The ΔF508 Mutation Disrupts Packing of the Transmembrane Segments of the Cystic Fibrosis Transmembrane Conductance Regulator*

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The most common mutation in cystic fibrosis (deletion of Phe-508 in the first nucleotide binding domain (ΔF508)) in the cystic fibrosis transmembrane conductance regulator (CFTR) causes retention of the mutant protein in the endoplasmic reticulum. We previously showed that the ΔF508 mutation causes the CFTR protein to be retained in the endoplasmic reticulum in an inactive and structurally altered state. Proper packing of the transmembrane (TM) segments is critical for function because the TM segments form the chloride channel. Here we tested whether the ΔF508 mutation altered packing of the TM segments by disulfide cross-linking analysis between TM6 and TM12 in wild-type and ΔF508 CFTRs. These TM segments were selected because TM6 appears to line the chloride channel, and cross-linking between these TM segments has been observed in the CFTR sister protein, the multidrug resistance P-glycoprotein. We first mapped potential contact points in wild-type CFTR by cysteine mutagenesis and thiol cross-linking analysis. Disulfide cross-linking was detected in CFTR mutants M548C(TM6)/T1142C(TM12), T351C(TM6)/T1142C(TM12), and W569C(TM6)/W1145C(TM12) in a wild-type background. The disulfide cross-linking occurs intramolecularly and was reducible by dithiothreitol. Introduction of the ΔF508 mutation into these cysteine mutants, however, abolished cross-linking. The results suggest that the ΔF508 mutation alters interactions between the TM domains. Therefore, a potential target to correct folding defects in the ΔF508 mutant of CFTR is to identify compounds that promote correct folding of the TM domains.

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in the Caucasian population. It affects 1 in every 2500 live births, and 1 in every 25 people is a carrier (1). It is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (cfrtr). The gene product, CFTR, is a member of the ABC (ATP-binding cassette) transporter family. It is composed of 1480 amino acids and has the putative structure of an N-terminal cytosolic domain, two transmembrane domains (TMDs), two nucleotide binding domains (NBDs), one regulatory domain, a C-terminal cytosolic tail, and two active N-glycosylation sites on the fourth extracellular loop (2). To date there are more than 1300 mutations identified in the gene (CF mutation data base: www.genet.sickkids.on.ca/cftr). The mutations occur throughout the gene and are classified according to their molecular consequences (for review, see Refs. 3 and 4) such as null-production, processing defect, regulation defect, conductance defect, and promoter defect.

Deletion of phenylalanine 508 (ΔF508) is the most common CF mutation as it accounts for more than 90% of the clinical cases. ΔF508 is a processing-defective mutation. CFTR is first synthesized in the endoplasmic reticulum (ER), where it is core-glycosylated at its N-glycosylation sites and folds with the aid of molecular chaperones. Upon proper folding and passing the ER quality control system, the CFTR protein is then exported into the Golgi apparatus where CFTR and its sugar moieties undergo further modification before being targeted onto the cell surface. Processing defective CFTRs such as ΔF508-CFTR do not make it past the ER quality control system. Instead, they are retained in the ER in an inactive form (5) before rapid degradation by cytosolic proteasomes (6, 7).

The lack of processing and maturation onto the cell surface by processing mutants such as ΔF508 is the most common cause of severe CF. Other mutations located through out the molecule also cause defective maturation (8–11). Correction of defective processing of CFTR mutants would benefit patients with CF. An important observation is that the ΔF508 mutant has been shown to be functional if it can be induced to mature and be delivered to the cell surface. Maturation of ΔF508-CFTR can be promoted by incubation at low temperature, expression in the presence of high concentrations of glycerol, or by the co-expression in the presence of high levels of the N-terminal of CFTR (12–15). All of these rescue methods, however, are inefficient and are not feasible in a clinical setting. To devise an improved strategy to induce efficient maturation of CFTR-processing mutants, we must first understand the molecular basis of their defects. There is evidence that there are structural differences between wild-type and mutant ΔF508 CFTRs. Mutant ΔF508 as well as immature wild-type CFTR are more susceptible to protease digestion compared with mature protein (5, 16). Results from these protease digestion studies suggest that CFTR-processing mutants are not grossly misfolded but are trapped as partially folded intermediates that are...
structurally similar to immature wild-type protein.

