Localization of Sequences within the C-terminal Domain of the Cystic Fibrosis Transmembrane Conductance Regulator Which Impact Maturation and Stability*

Martina Gentzsch and John R. Riordan‡

From the Mayo Foundation, S. C. Johnson Medical Research Center, Mayo Clinic Scottsdale, Scottsdale, Arizona 85259

Some disease-associated truncations within the 100-residue domain C-terminal of the second nucleotide-binding domain destabilize the mature protein (Haardt, M., Benharouga, M., Lechardeur, D., Kartner, N., and Lukacs, G. L. (1999) J. Biol. Chem. 274, 21873–21877). We now have identified three short oligopeptide regions in the C-terminal domain which impact cystic fibrosis transmembrane conductance regulator (CFTR) maturation and stability in different ways. A highly conserved hydrophobic patch (region I) formed by residues 1413–1416 (FLVI) was found to be crucial for the stability of the mature protein. Nascent chain stability was severely decreased by shortening the protein by 81 amino acids (1400X). This accelerated degradation was sensitive to proteasome inhibitors but not influenced by brefeldin A, indicating that it occurred at the endoplasmic reticulum. The five residues at positions 1400 to 1404 (region II) normally maintain nascent CFTR stability in a positional rather than a sequence-specific manner. A third modulating region (III) constituted by residues 1390 to 1394 destabilizes the protein. Hence the nascent form regains stability on further truncation back to residues 1390 or 1380, permitting some degree of maturation and a low level of cyclic AMP-stimulated chloride channel activity at the cell surface. Thus while not absolutely essential, the C-terminal domain strongly modulates the biogenesis and maturation of CFTR.

The cystic fibrosis transmembrane conductance regulator (CFTR) is a large multidomain membrane protein that forms a tightly regulated chloride channel in the apical membrane of many chloride secreting and reabsorbing epithelial cells (1, 2). As an adenine nucleotide binding cassette (ABC) protein it contains two nucleotide-binding domains (NBD1 and NBD2) and two transmembrane domains (TMD1 and TMD2), each spanning the membrane several times. CFTR also has a large R-domain between NBD1 and TMD2 which is not common to other ABC proteins and is the site of regulatory phosphorylation and dephosphorylation (3–6). Additionally there are N- and C-terminal cytoplasmic domains of less than 100 amino acids each that precede TMD1 and follow NBD2, respectively. The former has recently been shown to participate in channel regulation by interacting with the R-domain (7). The C-terminal domain, however, is apparently not essential to channel function (8–10) but the very C terminus can tether the protein to PDZ domain containing proteins (11, 12), possibly to localize CFTR within regulatory complexes.

Wild-type CFTR matures very inefficiently following synthesis on membrane-bound ribosomes (13) and many disease-associated mutations in different domains preclude the formation of any mature protein that can be transported to the cell surface. Hence, it is important to understand the role of each domain of the molecule in achieving and maintaining a mature and stable conformation. Frameshift or premature stop mutations found in patients with cystic fibrosis that cause truncations at several locations in the C-terminal domain were shown to destabilize the mature CFTR protein so that its lifetime was greatly shortened (10). We have now made systematic stepwise truncations as well as deletions and substitutions across the entire C-terminal domain and identified different short sequences which strongly influence not only the stability of the mature protein but also the maturation and stability by the nascent chain. The major determinant of the steady state amount of mature protein is a hydrophobic “patch” formed by residues 1413 to 1416 whereas there are strong “positional effects” on the nascent chain of sequences closer to NBD2. Truncation back to residue 1400 resulted in an extremely unstable nascent chain, degraded at the endoplasmic reticulum (ER) by the proteasome. Strikingly, on further truncation back to residues 1390 or 1380 the nascent protein regains stability such that some mature protein is again formed that mediates a low but detectable level of eAMP-stimulated chloride efflux. These findings reveal that C-terminal motifs and their positioning have a major impact on the assembly and stability of the CFTR ion channel.

