

The Use of Small Molecules to Correct Defects in CFTR Folding, Maturation, and Channel Activity

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Abstract: Cystic Fibrosis, one of the most common inherited lethal disease among Caucasians, is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The CFTR protein acts as a gated Cl⁻ channel at the apical membrane of epithelial cells, thereby facilitating proper hydration of mucosal linings. Disease causing mutations in the CFTR protein can affect a variety of steps in the biogenesis of a functional protein including the folding and trafficking of CFTR as well as the channel activity of plasma membrane-localized protein. Therefore, current research is focused on the use of small molecules to not only correct folding defects but also to enhance channel activity of mutant CFTR proteins. This review discusses the current knowledge of the folding, trafficking, and gating defects caused by CFTR mutations, the manner by which these defects are monitored by the cell, as well as the strategies which are currently being utilized to develop and screen for small molecule therapeutics.

Keywords: Cystic fibrosis, chaperones, ER Quality control, correctors, potentiators.

CYSTIC FIBROSIS

Cystic Fibrosis (CF) is one of the most common genetic diseases in the U.S. affecting approximately 30,000 children and adults in the U.S. and approximately 70,000 people worldwide (www.cff.org). The disease is most prevalent among the Caucasian population. Symptoms of CF include the accumulation of abnormally viscous mucous in the secretory epithelia, which is resultant from defective ion transport. The disease CF is caused by mutations in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene, which was discovered in 1989 [1]. Normally, the CFTR protein is localized to the apical surface of epithelial cells and acts as a cAMP activated chloride ion channel [2, 3]. The activity of this ion channel causes anion secretion which then drives liquid secretion and allows for the proper hydration of mucosal membranes that line the epithelial cell surfaces. Defective hydration of the mucous membranes and accumulation of abnormally viscous mucous causes many of the symptoms of CF such as chronic lung infections and inflammation as well as a failure to thrive due to the obstruction of pancreatic ducts [4]. Defective transepithelial ion transport in the sweat glands also results in the increased saltiness of sweat, which has historically been used to diagnose the presence of CF, and is currently still used for diagnosis in combination with genetic testing [5].

More than 1500 mutations have been identified in the CFTR gene, and research has shown that the severity of the disease symptoms is at least partially linked to the type of mutation. CFTR mutations have been classified into 6 different groups depending on the type of defect, and the stage of CFTR biogenesis that is affected (Fig. (1)) [4, 6]. Class I

mutations result in defective protein production due to the presence of a premature stop codon or instability of the mRNA. Class II mutations result from the defective processing of the CFTR protein from the endoplasmic reticulum (ER) where the protein is translated and folds, to the Golgi apparatus where the protein undergoes complex glycosylation and is packaged into vesicles for transport to the plasma membrane. If the CFTR protein reaches the plasma membrane but either exhibits defective channel regulation or reduced ion transport activity, then it results in Class III or IV mutations, respectively. Finally, a reduced level of CFTR transcription due to a mutation in the promoter region or an abnormal splicing event characterizes Class V mutations, and reduced stability at the plasma membrane falls into the Class VI category. Class I and II mutations are generally the most severe as they can result in a complete lack of CFTR at the plasma membrane, while Class III, IV, and V, and VI mutations generally present with milder symptoms. Current CF research is focused on the development of small molecules to act as “correctors” of mutant CFTR processing and “potentiators” to enhance mutant CFTR ion channel activity [7].

CFTR FOLDING AND MISFOLDING

The CFTR protein, an ABC transporter family member, is a 1480 amino acid complex polytopic protein [1, 8]. Similar to other ABC transporters, CFTR is comprised of 2 membrane-spanning domains (MSD1 and MSD2) that consist of 6 transmembrane helices each, and 2 cytosolic nucleotide binding domains (NBD1 and NBD2) [1, 9]. Specific to CFTR, in comparison to other ABC transporters, is the addition of a cytoplasmic regulatory domain (R domain) (Fig. (2)). The NBDs are thought to form an ATP binding sandwich, with residues from both NBDs contributing to the enzymatic ATP hydrolysis activity [10-12]. The binding and hydrolysis of ATP by the NBDs is necessary for channel opening. Furthermore, CFTR channel opening requires the PKA dependent phosphorylation of the R domain at multiple

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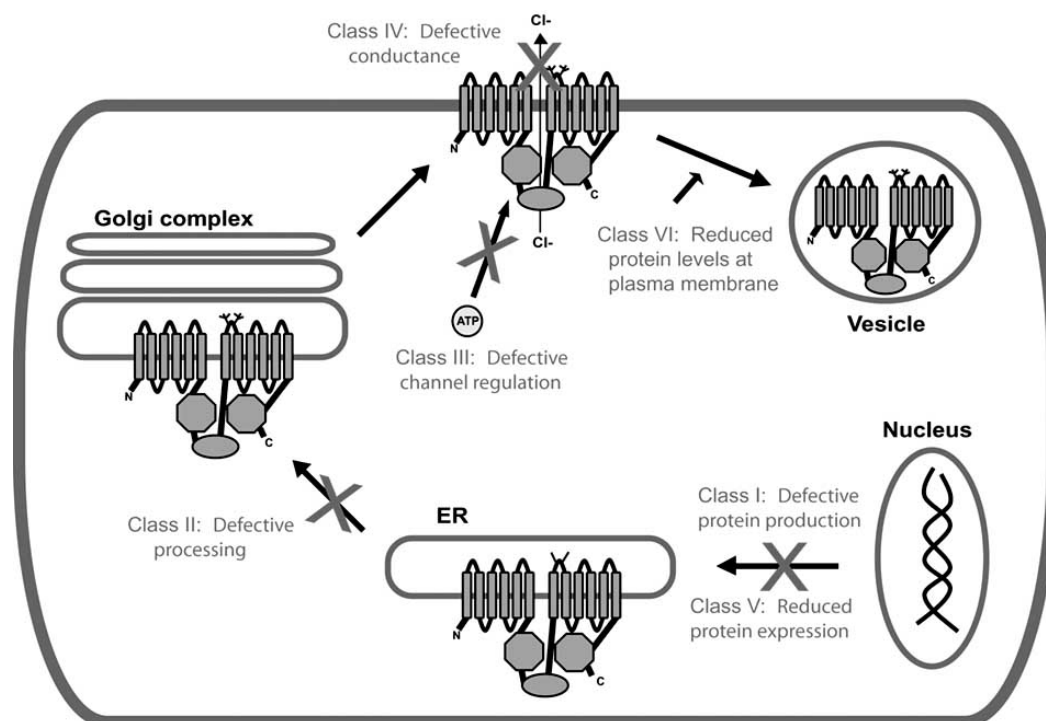


Fig. (1). Classification of CF Causing Mutations.

Disease causing mutations in the CFTR protein are grouped into 6 classes based on the affected stage of biogenesis. A full description of each class is included in the text.

sites [13]. The exact mechanism of R-domain regulation is not completely understood as there are data which support models in which the unphosphorylated R domain has an inhibitory action [14], as well as data which support a model in which R domain phosphorylation regulates domain-domain interactions within the CFTR molecule that then result in channel activation [15-18]. Protein phosphatases are thought to counteract channel activation, and to date interactions between CFTR and protein phosphatase-2C as well as protein-phosphatase-2A have been identified [19, 20].

Folding of CFTR is thought to occur both co- and post-translationally, and involves the formation of multiple intra-

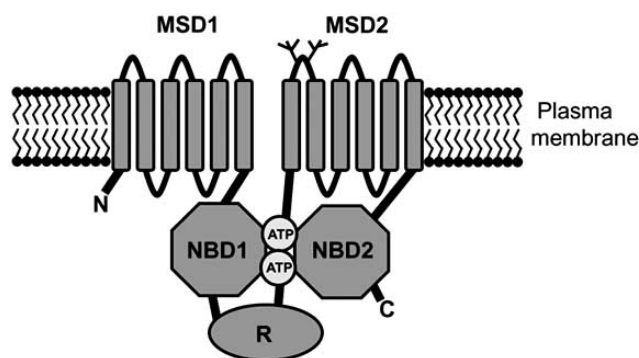


Fig. (2). Domain Structure of CFTR.