Studies on the CFTR sister protein, P-glycoprotein (P-gp), suggest that one structural alteration caused by the presence of the ΔF508 mutation is incomplete packing of the transmembrane (TM) segments located at the interface between TMD1 and TMD2 (17). P-gp is another ABC transporter that functions as an ATP-dependent pump. It has been used as a model system to learn about how processing mutations affect CFTR because introduction of CF-type mutations into P-gp also cause the mutant proteins to be retained in the ER in a protease-sensitive state (18). The folding defects in P-gp, however, can be corrected by expressing the mutants in the presence of drug substrates (19). Characterization of the ΔF508-type P-gp mutant suggested that deletion of this residue in NBD1 disrupted packing of the TM segments by affecting the interaction between NBD1 and the first cytoplasmic loop that connects TM segments 2 and 3. This in turn disrupted interactions between TMD1 and TMD2 of P-gp.

Interactions between the two TMDs also appear to be critical for proper folding of CFTR since they appear to be the main contact points between the two halves of the molecule (20). In this study we used disulfide cross-linking analysis to map contact points between TMD1 and TMD2 of CFTR and then tested whether packing of the TM segments was altered by the ΔF508 mutation.

EXPERIMENTAL PROCEDURES

Construction of Mutants—The CFTR point mutations were constructed in fragments using PCR mutagenesis. The fragments were then fully sequenced before insertion to create full-length cDNA either constructed in fragments using PCR mutagenesis. The fragments were corrected by expressing the mutants in the presence of drug sensitive state (18). The folding defects in P-gp, however, can be tested whether packing of the TM segments was altered by the ΔF508 mutation.

Measurement of cAMP-stimulated Iodide Efflux—Stable CHO cell lines were grown to 90% confluence in triplicates in 6-well cluster tissue culture plates (Costar). Cells were incubated in the presence of 2 μM forskolin (Sigma) for 1 h before the levels of cell surface expression. After aspiration of the growth media, the cells were washed 3 times with 2 ml of loading buffer (136 mM Na+, 4 mM KNO3, 2 mM Ca(NO3)2, 2 mM Mg(NO3)2, 11 mM glucose, 20 mM HEPES, pH 7.4). The cells were then loaded with NaI by incubation at room temperature with 2 ml of loading buffer for 1 h in the dark. The cells were then washed 13 times at 1-min intervals with 0.5 ml of efflux buffer (136 mM NaNO3, 4 mM KNO3, 2 mM Ca(NO3)2, 2 mM Mg(NO3)2, 11 mM glucose, 20 mM HEPES, pH 7.4). Solutions from the first 10 washes were discarded. The last three samples were collected and used to create a prestimulation base line. Stimulation buffer (0.5 ml of efflux buffer plus 10 μM forskolin (Sigma)) was added to the cells and removed at 1-min intervals for 12 min. The aspirated solutions were collected and stored in the dark until all samples had been collected. The relative voltages of the samples were subsequently measured using an iodide-specific electrode (Analytical Sensors Inc.). Standard iodide curves were generated using NaI dissolved in efflux buffer to determine the iodide concentration in the samples for data analysis.