EXPERIMENTAL PROCEDURES

Plasmid Construction—All C-terminal truncations were constructed by polymerase chain reaction introducing a TAG stop codon flanked by an ApaI restriction site. The polymerase chain reactions were performed on pBQ4.7 CFTR plasmid DNA. The antisense primers introducing the stop codon were the following: A1440X, 5′-TGATATCGGGCCCTATTGGGAAAGGGTCTCTCGT-3′; S1435X, 5′-TGATATCGGGCCCTACCTCTCTCTCAGAGTCTTGAGTGATGG-3′; L1430X, 5′-TGATATCGGGCCCTATTTTCTGGATGAGGACTGACTGCCGACC-3′; D1425X, 5′-TGATATCGGGCCCTACTAGACTGGCGCCAGTCTTCACTTCTTGATG-3′; K1420X, 5′-TGATATCGGGCCCTACTTCTCCTCTCTTTATGACAAAAATTGGTTGGGC-3′; V1415X, 5′-TGATATCGGGCCCTACTTCTCCTCTCTTTATGACAAAAATTGGTTGGGC-3′; C1410X, 5′-TGATATCGGGCCCTACTTCTCCTCTCTTTATGACAAAAATTGGTTGGGC-3′;
Stable Expression of Mutant and Wild-type CFTR in BHK Cells—

Metabolic Pulse-Chase Labeling—Stably transfected BHK-21 cells were starved in methionine-free medium for 30 min, labeled for 20 min with 0.1 μCi/ml [35S]methionine, and chased with Dulbecco’s modified Eagle’s medium’s F-12 supplemented with 1 μM methionine and 5% fetal bovine serum. Cell lysates and immunoprecipitations were performed as described before (15). With the exception of the 1380X CFTR mutant, all immunoprecipitations were performed using the monoclonal antibody M37 (16). Since the molecule 1380X does not contain the complete M37 epitope, it was immunoprecipitated with the L12B4 antibody (16). The amount of 35S-radioactivity in each band was quantified by electronic autoradiography using a Packard Instant Imager.

Treatment with Brefeldin A and Protease Inhibitors—Brefeldin A (BFA), lactacinin, N-acetyl-leucinyl-leucinyl-norleucinal (ALLN), N-acetyl-leucinyl-leucinyl-norleucinal (NLVS), and 4-hydroxy-5-iodo-3-nitrophenylacetyl-leucinyl-leucinyl-vinylsulfone (NLVS) were added to cells 90 min prior to methionine starvation to a final concentration of 10 μM (BFA) and 50 μM (NLVS) or 50 μM (lactacinin, MG132, and ALLN) and were present during the pulse labeling and chase period.

Immunofluorescence Microscopy—Cells were grown on coverslips and fixed in 100% methanol at −20 °C for 10 min. CFTR was detected by indirect immunofluorescence as described previously (17) using the mouse monoclonal antibody M37 (16) or BJ570 which recognizes the R-domain and visualized with Alexa Fluor 488 goat anti-mouse IgG conjugate (dilution 1:250, Molecular Probes). The plasma membrane Ca2+-ATPase was detected by the mouse monoclonal antibody 5F10 (Affinity Bioreagents, Inc.) and visualized with Alexa Fluor 594 goat anti-mouse IgG conjugate (dilution 1:250, Molecular Probes). To visualize CFTR and the plasma membrane ATPase simultaneously, the mouse monoclonal anti-CFTR antibody BJ570 was biotinylated with NHS-biotin (Pierce) as described by Harlow and Lane (18) and detected with streptavidin-Alexa Fluor 488 conjugate (dilution 1:1500, Molecular Probes).

RESULTS

Influence of C-terminal Truncations on Steady State Amounts of Immature and Mature CFTR—Haardt et al. (10) had previously shown that truncations at several positions within the C-terminal domain destabilized mature CFTR. We decided to further elucidate sites within the C-terminal domain that impact maturation and stability of both nascent and mature CFTR. As shown in Fig. 1A, there is an apparent effect on the steady state amounts of the immature, core-glycosylated form of CFTR (laborer band) or the mature form with complex oligosaccharide chains (upper band). Apparently no major changes have occurred between the 1200 and 1400 residue positions.

