A complex involving Derlin-1 and p97 mediates the retrotranslocation and endoplasmic reticulum (ER)-associated degradation of misfolded proteins in yeast and is used by certain viruses to promote host cell protein degradation (Romisch, K. (2005) Annu. Rev. Cell Dev. Biol. 21, 435–456; Lilley, B. N., and Ploegh, H. L. (2004) Nature 429, 834–840; Ye, Y., Shibata, Y., Yun, C., Ron, D., and Rapoport, T. A. (2004) Nature 429, 841–847). We asked whether the components of this pathway are involved in the endoplasmic reticulum-associated degradation of the mammalian integral membrane protein, the cystic fibrosis transmembrane conductance regulator (CFTR), a substrate for the ubiquitin-proteasome system. We report that Derlin-1 and p97 formed complexes with CFTR in human airway epithelial cells. Derlin-1 interacted with nonubiquitylated CFTR, whereas p97 associated with ubiquitylated CFTR. Exogenous expression of Derlin-1 led to its co-localization with CFTR in the ER where it reduced wild type (WT) CFTR expression and efficiently degraded the disease-associated CFTR folding mutants, ΔF508 and G85E (>90%). Consistent with this, Derlin-1 also reduced the amount of WT or ΔF508 CFTR appearing in detergent-insoluble aggregates. An ~70% knockdown of endogenous Derlin-1 by RNA interference increased the steady-state levels of WT and ΔF508 CFTR by 10–15-fold, reflecting its significant role in CFTR degradation. Derlin-1 mediated the degradation of N-terminal CFTR fragments corresponding to the first transmembrane domain of CFTR, but CFTR fragments that incorporated additional domains were degraded less efficiently. These findings suggest that Derlin-1 recognizes misfolded, nonubiquitylated CFTR to initiate its dislocation and degradation early in the course of CFTR biogenesis, perhaps by detecting structural instability within the first transmembrane domain.

The endoplasmic reticulum (ER) provides mechanisms that facilitate the appropriate folding of newly synthesized secretory and integral membrane proteins, as well as processes that eliminate proteins that do not achieve their native folded state (1). A proper balance between the events that promote productive folding and ER-associated degradation (ERAD) of proteins is vital for cellular homeostasis. The accumulation of aberrant proteins evokes ER stress and an unfolded protein response, which induces the expression of chaperones to maintain protein solubility as well as additional ERAD components to accelerate protein elimination (4). The first step in ERAD involves the recognition and removal of misfolded proteins from the ER, followed by their cytoplasmic ubiquitylation and ubiquitin-dependent digestion, which is conducted largely by the cytosolic 26 S proteasome (5–7).

CFTR was the first integral membrane mammalian protein to be implicated as an ERAD substrate (8, 9), and it has served as a model for the growing list of diseases of protein conformation that manifest in a diverse set of human pathologies (10). Because of its complex folding scheme, the majority of WT CFTR and nearly 100% of the common folding mutant, ΔF508 CFTR, are degraded by ubiquitin-proteasome-mediated processes. Although WT CFTR processing is more efficient in epithelial cells relative to heterologous expression systems (11), epithelial cells remain unable to fold and mature the ΔF508 mutant, and as a result, functional CFTR is absent from their apical membranes. This leads to a lack of regulated salt and water secretion, luminal dehydration, and secondary events that produce inflammation and fibrosis in the lungs and pancreas (12, 13).

A number of chaperones that interact with and assist in the folding of WT and ΔF508 CFTR during their biogenesis have been identified (14), including the cytosolic Hsp70, Hsp90, and Hsp40 chaperones and their cooperating proteins (15–19), and the ER luminal chaperone calnexin (14, 20). However, when CFTR folding and ER export do not succeed, ubiquitin ligases are recruited to divert the protein to degradation pathways (18, 21). Although the limiting steps in the folding and assembly of this multidomain protein are coming to light (22–24), the ERAD components that recognize misfolded CFTR for ER extraction and ubiquitylation have been largely unclarified.

