Fixing cystic fibrosis by correcting CFTR domain assembly

Tsukasa Okiyoneda[1] and Gergely L. Lukacs[1,2]

[1]Department of Physiology and [2]Groupe de Recherche Axé sur la Structure des Protéines (GRASP), McGill University, Montréal, Quebec H3E 1Y6, Canada

For cystic fibrosis (CF) patients most therapies focus on alleviating the disease symptoms. Yet the cellular basis of the disease has been well studied; mutations in the CF gene can impair folding, secretion, cell surface stability, and/or function of the CFTR chloride channel. Correction of these basic defects has been a challenge, but indicates that a deeper understanding of the molecular and cellular mechanism of mutations is a prerequisite for developing more efficient therapies.

CF is an autosomal recessive genetic disease with incidence of ∼1 in 2,500 Caucasians, affecting ∼70,000 people in North America and Europe (Riordan, 2008). The clinical features include pancreatic insufficiency, male infertility, meconium ileus in the newborn, and chronic lung infection with excessive inflammation, leading to progressive deterioration of lung function (Zielenski, 2000). The loss of lung function is the main cause of death in CF patients. Most current therapies treat the symptoms of these aspects of the disease and have increased the median life expectancy for individuals with CF to ∼39 years (Ashlock and Olson, 2011).

In 1989, the CF gene that encodes the CF transmembrane conductance regulator (CFTR), a member of the ABC transporter superfamily, was isolated (Rommens et al., 1989). More than 1,900 mutations have been identified in the CF gene (http://www.genet.sickkids.on.ca/cftr). CFTR, a polytopic membrane protein, is composed of five domains: two nucleotide-binding domains (NBDs), two membrane-spanning domains (MSDs) and a regulatory (R) region (Riordan, 2008). Biochemical, cell biological, and functional studies have shown that CFTR is an ATP- and phosphorylation-regulated chloride channel (Riordan et al., 1989). CFTR is confined to the apical plasma membrane of secretory epithelia in the airways, intestine, pancreas, testis, and exocrine glands and besides chloride, transports bicarbonate and regulates other ion transporters (Gadsby et al., 2006).

What is wrong with mutant CFTRs?

CF mutations have been grouped into six categories based on their cellular/molecular pathogenesis (Zielenski, 2000). Class I mutations include nonsense mutations (G542X and R553X), generating premature termination codons and frame-shift mutations that lead to truncated and/or and nonfunctional protein (Fig. 1). Class V mutations cause mRNA mis-splicing or interfere with the promoter activity. Both classes impair CFTR protein production and plasma membrane expression, causing a severe CF phenotype.

Class II mutations, despite normal transcript levels, have little or no detectable CFTR at the plasma membrane as a consequence of misfolding of the newly translated polypeptide. This category includes the most common mutation, deletion of phenylalanine 508 (ΔF508) in the NBD1, identifiable in one or both alleles in ∼90% of CF patients (Riordan, 2008). ΔF508-CFTR is largely retained in the ER and degraded by the ubiquitin–proteasome system (Fig. 1; Cheng et al., 1990; Ward et al., 1995).

Class III (e.g., G551D, ∼4%) and class IV (e.g., R117H) mutations impair the CFTR channel opening-closing (or gating) cycle and conductance, respectively, without recognizable conformational or trafficking defects. Class III mutations are primarily associated with NBD1-2, whereas class IV mutations are localized to the channel pore (Riordan, 2008). Class VI mutations reduce CFTR expression by facilitating the channel removal from the plasma membrane. Notably, some mutations have a mixed phenotype. For example the ΔF508 mutation causes folding, and gating, as well as plasma membrane stability impairments (Dalemans et al., 1991; Denning et al., 1992; Lukacs et al., 1993).

Correction of the basic defects

Because CF is a monogenic disease, it is postulated that the clinical phenotype would be alleviated by correcting the basic defects caused by various mutations impeding or preventing CFTR function, expression, or both (Cai et al., 2011). Efforts to correct the basic defects of CFTR biogenesis and function have been primarily focused on the most prevalent mutations: ΔF508, G551D, and premature termination codons (Fig. 1).

Correspondence to Gergely L. Lukacs: gergely.lukacs@mcgill.ca

Abbreviations used in this paper: CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; MSD, membrane-spanning domain; NBD, nucleotide-binding domain; PM, plasma membrane.

© 2012 Okiyoneda and Lukacs This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-sa/3.0/).

The Rockefeller University Press
J. Cell Biol. Vol. 199 No. 2 199–204
www.jcb.org/cgi/doi/10.1083/jcb.201208083