2 X.-B. Chang and J. R. Riordan, unpublished data.

3 T. J. Jensen, B. G. Bone, and J. R. Riordan, unpublished data.
in these amounts occur when truncation of as many as 61 residues occurs, \textit{i.e.} to form 1420X. However, truncation of a further five residues from this point resulted in a major reduction in the amount of the mature band (1415X). Shortening the C-terminal region by an additional five residues (1410X) nearly eliminated the mature protein band. Hence a portion of the polypeptide between residues 1410 and 1419 appears to play an important role in the formation or maintenance of mature CFTR. The intensity of the immature band also appeared to be significantly reduced on shortening from 1415X to 1410X (Fig. 1C). The next three incremental shortenings to 1405X, 1400X, and 1395X were without apparent additional effect; the mature band was barely detectable and the immature band remained fainter when compared with 1415X and was weakest at 1400X.

Strikingly, deeper truncation to 1390X resulted in a significant increase in the amounts of both bands, the immature band appearing nearly as strong as in the much longer constructs and the mature band as strong as at 1415X. Further shortening to 1380X resulted in a still larger amount of the mature band. To ensure that the mature form of CFTR reach the plasma membrane surface, labeling was carried out with the membrane-impermeable reagent biotin-LC-hydrazide (Fig. 1D). Mature forms of 1390X CFTR and 1380X CFTR were found to be biotinylated. These observations suggest that some sequence C-terminal of residue 1390 is actually destabilizing and emphasizes the fact that CFTR biogenesis and maturation can occur reasonably effectively in the complete absence of the C-terminal domain beyond NBD2. However, certain short regions within the domain have strong modulating effects, both positive and negative. At least three different short segments of the C-terminal domain have significant impact on the steady state amounts of immature and mature CFTR observed.

\textbf{Immunofluorescence Localization of C-terminal Truncated CFTR Variants—} The influence of these truncations on the intracellular localization of CFTR in stably transfected BHK-21 cells was evaluated by immunofluorescence microscopy. Simultaneous staining with an antibody to the plasma membrane Ca\textsuperscript{2+}-ATPase showed that wild-type CFTR is detectable as a uniform staining over the entire cell surface, some of which is punctate in nature (Fig. 2A). Clearly visible beneath the surface staining was a more intense perinuclear pattern corresponding well with ER localization in these cells. Overall this distribution was similar to that observed with CFTR expression in other nonpolar mammalian cells and the same as that observed in BHK cells expressing a CFTR-green fluorescent protein fusion (15). Truncation to remove the last 41 C-terminal amino acids (1440X) did not change the wild-type picture (Fig. 2B). However, shortening by 20 more residues to 1420X resulted in some decrease in cell surface staining while perinuclear staining remained intense. With the 1400X truncation only a very circumscribed perinuclear pattern was seen, consistent with the presence of primarily the core-glycosylated immature band seen in immunoblots (Fig. 1) and immunoprecipitates (Fig. 3). Shortening by a further 10 or 20 residues to...
1390 or 1380 caused the reappearance of weak staining over the cell surface. Hence there was good correspondence between the relative amount of the mature CFTR band detected in Western blots of whole cell lysates (Fig. 1C) and cell surface staining by immunofluorescence among the different truncations (Fig. 2).

