively), in cell lines expressing either endogenous (Calu-3 and T-84), or recombinant CFTR (HeLaΔΔ). After treating cells with one or both of the inhibitors for, we induced ER stress (TM for 16 h) and measured the relative CFTR mRNA levels. In unstressed cells, we observed an increase in basal CFTR mRNA levels following 5’-azacytidine or trichostatin A treatment alone, but neither of the inhibitors eliminated the decrease in CFTR mRNA levels during ER stress (Fig. 7A). When cells were treated with both 5’-azacytidine and

**TABLE 1**

Localization and methylation stage of CpG sites in the human CFTR minimal promoter

| Location | Control | ER stress |
|----------|---------|-----------|
| CpG-357  | 100     | 9         |
| CpG-312  | 100     | 7         |
| CpG-327  | 100     | 9         |
| CpG-323  | 100     | 11        |
| CpG-272  | 100     | 12        |
| CpG-247  | 100     | 11        |
| CpG-212  | 100     | 10        |
| CpG-186  | 100     | 10        |
| CpG-154  | 100     | 10        |
| CpG-110  | 100     | 11        |
| CpG-86   | 100     | 10        |
| CpG-47   | 100     | 12        |
| CpG-10   | 100     | 13        |

a CpGs that were methylated under basal conditions.
b CpGs sites methylated only under ER stress.

soluble ATF6 from the Golgi membrane. ATF6 is a key factor of the mammalian UPR (44, 57–60). Inhibition of ATF6 cleavage by the serine protease inhibitor AEBSF (40), or knockdown by small interference RNA (60), both abolish the UPR. In the present study, we inhibited the Golgi membrane cleavage of ATF6 by AEBSF while inducing ER stress using TM. We chose this approach over small interference RNA knockdown, because we wanted to test the immediate effects of ATF6 on CFTR transcription during the first 6–8 h of ER stress. The effects of AEBSF treatment on ATF6 and the UPR were assessed by measuring BiP mRNA levels as described previously (40). This readout was chosen because BiP is a major reporter of the UPR, which is strongly activated during TM-induced ER stress (17, 40) and ATF6 is the main transcriptional inducer of BiP (44, 61). Calu-3 cells were treated with AEBSF (300 μM) and TM (5 μg/ml) followed by measurements of BiP and CFTR mRNA levels. Inhibition of ATF6 cleavage by AEBSF eliminated both its inducer effect on BiP and repressor effect on CFTR transcription (Fig. 5D). These results support the ChIP assays and indicate that ATF6 is a key component in CFTR transcriptional repression under ER stress.

**Hypermethylation of the CFTR Minimal Promoter under ER Stress—**

Transcriptional repression mechanisms may involve methylation of CpG sites within the regulatory sequences of a gene. It has been demonstrated that methylation of CpG sites can inhibit transcription factor binding, or it can recruit methyl binding proteins and histone deacetylases associated with repressor complexes (62–66). The CFTR minimal promoter contains three CpG-rich regulatory sequences, CRE, Sp1 (basal transcription regulator binding site), and MAZ (Myc-associated zinc finger protein binding) (67), that regulate transcription for other genes (68, 69). For this reason, we hypothesized that, during the UPR, methylation of one or more of these regions might recruit methyl binding proteins and/or HDACs to mediate repression of the CFTR gene.

**FIGURE 6. Location of CpG sites in the human CFTR minimal promoter.** Numbering is based on the transcription start site (0). CpGs that are methylated under both control and ER stress conditions are underlined. CpG islands that are only methylated under ER stress conditions are overlined. Locations for MAZ binding, Y-box, and CRE (cAMP-responsive element) are also shown.
trichostatin-A, however, CFTR mRNA levels did not decrease in response to ER stress; instead, we observed a 2-fold increase in CFTR mRNA levels compared with non-ER-stressed cells treated with both inhibitors (Fig. 7B). Importantly, we observed this response to DNA MTase and HDAC inhibition only in Calu-3 and T84 cells, which express endogenous CFTR. In contrast, identical conditions did not affect recombinant CFTR mRNA levels in HeLaΔF cells (data not shown). Taken together, these results imply that CFTR transcriptional repression during ER stress is associated with the combined activities of DNA MTase and HDAC, and that this repression is specific to the endogenous CFTR promoter.