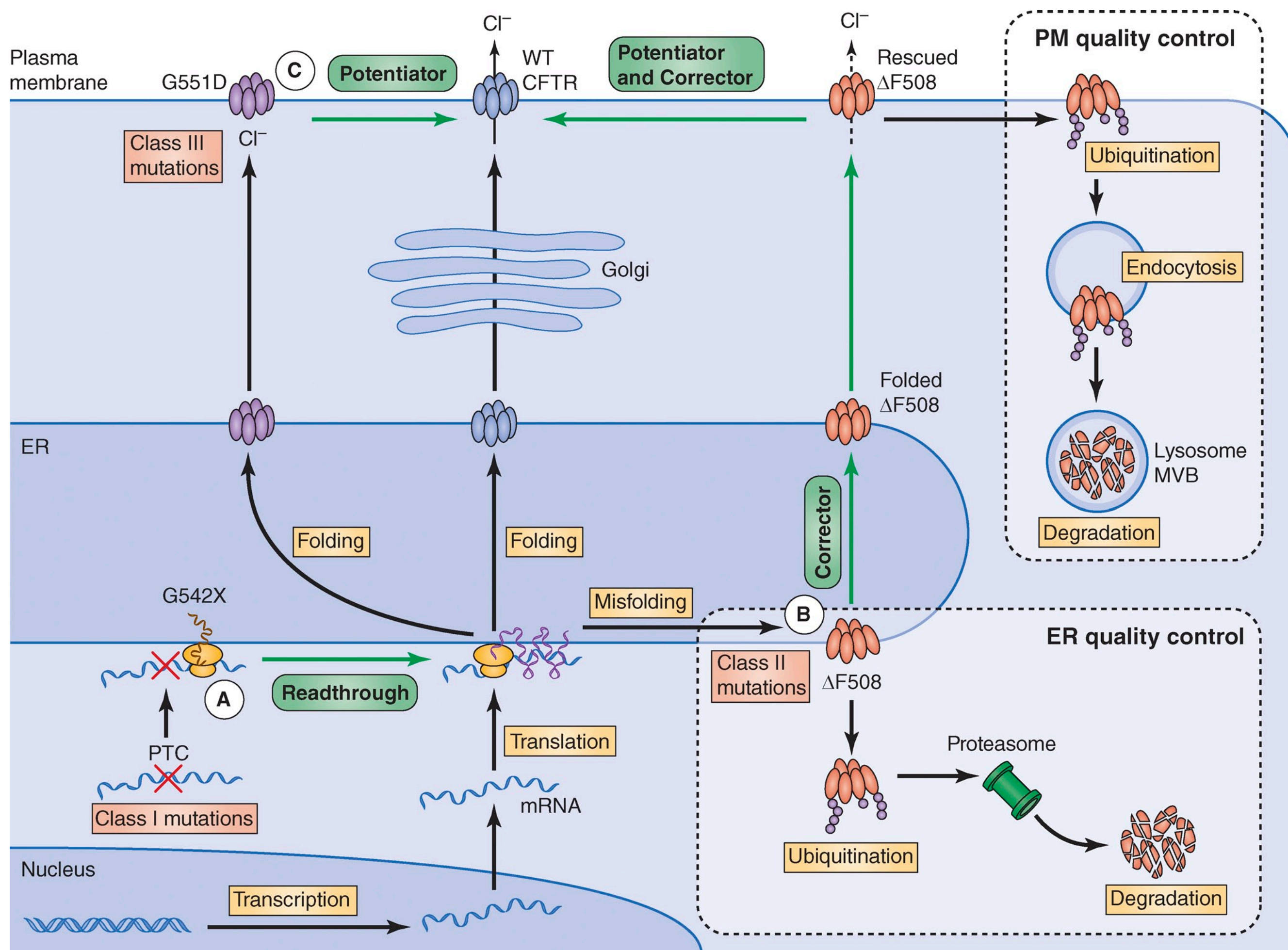
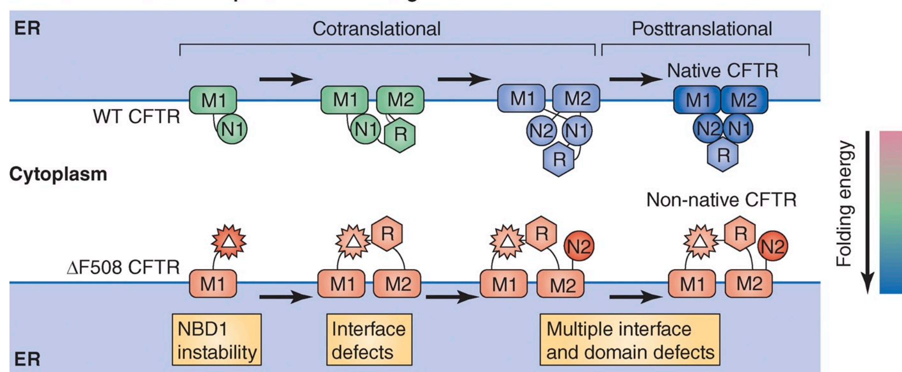
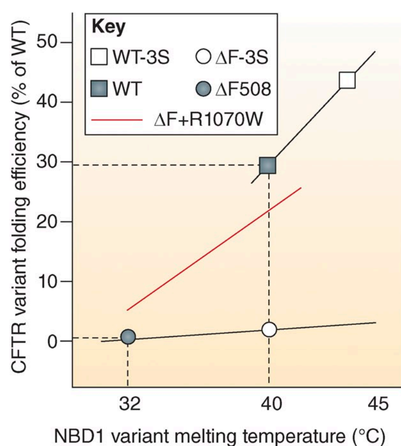


Figure 1. **Cellular mechanism and therapeutics of prevalent classes of CF-causing mutations.** (A) Class I mutations (e.g., G542X) impair production of CFTR full-length protein by induction of premature termination codons (PTC). Aminoglycosides and an investigational drug, Ataluren, can rescue this phenotype by inducing read-through of the PTC and allow translation of full-length CFTR protein. (B) The most common ΔF508 mutation (class II) impairs the channel conformational maturation and misfolded CFTR is recognized by the endoplasmic reticulum (ER) quality control system and is targeted for degradation via the ubiquitin–proteasome system. Correctors (e.g., VX-809) can partially rescue the misprocessing, probably by improving folding at the ER and delaying turnover at the plasma membrane (PM) with a presently poorly understood mechanism. Although rescued ΔF508-CFTR retains partial Cl^- channel function, it is conformationally unstable and eliminated by the PM QC system via ubiquitination-dependent lysosomal delivery (Okiyoneda et al., 2010). (C) Class III mutations (e.g., G551D) do not affect CFTR biosynthesis and PM expression, but impair the channel gating. CFTR potentiators, including the FDA-approved Ivacaftor, correct this phenotype.