RESULTS

CFTR is synthesized in the endoplasmic reticulum (ER) (Fig. 1A) as a core-glycosylated immature protein (Fig. 1C, band B) that then matures as it passes through the Golgi where complex carbohydrate is added (Fig. 1C, band C). The protein is then delivered to the cell surface. The processing defective mutant ΔF508, however, is retained in the ER (Fig. 1B) as a core-glycosylated immature protein (Fig. 1C). Our goal was to determine whether the ΔF508 mutation in CFTR altered packing of the TM segments at the interface between domains TMD1 and TMD2 since this is the major contact point between the two halves of the molecule (20). Each domain is predicted to contain six TM segments, and the chloride channel through the membrane is predicted to lie at the interface of TMD1 and TMD2 (22). The first step was to map a contact point between the two domains. Our approach to map a contact point between TMD1 and TMD2 was to employ cysteine mutagenesis and reaction with thiol cross-linkers. We selected a disulfide cross-linking approach because it has successfully been employed to...
map contact points between the TM domains of P-gp, the CFTR sister protein (17, 23–27). Disulfide cross-linking analysis is very useful to examine contact points between the various domains of ABC transporters because the presence of an interdomain disulfide bond causes the protein to migrate slower on non-reducing SDS-PAGE gels (17, 28–30). Therefore, it is relatively easy to assay for the presence of a disulfide bond. A potential problem with performing disulfide cross-linking analysis with CFTR, however, is that it contains 18 endogenous cysteines. Mutation of such a large number of cysteines to serines or alanines could alter the structure and properties of the protein. In preliminary studies we found that mutation of all 18 cysteines to serines caused defective maturation (Fig. 1C). It appeared, however, that it would be possible to conduct cross-linking studies in a wild-type CFTR background because no evidence of cross-linking was observed when it was treated with various methanethiosulfonate cross-linkers (see below). Promiscuous cross-linking agents such as copper phenanthroline, however, could not be used because they induced cross-linking in wild-type CFTR (data not shown).

To examine inter-TMD interactions in CFTR using disulfide cross-linking, we first had to mutate individual residues in the TMDs to cysteines. We chose TM6 because there is strong evidence that TM6 plays a crucial role in the formation of the pore for chloride conductance (31, 32). There is, however, little work done on the other TMs of CFTR. Studies on the TM packing of P-gp, the sister protein of CFTR, however, show interactions between TM6 and TM12 (25, 26). Thus, residues in the cytosolic halves of TM6 and TM12 of CFTR were individually mutated to cysteine using site-directed mutagenesis (see Fig. 2A). The positions of mature (C) and immature (B) CFTRs are shown.

![Fig. 1. Biosynthesis of wild-type and processing defective mutants of CFTR.](image1)

**A. Normal Biosynthesis**

WT CFTR is co-translationally inserted into the ER membrane where it is core-glycosylated. Upon proper folding and passing the ER quality control system, it progresses onto the Golgi apparatus, where it is further processed, and its sugar moieties are modified. Subsequently, CFTR is targeted onto the cell surface to function as a cAMP-dependent chloride channel. B, processing defective CFTR mutants are also co-translationally inserted into the ER membrane where they are core-glycosylated. The mutants, however, do not pass the ER quality control system to progress onto the Golgi apparatus. Instead, they are targeted for cytosolic degradation, and their sugar moieties remain core-glycosylated. C, expression of CFTR mutants. HEK293 cells transiently transfected with the ΔF508, WT, or Cys-less CFTR cDNAs were lysed and subjected to SDS-PAGE and immunoblot analysis using rabbit polyclonal antibody against the NBD2 of CFTR. Band B is the core-glycosylated form of CFTR. Band C is the complex-glycosylated form of CFTR formed upon progression into the Golgi apparatus.

**Fig. 2. Generation of cysteine mutations in TM segments 6 and 12.** A, predicted structure of CFTR showing the relative positions of the cysteine residues in TM6 and TM12 used in this study. The model was based on that proposed by Riordan et al. (2). The cylinders represent TM segments. B, immunoblot analysis of cells expressing the constructs used in this study. HEK293 cells transiently transfected with the CFTR cDNAs were lysed and subjected to SDS-PAGE and Western blot analysis using rabbit polyclonal antibody against the NBD2 of CFTR. The positions of mature (C) and immature (B) CFTRs are shown.