**Influence of C-terminal Truncations on the Turnover of Immature and Mature Forms of CFTR—**Pulse-chase experiments were performed to determine the kinetic effects of each of these truncations on nascent chain maturation or degradation and on the lifetime of the mature molecule once it was formed (Fig. 3). As has been consistently observed in a variety of different mammalian cell types, wild-type CFTR matures inefficiently with 40% or less of the pulse-labeled immature band converted to the mature band during the chase. This maximal conversion occurs in ~2 h after which little or no immature band is detectable and the amount of radioactively labeled mature band remains nearly constant until 4 h since it has a half-time of ~16 h in these cells (13, 24). Other observations showed that the rate of disappearance of the immature band is slowed by proteasome inhibitors without augmenting the amount that is converted to the mature product (25, 26) suggesting that a large proportion (in this case ~60%) of nascent chain was degraded by the proteasome. Truncations to produce 1440X, 1435X, 1430X, and 1425X did not drastically change this precursor-product relationship of nascent and mature CFTR. Notably the maximal proportion of the pulse-labeled nascent 1420X that matured was closer to 30% than 40% and the mature band decayed somewhat more rapidly than wild-type. As was already apparent from the Western blots (Fig. 1) truncation to 1415X had a more major impact. In the pulse-chase experiment the immature band disappeared somewhat more slowly, less mature band appeared and it then turned over more rapidly. The rates of nascent chain disappearance and mature form appearance and disappearance were similar to that of 1410X and 1405X, although the maximum proportion of mature protein formed decreased progressively with shortening. By 1400X this proportion was minimal (~7% at 1 h of chase). Strikingly, despite the fact that conversion to the mature form was barely detectable, the immature 1400X band disappeared extremely rapidly, i.e. shortening from 1405X to 1400X greatly accelerated the rate of disappearance of the nascent chain.

Unexpectedly, removal of a further 5 residues to produce 1395X seemed to cause a reversion to a situation more similar to 1405X than 1400X. A more pronounced restabilization and maturation was exhibited by 1390X. In fact the curve showing the rates of disappearance of the immature band were again quite similar to much longer variants including the wild-type as if residues C-terminal of 1390 destabilized the nascent chain. Formation and maturation of mature 1390X, however, remained considerably depressed. Further shortening to 1380X, essentially to the C-terminal end of NBD2, had little additional effect.

Overall these kinetic data confirm and extend the detection by the immunoblots of three different short segments, within the N-terminal portion of the C-terminal 100 residue domain of CFTR, which have major influences on the stability of the nascent and mature forms of the molecule. These segments lay within a 40-residue stretch immediately C-terminal of NBD2; the most C-terminal 60 residues have little influence on the turnover of immature and mature CFTR and presumably play other roles.

The three short segments delineated by the deletions are highlighted in Fig. 4A and are seen to be reasonably well conserved in CFTRs from different species. Numbering from the C-terminal end, these segments include region I, which when removed greatly reduced mature CFTR, consisting of amino acids 1413 to 1417, all of which except 1417E are hydrophobic, region II (1400–1404) which seems necessary for nascent chain stability, and region III (1390–1394) which has the opposite effect, i.e. destabilizes the nascent molecule.

The "Hydrophobic Patch" of Residues 1413–1416 in Region I Is Essential for the Formation and Maintenance of Mature CFTR—Haardt et al. (10) reported that naturally occurring C-terminal truncations increased the rate of turnover of the mature form. We observed that C-terminal truncation of 66 amino acids or more decreased the amount of mature protein drastically. Our truncations in steps of five residues suggested the involvement of region I. When all five residues from 1413 to

**Fig. 2.** Immunofluorescence localization of wild-type and C-terminal truncations of CFTR. Stably transfected BHK-21 cells grown on glass slides were fixed in 100% methanol, permeabilized in 0.1% saponin, and visualized by indirect immunofluorescence as described previously (17). A, CFTR localizes to plasma membrane and ER. CFTR was detected with biotinylated mouse monoclonal antibody BJ570 (footnote 3) and streptavidin Alexa Fluor 488 conjugate (Molecular Probes). The plasma membrane Ca2+-ATPase was visualized with the mouse monoclonal antibody SF10 (Affinity Bioreagents Inc.) and Alexa Fluor 594 goat anti-mouse IgG conjugate (Molecular Probes). B, C-terminal truncations influence localization of CFTR. CFTR was detected with the mouse monoclonal antibody BJ570 and visualized with Alexa Fluor 488 goat anti-mouse IgG conjugate (Molecular Probes).
1417 (FLVIE) were replaced by alanine, the effect was essentially the same as truncation after position 1410, i.e. no mature CFTR band was detected by Western blotting (Fig. 4B). To determine the crucial residues within region I, alanine substitutions were also made in pairs and individually. Each of the four contiguous hydrophobic residues appeared to contribute and substitution of the Phe1413-Leu1414 pair by alanines was nearly as detrimental as removing all four. Hence a short hydrophobic patch of at least two residues seems to be required for the appearance of the mature protein. Substitution of the glutamic acid at position 1417 was entirely without effect as was replacement of the nonconserved glutamate at 1418 and substitution of the two glutamines flanking this patch on the N-terminal side (Fig. 4A). In pulse-chase experiments just traces of the large band with complex oligosaccharides could be detected (less than 5%) which turned over rapidly (Fig. 4D). The nascent chain, however, persisted with a half-life longer than the wild-type protein. The absence of mature CFTR on substitution of this patch of hydrophobic residues was also observed by immunofluorescence microscopy where no cell surface staining could be detected (Fig. 4C).