Read-through of premature termination codons. Aminoglycosides such as gentamycin interact with eukaryotic rRNA within the ribosomal subunits and reduce the fidelity of translation by interrupting the normal proofreading function (Burke and Mogg, 1985). Consequently, aminoglycosides allow insertion of a near-cognate amino acid at a premature termination codon and the translation of the entire coding region. Aminoglycosides have been used to suppress premature termination codons, resulting in the synthesis of full-length CFTR in CF patients with class I mutations (Wilschanski et al., 2003). Ataluren (PTC124), an orally bioavailable drug with diminished toxicity, was developed by a cell-based high-throughput screening assay (Welch et al., 2007). Although Ataluren selectively suppresses the premature termination codon in a mouse model (Welch et al., 2007), it showed variable efficiency among patients with different genotypes (Rowe et al., 2007). Orally administered Ataluren has been reported to rescue the activity of CFTR with premature termination codons in phase II trials (Kerem et al., 2008; Sermet-Gaudelus et al., 2010). A phase III clinical study is currently underway to evaluate long-term efficacy and safety (http://clinicaltrials.gov/ct2/show/NCT00803205).

Are ΔF508-CFTR folding, processing, and functional defects correctable? In principle, the CFTR folding defect could be counteracted by pharmacological chaperones (PCs), similar to other misfolding diseases, where a variety of ligands or substrates can stabilize the target protein functional conformation (Bernier et al., 2004). Although this approach would be highly specific, and maintain the endogenous regulation and expression pattern of CFTR, high affinity CFTR ligands are not available. Altering the cellular folding environment could also be exploited to overcome the mutant misfolding/misprocessing and has shown some success in preclinical settings (Balch et al., 2011). Chemical chaperones similar to





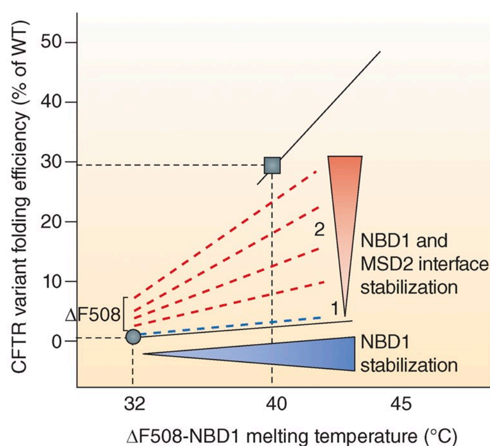


Figure 2. **Working models of CFTR folding, misfolding, and mechanism of ΔF508-CFTR correction by pharmacological chaperones.** (A) Hypothetical folding and misfolding models of the multidomain CFTR channel. Each CFTR domain, such as MSD1, NBD1, MSD2, and NBD2 (M1, N1, M2, and N2), folds to variable extents cotranslationally to form metastable states. Formation of domain–domain interfaces energetically facilitates further coupled-domain folding and assembly, a prerequisite for CFTR native tertiary structure. Progressive enthalpic stabilization of individual domains during co- and posttranslational folding is indicated by pseudocolors. ΔF508 mutation (Δ) impairs both NBD1 energetics and domain–domain interactions (especially via the NBD1–MSD2 interface) due to conformational and topological defects, rendering all four major domains structurally impaired in the ΔF508-CFTR. Adapted from Rabeh et al. (2012) with permission from Elsevier. (B) Genetic rescue of ΔF508-CFTR folding defect. Progressive stabilization of ΔF508-NBD1 by a panel of suppressor mutations (e.g., 3S) achieves only modest improvement in the marginal folding efficiency of ΔF508-CFTR (~0.4%). Representative data points and correlations between NBD1 stability and CFTR folding were obtained from Rabeh et al. (2012). Comparable changes in the conformational stability of the WT NBD1 (e.g., 3S) caused nearly twofold increase in WT CFTR folding efficiency. Stabilization of the NBD1–MSD2 interface by second site suppressor mutations (e.g., R1070W) largely restored the WT-like coupling efficiency between NBD1 stability and ΔF508-CFTR folding (Rabeh et al., 2012). This indicates that correction of two distinct structural defects is essential to achieve robust restoration of ΔF508-CFTR folding and function. (C) Predicted features of ΔF508-CFTR pharmacological rescues by structural defect-specific correctors. We speculate that a subset of correctors, yet to be identified, as pharmacological chaperones may either stabilize the NBD1 (1, blue dashed line) and/or the NBD1–MSD2 interface (2, red dashed lines) via direct binding to ΔF508-CFTR. Individual compound would result in modest increase in the mutant folding efficiency, but complementary pairs targeting both primary structural defects would synergistically improve the ΔF508-CFTR folding, PM expression, and function similar to suppressor mutations. For reference the WT- and ΔF508-CFTR folding efficiency are indicated (black lines).

reduced temperature can also counteract the ΔF508-CFTR misfolding and elicit modest accumulation of partially functional but unstable channels at the plasma membrane (Denning et al., 1992; Sato et al., 1996; Sharma et al., 2001).

The revelation that ΔF508-CFTR misfolding can be rescued prompted the development of a cell-based high-throughput screening assay using the yellow fluorescent protein–based halide indicator, which monitors the cAMP-activated plasma membrane chloride permeability, including CFTR activity, by sensing changes in the cytoplasmic halide concentration (Galietta et al., 2001). This assay became instrumental in the identification of not only "correctors" that improve ΔF508-CFTR plasma membrane expression, but also "potentiators" to activate plasma membrane–resident CFTR channels. Interestingly, the assay also identified inhibitors of chloride channels, which may be potential therapeutics in secretory diarrheas (Verkman and Galietta, 2009).