Single substitution of the TM residues with cysteine had little deleterious effect on the maturation of CFTR as most single cysteine mutants of TMs 6 and 12 had similar levels of...
maturation as that of WT CFTR (data not shown). Subsequently, paired-cysteine CFTR mutants containing a cysteine in TM6 and another in TM12 were constructed and tested for cross-linking with methanethiosulfonate cross-linkers (Fig. 3A). These methanethiosulfonate cross-linkers have thiol-reactive groups at both ends that are linked by spacer arms of various lengths (27). Alkylthiosulfonates react selectively with cysteines in protein, resulting in a disulfide attachment of the spacer arm and release of a sulfinic acid byproduct (37, 38). The methanethiosulfonate compounds are generally more reactive than other thiol-specific compounds such as maleimides or iodoacetates (38).

For our cross-linking assays, HEK293 cells transiently transfected with individual constructs of CFTR cDNA were harvested and treated with or without the thiol-reactive cross-linkers, M5M, M8M, or M17M. The cells were incubated with the cross-linkers for 15 min at room temperature, then lysed with the 2× SDS sample buffer and subjected to immunoblot analysis. Cross-linking of CFTR would cause the protein to migrate slower on SDS-PAGE gels. As shown in Fig. 3B, the addition of the thiol-reactive cross-linkers to cells expressing WT CFTR does not lead to cross-linking.

Three positive cross-linking mutants, M348C/T1142C, T351C/T1142C, and W356C/W1145C were identified (see Fig. 3B, band X) and selected for further study. These were useful mutants because none of the mutations appeared to affect maturation of the protein. Fig. 2B shows the expression of WT CFTR, the single cysteine mutants M348C, T351C, W356C, T1142C, and W1145C, and the double cysteine mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C. All of the mutants yielded relatively high levels of mature CFTR proteins (band C) relative to the core-glycosylated form of the protein (band B).

The cross-linking patterns of mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C showed differences when treated with different cross-linkers. Mutant M348C/T1142C, for example, showed cross-linking with M5M and M8M but not with M17M. Mutant T351C/T1142C, on the other hand, shows extensive cross-linking with M8M but not with M5M or M17M. It is interesting to note that both M348C and T351C in TM6 showed cross-linking to T1142C in TM12. Residue Met-348 is three residues away from Thr-351, which would put them on the same face of an α-helix. Therefore, it is not surprising that the substituted cysteines at both of these positions would cross-link to the same residue, T1142C. The positive mutant, W356C/W1145C, showed cross-linking with all three cross-linkers (Fig. 3B). Most of the other mutants tested did not show cross-linking with M5M, M8M, and M17M. An example of a mutant that did not show cross-linking, T351C/L1143C, is shown in Fig. 3B.