Residues 1400–1404 (CEHRI) in Region II Contribute to Nascent CFTR Stability in a Positional Rather than a Sequence-specific Manner—The pulse-chase experiments in Fig. 3 had shown that nascent CFTR became extremely unstable (short-lived) on truncating at residue 1400. Further evidence that this truncation influences nascent CFTR turnover at the ER came from the fact that its rapid disappearance was not significantly influenced by treatment of cells with BFA (Fig. 5A) which is known to inhibit the trafficking of CFTR out of the ER without blocking conformational maturation (27, 28). The ubiquitin-proteasome pathway was shown to be responsible for the degradation of immature wild-type CFTR (25, 26). To study the role of the 26 S proteasome in the degradation of 1400X CFTR, we investigated the turnover of the truncated protein in the presence of various proteasome inhibitors in BFA-treated cells (Fig. 5B). The highly specific proteasome inhibitor lactacystin strongly stabilized the truncated protein. A significant delay in the turnover of 1400X CFTR was also achieved by the reversible peptide aldehyde inhibitors ALLN or MG132 and by the peptide vinyl sulfone NLVS, which recently has been demonstrated to be a useful inhibitor of the proteasome (29). In direct contrast to the influence of truncation after residue 1400, replacement of amino acids 1400 to 1404 with alanines allowed the nascent chain to be nearly as stable and mature nearly as effectively as wild-type (Fig. 5C). Thus these five residues must play an essential positional role rather than providing a precise sequence. This interpretation was supported by substituting these residues with alanines pairwise which yielded steady state amounts of immature and mature protein similar to wild-type and is also strengthened by the observation that deletion of this region (3CEHRI) impaired the processing of the protein (Fig. 5D). Although the relatively high degree of conservation of these five amino acids may seem surprising if they serve only as a spacer, from the point of view of protein stability alanines appear to adequately replace the native sequence.

Residues 1390–1394 (QAFAD) in Region III Destabilize Nascent CFTR—The continued incremental truncation of CFTR beyond residue 1395 had the unexpected effect of partially restoring the stability and maturation of nascent CFTR (Figs.
C-terminal CFTR Sequences Influencing Processing

FIG. 4. A, alignment of C-terminal sequence of CFTR from different species showing three regions which impact the stability of the protein. Each of the 3 shaded regions were those identified by the deletion mutants. In addition, this portion of CFTR contains two consensus sequences known to play a role in trafficking. This is a tyrosine-based signal at amino acid position 1424, defined as YXX*, where * is a hydrophobic amino acid, and a dileucine (LL) motif at amino acid position 1430/1431. Both sequences are endocytic sorting signals and mediate protein internalization from the cell surface (38, 39, 43). B, Western blot showing requirement of region I (hydrophobic residues 1413–1416) for mature CFTR stability. The amino acids indicated were replaced by alanine residues, wild-type CFTR (WT) is shown as a control. 10 μg of whole cell lysates were separated by 6% SDS-PAGE and analyzed by Western blotting using the antibody M3A7 (16). C, immunofluorescence staining showing the absence of CFTR at the cell surface on alanine substitution of these hydrophobic residues. Immunocolocalization was performed as described in the legend to Fig. 2 using the antibody B570. D, pulse-chase experiments showing that this substitution causes persistence of the immature form and lack of appearance of the mature form of CFTR. Pulse-chase experiments were performed as described in the legend to Fig. 3.