The Cystic Fibrosis Conductance Regulator is comprised of 2 membrane spanning domains (MSD), 2 cytosolic nucleotide binding domains (NBD), and a cytoplasmic R domain (R). The N-terminus of CFTR encodes a cytoplasmic tail, which is then followed by MSD1, NBD1, the R domain, MSD2, and NBD2 in that order.

domain contacts [21-23]. Structural studies on the ABC transporter family of proteins have revealed a complicated folding pathway in which the MSDs of CFTR likely interact with one another to form a 2-winged pore where each wing contains helices from both MSDs [24]. As well, it appears that there is crosstalk between the cytosolic nucleotide binding domains with both membrane-spanning domains [24-26]. In this manner, the proper co- and post-translational folding and assembly of these domains is essential for both the trafficking and function of the CFTR protein.

The folding and processing of CFTR is a complex but inefficient process [27] (Fig. (3)). The nascent polypeptide is concomitantly folded and inserted into the ER lipid bilayer, where both cytoplasmic and ER luminal chaperones assist in the folding process [28-32]. Proteomic approaches have identified interactions with the "core" chaperones, Hsc-Hsp40/70, Hsp90, and calnexin [33]. Furthermore, the Hsp40, Hdj-2, and Hsc70 chaperones have been shown to play a role in the early steps of the folding pathway [32]. However, despite the assistance of the chaperones, approximately 55-80% of newly synthesized wild-type CFTR protein is improperly folded, recognized by the ER quality control system (ER QC) and targeted to the cytoplasmic proteasome for degradation [34]. The properly folded ER membrane inserted CFTR, referred to as the immature B-form [27], leaves the ER *via* coat protein complex II (COPII) coated vesicles [35]. CFTR then enters the Golgi apparatus where two of the Asn-linked glycans in the fourth extracellular loop are converted from an immaturely glycosylated high-mannose form to mature complex oligosaccharides, creating the mature C-form CFTR [27]. The mature CFTR protein is subsequently delivered to the plasma membrane where it functions as a chloride ion channel. Once at the api-

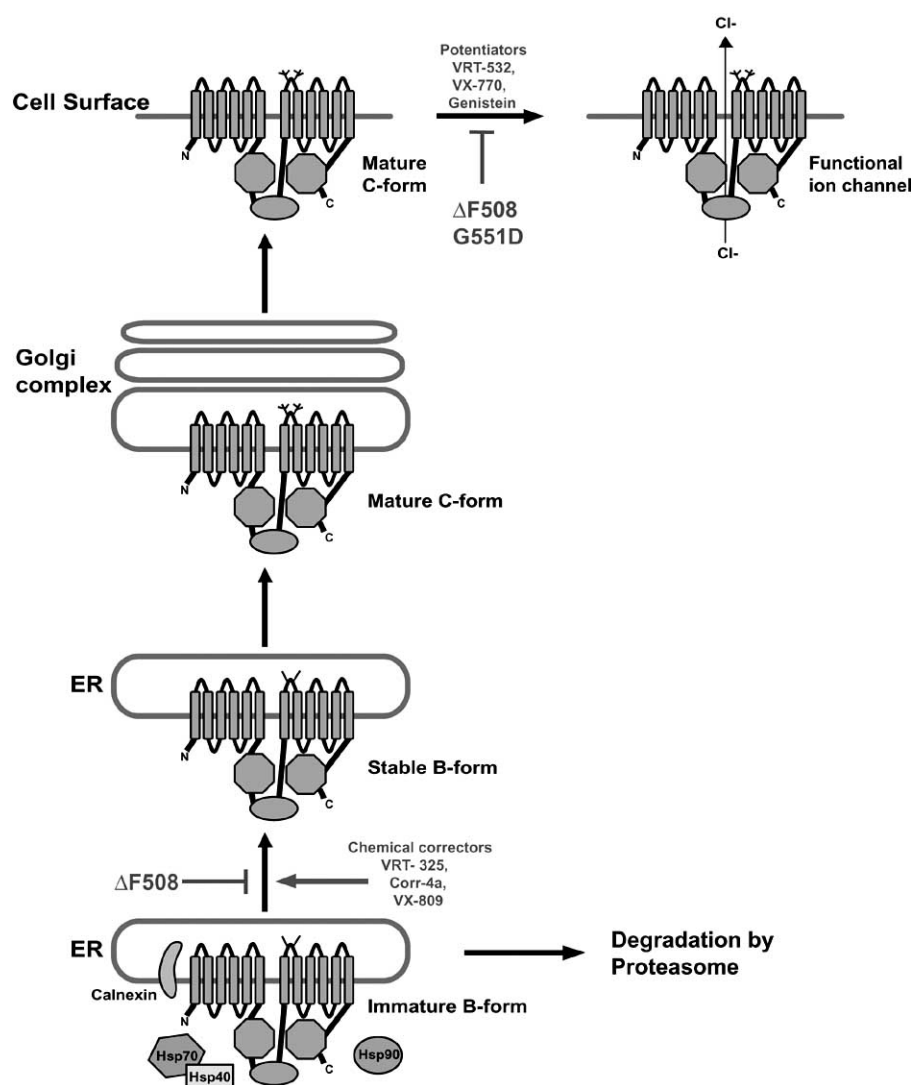


Fig. (3). Biogenesis of the CFTR protein.

The CFTR protein is translated and inserted into the ER membrane where its folding is assisted by molecular chaperones. This form of immaturely glycosylated CFTR is referred to as B band. Class II mutations such as $\Delta F508$ block the folding of CFTR and thereby prevent its exit from the ER. Misfolded WT or mutant CFTR which is trapped in the ER is then degraded by the ubiquitin proteasome system. However, upon adoption of a correctly folded structure, CFTR traffics through the Golgi where it undergoes sugar modification. This complex glycosylated form of CFTR is known as the C band, and is trafficked from the Golgi to the plasma membrane where it can act as a functional chloride ion channel. If the folding of the mutant $\Delta F508$ CFTR is rescued by low temperature, or by addition of chemical chaperones, then it can also traffic to the plasma membrane. However, rescued forms of class II mutant CFTR molecules as well as Class III mutants such as G551D also show defects in channel gating. These gating defects can be partially overcome by the addition of potentiator molecules.

cal membrane, CFTR levels are regulated by internalization to sub-apical vesicles resulting in two fates: recycling to the plasma membrane or lysosomal degradation [36].

Mutations in CFTR that affect the folding process can be very deleterious. For example, the $\Delta F508$ mutation, a Class II mutation, is responsible for approximately 70% of all CF cases [37, 38]. The misfolded $\Delta F508$ CFTR is retained in the ER, and approximately 99% of this protein is prematurely degraded by the ubiquitin proteasome system [39]. The F508 residue is localized to a solvent exposed loop of the NBD1 domain, but deletion of this residue does not appear to cause the gross misfolding of the NBD1 domain itself, and instead may play an important role in maintaining intra-domain contacts [10, 23, 40]. Interestingly, the folding defect of CFTR $\Delta F508$ is temperature sensitive, and can be corrected

by the addition of chemical chaperones or by treatment at low temperature in cultured cells [41, 42]. Therefore, understanding of the misfolding events which occur with this mutation could greatly aid in the development of therapeutics for CF. While there have been great strides made into understanding the folding pathway of CFTR and $\Delta F508$ CFTR, there still remains much work to be done.

CFTR DEGRADATION

As mentioned above, improperly folded CFTR protein is targeted for degradation by the ER quality control and the ubiquitin proteasome systems [34, 39]. These systems play very important cellular roles by recognizing and selecting misfolded proteins for degradation, and in doing so preventing the toxic accumulation of misfolded protein conformers.

When a membrane protein such as CFTR misfolds, the protein must be first recognized as misfolded, second targeted for degradation by addition of a polyubiquitin chain, and finally retrotranslocated to the cytosol and degraded by the cytosolic proteasome. In general, an enzymatic cascade involving the E1 ubiquitin activating enzyme, the E2 ubiquitin conjugating enzyme, and the E3 ubiquitin ligase enzyme carries out the conjugation of the polyubiquitin chains [43]. The conjugation of multiple ubiquitin molecules into one polyubiquitin chain then acts as a flag for the misfolded protein to be directed to the proteasome. The retrotranslocation step of membrane proteins has been shown to be at least partially dependent on the AAA ATPase, p97, along with its cofactors Ufd1, Npl4, and VIMP [44, 45]. In fact, p97 has been shown to stimulate the degradation of CFTR transmembrane domains, and co-expression of mutant p97 and $\Delta F508$ CFTR in human cells results in stabilization of the mutant CFTR [46, 47].