Because the cross-linkable mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C also contained the 18 endogenous cysteines, it was important to test whether any of the single cysteine mutants M348C, T351C, W356C, T1142C, and W1145C had shown evidence of cross-linking with endogenous cysteines. Accordingly, cells expressing each of the single cysteine mutants were treated with M5M, M8M, or M17M, and samples were subjected to immunoblot analysis. Fig. 3B, right panel, shows that none of the single cysteine mutants showed cross-linking. Reduced levels of the T352C and W353C mutants, however, were observed with M8M, and this may be due to aggregation. Aggregation of CFTR protein is normally observed in cells (39) and caused some problems in these studies. In the immunoblot analysis of cross-linking only 10 µl of the assay samples were loaded in the negative control lanes (i.e. no cross-linker added) compared with the 30 µl that was loaded in the cross-linker lanes. This is because the cross-linkers, particularly M5M and M8M, also caused nonspecific cross-linking that leads to aggregation of the CFTR protein as shown in Fig. 4. Fig. 4 shows an overexposed immunoblot of the effect of cross-linkers on WT and ΔF508 CFTR. The presence of the M5M and M8M cross-linkers led to accumulation of aggregates in the stacking gel. This is similar to the nonspecific aggregates we observed when we treated the cells with oxidants such as copper phenanthroline. The aggregates are likely due to reaction of the thiol-reactive cross-linkers with endogenous cysteines between CFTR molecules or between CFTR and other proteins. In addition, the studies were complicated by the presence of aggregates even in the absence of cross-linker (Fig. 4, lanes 0). It is also worth noting that it is possible that the efficiency of intramolecular cross-linking would be reduced if a separate cross-linker labels each of the introduced cysteines. Attachment of separate cross-linker labels to each cysteine or incomplete cross-linking (cross-linker only attached to one of the cysteines) may also promote aggregation when the reaction is stopped. It is also possible that formation of an intramolecular disulfide bond between TM6 and TM12 blocks aggrega-
tion. Despite the problems with aggregation, cross-linking analysis still appeared to be a useful assay because the putative cross-linked products were specific to the double cysteine mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C (Fig. 3B, band X).

To ensure that band X was indeed the product of disulfide cross-linking between the introduced cysteines of mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C, we added DTT after cross-linking. As shown in Fig. 5, the addition of the reducing agent DTT caused the slow-migrating band X to disappear. Therefore, formation of band X after treatment with cross-linker was due to the formation of a disulfide bond.

We then tested to see if cross-linking occurs intramolecularly or intermolecularly. To do this we co-transfected HEK293 cells with two CFTR cDNAs. Each cDNA contained one of the cysteine mutations M348C, T351C, W356C, T1142C, or W1145C. It was found that co-expression of the single cysteine mutants M348C plus T1142C, T351C plus T1142C or W356C plus W1145C followed by treatment with the cross-linkers M5M, M8M, or M17M did not lead to cross-linking (formation of band X) (data not shown). This indicates that cross-linking occurs intramolecularly and not intermolecularly.

To compare the inter-TMD interactions between WT and misprocessed CFTRs, the ΔF508 mutation was introduced into the positive cross-linking double cysteine constructs M348C/T1142C, T351C/T1142C, and W356C/W1145C. The mutants were then tested for disulfide cross-linking. As shown in Fig. 6A, incorporation of the ΔF508 mutation into mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C abolished cross-linking. The disappearance of the CFTR protein band on the blot after treatment with M5M and M8M is likely due to extensive aggregation. We previously observed (Fig. 4) that the majority of band B disappeared in both WT and ΔF508 CFTR. Aggregation was not a problem with M17M.

One potential problem, however, was that mature CFTR resides in the plasma membrane, whereas the immature protein resides in the ER. To test whether the lack of cross-linking in the ΔF508 series of double cysteine mutants was due to inaccessibility of thiol-reactive cross-linkers to the ER membrane, we tested whether mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C (lacking ΔF508 mutation) would still show cross-linking then they were located in an intracellular membrane. To block trafficking of the mutants to the cell surface, we pretreated cells expressing mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C with 10 μg/ml brefeldin A to block trafficking of CFTR to the cell surface and then treated without (0) or with the indicated cross-linker. Samples were then incubated in the presence or absence of 33 mM DTT and then subjected to immunoblot analysis. The positions of immature (B), mature (C), and cross-linked (X) CFTRs are indicated.

**Fig. 4.** Aggregation of CFTR. An immunoblot analysis of cells expressing WT or ΔF508 CFTR treated without (0) or with the indicated cross-linker is shown. The blot shows both the stacking and resolving parts of the gel. The positions of immature (B), mature (C), and cross-linked (X) CFTRs are indicated.