The AAA-ATPase, mammalian p97 (Cdc48 in yeast), together with its co-factors, Ufd1 and Npl4, are thought to play a central role in dislodging ubiquitylated proteins from the ER and transferring them to the cytosol for proteasome-mediated ERAD, ER associated degradation; HEK, human embryonic kidney; Hsp, heat shock protein; shRNA, short hairpin RNA; GFP, green fluorescent protein; DMEM, Dulbecco’s modified Eagle’s medium; IP, immunoprecipitation; E3, ubiquitin-protein isopeptide ligase; HA, hemagglutinin.
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degradation (1). The Cdc48-interacting protein, Der1p, mediates the degradation of a class of misfolded proteins in yeast (25). Its counterpart in mammalian cells, Derlin-1, couples cytomegalovirus-induced degradation of major histocompatibility class I heavy chain to p97, assisting the virus in avoiding detection by the immune system (2, 3). Thus, these components link substrate recognition in the ER to protein degradation in the cytosol. Despite the significance of Derlin-1 in virus-induced heavy chain degradation, however, physiologically relevant ERAD substrates for this pathway have not been described in mammalian cells.

Here we show that CFTR is targeted to proteasome-mediated degradation by Derlin-1 with high efficiency; this is particularly true for the common disease-causing mutant ΔF508 CFTR. The preference of Derlin-1 for degradation of N-terminal CFTR fragments corresponding to its first transmembrane (TM1) domain and its ability to degrade a CFTR disease mutant within TM1 suggest that Derlin-1 and its associated proteins constitute an early checkpoint that identifies misfolded CFTR (TM1) domain and its ability to degrade a CFTR disease mutant.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Calu-3 cells were cultured in Dulbecco’s modified Eagle’s (DMEM)/Ham’s F-12 media (Sigma) supplemented with 15% fetal bovine serum. HEK 293 and COS-1 cells were cultured in DMEM with 10% fetal bovine serum. All cells were maintained in a humidified atmosphere containing 5% CO₂ at 37 °C.

**Antibodies**—CFTR antibodies (M3A7, MM13-4, and 24-1) were purchased from Chemicon and R & D Systems. The antibody against Derlin-1 was described previously (3) and was a generous gift of Drs. Yihong Ye (National Institutes of Health) and Tom Rapoport (Harvard University). Antibodies against p97, the HA epitope, GFP, and ubiquitin were obtained from RDI, Abcam, Convance, Invitrogen, and Stressgen, respectively. All other antibodies were obtained from Sigma.

**Plasmid Constructs and Transfections**—N-terminal Myc-tagged or C-terminal HA-tagged Derlin-1 was amplified from an expression vector and subsequently cloned into pcDNA3.1. The results obtained using these tagged Derlin-1 constructs did not differ significantly from one another or from those obtained with the untagged protein. Wild type and ΔF508 CFTR constructs were described previously (19). CFTR C-terminal deletion constructs, denoted by the amino acid at which they terminate, were amplified by PCR and cloned into pcDNA3.1. The primers used to generate CFTR C-terminal deletions were as follows: 5’-GGTACCAGACATGCAGAGGTCGCCCTCTG (forward), 5’-CTCGAGCTACCAAGTTAGC CCCATG (216X, reverse), 5’-CTCGAGCTATTGTATTTAT GCTCCAAAG (370X, reverse), 5’-CTCGAGCTATATTTCT TTTTCTGTTAAAAC (588X, reverse), and 5’-CTCGAGCTA CTCCTTTTAAGCTTCTCTTTC (831X, reverse). CFTR G85E was generated using the site-directed mutagenesis kit (Stratagene) and the forward primer 5’-GAGGTATTTGTTCTATGAAATCT TTTTATATTTAGGG (where the underlined GAA represents the base change that produces the mutation from glycine to glutamine at position 85). All plasmid constructs were confirmed by DNA sequencing.

**Transfections and RNA Interference**—Cells were transiently transfected using Lipofectamine 2000 (Invitrogen). Transfections involving co-expression were carried out at 1:1 plasmid molar ratio. For the RNA interference experiments, HEK 293 cells were transiently transfected with a vector containing a short hairpin RNA (shRNA) targeting Derlin-1 (30), which was kindly provided by Dr. Hidde Ploegh (Whitehead Institute). Two days after shRNA transfection, the cells were retransfected with the shRNA vector and WT or ΔF508 CFTR cDNAs. Cell lysates were prepared 2 days after retransfection and subjected to immunoblotting.