1–3), i.e. 1390X was more like wild-type than 1395X. Alanine substitutions en bloc were again used to assess the nature of the destabilizing effect of the residues between these points. The alanines seemed to serve as effectively as the native sequence to support stability and maturation (Fig. 6). Hence it appears that termination of the polypeptide with the native 1390–1394 sequence has a very negative effect which is not manifest when it is followed by more of the normal sequence. The fact that reasonable maturation and stability can be achieved in the entire absence of the last 91 or 101 residues of CFTR probably means that parts of this domain play different essential roles not fundamentally involved with determining the lifetime of either form of the protein.

Chloride Channel Activity of C-terminal Mutants—As mentioned, earlier work had already shown that CFTR retained some chloride channel activity after truncations of substantial portions of the C-terminal domain (8–10). However, we wished to determine whether those mutations having greatest impact on processing and stability had effects on channel function other than what might be expected from the steady state amounts of protein present in each case. Hence 36Cl efflux experiments were performed. Fig. 7 shows that the rates of efflux following elevation of cellular cyclic AMP levels were as great after truncation of the final 61 residues (1420X) as with wild-type, indicating that this portion of the protein is not directly involved in regulated channel function. Replacement of residues 1413–1416 (FLVI), however, resulted in only a slight efflux response that was delayed, consistent with the minimal amount of mature protein formed. A very similar result was obtained with the 1400X truncation. Interestingly small but slightly elevated responses were detected with the 1390X and 1380X variants where detectable amounts of mature protein were again formed. However, the amount of channel activity is considerably less than would be expected just on the basis of the amounts of mature protein produced by these two variants. It may not be surprising that truncation so close to NBD2 impairs regulated channel function.

DISCUSSION

The function of the 100-amino acid domain of CFTR, C-terminal of the second nucleotide-binding domain, has not been defined. Notably it extends ~40 residues beyond the C terminus of other human ABC proteins, even those of the same subfamily (Fig. 1B). The final four residues, DTRL bind to PDZ domain containing proteins (11, 12). This interaction has been proposed to position CFTR within multimolecular regulatory complexes (22, 23), possibly contributing to its control by protein kinase A (30–32) and enabling it to influence or respond to other proteins involved in epithelial salt transport such as epithelial Na+ conductance, the epithelial sodium channel (33, 34). Removing or substituting the terminal PDZ domain-binding amino acids has also been reported to prevent apical localization in epithelial cells (21, 35), although another ABC protein, P-glycoprotein, localizes to apical membranes (36, 37) without having a PDZ domain binding terminus. The studies that we have reported here indicate that neither the final four residues nor the remainder of the 40-residue extension play an important role in the biosynthetic processing and stability of wild-type CFTR.

Furthermore, the next 20 residues in the N-terminal direction similarly have little influence despite the fact that they include two trafficking signals. The tyrosine residue at position 1424 has been reported to be involved in CFTR endocytosis (38, 39) and the dileucine pair at positions 1430 and 1431 is comparable to those of the structurally related SUR1 protein and has been described as essential to its maturation and movement from the ER to the cell surface (40). In addition to the lack of influence of truncation at residue 1420, alanine substitution of Tyr1424 and Leu1430-Leu1431 also did not alter the steady state amount of nascent or mature CFTR (not shown). Hence the final 61 residues of CFTR apparently are not crucially involved in its biosynthetic assembly and stability.

