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Targets for cystic fibrosis therapy: proteomic analysis and correction of mutant cystic fibrosis transmembrane conductance regulator

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Abstract

Proteomic analysis has proved to be an important tool for understanding the complex nature of genetic disorders, such as cystic fibrosis (CF), by defining the cellular protein environment (proteome) associated with wild-type and mutant proteins. Proteomic screens identified the proteome of CF transmembrane conductance regulator (CFTR), and provided fundamental information to studies designed for understanding the crucial components of physiological CFTR function. Simultaneously, high-throughput screens for small-molecular correctors of CFTR mutants provided promising candidates for therapy. The majority of CF cases are caused by nucleotide deletions ($\Delta F508$ CFTR; >75%), resulting in CFTR misfolding, or insertion of premature termination codons (~10%), leading to unstable mRNA and reduced levels of truncated dysfunctional CFTR. In this article, we review recent results of proteomic screens, developments in identifying correctors for the most frequent CFTR mutants, and comment on how integration of the knowledge gained from these studies may aid in finding a cure for CF and a number of other genetic disorders.

Keywords

chemical chaperones; cystic fibrosis transmembrane conductance regulator; protein biogenesis; proteome; readthrough

Cystic fibrosis (CF) is a multiorgan genetic disorder caused by the loss of function of the CF transmembrane conductance regulator (CFTR) [1]. CF is the most common autosomal recessive disease in people of European ancestry, with more than 1700 different mutations identified in the *CFTR* gene [201]. The CFTR protein is expressed in epithelial cells and is a member of the ATP-binding cassette (ABC) transporter superfamily, which also includes the multidrug-resistance protein (reviewed in [1]). CFTR consists of two membrane-spanning domains, two nucleotide-binding domains (NBDs), and a regulatory domain, which controls channel activity (Figure 1). CFTR is a chloride channel and regulator of other transporters [2,3].

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Loss of function of CFTR results in viscous secretions of the exocrine glands in multiple organ systems and elevated sweat chloride levels. The most prevalent symptoms develop in the gastrointestinal and respiratory tracts. While administration of pancreatic enzyme supplements have significantly reduced the gastrointestinal complications and extended the lifespan of patients to more than 35 years, the chronic bacterial airway infections lead to respiratory failure. At present, the respiratory complications are responsible for the majority of mortality associated with this disease. In spite of the fact that symptomatic combination therapies have been employed, including antibiotic, anti-inflammatory and mucolytic medications, the lung function often declines to less than 30%, and transplantation remains the last treatment option. Since the discovery of the *CFTR* gene in 1989 [4], a major effort has been directed to develop causative therapies for CF. These included gene-replacement strategies, based on both viral and nonviral vectors (reviewed in [5]), and a number of alternative approaches [6]. In this article, we will focus on the alternative therapeutic efforts designed to cure the majority of CF patients, and how proteomics may help both in designing and testing the efficacy of these methods.

CF as a protein-folding disease

The most common mutation in *CFTR* is the loss of phenylalanine at the 508 position of the CFTR protein, and results in CFTR misfolding, misprocessing [7] and degradation by the endoplasmic reticulum (ER) quality control [8,9]. $\Delta F508$ CFTR has garnered significant attention from cell biologists monitoring how mis-folded proteins are recognized and eliminated by ER-associated degradation (ERAD) [10]. $\Delta F508$ CFTR is a temperature-sensitive mutation and when cells expressing $\Delta F508$ CFTR are cultured at lower temperatures (27°C) for 24 h or more, some CFTR molecules escape from ERAD, and traffic to the cell surface [11]. Analysis of the rescued $\Delta F08$ CFTR revealed at least three defects in the $\Delta F508$ CFTR protein: failure to fold properly and exit the ER, reduced stability once $\Delta F508$ CFTR reaches the cell surface, and compromised channel activity. These observations prompted investigators to search for compounds that not only rescue $\Delta F508$ CFTR from ERAD, but also eliminate the protein instability and functional defects. In the next sections, we will discuss these strategies in more detail.