The folding of CFTR appears to be monitored via two distinct systems in human cells: the Hsc70/CHIP ubiquitin ligase complex [28], and the Derlin-1/RMA-1 ubiquitin ligase complex [48] (Fig. (4)). The Hsc70 chaperone, a cytosolic chaperone that interacts with CFTR, is thought to sense misfolding events in the cytoplasmic domains of CFTR and maintain those domains in a soluble state. Upon interaction of Hsc70 with the Hsc70 co-chaperone and E3 ubiquitin ligase, CHIP, the CFTR protein is diverted from a folding pathway to the degradation pathway [49]. In conjunction with the E2, UbcH5a, the CHIP E3 ligase then polyubiquitinates the CFTR protein as has been demonstrated by the *in vitro* reconstitution of the ubiquitination reaction, as well as by overexpression and pulse chase analysis in human cells [50].

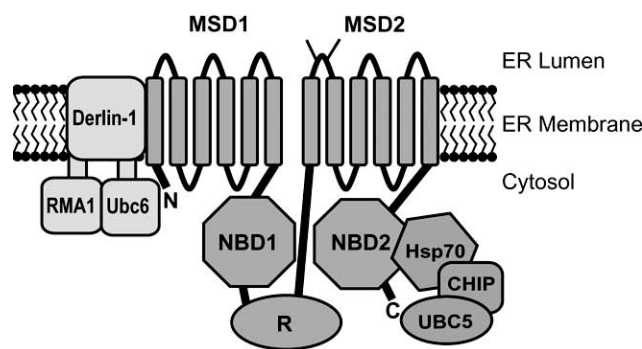


Fig. (4). The E3 Ubiquitin Ligase Complexes which Monitor CFTR Folding.

CHIP and Hsc70 are cytoplasmic proteins which are thought to monitor the folding of cytoplasmic domains of CFTR. Hsc70 is a chaperone which binds misfolded regions of CFTR. CHIP is an E3 ubiquitin ligase that binds Hsc70 through its TPR domains. In the case of CFTR degradation, CHIP has been shown to cooperate with the E2, UbcH5, to add polyubiquitin chains to misfolded CFTR. Derlin-1, RMA-1, and Ubc6 are all localized to the ER membrane. RMA-1 acts as an E3 ligase in concert with the E2, Ubc6, to promote degradation of CFTR. It has been proposed that Derlin-1 may act as a substrate selector which monitors the folding of CFTR transmembrane domains.

The important role of CHIP in the degradation of CFTR is also made obvious by studies in which CHIP's activity is inhibited by one means or another. For example, CHIP's E3 ubiquitin ligase activity can be regulated by other Hsp70 co-chaperones such as Bag-2 and HspBP1 [51-53]. BAG-2 inhibits the ubiquitin ligase activity of CHIP by abrogating the CHIP/E2 cooperation and enhances the chaperone-assisted maturation of CFTR [51]. Likewise, overexpression of HspBP1, which has been shown to inhibit CHIP's activity and is a nucleotide exchange factor that can promote the release of substrates from Hsp70, stimulates the maturation of CFTR [52]. Furthermore, inhibition of the CHIP-Hsp70 E3 ubiquitin ligase complex by overexpression of a CHIP mutant results in the accumulation of a folding competent stable B-form of CFTR [50].

As the CHIP/Hsp70 complex monitors the cytosolic domains of CFTR, there are also a variety of ER-membrane anchored proteins that can potentially monitor the assembly of CFTR membrane domains. In fact, aberrant CFTR folding within the ER lipid-bilayer is proposed to be identified by the transmembrane ER quality control factor Derlin-1 and then targeted for degradation in conjunction with the ER membrane bound E2 and E3 enzymes, Ubc6e and RMA-1 [54]. Both Ubc6e and RMA1 are localized to the cytosolic face of the ER membrane via their C-terminal domains and therefore are likely to ubiquitinate cytosolic regions of CFTR that are exposed in its misfolded form [55, 56]. In human cells Derlin-1 can be isolated in complex with a CFTR biogenic intermediate that corresponds to MSD1, and overexpression of Derlin-1 leads to the retention of CFTR in the ER, while RNAi mediated knock down of Derlin-1 leads to an increase in steady state levels of mutant CFTR [37, 54]. These results suggest that Derlin-1 may participate in the selection of misfolded membrane proteins such as CFTR for ERAD. Derlin-1 has also been shown to interact with a variety of proteins involved in ER quality control, including the E2 enzyme, Ubc6, E3 enzymes, RMA-1, HRD1, and gp78, the retrotranslocation factors, p97 and VIMP, and the deglycosylating enzyme, peptide N-glycanase [44, 54, 57-59]. Pulse chase analysis has shown that the Ubc6 and RMA-1 enzymes act in concert with each other to promote the degradation of CFTR in a co-translational manner [54]. Recently, gp78 has also been identified as a factor in the Derlin/RMA-1 complex, where it is thought to play a role in ubiquitin chain elongation on CFTR [60]. Finally, Derlin-1 may also play an essential role in the retrotranslocation process. This has been shown to be the case for the retrotranslocation of MHC Class I heavy chain molecules [61] as well as in a cell-free assay which measures retrotranslocation of a fluorescently labeled substrate packaged in ER vesicles [62].

While the CHIP/Hsc70 and Derlin/RMA-1 complexes appear to be the main proteins involved in the degradation of CFTR, the involvement of other proteins has not been ruled out. For example, studies in yeast have suggested a role for sHSPs in the degradation of CFTR [63]. Furthermore, a proteomics approach has identified a set of proteins which are found in association with $\Delta F508$ CFTR but not WT CFTR [33]. These proteins may play a role in the folding or recognition of the misfolded mutant CFTR. For example, many of the Hsp90 co-chaperones were found in a complex with $\Delta F508$ CFTR. Upon further analysis, it was found that the RNAi mediated silencing of the Hsp90 co-chaperone, Aha-1, rescued $\Delta F508$ CFTR such that a greater amount of protein

was able to fold and be exported to the plasma membrane [33]. Whether this represents an affect on the chaperone-assisted folding pathway, or the chaperone-assisted degradation pathway remains to be determined.

SMALL MOLECULE THERAPEUTICS FOR CF

Early therapies developed to treat CF were originally aimed at the treatment of symptoms, and not at the restoration of CFTR protein function. These therapies included the use of antibiotics, mucus-clearing agents, and digestive supplements. Many of these therapies are still being used and actively improved upon today [64]. However, research has shown that restoration of just 5-10% of wild type CFTR function dramatically improves lung and gut function in CF patients [65, 66]. For many CF causing mutations, restoration of function necessitates the correction of folding and trafficking as well as the correction of gating defects. For example, the most common mutation of CF, $\Delta F508$, is classified as a Class II mutation because it misfolds and does not traffic from the ER to the plasma membrane. However addition of chemical correctors or treatment at low temperature allows $\Delta F508$ CFTR to localize to the plasma membrane, but the mutant CFTR still exhibits a variety of defects including gating defects, and reduced stability at the plasma membrane [67, 68]. Recently, high throughput screens have been used to identify drugs that can act in protein rescue or in correction of the channel gating defects [69-73]. Protein rescue drugs are referred to as correctors as they are thought to correct the folding defect of Class II mutants such that the mutant CFTR molecules can fold and be exported to the plasma membrane (see (Table 1) for structures of corrector compounds discussed below). Drugs that act to correct the gating defects of CFTR at the plasma membrane are referred to as "potentiators" as they potentiate channel activity (see (Table 2) for structures of potentiator compounds discussed below). It is likely a combination of corrector and potentiator drugs that will prove useful in the treatment of CF. Below, the current knowledge on the correctors and potentiators that have been made available to the research community and the mechanisms by which they function is summarized. A current description of drugs moving through clinical trials can also be found at www.cff.org.