Screening of diverse chemical libraries has produced several classes of small-molecule ΔF508-CFTR correctors, including corr-4a and its analogues (Pedemonte et al., 2005a). Additional correctors were also obtained by high-throughput screening and computational methods (Kalid et al., 2010; Robert et al., 2010; Sampson et al., 2011). However, the efficacy of these correctors in restoring chloride conductance was limited and reached only <10% of normal human primary epithelia, which is significantly lower than the predicted requirement for therapeutic efficiency (Pedemonte et al., 2005a, 2010; Van Goor et al., 2006). A similar approach by Vertex Pharmaceuticals, Inc. with Cystic Fibrosis Foundation Therapeutics's (CFFT) support has yielded new classes of correctors, including VX-809, the most promising compound being evaluated in phase III clinical trials (Van Goor et al., 2006, 2011). VX-809 restores ~15% CFTR channel activity in primary respiratory epithelia expressing ΔF508-CFTR, but appears to have marginal clinical benefits (Van Goor et al., 2011; Clancy et al., 2012).

Reactivation of defective plasma membrane CFTR channels. Cell-based functional high-throughput screening assays have also isolated several potentiators that improve the channel function of class II and III mutants (Pedemonte et al., 2005b; Van Goor et al., 2006). The most promising potentiator, VX-770 (Ivacaftor), isolated by Vertex Pharmaceuticals, Inc., restores G551D-CFTR activity to ~50% of wild-type level (Van Goor et al., 2009). Clinical studies

confirmed short-term safety and clinical benefits, including 55% reduced pulmonary exacerbation frequency and 10% increased lung function (Ramsey et al., 2011). Ivacaftor is the first FDA-approved drug for treatment of G551D-CFTR patients (~4% of CF population), representing a landmark translational achievement, exploiting the basic biology of CFTR and years of research and development in both academia and industry. Remarkably, Ivacaftor also restores the gating defect of several other class II mutations; therefore, it may benefit ~10% of CF patients (Yu et al., 2012).

Combination therapy of potentiators and correctors could be useful for improving ΔF508-CFTR function given the persisting gating defect of rescued ΔF508-CFTR at the plasma membrane. Indeed, interim results of a phase II clinical trial suggest that ΔF508 CF patients treated with a combination of VX-809 and Ivacaftor seem to display better lung function than those treated with either drug alone (http://clinicaltrials.gov/ct2/show/NCT01225211).

Challenges ahead: Efficacious therapy of ΔF508-CFTR

Despite recent advances of CFTR research, further improvement in functional expression of ΔF508-CFTR, the most common mutation in CF patients, is necessary because correction of the CF phenotype likely requires restoring ~35% of wild-type CFTR plasma membrane activity. Elucidating CFTR folding/misfolding and the available corrector mechanisms should help to achieve this goal. Here, we focus on efforts to understand and correct the folding defects of the ΔF508-CFTR.

CFTR domain folding and misfolding. Compelling evidence supports the coupled domain-folding model of CFTR. Accordingly, individual domains can fold cotranslationally to metastable states; but attaining the CFTR native fold requires post-translational domain assembly and interdomain interactions that are critical to proper folding (Fig. 2 A; Du et al., 2005; He et al., 2008, 2010; Du and Lukacs, 2009). The slow post-translational conformational maturation is assisted by chaperones (Rosser et al., 2008) and reflected by the delayed formation of NBD–MSD interfaces in the mature wild-type CFTR (He et al., 2008; Serohijos et al., 2008). The energetic instability of individual domains and the slow domain assembly with the fast ER-associated degradation kinetics of folding intermediates all contribute to the inefficient folding (~30%) of wild-type CFTR (Fig. 2 B) and are further sensitized by point mutations in CF (Rabeh et al., 2012).

How does the ΔF508 mutation affect the channel? Homology modeling and cysteine cross-linking experiments have revealed the unique three-dimensional architecture of CFTR and the possible role of F508 (He et al., 2008; Mornon et al., 2008; Serohijos et al., 2008). In native CFTR, the F508 residue and surrounding area in the NBD1 forms an interface with the coupling helix of cytoplasmic loops 4 (CL4) and 1 (CL1) in MSD2 and MSD1, respectively, which creates a hydrophobic patch. NBD2 associates with CL2 and CL3 of MSD1 and MSD2, respectively. These interfaces relay conformational changes of the NBDs to the MSDs during channel gating, and are essential for CFTR folding (Wang et al., 2007; He et al., 2008, 2010; Mornon et al., 2008; Serohijos et al., 2008; Loo et al., 2010; Thibodeau et al., 2010; Grove et al., 2011). Destabilization of the interface by missense mutations in the CLs or by mutagenesis of the F508 side chain disrupts folding (Du et al., 2005; Mornon et al., 2008; He et al., 2010; Loo et al., 2010; Thibodeau et al., 2010). These observations are in support of the emerging model of co- and post-translational conformational maturation of CFTR that involves energetic and/or kinetic domain stabilization during coupled-domain folding (Du and Lukacs, 2009), similar to that of certain soluble multi-domain proteins and the BtuCD transporter (Fig. 2 A; Han et al., 2007; DiBartolo and Booth, 2011).