Defects in $\Delta F508$ CFTR biogenesis & ER exit

In a recent study from Roy *et al.*, an interesting question was posed [12]. Is $\Delta F508$ CFTR misprocessing the result of ER retention [13] or a failure to interact with the coat protein complex (COP)II-based export machinery [14]? While operationally these two have the same consequence, understanding the exact mechanism is important for potential therapeutic interventions. In support of the retention model, several studies have demonstrated that removal of the RXR motifs in $\Delta F508$ CFTR resulted in the release of CFTR from the ER [13,15–17]. In the alternative model, the data suggest that $\Delta F508$ CFTR has a reduced association with the Sec24 protein (part of the COPII complex necessary for ER exit), and lowering the temperature to 27°C reversed this effect [14]. To test these two possibilities the authors analyzed two mutations, $\Delta F508$ and D567A. The D567A mutation disrupts a diacidic ER exit signal (DAD567) [14] and inhibits wild-type CFTR ER exit [12,14]. Therefore, the DAA567 mutant serves as a sorting mutation in these studies. Using DAA567 and $\Delta F508$ CFTR, the authors demonstrate that low-temperature rescue requires a functional exit code, since the $\Delta F508$ /DAA567 mutation has a more severe block at low temperature (30°C) compared with $\Delta F508$ mutation alone. Therefore, the data suggest that the conformational alterations in $\Delta F508$ inhibit its ability to exit the ER, but retain its ability to interact with the ER exit machinery. However, it is difficult to differentiate between ablating a sorting signal versus slightly altering the structure near the $\Delta F508$ mutation.

This concern is particularly relevant given the data on second-site suppressors, which correct the folding defects caused by the original mutation. In an elegant study, Teem and colleagues replaced a part of the yeast ABC transporter sterile 6 (STE6) NBD1 domain with a Δ F508 CFTR NBD1 fragment. Since STE6 functions as peptide α -factor transporter during mating, expression of the chimera construct resulted in a sterile phenotype. The authors screened for revertants in the CFTR portion of the chimera that restored the mating defect. The yeast-suppressor mutations were then engineered back into Δ F508 CFTR and tested in mammalian cells [15]. A number of suppressors were identified, which corrected Δ F508 to various degrees.

Using yeast chimera-based results as a starting point, He *et al.* (2010) analyzed the influence of these suppressors on the folding of NBD1, and on NBD1 interactions with other domains of CFTR [18]. Notably, while the Δ F508 CFTR mutation is localized to NBD1, previous studies indicated that there was only a minimal influence of the mutation on the structure of NBD1 [19], but a more significant effect on the proper folding and intramolecular associations of other domains [20,21]. The first point addressed by these new studies was that the suppressors in NBD1 only influenced mutations within NBD1, without affecting mutations found in other domains. Importantly, the results indicated that the suppressor mutations restored the interactions of NBD1 with cytoplasmic loop (CL)4, and NBD2 with CL2 [18]. Two other important issues were also resolved in these studies. First, the success of the suppressor depended on the presence of NBD2; second, it required the intact CFTR molecule rather than the expression of two half molecules. These findings suggested that the sequential and timed assembly of the molecule is critical for proper biogenesis [18], and that the phenylalanine 508 of CFTR is a key residue, which locks the domains into a compact and stable structure. Presumably, any sort of pharmaceutical rescue will require slight alterations in NBD1 structure that enhance these domain–domain interactions.

Another interesting point about Δ F508 CFTR folding is that the cellular background appears to play an important role in the ability of Δ F508 CFTR to be rescued [22]. In a recent study, Wang *et al.* examined the ability of two different cell lines, human embryonic kidney (HEK) 293 and baby hamster kidney (BHK), to deliver Δ F508 CFTR to the cell surface at low temperature. While HEK 293 cells were able to export Δ F508 CFTR very effectively at low temperatures, Δ F508 CFTR rescue in BHK cells was inefficient. Furthermore, a mild heat shock enhanced the rescue of Δ F508 CFTR in the HEK 293 cells, but had no effect in the BHK cells [22]. These results suggest that the cellular background and chaperone/folding environmental differences profoundly affect the rescue of Δ F508 CFTR. Furthermore, it is evident that there are cell type-specific differences in Δ F508 CFTR rescue using pharmacological chaperones [23,24].

Whether the rescue of Δ F508 CFTR occurs through the alteration of the cell-specific chaperone environment or other mechanisms is not yet clear. However, proteomic analysis of CFTR-interacting proteins in the presence or absence of correctors and different culture conditions may help answer this important question.