CFTR CORRECTORS

The first generation of chemical chaperones that were identified to assist in $\Delta F508$ folding and trafficking were non-specific osmolytes, such as glycerol (I) and trimethylamine N-oxide (TMAO) (II) [42]. These chemicals protect proteins against both thermal and chemical denaturants and stabilize the native conformation of proteins. The cellular organic solute, myo-inositol (III), was also shown to act in a similar manner to promote $\Delta F508$ folding [74]. However, extremely high doses of these chemicals are needed to obtain correction, thereby making these drugs unlikely candidates for therapeutic development. 4-phenylbutyrate (IV) is another chemical which has been shown to produce a small increase in the trafficking of mutant $\Delta F508$ CFTR to the cell membrane and in chloride secretion [75]. Interestingly, this drug has already been approved by the FDA to treat urea-cycle disorders, but again, the large doses required and the limited efficacy of this drug leaves the field still searching for more potent correctors. Recently, there was excitement

over the initial discovery that the SERCA pump inhibitors, curcumin (V) and thapsigargin (VI), could promote mutant CFTR trafficking by depleting ER calcium stores [76, 77]. However, it appears that the effects of these drugs are variable among cell types and mouse strains [78].

Researchers have now turned to the use of high throughput screening to identify small molecules that will aid in the correction of the $\Delta F508$ -folding defect. Verkman's group performed one such screen in 2005 [70]. In this screen, over 150,000 chemically diverse molecules were analyzed for their ability to correct $\Delta F508$ trafficking. The initial screen for $\Delta F508$ trafficking was performed in Fisher rat thyroid (FRT) epithelial cells which co-expressed $\Delta F508$ CFTR along with a functional fluorescent halide indicator.

This screen resulted in the identification of 4 different classes of corrector molecules which were grouped based on their chemical structure: Class 1 molecules were 2-aminobenzothiazoles (VII), Class 2 were 2-amino-4-arylthiazoles (VIII), Class 3 were 2-quinazolinyl-4-aminopyrimidinones (IX) and Class 4 molecules were bisaminomethylbithiazoles (X). The identified corrector molecules all increased the abundance of $\Delta F508$ CFTR at the plasma membrane, and a proportionate increase in the forskolin-genistein stimulated apical membrane chloride current was also observed. However, upon further analysis the class 4 bisaminomethylbithiazoles showed the most promise for further development due to the fact that they showed the highest functional correction of the mutant $\Delta F508$ CFTR upon measurement of Γ influx [70]. Furthermore, class 4 correctors were shown by pulse chase analysis to increase the folding efficiency of $\Delta F508$ CFTR by 2-3 fold and that of WT CFTR by 5-70% while the other classes of corrector molecules showed no significant increase [70]. Likewise, when corrector molecules were tested for their effects on human bronchial epithelial cells isolated from patients homozygous for the $\Delta F508$ mutation, only the class 4 molecule resulted in a significant increase in the CFTR-dependent Cl^- secretion; the Cl^- secretion was only 8% of that measured in non-CF epithelia, but this level of correction may actually be sufficient for treatment [70].

How the class 4 compounds facilitate the folding of $\Delta F508$ CFTR is not completely understood, but insight into their mechanism of action has been gained through studying the Corr-4a derivative (X). Interestingly, the effects of Corr-4a are not specific to mutant CFTR proteins as a misprocessed mutant of the sister protein, P-glycoprotein (P-gp), can also be rescued [79]. However, it is possible that the CFTR and P-gp folding defects may be corrected by a similar molecular mechanism, and interestingly, Corr-4a was not able to rescue the processing of the unrelated mutant dopamine receptor 4 [70]. Current literature suggests that Corr-4a may exert its effect on $\Delta F508$ CFTR folding by binding to the transmembrane regions of the protein [80], although direct evidence of a drug-binding pocket on CFTR is lacking. In addition, Corr-4a not only improves trafficking of $\Delta F508$ CFTR to the plasma membrane, but it was also found to enhance the cell surface stability of the rescued mutant protein [70, 81]. Overall, Corr-4a appears to affect $\Delta F508$ CFTR folding in such a way as to allow this mutant CFTR protein to bypass multiple quality control checkpoints.

Table 1.

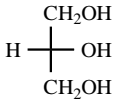
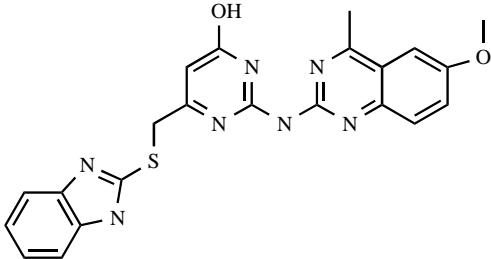
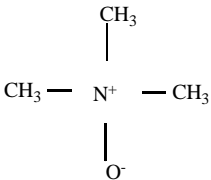
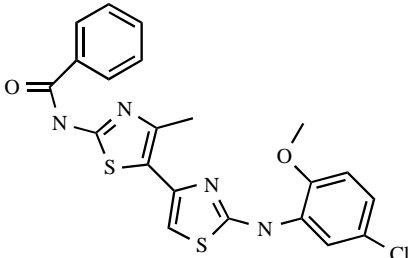
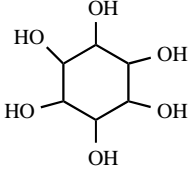
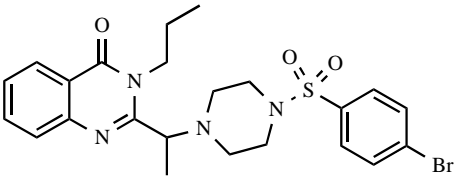
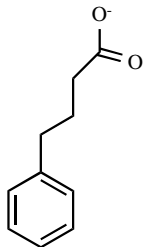
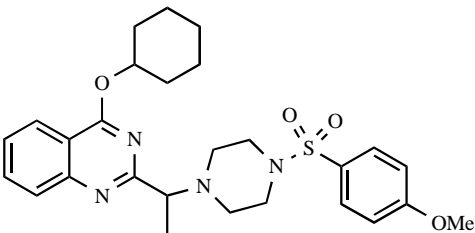
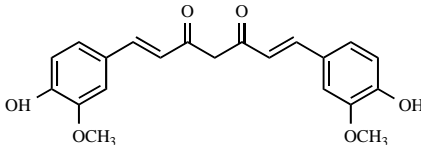
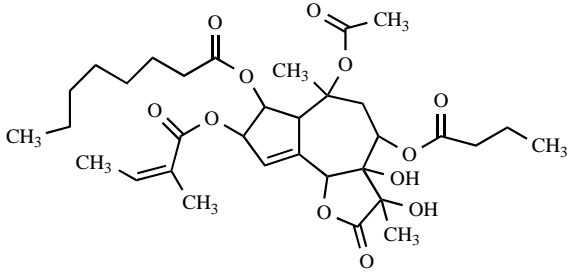
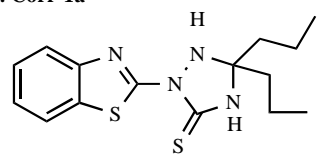
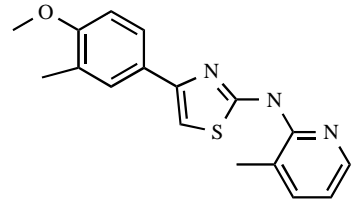
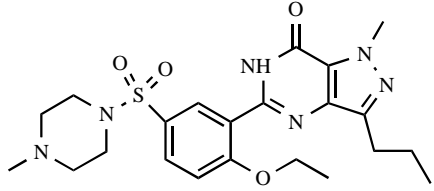
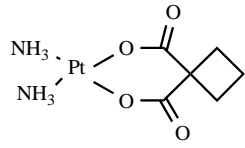
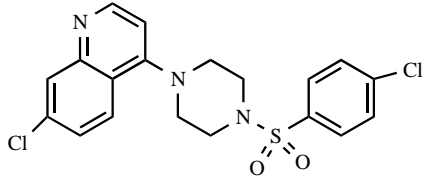
CFTR CORRECTORS:		
Compound Name/Structure		Compound Name/Structure
I	Glycerol 	IX Class 3: 2-quinazoliny-4-aminopyrimidinones Example: Corr-3a 
II	TMAO 	X Class 4: bisaminomethylthiazoles Example: Corr-4a 
III	Myoinositol 	XI VRT-422 
IV	4-phenylbutyrate 	XII VRT-325 (CFcor-325) 
V	Curcumin 	XIII VX-809 UNAVAILABLE

Table 1. Contd....