**Detergent Solubility and Microsome Fractions**—Soluble and insoluble fractions from transfected HEK 293 cells were described previously (8). Briefly, cells were lysed with buffer A (10 mM Tris-HCl (pH 7.4), 5 mM EDTA, 1% Triton X-100 with protease inhibitors), and the lysates were centrifuged at 15,000 × g for 20 min. The supernatant (soluble fraction) was collected, and the pellet (insoluble fraction) was washed twice with buffer A and then solubilized in SDS sample buffer with a brief sonication. For preparation of microsome and cytoplasmic fractions, transfected HEK 293 cells were fractionated as described previously (26). Briefly, the cells were suspended in hypotonic buffer (10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 1 mM EDTA, and protease inhibitors) and homogenized. Postnuclear supernatants were obtained by centrifugation (14,000 × g for 5 min). The postnuclear supernatants were then subjected to centrifugation at 100,000 × g for 60 min to collect the supernatant (cytosolic fraction) and pellet (microsomal fraction). Cytosolic proteins were concentrated by precipitation using 10% trichloroacetic acid and then resuspended in lysis buffer (50 mM HEPES (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 10% glycerol) with 1 mM NaOH. Membrane fractions were resuspended in lysis buffer directly.

**Pulse-Chase Assays**—Transfected HEK 293 cells were starved in methionine- and cysteine-free DMEM for 30 min, and then metabolically labeled with Redivue Pro-mix L-[35S]Met and -Cys (120 μCi/ml; Amersham Biosciences) for 30 min at 37 °C. Cells were washed twice with phosphate-buffered saline and lysed immediately or incubated in complete DMEM for the indicated chase times. Cell lysates were pre-cleared with protein A/G-agarose, and immunoprecipitation was performed as described (27) using CFTR antibodies M3A7 and 24-1, which are directed at epitopes within the second nucleotide binding domain (NBD2) and the CFTR C terminus, respectively. The immunoprecipitates were analyzed using SDS-PAGE and autoradiography.

**Immunoprecipitations and Immunoblots**—The immunoblot and the immunoprecipitation (IP) protocols were described previously (27). For re-immunoprecipitations, immunocomplexes from the first IP were dissolved in 30 μl of sample buffer with 2% SDS to dissociate the complex. After 10 min of incubation at 37 °C, the samples were diluted in 600 μl of lysis buffer with 2 mg/ml bovine serum albumin (final SDS concentration is ~0.1%). The diluted samples were then subjected to a second IP with the indicated antibody.
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Confocal Microscopy—Immunofluorescent staining of COS-1 cells was performed as described previously (26, 27). After fixation in 2% paraformaldehyde and permeabilization with 2% paraformaldehyde plus 0.1% Triton X-100, cells were washed three times with buffer B (0.5% bovine serum albumin and 0.15% glycine at pH 7.4) in phosphate-buffered saline. After blocking with purified goat serum, cells were incubated with the appropriate primary antibodies for 1 h, followed by three washes in buffer B and subsequent incubation with Alexa 488- (green) or Alexa568 (red)-labeled secondary antibodies (Molecular Probes) for 1 h. After washing with buffer B, the coverslips were mounted for confocal microscopy.

RESULTS

CFTR Interacts Physically with Derlin-1 and p97 in Airway Cells—To determine whether CFTR is a substrate for Derlin-1/ p97-mediated degradation, we first asked if these components interact physically in human airway cells that endogenously express these proteins. Both Derlin-1 and p97 were present in CFTR immunoprecipitates obtained from Calu-3 airway cell lysates (Fig. 1, A and B) and not in those obtained using an irrelevant IgG. Reciprocal immunoprecipitations confirmed the presence of CFTR in the Derlin-1 and p97 precipitates (Fig. 1, C and D) and showed that these proteins associated with different ubiquitylated forms of CFTR. Derlin-1 interacted with immature (band B) CFTR (Fig. 1C), despite the predominant expression of mature, glycosylated CFTR (band C) in these cells (lower panel, Fig. 1A). The lower molecular mass CFTR species present in the Derlin-1 precipitate likely represent degradation product(s) (see also Fig. 3B) (21).