Targeting more than one folding defect in ΔF508-CFTR. Recent studies revealed that the ΔF508-NBD1 is thermodynamically and kinetically destabilized at physiological temperature and suggested that the NBD1 stabilization would effectively counteract ΔF508-CFTR misprocessing (Protasevich et al., 2010; Wang et al., 2010). Surprisingly, this was not the case. Even substantial conformational stabilization of ΔF508-NBD1 by second site mutations led to modest rescue (<20%) of ΔF508-CFTR processing, plasma membrane expression, and function, and failed to reinstate coupled domain folding (Fig. 2 B; Mendoza et al., 2012; Rabeh et al., 2012). Likewise, reversing the NBD1–MSD2 interface instability by second site mutations (e.g., R1070W) only marginally rescued the ΔF508-CFTR phenotype (Fig. 2 B). Remarkably, simultaneous genetic stabilization of NBD1 energetics and the NBD1–MSD2 interface led to robust, synergistic rescue (65–80%) of ΔF508-CFTR folding and function (Fig. 2 B; Rabeh et al., 2012). A similar conclusion was reached by the analysis of evolved sequences coupled to the F508 residue (Mendoza et al., 2012). These unexpected findings suggest that correction of two primary structural defects is necessary and sufficient to restore CFTR function in most CF patients.

Translational implications of the ΔF508-CFTR misfolding mechanism

The discovery of two primary folding defects in ΔF508-CFTR highlighted three pharmacological implications (Fig. 2 C): (1) the plural folding defects provide a reasonable explanation for the modest efficacy of single correctors that may target only one of them with a presently unknown mechanism(s) (Sampson et al., 2011; Van Goor et al., 2011); (2) second site mutations counteracting one of the primary folding defects could promote mechanistic classification of existing corrector molecules, as well as the identification of new ones by second generation of structural defect–targeted high-throughput screening assays; (3) correction of both NBD1 energetic and interface instability is likely required to robustly normalize ΔF508-CFTR processing, expression, and function (Fig. 2 C). Whether this could be achieved by one or two small molecules alone, or in combination with proteostasis network regulators that indirectly modulate ΔF508-CFTR folding, trafficking, and function (Balch et al., 2011) awaits further experimentation.

Other major challenges to translation

In this paper we have described the cellular consequences of CFTR mutations and recent efforts to understand the folding defects underlying the ΔF508-CFTR mutation in order to improve channel folding, stability, and function. However, many critical issues remain. Our understanding of how the loss of channel function results in CF, particularly the lung symptoms, is incomplete (Ashlock and Olson, 2011) and compounded by the fact that mouse models fail to recapitulate the CF lung disease, though the development of transgenic pig and ferret might address this issue (Rogers et al., 2008; Sun et al., 2010). Mechanistic studies of the channel are hampered by the low copy number and instability of mutants and the difficulties to monitor their structural alteration, protein–protein interaction, and trafficking at high spatiotemporal resolution in the appropriate cellular environment (Riordan, 2008; Balch et al., 2011). In addition to the drug discovery programs described in this paper, there are ongoing efforts to replace the mutant by gene therapy and/or activating alternative chloride secretion. However, multiple cellular mechanisms impede the nuclear delivery of CFTR transgene packaged either into cationic lipid complexes or viruses, though overcoming these processes may allow genotype-independent therapy (Griesenbach and Alton, 2009). Identification of the epithelial isoform of the Ca^{2+}-activated chloride channel (TMEM16A) has opened the possibility to pharmacological activation of an alternative chloride secretory pathway (Ferrera et al., 2010).

The ultimate success of translational research most often relies on our detailed understanding of the basic biological problem at hand. We hope that this short perspective will help inspire further biological research, a prerequisite for translational successes in curing basic defects in CF and other genetic diseases such as diabetes insipidus and familial hypercholesterolemia.

We apologize to those authors whose contribution could not be cited in this manuscript owing to space limitation.

We are thankful for the generous support of NIH-NIDDK, Cystic Fibrosis Foundation Therapeutics Inc., Cystic Fibrosis Canada, Canadian Institute of Health Research, and the Canadian Foundation for Innovation. G. Lukacs is holder of a Canada Research Chair. Illustrations were provided by Neil Smith, www.neilsmithillustration.co.uk.

Submitted: 6 August 2012
Accepted: 19 September 2012

References

Ashlock, M.A., and E.R. Olson. 2011. Therapeutics development for cystic fibrosis: a successful model for a multisystem genetic disease. *Annu. Rev. Med.* 62:107–125. http://dx.doi.org/10.1146/annurev-med-061509-131034

Balch, W.E., D.M. Roth, and D.M. Hutt. 2011. Emergent properties of proteostasis in managing cystic fibrosis. *Cold Spring Harb. Perspect. Biol.* 3. http://dx.doi.org/10.1101/cshperspect.a004499

Bernier, V., M. Lagacé, D.G. Bichet, and M. Bouvier. 2004. Pharmacological chaperones: potential treatment for conformational diseases. *Trends Endocrinol. Metab.* 15:222–228. http://dx.doi.org/10.1016/j.tem.2004.05.003

Burke, J.F., and A.E. Mogg. 1985. Suppression of a nonsense mutation in mammalian cells in vivo by the aminoglycoside antibiotics G-418 and paromomycin. *Nucleic Acids Res.* 13:6265–6272. http://dx.doi.org/10.1093/nar/13.17.6265

Cai, Z.W., J. Liu, H.Y. Li, and D.N. Sheppard. 2011. Targeting F508del-CFTR to develop rational new therapies for cystic fibrosis. *Acta Pharmacol. Sin.* 32:693–701. http://dx.doi.org/10.1038/aps.2011.71

Cheng, S.H., R.J. Gregory, J. Marshall, S. Paul, D.W. Souza, G.A. White, C.R. O'Riordan, and A.E. Smith. 1990. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell.* 63:827–834. http://dx.doi.org/10.1016/0092-8674(90)90148-8

Clancy, J.P., S.M. Rowe, F.J. Accurso, M.L. Aitken, R.S. Amin, M.A. Ashlock, M. Ballmann, M.P. Boyle, I. Bronsveld, P.W. Campbell, et al. 2012. Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation. *Thorax.* 67:12–18. http://dx.doi.org/10.1136/thoraxjnl-2011-200393