CFTR Correctors:		
VI	Thapsigargin	
VII	Class 1: 2-aminobenzothiazoles Example: Corr-1a	
VIII	Class 2: 2-amino-4-arylthiazoles Example: Corr-2a	
XIV	Sildenafil	
XV	Carboplatin	
XVI	KM11060	

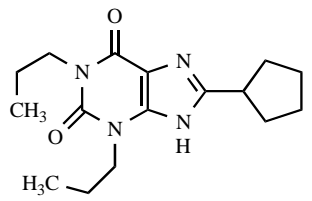
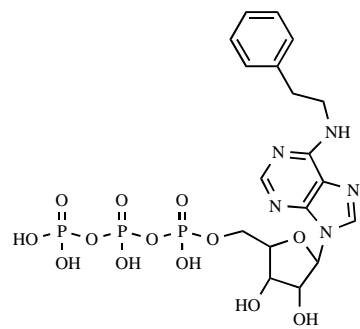
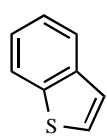
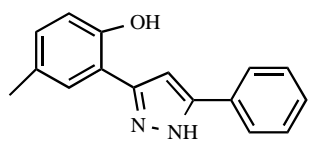
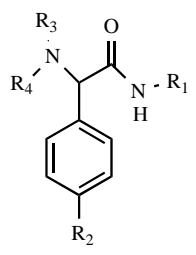
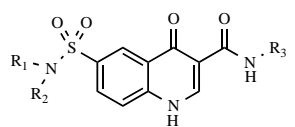
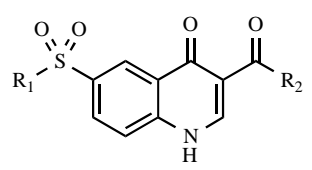
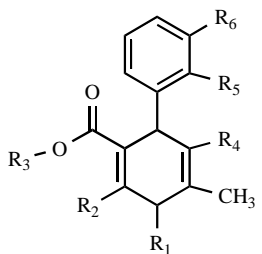
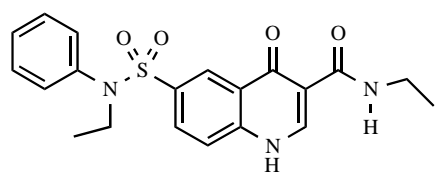
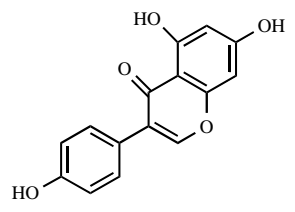
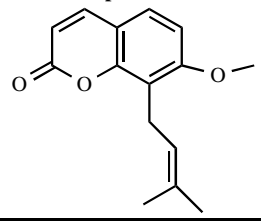
Vertex Pharmaceuticals also performed a high throughput screen for corrector molecules that could restore the function of $\Delta F508$ CFTR [72]. This screen was performed in NIH/3T3 cells expressing $\Delta F508$ CFTR by using fluorescence resonance energy transfer (FRET)-based membrane potential probes. In this screen, the corrector molecules identified were further tested for their activity in FRT epithelial cultures expressing $\Delta F508$ CFTR. The most potent hit from this screen was a quinazolinone compound named VRT-422 (**XI**). This molecule was then further optimized for corrector activity by chemical modification, and out of this optimization came VRT-325 (CFcor-325) (**XII**) [72]. When tested in human bronchial epithelial cultures, VRT-325 restored the $\Delta F508$ -mediated chloride transport to more than 10% of that observed with cells expressing WT CFTR [72]. This molecule has also been shown to correct mutations in the cytoplasmic loop 4 of CFTR, which is a hotspot for mutations that result in a severe CF phenotype [82]. It is thought that VRT-325 may act by binding directly to CFTR and correcting transmembrane domain packing [80, 82]. Overall, this molecule shows great potential for therapeutic studies. Vertex also identified a corrector molecule named VX-809 (**XIII**). While the preliminary data for this molecule is not publicly available at this time, the molecule is currently in phase I clinical trials for treatment of CF (www.cff.org).

Recent studies published by Carlile *et al.* [73] and Robert *et al.* [83] also describe a novel screening assay for CF correctors. This screen focused on identifying molecules based on their ability to promote delivery of 3-HA tagged $\Delta F508$ CFTR to the plasma membrane of BHK cells. Instead of

initially screening molecules based on halide conductance, this screen measured the transport of $\Delta F508$ CFTR to the cell surface based on the accessibility of the HA tag as monitored by a three stage ELISA assay. Upon an initial screening of a 2000 compound library [73], there were several classes of trafficking correctors identified, some of which had been previously identified to play a role in CFTR trafficking and some of which were novel. The 2 hits which the authors chose for further investigation were sildenafil (**XIV**) and carboplatin (**XV**) [73].

Sildenafil, commonly known as Viagra, was previously identified to correct CFTR trafficking when applied at high micromolar doses [84]. However, the screen described above identified this molecule as a hit at a 20 μM dose. Further analysis showed that while the low dose of sildenafil was sufficient to promote trafficking, it did not result in the accumulation of functional channels at the plasma membrane [73]. Upon increasing the dosage to the mM range, addition of this compound did result in a strong increase in iodide efflux responses. Further screening of sildenafil derivatives identified a molecule named KM11060 (**XVI**) [83]. This molecule was shown to be more potent in its ability to increase both the amount of mature $\Delta F508$ C band as well as the function of $\Delta F508$ CFTR in iodide efflux assays. Similar results were obtained for KM11060 in both BHK cells and in the human airway epithelial cell line, CFBE41o⁻ [83]. Carboplatin, on the other hand, was a novel hit and while able to increase maturation of $\Delta F508$ CFTR it only resulted in very slight increases in iodide efflux regardless of dosage [73].

Table 2.

CFTR Potentiators:		CFTR Potentiators:	
Compound Name/Structure		Compound Name/Structure	
XVII	Class: Alkylxanthines Example: CPX 	XXIII	N6-(2-phenylethyl)-ATP 
XVIII	Benzothiophenes 	XXIV	VRT-532 (CFpot-532) 
XIX	Phenylglycines 	XXV	VX-770 UNAVAILABLE
XX	Sulfonamides 	XXVI	Sulfamoyl-4-oxoquinoline-3-carboxamides 
XXI	1,4-dihydropyridines 	XXVII	6-(ethylphenylsulfamoyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (7b) 
XXII	Genistein 	XXVIII	Coumarin Compounds Example: Osthole 

CFTR POTENTIATORS

While corrector molecules are important for rescuing the folding defects of $\Delta F508$ CFTR thereby allowing the mutant

protein to traffic to the plasma membrane, other defects of this mutant and other CFTR mutants which exacerbate the disease state exist. For example, rescued $\Delta F508$ CFTR has

been shown to have a reduced gating activity at the plasma membrane [85]. As well, Class III mutants of CFTR exhibit defective channel regulation such that channel activity is greatly reduced. An example of a Class III mutant is G551D CFTR which accounts for approximately 3% of CF cases and is the third most common disease causing mutation of CF [86]. The G551D mutation abolishes ATP-dependent gating and results in a channel open probability that is approximately 100-fold lower than that of wild-type channels [87]. Therefore, there has been great interest in the development of potentiator drugs that function to increase the open state of mutant CFTR channels thereby promoting better ion and fluid trafficking through epithelia to relieve CF patient symptoms.

There have been numerous CFTR potentiators identified which will activate both wild type and mutant CFTR channel activity. These molecules include alkylxanthines (**XVII**) such as CPX, as well as benzothiophene (**XVIII**), phenylglycine (**XIX**) and sulfonamide drugs (**XX**) [71, 88, 89]. Anti-hypertensive 1,4-dihydropyridines (**XXI**), previously shown to block voltage-dependent calcium channels, have been shown to act as effective potentiators of CFTR gating [90]. Genistein (**XXII**), an isoflavone found in a number of plants such as soybeans, acts as a CFTR potentiator, and has been one of the most widely used potentiators by the research community [91]. It was originally found to act as a tyrosine kinase inhibitor [91] but may act on CFTR by binding directly to the NBDs of CFTR thereby stabilizing their dimerization [92]. However, recent studies with genistein have shown that it not only modulates channel gating defects but also affects CFTR maturation [93]. Other studies have also shown that the ATP analog, N₆-(2-phenylethyl)-ATP (P-ATP) (**XXIII**) increases the channel currents measured with G551D CFTR [87]. It is thought that this analog acts as a potentiator by binding to the NBDs of CFTR and increasing the channel's open time.