Defects in Δ F508 CFTR surface stability

Low-temperature-rescued Δ F508 CFTR is unstable at the cell surface [25–30], and is rapidly internalized and degraded in the lysosomal compartment [28,31]. Given that there are cell type-specific differences in the rescue of Δ F508 CFTR [22], it is important to understand the trafficking of the mutant protein in human airway epithelial (HAE) cells. Cholon *et al.* examined this in HAE cells isolated from the CF and normal lung. In these studies, the HAE cells were transduced with adenoviral vectors expressing epitope-tagged wild-type and Δ F508 CFTR. The results indicated that wild-type CFTR internalization is slower in

polarized HAE cultures [30] than in nonpolarized cells, which is consistent with the results in HAE cell lines [32]. In these HAE cultures, the authors show that $\Delta F508$ CFTR is low-temperature correctable, but unstable at the cell surface. Significantly, the surface instability could not be corrected with small-molecule correctors, such as VRT-325 or Corr-4a [30]. Surface $\Delta F508$ CFTR could, however, be stabilized by keeping the cells at low temperature [30,33], with a dynamin inhibitor, dynasore [30,34], with a proteasome inhibitor, MG-132, or with extracting membrane cholesterol, methyl- β -cyclodextrin [30]. These methods, as the authors indicate, lack specificity and, therefore, emphasize the need for identifying reagents that stabilize $\Delta F508$ CFTR itself.

CFTR proteomics

Given the delicate balance between protein folding and degradation in the ER, it is not surprising that a number of different proteomics-based approaches have been employed to understand ‘what is wrong with $\Delta F508$ CFTR?’ or, more pragmatically, ‘how do you fix it?’ An excellent example is the study by Wang and colleagues to identify binding partners of wild-type and $\Delta F508$ CFTR [35]. In this study, the authors examined the differences between wild-type and $\Delta F508$ CFTR binding partners by MudPIT [36] after coimmunoprecipitation. Their analysis yielded what they refer to as the ‘CFTR interactome’ (i.e., those proteins required for the biogenesis, trafficking and function of CFTR). A major finding of this study is that the heat shock protein (Hsp)90 cochaperone interactions are different between wild-type and $\Delta F508$ CFTR. Reduction in the expression of one of these cochaperones, Aha1, led to the export of $\Delta F508$ CFTR from the ER (an increase in C-band formation). Furthermore, overexpression of Aha1 decreased the ER export of $\Delta F508$ CFTR. These results suggested that altering the chaperone/cochaperone composition of the ER might be used to facilitate $\Delta F508$ CFTR biogenesis.

Using an alternative strategy, Gomes-Alves and colleagues examined the protein profiles of BHK cells expressing either wild-type, $\Delta F508$ CFTR, or a partially corrected $\Delta F508$ CFTR mutant, which had its four RXR ER-retention motifs inactivated ($\Delta F508$ -4RK) [37]. Rather than examine the CFTR protein interactions, the authors examined how the expression of the various forms of CFTR affected the global protein-expression patterns. Cell lysates from each of the cell types were analyzed by 2D gel electrophoresis-based proteomics, and confirmed by western blot analysis. The results indicated that the $\Delta F508$ -4RK and $\Delta F508$ CFTR induce an unfolded-protein response (UPR), with a greater effect found with the $\Delta F508$ -4RK construct [37]. This is consistent with previous studies showing that significant overexpression of $\Delta F508$ CFTR induces the UPR [38]. Furthermore, the fact that the $\Delta F508$ -4RK mutant enhances the UPR is consistent with the idea that the 4RK substitutions do not correct the folding defect of $\Delta F508$ CFTR [39] but, rather, inhibit the retention of a misfolded protein [37,39]. A major point made in these studies is that cells expressing the 4RK revertant had a protein-expression profile similar to wild-type CFTR-expressing cells, suggesting that cellular protein-expression pattern changes could occur during $\Delta F508$ CFTR rescue. This idea is consistent with the study by Singh *et al.*, demonstrating that chaperone network identified in chemically rescued $\Delta F508$ CFTR using sodium 4-phenylbutyrate in IB3-1 bronchial epithelial cells is similar to that of genetically repaired IB3-1 cells expressing wild-type CFTR [40].