Dalemans, W., P. Barbry, G. Champigny, S. Jallat, K. Dott, D. Dreyer, R.G. Crystal, A. Pavirani, J.P. Lecocq, and M. Lazdunski. 1991. Altered chloride ion channel kinetics associated with the delta F508 cystic fibrosis mutation. *Nature.* 354:526–528. http://dx.doi.org/10.1038/354526a0

Denning, G.M., M.P. Anderson, J.F. Amara, J. Marshall, A.E. Smith, and M.J. Welsh. 1992. Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. *Nature.* 358:761–764. http://dx.doi.org/10.1038/358761a0

DiBartolo, N., and P.J. Booth. 2011. Unravelling the folding and stability of an ABC (ATP-binding cassette) transporter. *Biochem. Soc. Trans.* 39: 751–760.

Du, K., and G.L. Lukacs. 2009. Cooperative assembly and misfolding of CFTR domains in vivo. *Mol. Biol. Cell.* 20:1903–1915. http://dx.doi.org/10.1091/mbc.E08-09-0950

Du, K., M. Sharma, and G.L. Lukacs. 2005. The DeltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. *Nat. Struct. Mol. Biol.* 12:17–25. http://dx.doi.org/10.1038/nsmb882

Ferrera, L., A. Caputo, and L.J. Galietta. 2010. TMEM16A protein: a new identity for Ca(2+)-dependent Cl^- channels. *Physiology (Bethesda).* 25:357–363. http://dx.doi.org/10.1152/physiol.00030.2010

Gadsby, D.C., P. Vergani, and L. Csanády. 2006. The ABC protein turned chloride channel whose failure causes cystic fibrosis. *Nature.* 440:477–483. http://dx.doi.org/10.1038/nature04712

Galietta, L.J., P.M. Haggie, and A.S. Verkman. 2001. Green fluorescent protein-based halide indicators with improved chloride and iodide affinities. *FEBS Lett.* 499:220–224. http://dx.doi.org/10.1016/S0014-5793(01)02561-3

Griesenbach, U., and E.W. Alton; UK Cystic Fibrosis Gene Therapy Consortium. 2009. Gene transfer to the lung: lessons learned from more than 2 decades of CF gene therapy. *Adv. Drug Deliv. Rev.* 61:128–139. http://dx.doi.org/10.1016/j.addr.2008.09.010

Grove, D.E., C.Y. Fan, H.Y. Ren, and D.M. Cyr. 2011. The endoplasmic reticulum-associated Hsp40 DNAJB12 and Hsc70 cooperate to facilitate RMA1 E3-dependent degradation of nascent CFTRDeltaF508. *Mol. Biol. Cell.* 22:301–314. http://dx.doi.org/10.1091/mbc.E10-09-0760

Han, J.H., S. Batey, A.A. Nickson, S.A. Teichmann, and J. Clarke. 2007. The folding and evolution of multidomain proteins. *Nat. Rev. Mol. Cell Biol.* 8:319–330. http://dx.doi.org/10.1038/nrm2144

He, L., A.A. Aleksandrov, A.W. Serohijos, T. Hegedus, L.A. Aleksandrov, L. Cui, N.V. Dokholyan, and J.R. Riordan. 2008. Multiple membrane-cytoplasmic domain contacts in the cystic fibrosis transmembrane conductance regulator (CFTR) mediate regulation of channel gating. *J. Biol. Chem.* 283:26383–26390. http://dx.doi.org/10.1074/jbc.M803894200

He, L., L.A. Aleksandrov, L. Cui, T.J. Jensen, K.L. Nesbitt, and J.R. Riordan. 2010. Restoration of domain folding and interdomain assembly by second-site suppressors of the DeltaF508 mutation in CFTR. *FASEB J.* 24:3103–3112. http://dx.doi.org/10.1096/fj.09-141788

Kalid, O., M. Mense, S. Fischman, A. Shitrit, H. Bihler, E. Ben-Zeev, N. Schutz, N. Pedemonte, P.J. Thomas, R.J. Bridges, et al. 2010. Small molecule correctors of F508del-CFTR discovered by structure-based virtual screening. *J. Comput. Aided Mol. Des.* 24:971–991. http://dx.doi.org/10.1007/s10822-010-9390-0

Kerem, E., S. Hirawat, S. Armoni, Y. Yaakov, D. Shoseyov, M. Cohen, M. Nissim-Rafinia, H. Blau, J. Rivlin, M. Aviram, et al. 2008. Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospective phase II trial. *Lancet.* 372:719–727. http://dx.doi.org/10.1016/S0140-6736(08)61168-X

Loo, T.W., M.C. Bartlett, and D.M. Clarke. 2010. The V510D suppressor mutation stabilizes DeltaF508-CFTR at the cell surface. *Biochemistry.* 49:6352–6357. http://dx.doi.org/10.1021/bi100807h

Lukacs, G.L., X.B. Chang, C. Bear, N. Kartner, A. Mohamed, J.R. Riordan, and S. Grinstein. 1993. The delta F508 mutation decreases the stability of cystic fibrosis transmembrane conductance regulator in the plasma membrane. Determination of functional half-lives on transfected cells. *J. Biol. Chem.* 268:21592–21598.