CFTR immunoreactivity in the p97 immunoprecipitate demonstrated its association with immature CFTR (Fig. 1D, left panel) and also with CFTR derivatives of higher molecular mass. The higher mass CFTR species associating with p97 were ubiquitylated, as was indicated when the precipitate was re-solubilized, re-precipitated with anti-CFTR, and blotted with an antibody to ubiquitin (Fig. 1D, right panel). Thus, by virtue of its interactions with Derlin-1 and p97, CFTR has the potential to be a substrate for this degradation pathway. Because Derlin-1 is an integral membrane ER protein (2, 3), it recognizes CFTR in the ER membrane, whereas p97 associates with ubiquitylated CFTR, a modification that occurs in the cytosol (8, 9). Therefore, these data suggest that CFTR ubiquitylation takes place at steps intervening in its association with Derlin-1 and p97.

Derlin-1 Interferes with WT and ΔF508 CFTR Biogenesis—Because Derlin-1 associated with immature CFTR (Fig. 1D, left panel), we evaluated its ability to initiate WT and ΔF508 CFTR degradation by expressing both proteins in HEK 293 cells. Representative immunoblots for WT and ΔF508 CFTR are illustrated in Fig. 2A, and quantitation of data from three experiments is provided in Fig. 2B. On average, Derlin-1 reduced the expression of immature and mature WT CFTR by 60 and 80%, respectively. Moreover, Derlin-1 co-expression had a profound effect on steady-state ΔF508 CFTR, reducing levels of the immature protein by 95% (Fig. 2, A and B). These findings suggest that Derlin-1 degrades misfolded CFTR and that it very efficiently disposes of the ΔF508 folding mutant. The Derlin-1-induced reductions in CFTR expression were offset by treatment of the cells with the proteasome inhibitor MG132 (Fig. 2A, right panel). This finding indicates that Derlin-1 couples CFTR degradation to the ubiquitin-proteasome pathway, as observed for yeast and virus-mediated protein degradation mediated by Derlin-1 (1–3).

Derlin-1 Promotes the Rapid Degradation of ΔF508 CFTR—The effect of Derlin-1 on the kinetics of WT and ΔF508 CFTR biogenesis was examined in metabolic labeling, pulse-chase experiments (Fig. 2C). Under control conditions, WT CFTR appeared initially as the immature protein, and subsequently about 40% of the initially labeled, core-glycosylated protein was detected as the complex glycosylated, mature form, which traffics to the plasma membrane (Fig. 2C, right upper panel) (8, 9). In contrast, ΔF508 CFTR was quantitatively degraded without
detectable formation of a mature protein (Fig. 2C, right lower panel).

Derlin-1 co-expression virtually eliminated the glycolytic maturation of WT CFTR, markedly reducing the conversion of the protein from the immature to the mature form. This result suggests that CFTR is not encountering Golgi glycosylation sites in Derlin-1-expressing cells and is largely retained in the ER (Fig. 2C, upper panels). This outcome for WT CFTR is likely
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the result of two actions of Derlin-1. First, the amount of full-length CFTR synthesized during pulse labeling is reduced (discussed further below) so that less WT CFTR is available for maturation. Second, WT CFTR is retained in the ER, probably through its physical interaction with Derlin-1 (Fig. 1), as it awaits degradation. This conclusion is consistent with cellular localization data obtained from cells co-expressing Derlin-1 and CFTR (see Fig. 2D, below). For ΔF508 CFTR, Derlin-1 co-expression induced a more rapid degradation of the immature mutant protein (Fig. 2C, lower panels), as reflected by a reduction in its half-life from 60 to 30 min.

Another indication of rapid Derlin-1-mediated CFTR degradation was the significant decrease in the formation of full-length immature WT and ΔF508 CFTR observed at the completion of the 30-min pulse period in Derlin-1-expressing cells (chase time = 0) (Fig. 2C). Relative to their labeling in control cells, Derlin-1 reduced immature CFTR formation 65% for WT and 85% for ΔF508 CFTR. Because the epitopes of the antibodies used for the IPs lie at the CFTR C terminus (see “Experimental Procedures”), they detect only the full-length proteins. These data suggest that Derlin-1 interferes with the formation of full-length CFTR, perhaps by promoting the degradation of partial length CFTR fragments during their translation, but this will require further study. The present findings clearly indicate that immature ΔF508 CFTR is a preferred substrate for Derlin-1, as evidenced by the steady-state and pulse-chase data.