While the previous studies examined the differences between wild-type and $\Delta F508$ CFTR-expressing cell lines using proteomics, a recent study by Trzcinska-Daneluti *et al.* relied on a functional screen to identify proteins that correct $\Delta F508$ CFTR [41]. Using HEK 293 cell line expressing $\Delta F508$ CFTR, they individually coexpressed 450 unique proteins that were fused to an enhanced halide-sensitive yellow fluorescent protein (YFP). Using a high-throughput imaging system, they identified 13 proteins (signal transducer and activator of

transcription 1 [STAT1], endothelin 1, HIP A4, adaptor protein 2, regenerating islet-derived 3a variant 1, galectin-3, Trk-fused gene, caveolin 2, SAPK substrate 1, related to DYDC2–DPY30 domain-containing protein 2, T-complex 10-like, Golgi-reassembly stacking proteins 1 and prefoldin subunit 2), whose overexpression enhanced rescue better than Corr-4a treatment in the HEK293 cells. While each of these effects were confirmed in BHK cells, only one of them was better at rescuing Δ F508 CFTR than the Corr-4a treatment in the BHK cells, STAT1 [41]. Further support for the importance of STAT1 comes from siRNA knockdown of protein inhibitor of activated STAT1 (PIAS1), which also rescued Δ F508 CFTR [41], supporting the idea that the STAT1 activity was required for this effect. This assay is interesting, since it can be used to identify proteins that either enhance or inhibit Δ F508 CFTR maturation and, therefore, offers the ability to identify completely unanticipated protein targets and potential avenues for intervention. The potential protein targets that have been shown to influence Δ F508 CFTR biogenesis are listed in Table 1.

Identification of pharmacological/chemical chaperones & their effectiveness

One of the most heavily investigated and exciting areas of research for Δ F508 CFTR rescue comes from the high-throughput screening of chemical libraries. Initially, glycerol was shown to reverse the misfolding of Δ F508 CFTR [42]. Soon afterwards, a number of other compounds were reported to mimic the low-temperature effects. These included sarcoplasmic-reticulum Ca^{2+} -ATPase inhibitors (curcumin and thapsigargin) [43–45], nitric oxide donors [46,47], sodium 4-phenylbutyrate [40,48–50], and pharmacological chaperones that promote Δ F508 CFTR trafficking [51–55]. As a way of clarifying the nomenclature, chemicals, such as glycerol, that act nonspecifically, are often referred to as chemical chaperones. Pharmacological chaperones, on the other hand, are believed to act more specifically on the folding of Δ F508 CFTR. The functional distinction between these two types of chaperones, however, is not always entirely clear. For the purpose of this article, we will focus on pharmacological chaperones and how well the current compounds function.

In theory, an ideal pharmacological chaperone should correct all of the defects associated with Δ F508 CFTR. Operationally, however, the pharmacological chaperones have fallen into two categories, correctors and potentiators. The correctors rescue some Δ F508 CFTR from ERAD, whereas, the potentiators enhance defective channel activity at the cell surface.

Early high-throughput screens of small molecules concentrated on potentiators [56–58], since these screens could be performed on wild-type CFTR-expressing cells, G551D CFTR (a channel-defective mutant)-expressing cells, or even low-temperature-rescued Δ F508 CFTR-expressing cells, after a brief incubation with the molecule. Screening for correctors has proved to be much more of a challenge, since the requirements for success depended on enhancing folding/ER exit and promoting a reasonably stable level of Δ F508 CFTR at the cell surface for analysis. The first examples come from the work of Pedemonte *et al.* and Van Goor *et al.* [51,52].

From these two important studies, two of the most commonly used/tested correctors, Corr-4a [51] and VRT-325 [52], were identified, along with one of the most common potentiators, VRT-532 [52]. In the study by Pedemonte *et al.*, the authors screened 150,000 compounds and more than 1500 analogs. The screen relied on Fischer rat thyroid (FTR) cells coexpressing Δ F508 CFTR and a halide-sensitive indicator, YFP-H148Q/I152L. Compounds were tested at 10 μM concentration, cells were treated for 24 h, and Δ F508 CFTR-mediated halide influx was monitored [51]. A number of effective correctors, including Corr-4a, come from this screen. Several of them were shown to be more effective than low-temperature correction in the FTR cells and the correction after washout of the

drug lasted more than 12 h [51]. Importantly, Ussing chamber analysis of primary cultures of HBE cells from $\Delta F508$ CFTR patients indicated that Corr-4a was comparable to low-temperature correction and equivalent to approximately 8% of the CFTR-dependent chloride secretion found in non-CF HBEs [51].