**DISCUSSION**

Understanding cellular stress responses is critical for progress against a growing number of human disorders associated with cellular stress (73). The ER plays central role in protein synthesis and processing, and a variety of insults may cause ER stress and activate the UPR, resulting in UPR-specific regulation of gene expression (2). Although we had previously observed that induction of ER stress inhibited endogenous CFTR expression in epithelial cells (17), the functional consequences of this decreased CFTR expression remained questionable until the current studies were conducted. Our results demonstrate that ER stress decreased WT CFTR function (as measured by transepithelial chloride secretion) in two different human epithelial cell lines. We also demonstrate that the effects of ER stress on endogenous CFTR expression were measurable even in cells expressing very low levels of ΔF508 CFTR (CFPAC-1). In this cell line, the transcriptional repression during ER stress eliminated CFTR mRNA levels to below detection, suggesting that the effects of ER stress on CFTR expression could be significant in vivo. Because multiple environmental factors can cause or contribute to ER stress, our studies illustrate the need to consider the effects of cellular stress when designing therapies to rescue the mutant CFTR protein.

Even though CFTR message levels decreased after 3–6 h of ER stress (17), we chose to analyze CFTR protein levels and function after 12 h, because the half-life of WT CFTR in Calu-3 cells is >20 h (74). We chose two different human epithelial cell lines for these studies, Calu-3 (lung) and T84 (colon), because they express high levels of endogenous CFTR compared with human epithelial cells in primary culture (74).

Because primary epithelial cells express such low levels of CFTR under basal conditions, our findings suggest that they may be more susceptible to the CFTR transcriptional repression mediated by ER stress. This idea is supported by our studies of mRNA levels in CFPAC-1 cells, which express very low levels of ΔF508 CFTR. The experiments indicate that, in these cells, endogenous ΔF508 CFTR mRNA virtually disappears following induction of the UPR.

Decreases in chloride currents correspond to decreases in CFTR mRNA and protein levels in Calu-3 cells (17). However, our functional readout, forskolin-induced short circuit current (I(sc)), is affected by all cAMP-activated electrolyte transporters in the monolayer, not just CFTR. Therefore, we carefully optimized our experimental conditions to maximize the CFTR-mediated I(sc). These included the use of low chloride containing Ringer’s solution in the apical compartment, specific concentrations of forskolin, glybenclamide (28), and CFTR inhibitor-172 (45). The biochemical data also validated the functional readout, because the I(sc) decreases we observed in ER-stressed cells were comparable to the changes observed in CFTR protein and mRNA levels. Nevertheless, we cannot fully exclude the possibility that the net decrease in I(sc) was a combination of basolateral and apical transport activities in the cell lines tested.

Because ER stress and UPR activation affected only endogenous CFTR mRNA levels, not recombinant CFTR mRNA levels, we explored the differences between the endogenous and recombinant mRNA. A 3’ AU-rich untranslated sequence (3’- UTR) that was present on the endogenous CFTR mRNA was missing from the recombinant mRNA, and this region has been shown to regulate CFTR mRNA levels post-transcriptionally through mRNA stability (75). Because decreases in mRNA may result from enhanced degradation, as opposed to transcriptional inhibition, it was important to measure the stability of the endogenous CFTR mRNA under ER stress conditions. Our results indicated that the half-life of endogenous CFTR mRNA did not change during ER stress, suggesting that decreased CFTR mRNA levels were not a result of mRNA destabilization, but were rather due to transcriptional down-regulation.

Our next step was to explore the mechanism by which ER stress induced transcriptional repression of CFTR. The CFTR
ER Stress, the UPR, and CFTR Expression Regulation

FIGURE 8. Model for transcriptional inhibition of CFTR during ER stress. The diagram depicts the location of CpG sites and main regulatory regions within the human CFTR minimal promoter. CpGs that are hypermethylated under ER stress are overlined. CpGs that are methylated under control conditions are underlined. The putative methylation-dependent regulatory site (MAZ binding site) and the methylation-independent regulatory region (Y-box and CRE) are labeled with rectangles and enlarged. The composition of regulatory factors is based on previously published data for CFTR or other genes.