Derlin-1 and CFTR Co-localize in the ER—The cellular locations of WT CFTR and Derlin-1 were examined by immunofluorescence microscopy of HEK 293 cells co-expressing these proteins (Fig. 2D). When CFTR alone was expressed (Fig. 2D, panel a), it localized to the perinuclear region and plasma membrane. The co-expression of Derlin-1 limited CFTR expression to the perinuclear region, primarily to ER membrane dilations or puncta (Fig. 2D, panel b), whereas co-expressed Derlin-1 showed a broader ER distribution (panel c). Virtually all CFTR co-localized with Derlin-1 in the ER membrane dilations, as shown in the merged image (Fig. 2D, panel d). These structures resemble previously described ER sub-compartments that contain Derlin-1, p97, their binding partner VIMP, and the ER membrane-associated E3 ubiquitin ligase Hrd1 (3, 28). Thus, it is likely that the structures observed in Fig. 2D bring together not only CFTR and Derlin-1 but also p97 and one or more ubiquitin ligases in a degradation complex.

Derlin-1 Decreases CFTR Aggregation—CFTR is not folded efficiently in most cells (8, 9), and the nondegraded fraction accumulates as detergent-insoluble aggregates (29). To evaluate the effect of Derlin-1 on CFTR aggregate formation, lysates from HEK 293 cells expressing WT or ΔF508 CFTR were separated into Triton X-100-soluble and -insoluble fractions as described under “Experimental Procedures.” 25 μg of protein from the soluble (A) and equal amounts from the insoluble (B) fractions, normalized to the soluble fraction volume, were resolved by SDS-PAGE and subjected to immunoblot (IB) with the indicated antibodies. Higher molecular mass proteins likely represent ubiquitylated CFTR. Calnexin (CNX) and Hsp90 served as microsomal and cytosolic markers, respectively. The results are representative of data from three experiments.

We also prepared microsomal and cytosolic fractions from cells expressing WT or ΔF508 CFTR, without the use of detergent, to evaluate the possibility that Derlin-1 expression was recognizing CFTR in the ER and, together with its degradation complex partners, transferring it to the cytosol as a means of limiting aggregate formation. As shown in Fig. 3C, Derlin-1 decreased the high molecular mass CFTR species found in microsomes, and they did not appear in the cytosolic supernatants from Derlin-1-expressing cells. These findings further suggest that Derlin-1 promotes the efficient ER dislocation and degradation of misfolded WT and mutant CFTR, obviating the accumulation of ubiquitin-modified protein. The absence of CFTR in the cytosol of Derlin-1-expressing cells suggests also that the coupling between the Derlin-1-p97 complex and proteasome is tight, so that CFTR is not detected as a cytosolic intermediate in this process.

Derlin-1 Knockdown Increases CFTR Biogenesis—To examine the role of endogenous Derlin-1 in CFTR degradation, we used RNA interference to reduce its expression. shRNA targeting Derlin-1 was transfected at different doses into HEK 293 cells, together with WT or ΔF508 CFTR, and their expression levels were examined by immunoblot. This shRNA was shown previously to reduce the expression of Derlin-1 in U373 cells, without affecting other components of the Derlin-1-p97 complex (30). As illustrated in Fig. 4A, relative to control, this construct reduced the expression of endogenous Derlin-1 between 50 and 70%, depending on the dose employed. With this degree of Derlin-1 knockdown, the steady-state level of immature WT CFTR increased nearly 10-fold (Fig. 4B), whereas the level of
mature CFTR increased nearly 5-fold. The levels of immature ΔF508 CFTR increased 6- and 15-fold with Derlin-1 knockdown. Thus, Derlin-1 knockdown resulted in increases in WT and ΔF508 CFTR biogenesis in a dose-dependent manner. Although Derlin-1 knockdown markedly increased the levels of immature ΔF508 CFTR, this did not lead to significant maturation of the mutant protein. This finding is similar to results obtained from inhibition of proteasome-mediated ΔF508 CFTR degradation, which also does not rescue maturation of the mutant (8, 9). Nevertheless, these findings indicate that endogenous Derlin-1 is involved in the degradation of both WT and mutant CFTRs.