In the Van Goor study, the authors used NIH/3T3 mouse fibroblasts stably expressing $\Delta F508$ CFTR for the corrector screen [52]. To monitor CFTR function, they measured membrane potential changes in response to $\Delta F508$ CFTR activation in a fluorescence resonance energy-transfer assay [52]. In total, 164,000 compounds were tested at 10 μM concentration, and a secondary screen in FTR cells was performed to narrow the field of drugs. A quinazolinone compound, VRT-422, was identified as one of the most potent [52]. An analysis of more than 500 analogs of this compound resulted in the identification of the most effective second-generation compound, VRT-325. The rescue by VRT-325 was shown to be not specific for $\Delta F508$ CFTR, since another trafficking mutant of a hERG cardiac potassium channel (G601S) was also rescued from ER retention [52]. Analysis in primary CF airway cultures in Ussing chambers revealed that even uncorrected cells have approximately 5% activity (compared with wild type). This channel activity was attributed to $\Delta F508$ CFTR and correction with VRT-325 increased the CFTR currents to approximately 10% of wild type. Furthermore, with VRT-325, and the potentiator VRT-532, activity increased to 20% of that observed in non-CF HBEs [52]. These two studies are significant, since they provide the proof of concept that small-molecule correctors and potentiators can be used to correct many of the defects associated with $\Delta F508$ CFTR and, even more importantly, suggest that drug development and discovery of this sort may be useful in a number of other protein-folding diseases [51,59].

Premature termination codons & therapeutic approaches

Although the $\Delta F508$ CFTR mutation has received the most attention in experimental therapies because of its prevalence, approximately 10% of CF patients worldwide, and more than 50% of patients in Israel, carry premature termination codons (PTCs) in the CFTR mRNA [60]. PTCs (UAA, UAG or UGA) result from nonsense mutations, frame-shift deletions and insertions, or from splicing variants. These mutations produce truncated, nonfunctional or toxic proteins, as the result of dominant-negative or gain-of-function effects (Figure 1) [61]. PTCs are also associated with reduced levels of the affected mRNA transcripts [62], as a result of nonsense-mediated mRNA decay (NMD) [61,63]. Since approximately 12% of all point mutations result in PTCs [64] and cause more than 1800 known human disorders [65], approaches to develop therapies for these mutations are of great interest. During the past 15 years, great advances have been made in potential treatments based on the pioneering studies by Bedwell and colleagues designed to suppress termination codons in the yeast *STE6* [66] and the *CFTR* genes [67,68].

Early genetic studies indicated that insertion of a termination codon (UGA) at R553X or W1316X resulted in significantly reduced CFTR mRNA levels in the respiratory epithelial cells of CF patients [69,70]. Subsequent studies revealed additional PTCs in different regions of the *CFTR* gene caused CF (Figure 1). Interestingly, an identical premature translation termination mutation (UGA) was identified within a yeast ABC transporter, Ste6p [66]. The observation that suppression of the *STE6* termination codon resulted in the production of full-length protein [66] formed the foundation for studies to suppress termination codons in *CFTR* and other genes as a potential therapeutic approach.

Aminoglycoside antibiotics inhibit bacterial protein synthesis by efficiently binding to the decoding site of the rRNA [71]. In eukaryotes, antibiotic binding is less specific, and results in translational readthrough of termination codons by the insertion of an amino acid at the

stop codon [71–73]. Based on the ability of antibiotics to promote readthrough of the yeast mRNA, Bedwell and colleagues demonstrated that gentamicin and its derivative, geneticin (G418) suppress the termination codons in the *CFTR* mRNA harboring the G542X [67] and W1282X mutations [68]. Subsequently, aminoglycosides were shown to enhance the expression of a PTC mutant, dystrophin, in mdx mice, the animal model of Duchenne muscular dystrophy [74]. More importantly, gentamicin was also shown to be effective in suppressing PTCs of *CFTR* in subsequent clinical trials performed in Israel [75,76] and in the USA [77]. However, the considerable toxicity of repeated gentamicin administration advanced efforts to identify systemically potent, but less toxic compounds. The development of a transgenic mouse model carrying the *CFTR*-G542X mutation significantly aided these efforts [78]. With this animal model, amikacin was first identified as a more potent and less toxic alternative of gentamicin to suppress the G542X PTC [79].