In contrast, within the next 30 residues in the N-terminal direction there are at least three short stretches that strongly
impact the turnover of both immature and mature CFTR. Hydrophobic residues at positions 1413–1416 (region I) appear to be essential for the formation and maintenance of the mature protein. Substitution of as few as two of these residues prevents the appearance of mature CFTR. It is notable that hydrophobic residues are conserved at these four positions in all members of the CFTR/multidrug resistance-associated protein subfamily of ABC membrane proteins. Within the hydrophobic patch, the FL pair has the most striking impact. There are examples of pairs of hydrophobic residues mediating specific interactions of some membrane proteins with the secretory pathway machinery. The retrieval from the Golgi of type I proteins, especially those of the p24 family is dependent on binding to COPI mediated by a diphenylalanine pair in their cytoplasmic tails (41). Although the lipophilic residues in region I of CFTR might be needed for a crucial hydrophobic interaction, we have not yet determined if this is intra- or inter-molecular.

Truncation to residue 1400 or deletion of amino acids 1400–1404 caused lability of nascent CFTR but alanine substitutions of these residues did not. Hence this region must provide conservation of residues.
rect positioning of other parts of the domain rather than specific sequence information. In the absence of this positional effect, the nascent chain becomes more susceptible to degradation. The third short region comprised of residues 1390 to 1394 apparently also imparts a sequence-independent effect but in the opposite direction, i.e. termination of the molecule with these five residues causes it to be extremely unstable. When they are then removed, there is a degree of restoration of stability. A larger amount of nascent 1390X protein is present at steady state; it is degraded less rapidly than 1395X or 1400X and matures more effectively than they do.

Haardt et al. (10) considered disease-associated truncations at various sites in the C-terminal domain to be a novel class of mutations in which the defect is entirely due to accelerated degradation of the mature molecule. Indeed the premature stop, A1411X does remove region I and causes rapid turnover of mature CFTR. However, the frameshift 4326de1TC resulting in truncation at residue 1398 and the premature stop L1399X also remove region II and hence also compromise the stability and ability to mature of the nascent chain. We observed a stabilizing effect of truncations at residues 1390 or deeper. But even if a small amount of mature protein could be detected, the cAMP-stimulated chloride channel activity was very low and delayed when compared with wild-type responses. The small amount of mature protein may still be inadequate to allow sufficient chloride transport across the plasma membrane and this might explain why patients carrying frameshift 4279 insA, causing termination after residue 1384, have severe disease. It appears that all C-terminal truncation mutations do not fit into a single class. Our findings indicate that the effects are very much dependent on the precise location of the truncation. Another truncation detected in a family where the sweat gland may be the sole tissue effected, S1455X (42), would not influence the turnover of either form of CFTR. It does, however, remove the terminal PDZ domain binding motif and the loss of interactions mediated through it may be responsible for the phenotype (21).

In summary, these experiments have shown that although the C-terminal domain is not absolutely essential for the biosynthetic maturation of CFTR, several short sequences within the C-terminal domain to be a novel class of mutations in which the defect is entirely due to accelerated degradation of the mature molecule. Indeed the premature stop, A1411X does remove region I and causes rapid turnover of mature CFTR. However, the frameshift 4326de1TC resulting in truncation at residue 1398 and the premature stop L1399X also remove region II and hence also compromise the stability and ability to mature of the nascent chain. We observed a stabilizing effect of truncations at residues 1390 or deeper. But even if a small amount of mature protein could be detected, the cAMP-stimulated chloride channel activity was very low and delayed when compared with wild-type responses. The small amount of mature protein may still be inadequate to allow sufficient chloride transport across the plasma membrane and this might explain why patients carrying frameshift 4279 insA, causing termination after residue 1384, have severe disease. It appears that all C-terminal truncation mutations do not fit into a single class. Our findings indicate that the effects are very much dependent on the precise location of the truncation. Another truncation detected in a family where the sweat gland may be the sole tissue effected, S1455X (42), would not influence the turnover of either form of CFTR. It does, however, remove the terminal PDZ domain binding motif and the loss of interactions mediated through it may be responsible for the phenotype (21).

Acknowledgments—We thank April Mengos and Tim Jensen for technical assistance, Bradley Bone for antibody production and biontilylation, Susan Bond for preparation of the manuscript, and Marv Ruona for preparation of the graphics.