The promoter is not well defined compared with many genes, but the main regulatory elements have been identified (18). Using luciferase-based CFTR promoter reporter constructs containing different segments of the regulatory region, we confined the ER stress response element(s) to a region that was previously identified as the minimal promoter region (50). It is worth noting that, under ER stress conditions, the decrease in luciferase expression was greater from the 2.3-kb promoter than the other vectors tested (Fig. 7). However, the relative decrease following induction of ER stress was not significantly different between constructs, based on data from three individual experiments ($p > 0.05$). Because luciferase expression (based on relative light units) was consistently higher from this vector, it is possible that additional enhancer elements are present in this segment.

Our analysis of the minimal promoter revealed sequences that have previously been implicated in the repression of CFTR and other genes. These regulatory sequences include MAZ (68, 69), Y-box (54), and CRE (49). We also found direct and indirect binding sites for ATF6, a UPR-activated transcription factor that has been implicated in gene repression (51). Using ChIP assays, we confirmed that ATF6 interacted with the minimal promoter of CFTR during ER stress, which provided the first direct link between CFTR transcriptional repression and the UPR.

Inhibition of ATF6 cleavage using AEBSF further supported the role of ATF6 in CFTR repression. However, because ATF6 is a central activator of the mammalian UPR, inhibition of ATF6 cleavage also eliminated the UPR. Based on these results, and because ATF6 has previously been identified as a component of a repressor complex (51), we propose that the UPR is responsible for CFTR transcriptional repression through the activity of ATF6. Although this demonstrates a role for ATF6 in CFTR transcriptional repression, neither the ChIP assays nor the inhibitor studies can distinguish between direct and indirect binding of ATF6. If ATF6 is part of a repression complex, further studies will be necessary to identify members of the repressor complex.

Because promoter methylation and subsequent binding of repressor complexes to the methylated DNA are well known mechanisms for gene repression (66, 69, 76), we performed MSP studies to further unravel the mechanism of CFTR transcriptional repression during ER stress. Importantly, the results of the MSP studies indicate that the MAZ binding sequence within the CFTR promoter is hypermethylated during ER stress. We hypothesize that this hypermethylation is an important component of the mechanism of transcriptional repression of CFTR during ER stress. This hypothesis is supported by other studies indicating that Sp1 and MAZ may bind to the same cis-elements in a GC-rich promoter. These two transcription factors apparently share DNA binding sites, and the extent of binding corresponds to the regulation of gene expression. Despite sharing binding sites, the suppression activities mediated by Sp1 and MAZ were independent of each other; MAZ-dependent suppression involved HDAC, whereas Sp1-dependent suppression involved DNA MTase 1 (69). These results suggest that both deacetylation and methylation might be independently involved in regulating expression of a single gene, due to different zinc finger proteins (Sp1 and MAZ) binding to the same cis-elements in the promoter.

Because the CFTR minimal promoter contains putative binding sites for both Sp1 and MAZ, we hypothesized that CFTR expression might be regulated by such a mechanism. To test whether methylation of MAZ is followed by recruitment of HDACs, we performed studies in which we inhibited the activity of DNA MTases and HDACs, both alone and in concert. These inhibitor studies revealed that blocking both DNA MTase and HDAC functions can ameliorate the decrease in CFTR mRNA levels associated with ER stress. This result indicated that transcriptional repression by the UPR involves both methylation-dependent and -independent pathways.

Based on our results thus far, we proposed a model for transcriptional repression of CFTR under ER stress (Fig. 8). The model focuses on regulation through the minimal promoter of the human CFTR, because our promoter reporter construct studies confined the CFTR transcriptional repression to this region. Binding site analysis of the minimal promoter revealed putative direct and indirect binding sites for ATF6 and XBP1, two transcription factors associated with the UPR. The ChIP assays subsequently confirmed the binding of ATF6 to the minimal promoter, linking the observed CFTR repression to the UPR (Fig. 5). MSP studies demonstrated hypermethylation of specific CpG sites in the vicinity of the MAZ binding region...
under ER stress, which suggests methylation-dependent repression (Fig. 6). It is possible that methylation of the MAZ binding sequence and CpGs in its vicinity may recruit HDACs, resulting in both histone and transcription factor deacetylation. We hypothesize that the binding of these histones and deacetylated repressors to the CFTR minimal promoter mediates transcriptional repression. In support of this hypothesis, our DNA MTase and histone deacetylase inhibitor studies confirmed that the combined actions of DNA MTases and HDACs contributed to repression of CFTR transcription under ER stress.