**Fig. 5.** Effect of DTT on cysteine mutants treated with cross-linker. Cells expressing the indicated double cysteine mutant were treated without (0) or with the indicated cross-linker. Samples were then incubated in the presence or absence of 33 mM DTT and then subjected to immunoblot analysis. The positions of immature (B), mature (C), and cross-linked (X) CFTRs are indicated.

**Fig. 6.** The ΔF508 mutation but not incomplete glycosylation inhibits disulfide cross-linking between cysteine residues in TMs 6 and 12. A, cells expressing the indicated double cysteine mutants in a ΔF508 background were treated without (0) or with the indicated cross-linker. Samples were then treated with (right panel) or without (left panel) 33 mM DTT before immunoblot analysis. B, cells expressing the indicated double cysteine mutants in a wild-type background were grown in the presence of 10 μg/ml brefeldin A to block trafficking of CFTR to the cell surface and then treated without (0) or with the indicated cross-linker. Samples were then treated with (right panel) or without (left panel) 33 mM DTT before immunoblot analysis. The positions of immature (B), mature (C), and cross-linked (X) CFTRs are indicated.
and cAMP-stimulated iodide efflux was measured using an iodide-sensitive electrode that was calibrated with NaI standards. Fig. 7 shows that untransfected CHO cells show no stimulation of iodide efflux in the presence of forskolin. By contrast, CHO cells expressing WT CFTR exhibited increased iodide efflux upon stimulation by forskolin. The M348C/T1142C mutant showed a similar level of activity as WT CFTR. Both mutants T351C/T1142C and W356C/W1145C, however, exhibited ~40% reduction in activity compared with WT CFTR. This suggests that the mutants retained channel activity, but some of the mutations may have caused some change in the regulatory or conductance properties of CFTR. It was expected that there would be some changes in channel activity because residues in TM6 and TM12 appear to line the pore or lie close to it (31, 44–46).  

DISCUSSION

We have previously used the multidrug resistance P-gp as a model system to study the structure, biosynthesis, and maturation of ABC transporters such as CFTR using disulfide cross-linking analysis. P-gp appears to be a good model system for CFTR because it was recently shown that the two proteins are structurally quite similar when their structures were compared by electron microscopy of two-dimensional crystals (47). Indeed, a P-gp/CFTR chimera in which the third predicted α-helix of NBD1 was replaced with the corresponding segment from CFTR (contains Phe-508) remained active (17). Deletion of Phe-508 in the P-gp/CFTR chimera inhibited maturation of the protein. Cysteine-scanning mutagenesis (introduction of one cysteine in TMD1 and another in TMD2) followed by cross-linking analysis showed that cross-linked products observed in the P-gp/CFTR chimera were not detected in the P-gp/CFTR-∆F508 mutant. The presence of the ΔF508 mutation appeared to interfere with the ability of TMD1 and TMD2 to adopt a native conformation. The ΔF508 mutation did not appear to cause gross misfolding of the protein because cross-linking of the double cysteine mutants was restored when the mutants were rescued by carrying out expression in the presence of a chemical chaperone (drug substrate). An interpretation of the
results was that the presence of the ΔF508 mutation resulted in incomplete folding of the molecule and affected packing of the TM segments. Therefore, we predicted that the ΔF508 mutation in CFTR may also disrupt packing of the TM segments by causing the protein to pause at an early folding step before TMD1 and TMD2 have adopted a native conformation.