Mendoza, J.L., A. Schmidt, Q. Li, E. Nuvaga, T. Barrett, R.J. Bridges, A.P. Feranchak, C.A. Brautigam, and P.J. Thomas. 2012. Requirements for

efficient correction of ΔF508 CFTR revealed by analyses of evolved sequences. *Cell*. 148:164–174. http://dx.doi.org/10.1016/j.cell.2011.11.023

Mornon, J.P., P. Lehn, and I. Callebaut. 2008. Atomic model of human cystic fibrosis transmembrane conductance regulator: membrane-spanning domains and coupling interfaces. *Cell. Mol. Life Sci.* 65:2594–2612. http://dx.doi.org/10.1007/s00018-008-8249-1

Okiyoneda, T., H. Barrière, M. Bagdány, W.M. Rabeh, K. Du, J. Höhfeld, J.C. Young, and G.L. Lukacs. 2010. Peripheral protein quality control removes unfolded CFTR from the plasma membrane. *Science*. 329:805–810. http://dx.doi.org/ 10.1126/science.1191542

Pedemonte, N., G.L. Lukacs, K. Du, E. Caci, O. Zegarra-Moran, L.J. Galietta, and A.S. Verkman. 2005a. Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. *J. Clin. Invest.* 115:2564–2571. http://dx.doi.org/10.1172/JCI24898

Pedemonte, N., N.D. Sonawane, A. Taddei, J. Hu, O. Zegarra-Moran, Y.F. Suen, L.I. Robins, C.W. Dicus, D. Willenbring, M.H. Nantz, et al. 2005b. Phenylglycine and sulfonamide correctors of defective delta F508 and G551D cystic fibrosis transmembrane conductance regulator chloride-channel gating. *Mol. Pharmacol.* 67:1797–1807. http://dx.doi.org/10.1124/mol.105.010959

Pedemonte, N., V. Tomati, E. Sondo, and L.J. Galietta. 2010. Influence of cell background on pharmacological rescue of mutant CFTR. *Am. J. Physiol. Cell Physiol.* 298:C866–C874. http://dx.doi.org/10.1152/ajpcell.00404.2009

Protasevich, I., Z. Yang, C. Wang, S. Atwell, X. Zhao, S. Emtage, D. Wetmore, J.F. Hunt, and C.G. Brouillette. 2010. Thermal unfolding studies show the disease causing F508del mutation in CFTR thermodynamically destabilizes nucleotide-binding domain 1. *Protein Sci.* 19:1917–1931. http://dx.doi.org/10.1002/pro.479

Rabeh, W.M., F. Bossard, H. Xu, T. Okiyoneda, M. Bagdany, C.M. Mulvihill, K. Du, S. di Bernardo, Y. Liu, L. Konermann, et al. 2012. Correction of both NBD1 energetics and domain interface is required to restore ΔF508 CFTR folding and function. *Cell*. 148:150–163. http://dx.doi.org/10.1016/j.cell.2011.11.024

Ramsey, B.W., J. Davies, N.G. McElvaney, E. Tullis, S.C. Bell, P. Dřevínek, M. Griese, E.F. McKone, C.E. Wainwright, M.W. Konstan, et al; VX08-770-102 Study Group. 2011. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N. Engl. J. Med.* 365:1663–1672. http://dx.doi.org/10.1056/NEJMoa1105185

Riordan, J.R. 2008. CFTR function and prospects for therapy. *Annu. Rev. Biochem.* 77:701–726. http://dx.doi.org/10.1146/annurev.biochem.75.103004.142532

Riordan, J.R., J.M. Rommens, B. Kerem, N. Alon, R. Rozmahel, Z. Grzelczak, J. Zielenski, S. Lok, N. Plavsic, J.L. Chou, et al. 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science*. 245:1066–1073. http://dx.doi.org/10.1126/science.2475911

Robert, R., G.W. Carlile, J. Liao, H. Balghi, P. Lesimple, N. Liu, B. Kus, D. Rotin, M. Wilke, H.R. de Jonge, et al. 2010. Correction of the Delta phe508 cystic fibrosis transmembrane conductance regulator trafficking defect by the bioavailable compound glafenine. *Mol. Pharmacol.* 77:922–930. http://dx.doi.org/10.1124/mol.109.062679

Rogers, C.S., D.A. Stoltz, D.K. Meyerholz, L.S. Ostedgaard, T. Rokhlina, P.J. Taft, M.P. Rogan, A.A. Pezzulo, P.H. Karp, O.A. Itani, et al. 2008. Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs. *Science*. 321:1837–1841. http://dx.doi.org/10.1126/science.1163600

Rommens, J.M., M.C. Iannuzzi, B. Kerem, M.L. Drumm, G. Melmer, M. Dean, R. Rozmahel, J.L. Cole, D. Kennedy, N. Hidaka, et al. 1989. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science*. 245:1059–1065. http://dx.doi.org/10.1126/science.2772657

Rosser, M.F., D.E. Grove, L. Chen, and D.M. Cyr. 2008. Assembly and misassembly of cystic fibrosis transmembrane conductance regulator: folding defects caused by deletion of F508 occur before and after the calnexin-dependent association of membrane spanning domain (MSD) 1 and MSD2. *Mol. Biol. Cell*. 19:4570–4579. http://dx.doi.org/10.1091/mbc.E08-04-0357

Rowe, S.M., K. Varga, A. Rab, Z. Bebok, K. Byram, Y. Li, E.J. Sorscher, and J.P. Clancy. 2007. Restoration of W1282X CFTR activity by enhanced expression. *Am. J. Respir. Cell Mol. Biol.* 37:347–356. http://dx.doi.org/10.1165/rcmb.2006-0176OC

Sampson, H.M., R. Robert, J. Liao, E. Matthes, G.W. Carlile, J.W. Hanrahan, and D.Y. Thomas. 2011. Identification of a NBD1-binding pharmacological chaperone that corrects the trafficking defect of F508del-CFTR. *Chem. Biol.* 18:231–242. http://dx.doi.org/10.1016/j.chembiol.2010.11.016