As a result of high-throughput screening efforts, PTC Therapeutics, Inc. identified PTC-124, a 1,2,4-oxadiazole compound that is an orally active, small molecule, which can override nonsense translation termination signals to produce full-length proteins [80]. PTC-124 has been shown to be nontoxic and can correct both *CFTR* [81] and dystrophin [82] in transgenic animals, providing hope for patients with CF and Duchenne muscular dystrophy, respectively [83].

In spite of the significant progress made in this area, some studies in animal models of muscular dystrophy [74,84], and in CF patients [76,85], revealed significant variability in the responses to these drugs. We will now review the possible molecular mechanisms of this variability and the necessary factors for successful readthrough. Using an *in vitro* translation-based reporter system containing all three PTCs (UGA, UAG and UAA), Manuvakhova *et al.* demonstrated that the sequence context around the PTCs affects the efficiency of the aminoglycoside-dependent amino acid incorporation [86]. These studies indicate that the mRNA context influences the suppression of stop codons based on the delicate conformational differences caused by the aminoglycoside binding to the ribosomal decoding site. These studies are significant because they provide mechanistic reasons for the discrepancies observed in translational readthrough between *CFTR* and dystrophin, harboring identical PTCs (UGA) [74,76,84,85].

In addition to the genetic context of the PTCs, the chemical composition of the aminoglycosides (gentamicin, amikacin, tobramycin, neomycin and paromomycin) [78,87] and the brand (composition of the gentamicin components [C1, C1a and C2] in the product) [88] have also been proven to be important for PTC suppression. Unfortunately, the variability of drug metabolism between patients and the differences in the delivery methods also contribute to variability observed in clinical studies.

However, not all the variability can be explained based on the aforementioned factors. Importantly, none of the clinical studies monitored *CFTR* mRNA levels. Considering that adult CF patients participating in clinical trials are likely to suffer from inflammatory respiratory symptoms and hypoxia, it is necessary to consider additional mechanisms that may contribute to variability of responses to readthrough compounds. We review possible cellular mechanisms that may influence translational readthrough. Inflammation, hypoxia and environmental insults inflict cellular stress and activate cellular stress responses in order to re-establish homeostasis [89]. The ER stress response, the UPR, is a cellular stress response that can be activated by a number of insults [90,91], including inflammation [92] and hypoxia [93]. The UPR reduces the load and enhances the capacity of the ER in order to re-establish homeostasis [91]. Importantly, it has been demonstrated that the UPR inhibits *CFTR* transcription and translation and enhances the ERAD of *CFTR*, resulting in reduced *CFTR* function at the cell surface [94,95]. In other words, the UPR-associated

transcriptional repression of CFTR may reduce the availability of CFTR mRNA for readthrough. If this is coupled with reduced translation and enhanced degradation of CFTR, the efficiency of PTC suppression could be diminished. This hypothesis is supported by reports indicating that the UPR is activated in normal and cancerous lung cells of smokers [96], and there is a corresponding decrease in CFTR message levels in lung epithelial cells of smokers [97,98]. However, it is important to note that in studies using recombinant CFTR-expressing cells as models, the factors that regulate endogenous CFTR transcription are not being considered. Importantly, CF patients participating in clinical trials may have different levels of CFTR mRNA available for readthrough, resulting from UPR activity or other stress responses.