As yet, it is unclear to what extent the UPR mediates transcriptional repression in mammalian cells. Most UPR-associated mechanisms, such as increase in ER capacity, translation decrease, and ERAD increase, have been extensively studied (10, 12), but the transcription inhibitory component of the UPR has not been carefully examined. In yeast, only 15 genes have been identified as being transcriptionally down-regulated by the UPR, and most of them encode cell surface or extracellular proteins (6). Although the present studies concentrate on CFTR, we also measured mRNA levels of other cell surface proteins, such as TR, the insulin receptor, and multiple ENaC subunits (data not shown) and did not find a significant change in their message levels under ER stress. In fact, in our preliminary screen, we could not identify any additional genes that displayed transcriptional repression during ER stress. This finding suggests that transcriptional repression by the UPR is rather specific in mammalian cells, perhaps targeting a specific structural component within certain promoters. To further explore this hypothesis, we are currently designing gene array studies to examine the effect of ER stress on transcriptional regulation of multiple genes.

Our findings also have clinical relevance. Although we induced ER stress pharmacologically within the controlled environment of our studies, it is known that in vivo ER stress occurs as an important component of more generalized cellular stress. Interestingly, oxidative stress caused by butylhydroquinone exposure has been shown to decrease CFTR mRNA stability (77), most likely through enhanced cytosolic RNase activity. Moreover, cigarette smoke extract, a well known cause of ER and/or cytosolic stress, has also been implicated in decreasing chloride secretion in human bronchial epithelial cells (78). Furthermore, inflammatory cytokines have been shown to decrease CFTR mRNA stability by activating exosomes in the AU-rich element of the 3′-UTR (75). Although ER stress and UPR activity have not been directly investigated under the conditions described above, studies have reported that the UPR could be triggered by infection and inflammation in CF airway epithelia (79). Based on these studies, it is likely that the previously observed negative effects of cellular stress on CFTR expression, such as enhanced mRNA decay, may be combined with transcriptional suppression and enhanced ERAD under a variety of conditions. In such a situation, functional CFTR levels may decrease to pathologically low levels in epithelial cells as a result of cellular stress.

Our current observations are also relevant regarding CFTR function in compound heterozygote CF patients that present with “mild” cystic fibrosis. For these patients, it is possible that any source of cellular stress may disturb their partially balanced CFTR function, resulting in a detrimental decrease in CFTR expression.

The studies presented herein also provide additional information regarding CFTR transcriptional regulation in native tissues under pathological conditions. Most importantly, these results provide an unprecedented link between CFTR transcriptional regulation and cellular stress responses. Understanding cellular stress associated regulation of WT and ΔF508 CFTR biogenesis in native cells, and recognizing the difference between regulation of recombinant and endogenous CFTR expression, are essential to developing efficient therapies for CF.

In addition, our results will also aid in designing future studies to correct ΔF508 CFTR misprocessing. Several promising candidates have emerged as a result of the significant efforts directed toward identifying compounds that can rescue ΔF508 CFTR from degradation in the ER (80–85). However, the primary models for testing these compounds have chiefly employed recombinant ΔF508 CFTR-expressing cell lines, and to date only a few endogenous ΔF508 CFTR-expressing models have been tested (82, 84). The mechanisms by which any of these rescue agents work are not fully understood, and if any of these compounds initiate ER stress, any inhibitory effect on CFTR expression would only be apparent in endogenous CFTR expression models.

Taken together, these studies have demonstrated that ER stress and activation of the UPR inhibit endogenous CFTR function, and our results have defined the main mechanistic steps by which such repression occurs. These findings warrant further studies to reveal the detailed mechanisms and the extent of transcriptional repression by the UPR.

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