High throughput screening analysis has also been utilized to identify potentiator molecules. Vertex Pharmaceuticals performed a screen for potentiators using NIH 3T3 cells by assaying for an increase in halide conductance of temperature rescued Δ F508 CFTR [72]. This screen identified VRT-532 (CFpot-532) (**XXIV**) as a potent potentiator that achieves single-channel activity similar to that of wild-type CFTR. A recent study suggested that in addition to its role as a CFTR potentiator, VRT-532 also acts as a specific corrector for CFTR and promotes the maturation and trafficking of Δ F508 CFTR, but not that of the related P-glycoprotein mutant, to the plasma membrane in BHK cells [79]. This molecule is also thought to bind directly to CFTR based on its ability to block specific crosslinks between cysteines introduced in TM6 and 7 of CFTR [80]. VX-770 (**XXV**), another CFTR potentiator identified by Vertex Pharmaceuticals, has successfully completed phase I clinical dosing trials and recently began phase II trials with CF patients (www.cff.org).

Verkman's group has also performed multiple high throughput screens for CFTR potentiators [69, 71, 89]. These screens used a fluorescent halide indicator to measure channel activity of either WT CFTR [69], temperature rescued Δ F508 CFTR [69, 71, 89], or G551D CFTR [69, 89]. The original screen, performed in 2002, identified 57 "strong activators" of WT CFTR channel activity [69]. However, out

of these compounds, none activated G551D with any stronger potency than the already known low potency flavones. When these compounds were tested on FRT cells co-expressing Δ F508-CFTR and the halide indicator, they were inactive on cells grown at 37°C. Even after growth at 27°C for 2 days to rescue Δ F508 and allow for its trafficking to the plasma membrane, only 2 of the WT CFTR "strong activators" were functional in the near micromolar range. Because these results underscored the importance of developing mutation specific therapies for CFTR, another screen was performed with temperature rescued Δ F508 as the main therapeutic target of the screen [71]. This screen identified 6 novel classes of potentiators, all of which acted as strong potentiators for Δ F508 CFTR. Further screening of another 50,000 molecules with secondary analysis of 1000 compounds identified 2 more classes of potentiators which fell into the phenylglycine (**XIX**) and sulfonamide classes (**XX**) [89]. These compounds were able to fix gating defects caused by both Δ F508 as well as G551D mutations. In a follow-up study, researchers synthesized a collection of sulfamoyl-4-oxoquinoline-3-carboxamides (**XXVI**) [94]. While several of these molecules were found to correct CFTR gating with submicromolar potency, the most effective molecule identified from this collection was 6-(ethylphenylsulfamoyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (7b) (**XXVII**).

Recently another screen was performed to identify natural compounds from Chinese medicinal herbs which could act to correct the gating defect of Δ F508 CFTR [95]. The screen was performed in Fisher rat thyroid epithelial cells that co-expressed Δ F508 CFTR along with a cytoplasmic iodide sensitive fluorescent indicator. After screening a collection of 386 herbal compounds, the researchers identified 5 coumarin compounds (**XXVIII**), two of which acted with low micromolar affinity to both stimulate iodide influx as well as transepithelial short-circuit chloride ion currents, without elevating cAMP levels on their own. Overall, while the exact mechanism of action of the drugs discussed above is still unknown, it is the hope that one of the many potentiator drugs identified so far, or a derivative thereof, will be able to be used in combination with corrector molecules to treat both the trafficking and channel activity defects of Δ F508 CFTR.

SUMMARY

While great strides have been made over the past 10 years in the development of CFTR protein rescue drugs, future studies will hopefully focus on defining the mechanism of action of these lead compounds. By understanding the mechanism of action of these drugs, and by developing a better understanding of the defects caused by CFTR mutations, the possibilities for further development of the current lead compounds increases exponentially. It has been suggested that many of the current small molecule therapeutics bind directly to CFTR [80], but the binding pocket(s) in CFTR still need to be identified. Due to the fact that formation of proper intra-domain contacts is necessary for both the folding and trafficking of CFTR [22, 23, 96] as well as for proper channel regulation [97, 98], it may be that binding of small molecules to CFTR stabilizes certain domain structures and allows for the formation of proper domain contacts. Another possibility is that by binding to a particular region

of CFTR, these molecules may obviate the necessity of certain native contacts. The corrector molecules may also act by downregulating the quality control machinery that normally targets misfolded CFTR for degradation. Because different molecules will likely act to correct the defects associated with mutant CFTR in unique ways, the understanding of the mechanism of action of these drugs will also allow for proper combinatorial treatments to be identified.