Furthermore, in any clinical trial dealing with PTC readthrough, the role of NMD, and its regulation by the UPR, also needs to be considered. The NMD is responsible for the degradation of mRNAs containing PTCs, and this process is in place to reduce the production of truncated and often toxic proteins [99,100]. Indeed, it has been reported that the efficiency of NMD varies among different cells [101]. Importantly, inhibition of NMD by siRNA knockdown of key NMD regulators, such as UPF1 or UPF2, enhances the efficiency of CFTR PTC suppression by gentamicin [101,102]. Furthermore, while hypoxia can activate the UPR [93], resulting in CFTR expression reduction [94,95], it also downregulates CFTR mRNA levels through another pathway that involves HIF [103]. Interestingly, hypoxia also inhibits NMD through the activity of the α -subunit of eukaryotic initiation factor 2 (eIF2 α) [104], which is activated (phosphorylated) by the UPR regulator PERK (PKR [dsRNA-dependent protein kinase]-like ER kinase). eIF2 α attenuates protein synthesis in general (including NMD subunits), but promotes the translation of ATF4, a transcription factor that activates ER stress-response genes [105]. Based on these findings, the UPR and NMD can dynamically alter gene expression and the level of *CFTR* mRNA harboring PTCs will be the result of a balance between these different pathways. Nevertheless, a deeper understanding of cellular stress responses and how they communicate during pathological conditions will be necessary to fine-tune treatments, such as PTC readthrough.

To connect the discussed results with the main theme of this article, it is important to note that identification of the main pathways that regulate *CFTR* gene expression under both physiological and pathological conditions will help to provide important information that is necessary for evaluating any potential therapeutic strategy.

Expert commentary

In order to understand the complexity of genetic disorders caused by point mutations, it is fundamental to identify the physiological function of the wild-type gene product and the cellular environment in which it functions. Indeed, proteomics has provided a wealth of information about cell type-specific CFTR-interacting proteins, and how the loss of CFTR or expression of mutants may alter this environment known as the proteome. Importantly, understanding the differences in cellular protein-expression profiles between wild-type and mutant CFTR-expressing cells may provide a diagnostic tool to measure the efficacy of experimental treatments. To find a cure for CF, the most significant efforts have been directed toward high-throughput screens of chemical libraries to identify correctors of CFTR mutants. These screens have yielded attractive candidate drugs, such as VX-770 (a potentiator), VX-809 (a corrector) and GS-9411 (a sodium channel antagonist) to treat CF. Some of these are currently in various stages of clinical trials [106,107]. The safety of the compounds, and the best methods for delivery, will certainly be paramount issues in the clinical trials. For PTCs, high-throughput screens identified gentamicin analogs with minimal toxicity and efficient readthrough capacity [81]. An efficient treatment protocol

will either specifically correct the mutant CFTR (PTCs) and change the cellular environment to resemble wild-type CFTR-expressing cells or, alternatively, alter the cellular environment to accommodate the folding of the mutant protein ($\Delta F508$ CFTR). Importantly, the strategies employed in the CF field have provided a roadmap to navigate efforts directed towards the treatment of other genetic disorders.

Five-year view

Proteomic screens and high-throughput small-molecule/drug screens ushered in a new era of excitement, and added significant new prospects for developing causative treatments not only for CF, but for a large number of genetic disorders. Developments in automated screening and proteome identification techniques will provide opportunities to use proteomics as a useful diagnostic tool. Identification of mutation-specific small-molecular correctors and potentiators will provide targeted therapeutic options and eliminate the unpredictable effects of nonspecific approaches. However, it is likely that future generations of drugs will be discovered prior to wide-range therapeutic applications. In addition, recent studies indicate that the processing and functional correction of $\Delta F508$ CFTR appears to be different in different cell types. This suggests that correction in some tissues may be more efficient than in others. Therefore, understanding CFTR expression regulation and function in different cell types will be an important area of research. Furthermore, determining the complete mechanism of action and the indirect effects of the drugs identified in the high-throughput screens will continue to be a major effort in the field for years to come.

Key issues

- The majority of cystic fibrosis (CF) cases are from the $\Delta F508$ mutation or premature termination codons (PTCs) that result in unstable mRNA and truncated CF transmembrane conductance regulator (CFTR).
- Approximately 12% of all point mutations result in premature termination codons (PTCs) and cause 1800 known human disorders.
- Pioneering studies designed to promote readthrough of stop codons in the *CFTR* gene have provided a blueprint for the potential treatments in other diseases.
- New strategies for correcting CF have identified protein targets whose overexpression/siRNA knockdown promote $\Delta F508$ CFTR processing.
- The relative correction of $\Delta F508$ CFTR by either low temperature or pharmacological chaperones is cell type specific.
- Proteomics-based approaches have been utilized to identify the wild-type and mutant CFTR proteomes in different cell types, and have yielded valuable insights into potential therapeutic approaches.
- High-throughput screens of chemical libraries have provided a number of small molecules that:
 - Promote $\Delta F508$ CFTR biogenesis
 - Enhance $\Delta F508$ CFTR channel activity
 - Facilitate translational readthrough for PTCs
- Understanding the mechanism of action of the small molecules will be an important consideration in future studies.