The results of this study suggest that the ΔF508 mutation in CFTR also disrupts packing of the TM segments. We were able to identify three mutants, M348C/T1142C, T351C/T1142C, and W356C/W1145C, that showed disulfide cross-linking in the mature WT background but not in the ΔF508 background. Various control experiments were done to confirm that the mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C were indeed cross-linked through the introduced cysteines via the disulfide cross-linker. First, it was shown that cross-linking was sensitive to treatment with DTT. Second, cross-linking was not detected when only a single cysteine was present. Finally, cross-linking was not observed when the cysteines in mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C were co-expressed on separate CFTR molecules. Attempts to cross-link half-molecule forms of CFTR were not successful because the C-terminal half-molecule readily aggregated (data not shown). The ability to detect cross-linked products between TMD1 and TMD2 such as observed with mutants M348C/T1142C, T351C/T1142C, and W356C/W1145C could be particularly useful in future studies to monitor dynamic changes in the molecule associated with phosphorylation or ATP binding/ hydrolysis. Recent crystal structure studies suggest that CFTR can exist in an open or closed conformation (47). Cysteine mutagenesis and cross-linking analysis has been a very useful method for studying the dynamic changes in P-gp. Such studies have successfully been utilized to show that there is extensive cross-talk between the ATP-binding sites and the drug-binding sites located in the TM domains (25, 48–52).

As observed with P-gp (17), we found that introduction of the ΔF508 mutation into the double cysteine mutants inhibited cross-linking (Fig. 6A). Therefore, we predict that the ΔF508 mutation has the same effect on folding of CFTR (Fig. 8) as we previously postulated for P-gp (17). When WT CFTR is synthesized, the individual domains may be formed first. We predict that the individual domains can fold independently because similar domains in bacterial ABC transporters are synthesized as separate polypeptides before they associate to form the transport complex (53). In addition, the individual domains of mammalian ABC transporters, when expressed as separate polypeptides, can fold properly and bind substrates (18, 54).

A model of the domain interactions is shown in Fig. 8. The protein is first synthesized in the ER, where it adopts a “loosely folded” immature conformation that is very sensitive to proteases. At this stage, the WT and ΔF508 CFTR appear to be structurally similar, as both show a similar hypersensitivity to proteases (5, 16). WT CFTR can be trapped at the immature stage by carrying out synthesis in the presence of MG-132 (5). In the absence of MG-132, WT CFTR will undergo superfolding so that the individual domains establish their proper contacts, and the protein adopts a mature form that is more resistant to proteases (Fig. 8A). Mutant ΔF508, however, is trapped in the immature stage since the presence of the mutation results in the formation of a thermodynamic hurdle that inhibits maturation of the protein (Fig. 8B).

How does the ΔF508 mutation in NBD1 inhibit the proper association of TMD1 and TMD2 in CFTR? Results from cross-linking studies on P-gp suggest that the residue corresponding to residue Phe-508 lies close to the first intracellular loop in TMD1. Similarly the crystal structure of the bacterial ABC transporter BtuCD also shows that the residue corresponding to Phe-508 lies at the interface between NBD1 and TMD1 (55). The association of TMD1 with TMD2 may first require interaction of NBD1 with TMD1. This prediction is supported by the observation that the TMD1 and TMD2 “quarter-molecules” of P-gp do not adopt a protease-resistant conformation in the absence of the NBDs (18). Quarter-molecules are the protein products of truncated cDNAs that code for one domain of the molecule. TMD1 and TMD2 quarter-molecules of P-gp will adopt a protease-resistant complex, however, if they are expressed together in the presence of a chemical chaperone (drug substrate).

Recently, the crystal structure of NBD1 of mouse CFTR was solved (54). Alignment of this structure to the solved structures of other ABC transporters (55, 56) shows that the residue corresponding to Phe-508 in CFTR is exposed at the NBD1-TMD1 interface. In previous peptide studies, a peptide containing the ΔF508 mutation caused localized misfolding (57), and this may cause improper interaction between TMD1 and NBD1.

In the P-gp/CFTR-ΔF508 chimera, it was possible to promote maturation of the misprocessed mutant by carrying out expression in the presence of a specific chemical chaperone (drug substrate) (17). Drug substrates promoted maturation of the protein by promoting interaction between TMD1 and TMD2, as the drug-binding sites are located at the interface between these two domains (58). Therefore, the interface between TMD1 and TMD2 of CFTR should be a target for development of therapeutic compounds because any useful drug must induce proper packing of the TM segments.

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