Sato, S., C.L. Ward, M.E. Krouse, J.J. Wine, and R.R. Kopito. 1996. Glycerol reverses the misfolding phenotype of the most common cystic fibrosis mutation. *J. Biol. Chem.* 271:635–638. http://dx.doi.org/10.1074/jbc.271.2.635

Sermet-Gaudelus, I., K.D. Boeck, G.J. Casimir, F. Vermeulen, T. Leal, A. Mogenet, D. Roussel, J. Fritsch, L. Hanssens, S. Hirawat, et al. 2010. Ataluren (PTC124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 182:1262–1272. http://dx.doi.org/10.1164/rccm.201001-0137OC

Serohijos, A.W., T. Hegedus, A.A. Aleksandrov, L. He, L. Cui, N.V. Dokholyan, and J.R. Riordan. 2008. Phenylalanine-508 mediates a cytoplasmic-membrane domain contact in the CFTR 3D structure crucial to assembly and channel function. *Proc. Natl. Acad. Sci. USA*. 105:3256–3261. http://dx.doi.org/10.1073/pnas.0800254105

Sharma, M., M. Benharouga, W. Hu, and G.L. Lukacs. 2001. Conformational and temperature-sensitive stability defects of the delta F508 cystic fibrosis transmembrane conductance regulator in post-endoplasmic reticulum compartments. *J. Biol. Chem.* 276:8942–8950. http://dx.doi.org/10.1074/jbc.M009172200

Sun, X., H. Sui, J.T. Fisher, Z. Yan, X. Liu, H.J. Cho, N.S. Joo, Y. Zhang, W. Zhou, Y. Yi, et al. 2010. Disease phenotype of a ferret CFTR-knockout model of cystic fibrosis. *J. Clin. Invest.* 120:3149–3160. http://dx.doi.org/10.1172/JCI43052

Thibodeau, P.H., J.M. Richardson III, W. Wang, L. Millen, J. Watson, J.L. Mendoza, K. Du, S. Fischman, H. Senderowitz, G.L. Lukacs, et al. 2010. The cystic fibrosis-causing mutation deltaF508 affects multiple steps in cystic fibrosis transmembrane conductance regulator biogenesis. *J. Biol. Chem.* 285:35825–35835. http://dx.doi.org/10.1074/jbc.M110.131623

Van Goor, F., K.S. Straley, D. Cao, J. González, S. Hadida, A. Hazlewood, J. Joubran, T. Knapp, L.R. Makings, M. Miller, et al. 2006. Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 290:L1117–L1130. http://dx.doi.org/10.1152/ajplung.00169.2005

Van Goor, F., S. Hadida, P.D. Grootenhuis, B. Burton, D. Cao, T. Neuberger, A. Turnbull, A. Singh, J. Joubran, A. Hazlewood, et al. 2009. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. *Proc. Natl. Acad. Sci. USA*. 106:18825–18830. http://dx.doi.org/10.1073/pnas.0904709106

Van Goor, F., S. Hadida, P.D. Grootenhuis, B. Burton, J.H. Stack, K.S. Straley, C.J. Decker, M. Miller, J. McCartney, E.R. Olson, et al. 2011. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proc. Natl. Acad. Sci. USA*. 108:18843–18848. http://dx.doi.org/10.1073/pnas.1105787108

Verkman, A.S., and L.J. Galietta. 2009. Chloride channels as drug targets. *Nat. Rev. Drug Discov.* 8:153–171. http://dx.doi.org/10.1038/nrd2780

Wang, C., I. Protasevich, Z. Yang, D. Seehausen, T. Skalak, X. Zhao, S. Atwell, J. Spencer Emtage, D.R. Wetmore, C.G. Brouillette, and J.F. Hunt. 2010. Integrated biophysical studies implicate partial unfolding of NBD1 of CFTR in the molecular pathogenesis of F508del cystic fibrosis. *Protein Sci.* 19:1932–1947. http://dx.doi.org/10.1002/pro.480

Wang, Y., T.W. Loo, M.C. Bartlett, and D.M. Clarke. 2007. Correctors promote maturation of cystic fibrosis transmembrane conductance regulator (CFTR)-processing mutants by binding to the protein. *J. Biol. Chem.* 282:33247–33251. http://dx.doi.org/10.1074/jbc.C700175200

Ward, C.L., S. Omura, and R.R. Kopito. 1995. Degradation of CFTR by the ubiquitin-proteasome pathway. *Cell*. 83:121–127. http://dx.doi.org/10.1016/0092-8674(95)90240-6

Welch, E.M., E.R. Barton, J. Zhuo, Y. Tomizawa, W.J. Friesen, P. Trifillis, S. Paushkin, M. Patel, C.R. Trotta, S. Hwang, et al. 2007. PTC124 targets genetic disorders caused by nonsense mutations. *Nature*. 447:87–91. http://dx.doi.org/10.1038/nature05756

Wilschanski, M., Y. Yahav, Y. Yaacov, H. Blau, L. Bentur, J. Rivlin, M. Aviram, T. Bdolah-Abram, Z. Bebok, L. Shushi, et al. 2003. Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. *N. Engl. J. Med.* 349:1433–1441. http://dx.doi.org/10.1056/NEJMoa022170

Yu, H., B. Burton, C.J. Huang, J. Worley, D. Cao, J.P. Johnson Jr., A. Urrutia, J. Joubran, S. Seepersaud, K. Sussky, et al. 2012. Ivacaftor potentiation of multiple CFTR channels with gating mutations. *J. Cyst. Fibros.* 11:237–245. http://dx.doi.org/10.1016/j.jcf.2011.12.005

Zielenski, J. 2000. Genotype and phenotype in cystic fibrosis. *Respiration*. 67:117–133. http://dx.doi.org/10.1159/000029497