REFERENCES

- [1] Riordan JR, Rommens JM, Kerem B, *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; 245: 1066-73.
- [2] Anderson MP, Gregory RJ, Thompson S, *et al.* Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science* 1991; 253: 202-5.
- [3] Li M, McCann JD, Liedtke CM, Nairn AC, Greengard P, Welsh MJ. Cyclic AMP-dependent protein kinase opens chloride channels in normal but not cystic fibrosis airway epithelium. *Nature* 1988; 331: 358-60.
- [4] Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993; 73: 1251-4.
- [5] Wang L, Freedman SD. Laboratory tests for the diagnosis of cystic fibrosis. *Am J Clin Pathol* 2002; 117 (Suppl 1): S109-15.
- [6] Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. *N Engl J Med* 2005; 352: 1992-2001.
- [7] Rubenstein RC. Novel, mechanism-based therapies for cystic fibrosis. *Curr Opin Pediatr* 2005; 17: 385-92.
- [8] Hyde SC, Emsley P, Hartshorn MJ, *et al.* Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. *Nature* 1990; 346: 362-5.
- [9] Chang XB, Hou YX, Jensen TJ, Riordan JR. Mapping of cystic fibrosis transmembrane conductance regulator membrane topology by glycosylation site insertion. *J Biol Chem* 1994; 269: 18572-5.
- [10] Lewis HA, Buchanan SG, Burley SK, *et al.* Structure of nucleotide-binding domain I of the cystic fibrosis transmembrane conductance regulator. *EMBO J* 2004; 23: 282-93.
- [11] Vergani P, Lockless SW, Nairn AC, Gadsby DC. CFTR channel opening by ATP-driven tight dimerization of its nucleotide-binding domains. *Nature* 2005; 433: 876-80.
- [12] Smith PC, Karpowich N, Millen L, *et al.* ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer. *Mol Cell* 2002; 10: 139-49.
- [13] Cheng SH, Rich DP, Marshall J, Gregory RJ, Welsh MJ, Smith AE. Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. *Cell* 1991; 66: 1027-36.
- [14] Ma J, Tasch JE, Tao T, *et al.* Phosphorylation-dependent block of cystic fibrosis transmembrane conductance regulator chloride channel by exogenous R domain protein. *J Biol Chem* 1996; 271: 7351-6.
- [15] Ma J, Zhao J, Drumm ML, Xie J, Davis PB. Function of the R domain in the cystic fibrosis transmembrane conductance regulator chloride channel. *J Biol Chem* 1997; 272: 28133-41.
- [16] Winter MC, Welsh MJ. Stimulation of CFTR activity by its phosphorylated R domain. *Nature* 1997; 389: 294-6.
- [17] Chappel V, Irvine T, Liao J, Evagelidis A, Hanrahan JW. Phosphorylation of CFTR by PKA promotes binding of the regulatory domain. *EMBO J* 2005; 24: 2730-40.
- [18] Hegedus T, Serohijos AW, Dokholyan NV, He L, Riordan JR. Computational studies reveal phosphorylation-dependent changes in the unstructured R domain of CFTR. *J Mol Biol* 2008; 378: 1052-63.
- [19] Dahan D, Evagelidis A, Hanrahan JW, *et al.* Regulation of the CFTR channel by phosphorylation. *Pflugers Arch* 2001; 443 (Suppl 1): S92-6.
- [20] Thelin WR, Kesimer M, Tarran R, *et al.* The cystic fibrosis transmembrane conductance regulator is regulated by a direct interaction with the protein phosphatase 2A. *J Biol Chem* 2005; 280: 41512-20.
- [21] Riordan JR. Assembly of functional CFTR chloride channels. *Annu Rev Physiol* 2005; 67: 701-18.
- [22] Cui L, Aleksandrov L, Chang XB, *et al.* Domain interdependence in the biosynthetic assembly of CFTR. *J Mol Biol* 2007; 365: 981-94.
- [23] Du K, Sharma M, Lukacs GL. The DeltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. *Nat Struct Mol Biol* 2005; 12: 17-25.
- [24] Dawson RJ, Locher KP. Structure of a bacterial multidrug ABC transporter. *Nature* 2006; 443: 180-5.
- [25] Serohijos AW, Hegedus T, Aleksandrov AA, *et al.* Phenylalanine-508 mediates a cytoplasmic-membrane domain contact in the CFTR 3D structure crucial to assembly and channel function. *Proc Natl Acad Sci USA* 2008; 105: 3256-61.
- [26] Mendoza JL, Thomas PJ. Building an understanding of cystic fibrosis on the foundation of ABC transporter structures. *J Bioenerg Biomembr* 2007; 39: 499-505.
- [27] Cheng SH, Gregory RJ, Marshall J, *et al.* Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* 1990; 63: 827-34.
- [28] Meacham GC, Patterson C, Zhang W, Younger JM, Cyr DM. The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation. *Nat Cell Biol* 2001; 3: 100-5.
- [29] Pind S, Riordan JR, Williams DB. Participation of the endoplasmic reticulum chaperone calnexin (p88, IP90) in the biogenesis of the cystic fibrosis transmembrane conductance regulator. *J Biol Chem* 1994; 269: 12784-8.
- [30] Loo MA, Jensen TJ, Cui L, Hou Y, Chang XB, Riordan JR. Perturbation of Hsp90 interaction with nascent CFTR prevents its maturation and accelerates its degradation by the proteasome. *EMBO J* 1998; 17: 6879-87.
- [31] Farinha CM, Nogueira P, Mendes F, Penque D, Amaral MD. The human DnaJ homologue (Hdj)-1/heat-shock protein (Hsp) 40 co-chaperone is required for the *in vivo* stabilization of the cystic fibrosis transmembrane conductance regulator by Hsp70. *Biochem J* 2002; 366: 797-806.
- [32] Meacham GC, Lu Z, King S, Sorscher E, Tousson A, Cyr DM. The Hdj-2/Hsc70 chaperone pair facilitates early steps in CFTR biogenesis. *EMBO J* 1999; 18: 1492-505.
- [33] Wang X, Venable J, LaPointe P, *et al.* Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. *Cell* 2006; 127: 803-15.
- [34] Ward CL, Kopito RR. Intracellular turnover of cystic fibrosis transmembrane conductance regulator. Inefficient processing and rapid degradation of wild-type and mutant proteins. *J Biol Chem* 1994; 269: 25710-8.
- [35] Wang X, Matteson J, An Y, *et al.* COPII-dependent export of cystic fibrosis transmembrane conductance regulator from the ER uses a di-acidic exit code. *J Cell Biol* 2004; 167: 65-74.
- [36] Sharma M, Pampinella F, Nemes C, *et al.* Misfolding diverts CFTR from recycling to degradation: quality control at early endosomes. *J Cell Biol* 2004; 164: 923-33.
- [37] Bobadilla JL, Macek M, Jr., Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations--correlation with incidence data and application to screening. *Hum Mutat* 2002; 19: 575-606.
- [38] Rommens JM, Iannuzzi MC, Kerem B, *et al.* Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 1989; 245: 1059-65.
- [39] Gelman MS, Kannegaard ES, Kopito RR. A principal role for the proteasome in endoplasmic reticulum-associated degradation of misfolded intracellular cystic fibrosis transmembrane conductance regulator. *J Biol Chem* 2002; 277: 11709-14.
- [40] Thibodeau PH, Brautigam CA, Machius M, Thomas PJ. Side chain and backbone contributions of Phe508 to CFTR folding. *Nat Struct Mol Biol* 2005; 12: 10-6.
- [41] Denning GM, Anderson MP, Amara JF, Marshall J, Smith AE, Welsh MJ. Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. *Nature* 1992; 358: 761-4.
- [42] Brown CR, Hong-Brown LQ, Biwersi J, Verkman AS, Welch WJ. Chemical chaperones correct the mutant phenotype of the delta F508 cystic fibrosis transmembrane conductance regulator protein. *Cell Stress Chaperones* 1996; 1: 117-25.
- [43] Varshavsky A. The ubiquitin system. *Trends Biochem Sci* 1997; 22: 383-7.