**DISCUSSION**

This work demonstrates the involvement of Derlin-1 in the degradation of the physiologically relevant mammalian ERAD substrate, CFTR. Derlin-1 and its degradation complex partner, p97, interacted physically with WT CFTR and reduced its maturation, and Derlin-1 initiated the efficient ER degradation of two CFTR folding mutants, ΔF508 and G85E. The significance of endogenous Derlin-1 in CFTR degradation was demonstrated in RNA interference experiments, which showed that reducing Derlin-1 markedly increased the expression of WT and ΔF508 CFTR. These findings indicate that the Derlin-1/p97 pathway represents a significant early checkpoint in the recognition of disease-associated mutations that impair CFTR biogenesis. Recent data support the involvement of p97 in CFTR degradation, but they did not identify the ERAD pathway that provides for recognition of misfolded CFTR (33).

Prior studies of yeast (34–36) and virus-induced mamma-
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FIGURE 5. Derlin-1 promotes the degradation of N-terminal CFTR fragments. A, schematic diagram of the C-terminal CFTR truncation constructs. The numbers indicate the position of the start and terminal amino acid residues. TM1, transmembrane domain 1; NBD1, nucleotide binding domain 1 (WT or ΔF508); RD, regulatory domain. B, HEK 293 cells were transfected with the indicated plasmids. Equal amounts of total protein (25 μg) from each sample were resolved and subjected to immunoblot with an antibody against the CFTR N terminus (amino acids 25–36; clone MM13-4). C, quantitation of CFTR fragment expression; results are given as the percent CFTR fragment density with Derlin-1 co-expression relative to the GFP control (n = 3). D, CFTR folding mutant G85E was co-expressed with GFP or Derlin-1 in HEK 293 cells, as in Fig. 2A. Cell lysates were resolved by SDS-PAGE and immunoblotted (IB) with anti-CFTR.

This respect, Derlin-1 has been shown to interact with the membrane-associated E3 ligases Hrd1 and gp78 (28, 30). Thus, an associated E3 ligase is an anticipated component of the complex, acting to ubiquitylate CFTR once it is selected by Derlin-1 for degradation. During the review of this work, Younger et al. (40) provided evidence implicating RMA-1 and Ubc6 as ER resident E3 ubiquitin ligase and ubiquitin carrier protein that act at early steps in CFTR degradation, and these proteins were found to physically associate with Derlin-1. It is likely that the ER membrane puncta containing CFTR and Derlin-1 (Fig. 2D) represent degradation staging areas that have accumulated overexpressed CFTR and also contain additional components of the degradation machinery. The absence of cytosolic CFTR in Derlin-1-expressing cells suggests that these components are tightly linked to p97 and the proteasome. The preferred substrate for Derlin-1-mediated degradation was ΔF508 CFTR. Its steady-state levels were reduced 95% by Derlin-1 expression, and the rate of degradation of immature ΔF508 CFTR was doubled by Derlin-1 in pulse-chase experiments. Although decreases in Derlin-1 expression led to a marked accumulation of immature ΔF508 CFTR, they did not produce a corresponding progression of the protein to the mature form. This suggests that additional downstream checkpoints arrest the progression of ΔF508 CFTR when it passes the Derlin-1 filter, and that this step may not represent a viable target for the pharmacological rescue of folding mutants like ΔF508. Nevertheless, it is noteworthy that the interaction with Derlin-1 involves nonubiquitylated CFTR, indicating that the process of ubiquitin addition lies distal to the recognition step at which CFTR goes off-pathway. Unlike the results obtained with conditions that interfere with proteasome activity or with ubiquitin addition (9), however, Derlin-1 knockdown resulted in the accumulation of soluble CFTR, which in principle remains in a foldable state. This suggests that the actions of pharmacophores that assist with CFTR folding (41,
42) would be augmented by disrupting the interaction of CFTR with Derlin-1.

The structure-based principles responsible for recognition of misfolded proteins by Derlin-1 are as yet unknown. Within the extracellular loops of the TM1 of CFTR, few amino acids are predicted to reside within the ER lumen, so little structure is available to provide a luminal misfolding signal (43). Rather, the avid degradation of G85E and TM1 fragments by Derlin-1 suggests that the amino acid residue at position 508 in the membrane proteins.

Experiments involving amino acid substitutions at position 508 of CFTR NBD1 have suggested that the amino acid residue at this site may stabilize TM1 through an interdomain interaction. In agreement with this idea, the stabilizing effect of add-
tible) for shRNA constructs targeting Derlin-1 expression.

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