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Website

201. Cystic Fibrosis Mutation Database. www.genet.sickkids.on.ca/cftr

Figure 1. Cystic fibrosis transmembrane conductance regulator and location of the most frequent mutations

Red regions indicate the nucleotide binding domains (NBD1 and NBD2) and the green region is the regulatory (R) domain. The black rectangle represents the $\Delta F508$ mutation and 'X' represents premature termination codons.

Table 1

Protein targets for $\Delta F508$ cystic fibrosis transmembrane conductance regulator rescue.

Protein targets	Function	Effects of inhibition or siRNA knockdown	Effects of overexpression	Ref.
Ala1	Hsp90 cochaperone ATPase regulator	Increases the expression and maturation of $\Delta F508$ CFTR after depletion	Downregulates the expression and maturation when overexpressed	[35]
Nedd4-2	E3 ubiquitin ligase, binds both wild-type and $\Delta F508$ CFTR. Dexamethasone rescues functional expression of $\Delta F508$ CFTR, which can be blocked by siRNA depletion of SGK1	Correct $\Delta F508$ CFTR trafficking after siRNA depletion		[108]
STAT1	Signal transducer and activator of transcription 1		Rescues $\Delta F508$ CFTR function when co-expressed as discovered through high-content screen	[41]
ET-1	Endothelin 1			
HSPA4	Heat-shock protein A4			
AP2M1	Adapter protein 2, $\mu 1$ (AP2 $\mu 1$)			
PAP	Regenerating islet-derived 3 α (REG3 α) variant 1			
LGALS3	Galectin-3 (lectin, galactosidase-binding soluble protein 3)			
TFG	Trk-fused gene			
Cav2	Caveolin 2			
SAKS1	SAPK substrate 1			
MGC16186	Related to DYDC2-DPY30 domain-containing protein 2			
TCP10L	T-complex 10-like			
GORASP1	Golgi reassembly stacking proteins 1)(GRSP65 (amino acids 1–86)			
PFDN2	Prefoldin subunit 2			
USP19	A membrane associated deubiquitination enzyme		Rescues $\Delta F508$ CFTR when overexpressed	[109]
RMA1 CHIP	Ubiquitin ligases	Increases correctable pool of $\Delta F508$ CFTR when depleted		[110]
PA28 COP9	Presumably decrease $\Delta F508$ CFTR degradation when downregulated	Downregulated by low temperature		[37]
NYD-SP27	Has structural similarity to an isoform of phospholipase C	Antisense suppression of NYD-SP27 improves the trafficking of $\Delta F508$ CFTR		[111]
UCH-L1	Ubiquitin C-terminal hydrolase-L1		Increases CFTR expression when overexpressed	[112]

Protein targets	Function	Effects of inhibition or siRNA knockdown	Effects of overexpression	Ref.
HDAC7	Histone deacetylase 7	Reduction of HDAC7 activity by chemical inhibitors or siRNA knockdown restores ΔF508 CFTR function		[113]
STX6	Syntaxin 6	Silencing STX6 increases CFTR function	Overexpression of STX6 reduces the steady-state level and stability of CFTR	[114]
CAL	CFTR-associated ligand	Silencing CAL increases the cell surface expression of ΔF508 CFTR	Overexpression of CAL directs CFTR to lysosomal degradation and decreases the amount of CFTR	[115,116]
NHERF1/EBP50	Na ⁺ /H ⁺ exchanger regulatory factor 1	Knockdown of NHERF1 enhances the degradation of rescued ΔF508 CFTR	Overexpression increases F-actin assembly and CFTR-dependent Cl ⁻ secretion	[117,118]
TC10	Rho family small GTPase		Interacts with CAL, overexpression of TC10 induces CFTR surface expression by increasing recycling	[119]

CFTR: Cystic fibrosis transmembrane conductance regulator.