- [44] Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. *Nature* 2004; 429: 841-7.
- [45] Ye Y, Meyer HH, Rapoport TA. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 2001; 414: 652-6.
- [46] Carlson EJ, Pironzo D, Skach WR. p97 functions as an auxiliary factor to facilitate TM domain extraction during CFTR ER-associated degradation. *EMBO J* 2006; 25: 4557-66.
- [47] Dalal S, Rosser MF, Cyr DM, Hanson PI. Distinct roles for the AAA ATPases NSF and p97 in the secretory pathway. *Mol Biol Cell* 2004; 15: 637-48.
- [48] Younger JM, Fan CY, Chen L, Rosser MF, Patterson C, Cyr DM. Cystic fibrosis transmembrane conductance regulator as a model substrate to study endoplasmic reticulum protein quality control in mammalian cells. *Methods Mol Biol* 2005; 301: 293-303.
- [49] Hohfeld J, Cyr DM, Patterson C. From the cradle to the grave: molecular chaperones that may choose between folding and degradation. *EMBO Rep* 2001; 2: 885-90.
- [50] Younger JM, Ren HY, Chen L, *et al.* A foldable CFTR{Delta}-F508 biogenic intermediate accumulates upon inhibition of the Hsc70-CHIP E3 ubiquitin ligase. *J Cell Biol* 2004; 167: 1075-85.
- [51] Arndt V, Daniel C, Nastainczyk W, Alberti S and Hohfeld J. BAG-2 acts as an inhibitor of the chaperone-associated ubiquitin ligase CHIP. *Mol Biol Cell* 2005; 16: 5891-900.
- [52] Alberti S, Bohse K, Arndt V, Schmitz A and Hohfeld J. The co-chaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator. *Mol Biol Cell* 2004; 15: 4003-10.
- [53] Dai Q, Qian SB, Li HH, *et al.* Regulation of the cytoplasmic quality control protein degradation pathway by BAG2. *J Biol Chem* 2005; 280: 38673-81.
- [54] Younger JM, Chen L, Ren HY, *et al.* Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator. *Cell* 2006; 126: 571-82.
- [55] Matsuda N, Suzuki T, Tanaka K, Nakano A. Rma1, a novel type of RING finger protein conserved from Arabidopsis to human, is a membrane-bound ubiquitin ligase. *J Cell Sci* 2001; 114: 1949-57.
- [56] Chen P, Johnson P, Sommer T, Jentsch S, Hochstrasser M. Multiple ubiquitin-conjugating enzymes participate in the *in vivo* degradation of the yeast MAT alpha 2 repressor. *Cell* 1993; 74: 357-69.
- [57] Ye Y, Shibata Y, Kikkert M, van Voorden S, Wiertz E, Rapoport TA. Inaugural Article: Recruitment of the p97 ATPase and ubiquitin ligases to the site of retrotranslocation at the endoplasmic reticulum membrane. *Proc Natl Acad Sci U S A* 2005; 102: 14132-8.
- [58] Schulze A, Stander A, Buerger E, *et al.* The ubiquitin-domain protein HERP forms a complex with components of the endoplasmic reticulum associated degradation pathway. *J Mol Biol* 2005; 354: 1021-7.
- [59] Katiyar S, Joshi S, Lennarz WJ. The retrotranslocation protein Derlin-1 binds peptide:N-glycanase to the endoplasmic reticulum. *Mol Biol Cell* 2005; 16: 4584-94.
- [60] Morito D, Hirao K, Oda Y, *et al.* Gp78 Cooperates with RMA1 in ER-associated Degradation of CFTR{Delta}F508. *Mol Biol Cell* 2008; 19: 1328-1336.
- [61] Lilley BN, Ploegh HL. A membrane protein required for dislocation of misfolded proteins from the ER. *Nature* 2004; 429: 834-40.
- [62] Wahlman J, DeMartino GN, Skach WR, Bulleid NJ, Brodsky JL, Johnson AE. Real-time fluorescence detection of ERAD substrate retrotranslocation in a mammalian *in vitro* system. *Cell* 2007; 129: 943-55.
- [63] Ahner A, Nakatsukasa K, Zhang H, Frizzell RA, Brodsky JL. Small heat-shock proteins select deltaF508-CFTR for endoplasmic reticulum-associated degradation. *Mol Biol Cell* 2007; 18: 806-14.
- [64] Rowe SM, Clancy JP. Advances in cystic fibrosis therapies. *Curr Opin Pediatr* 2006; 18: 604-13.
- [65] Johnson LG, Olsen JC, Sarkadi B, Moore KL, Swanstrom R, Boucher RC. Efficiency of gene transfer for restoration of normal airway epithelial function in cystic fibrosis. *Nat Genet* 1992; 2: 21-5.
- [66] Amaral MD. Processing of CFTR: traversing the cellular maze--how much CFTR needs to go through to avoid cystic fibrosis? *Pediatric Pulmonol* 2005; 39: 479-91.
- [67] Sharma M, Benharouga M, Hu W, Lukacs GL. Conformational and temperature-sensitive stability defects of the delta F508 cystic fibrosis transmembrane conductance regulator in post-endoplasmic reticulum compartments. *J Biol Chem* 2001; 276: 8942-50.
- [68] Gentzsch M, Chang XB, Cui L, *et al.* Endocytic trafficking routes of wild type and DeltaF508 cystic fibrosis transmembrane conductance regulator. *Mol Biol Cell* 2004; 15: 2684-96.
- [69] Ma T, Vetrivel L, Yang H, *et al.* High-affinity activators of cystic fibrosis transmembrane conductance regulator (CFTR) chloride conductance identified by high-throughput screening. *J Biol Chem* 2002; 277: 37235-41.
- [70] Pedemonte N, Lukacs GL, Du K, *et al.* Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. *J Clin Invest* 2005; 115: 2564-71.
- [71] Yang H, Shelat AA, Guy RK, *et al.* Nanomolar affinity small molecule correctors of defective Delta F508-CFTR chloride channel gating. *J Biol Chem* 2003; 278: 35079-85.
- [72] Van Goor F, Straley KS, Cao D, *et al.* Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am J Physiol Lung Cell Mol Physiol* 2006; 290: L1117-30.
- [73] Carlile GW, Robert R, Zhang D, *et al.* Correctors of protein trafficking defects identified by a novel high-throughput screening assay. *Chembiochem* 2007; 8: 1012-20.
- [74] Zhang XM, Wang XT, Yue H, *et al.* Organic solutes rescue the functional defect in delta F508 cystic fibrosis transmembrane conductance regulator. *J Biol Chem* 2003; 278: 51232-42.
- [75] Lim M, McKenzie K, Floyd AD, Kwon E, Zeitlin PL. Modulation of deltaF508 cystic fibrosis transmembrane regulator trafficking and function with 4-phenylbutyrate and flavonoids. *Am J Respir Cell Mol Biol* 2004; 31: 351-7.
- [76] Egan ME, Pearson M, Weiner SA, *et al.* Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science* 2004; 304: 600-2.
- [77] Egan ME, Glockner-Pagel J, Ambrose C, *et al.* Calcium-pump inhibitors induce functional surface expression of Delta F508-CFTR protein in cystic fibrosis epithelial cells. *Nat Med* 2002; 8: 485-92.
- [78] Mall M, Kunzelmann K. Correction of the CF defect by curcumin: hopes and disappointments. *Bioessays* 2005; 27: 9-13.
- [79] Wang Y, Bartlett MC, Loo TW, Clarke DM. Specific rescue of cystic fibrosis transmembrane conductance regulator processing mutants using pharmacological chaperones. *Mol Pharmacol* 2006; 70: 297-302.
- [80] Wang Y, Loo TW, Bartlett MC, Clarke DM. Correctors promote maturation of cystic fibrosis transmembrane conductance regulator (CFTR)-processing mutants by binding to the protein. *J Biol Chem* 2007; 282: 33247-51.
- [81] Varga K, Goldstein RF, Jurkuvenaite A, *et al.* Enhanced cell-surface stability of rescued DeltaF508 cystic fibrosis transmembrane conductance regulator (CFTR) by pharmacological chaperones. *Biochem J* 2008; 410: 555-64.
- [82] Loo TW, Bartlett MC, Wang Y, Clarke DM. The chemical chaperone Cfcor-325 repairs folding defects in the transmembrane domains of CFTR-processing mutants. *Biochem J* 2006; 395: 537-42.
- [83] Robert R, Carlile GW, Pavel C, *et al.* Structural analog of sildenafil identified as a novel corrector of the F508del-CFTR trafficking defect. *Mol Pharmacol* 2008; 73: 478-89.
- [84] Dormer RL, Harris CM, Clark Z, *et al.* Sildenafil (Viagra) corrects DeltaF508-CFTR location in nasal epithelial cells from patients with cystic fibrosis. *Thorax* 2005; 60: 55-9.
- [85] Dalemans W, Barbry P, Champigny G, *et al.* Altered chloride ion channel kinetics associated with the delta F508 cystic fibrosis mutation. *Nature* 1991; 354: 526-8.
- [86] Cutting GR, Kasch LM, Rosenstein BJ, *et al.* A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis conductance regulator protein. *Nature* 1990; 346: 366-9.
- [87] Bompadre SG, Li M, Hwang TC. Mechanism of G551D-CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) Potentiation by a High Affinity ATP Analog. *J Biol Chem* 2008; 283: 5364-9.
- [88] Kerem E. Mutation specific therapy in CF. *Paediatr Respir Rev* 2006; 7 (Suppl 1): S166-9.
- [89] Pedemonte N, Sonawane ND, Taddei A, *et al.* Phenylglycine and sulfonamide correctors of defective delta F508 and G551D cystic fibrosis transmembrane conductance regulator chloride-channel gating. *Mol Pharmacol* 2005; 67: 1797-807.

- [90] Pedemonte N, Diena T, Caci E, *et al.* Antihypertensive 1,4-dihydropyridines as correctors of the cystic fibrosis transmembrane conductance regulator channel gating defect caused by cystic fibrosis mutations. *Mol Pharmacol* 2005; 68: 1736-46.
- [91] Illek B, Zhang L, Lewis NC, Moss RB, Dong JY, Fischer H. Defective function of the cystic fibrosis-causing missense mutation G551D is recovered by genistein. *Am J Physiol* 1999; 277: C833-9.
- [92] Moran O, Galiotta LJ, Zegarra-Moran O. Binding site of activators of the cystic fibrosis transmembrane conductance regulator in the nucleotide binding domains. *Cell Mol Life Sci* 2005; 62: 446-60.
- [93] Schmidt A, Hughes LK, Cai Z, *et al.* Prolonged treatment of cells with genistein modulates the expression and function of the cystic fibrosis transmembrane conductance regulator. *Br J Pharmacol* 2008; 153: 1311-23.
- [94] Suen YF, Robins L, Yang B, Verkman AS, Nantz MH, Kurth MJ. Sulfamoyl-4-oxoquinoline-3-carboxamides: novel potentiators of defective DeltaF508-cystic fibrosis transmembrane conductance regulator chloride channel gating. *Bioorg Med Chem Lett* 2006; 16: 537-40.
- [95] Xu LN, Na WL, Liu X, *et al.* Identification of natural coumarin compounds that rescue defective deltaf508-cftr chloride channel gating. *Clin Exp Pharmacol Physiol* 2008; 35: 878-883.
- [96] Chen EY, Bartlett MC, Loo TW, Clarke DM. The DeltaF508 mutation disrupts packing of the transmembrane segments of the cystic fibrosis transmembrane conductance regulator. *J Biol Chem* 2004; 279: 39620-7.
- [97] Baker JM, Hudson RP, Kanelis V, *et al.* CFTR regulatory region interacts with NBD1 predominantly via multiple transient helices. *Nat Struct Mol Biol* 2007; 14: 738-45.
- [98] Naren AP, Cormet-Boyaka E, Fu J, *et al.* CFTR chloride channel regulation by an interdomain interaction. *Science* 1999; 286: 544-8.

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