Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Potentiators Protect G551D but Not ΔF508 CFTR from Thermal Instability

Xuehong Liu* and David C. Dawson

Department of Physiology & Pharmacology, Oregon Health & Science University, Portland, Oregon 97239, United States

ABSTRACT: The G551D cystic fibrosis transmembrane conductance regulator (CFTR) mutation is associated with severe disease in ~5% of cystic fibrosis patients worldwide. This amino acid substitution in NBD1 results in a CFTR chloride channel characterized by a severe gating defect that can be at least partially overcome in vitro by exposure to a CFTR potentiator. In contrast, the more common ΔF508 mutation is associated with a severe protein trafficking defect, as well as impaired channel function. Recent clinical trials demonstrated a beneficial effect of the CFTR potentiator, Ivacaftor (VX-770), on lung function of patients bearing at least one copy of G551D CFTR, but no comparable effect on ΔF508 homozygotes. This difference in efficacy was not surprising in view of the established difference in the molecular phenotypes of the two mutant channels. Recently, however, it was shown that the structural defect introduced by the deletion of F508 is associated with the thermal instability of ΔF508 CFTR channel function in vitro. This additional mutant phenotype raised the possibility that the differences in the behavior of ΔF508 and G551D CFTR, as well as the disparate efficacy of Ivacaftor, might be a reflection of the differing thermal stabilities of the two channels at 37 °C. We compared the thermal stability of G551D and ΔF508 CFTR in Xenopus oocytes in the presence and absence of CFTR potentiators. G551D CFTR exhibited a thermal instability that was comparable to that of ΔF508 CFTR. G551D CFTR, however, was protected from thermal instability by CFTR potentiators, whereas ΔF508 CFTR was not. These results suggest that the efficacy of VX-770 in patients bearing the G551D mutation is due, at least in part, to the ability of the small molecule to protect the mutant channel from thermal instability at human body temperature.

The recent demonstration of efficacy of a CFTR potentiator in patients carrying at least one copy of G551D CFTR was a quantum leap for CF therapy, being the first instance of a therapeutic intervention based on a small molecule that directly targets the mutant gene product.1–4 The potentiator, VX-770, known as Ivacaftor or Kalydeco, did not exhibit similar efficacy in patients homozygous for the more common mutation, ΔF508, however.5 This difference could be attributed to the well-established difference in the molecular phenotypes of the two mutations, namely, a gating defect for G551D and a combined trafficking and gating defect for ΔF508,6 but we wondered if the two mutants might also differ with regard to the more recently established mutant CFTR phenotype of thermal instability.

Results from three laboratories provided strong evidence that the channel function of ΔF508 CFTR exhibits severe thermal instability. In Xenopus oocytes7 and HEK cells,8 conductance due to ΔF508 CFTR channels rescued at the surface by low temperature and activated by PKA and ATP rapidly decreased if the temperature was increased to 37 °C, an effect that could be traced to a reduction in open probability. A similar thermal instability was detected in ΔF508 CFTR channels reconstituted in planar bilayers.9,10 This severe gating defect, manifest at temperatures in excess of ~28 °C, was rescued to varying extents by single7 and multiple7,8 second-site suppressor mutations. The apparent disparity in clinical efficacy of VX-770 in compound heterozygotes (ΔF508/G551D) carrying one copy of G551D CFTR2,4 and a G551D homozygote carrying two copies of G551D CFTR,3 as well as the modest efficacy of VX-770 seen in ΔF508 homozygotes, suggested to us that ΔF508 CFTR channels and G551D CFTR channels might differ in their thermal stabilities. Might it be, for example, that the well-known trafficking defect seen with ΔF508 CFTR is, at least in part, a reflection of thermal instability apparent in a channel function assay, a thermal instability that might be lacking in the normally trafficked G551D channels?

We compared the thermal stability of G551D CFTR channels expressed in Xenopus oocytes with that previously reported by us for ΔF508 CFTR channels. We found, contrary to our initial expectations, that G551D CFTR channel function was thermally unstable at 37 °C, although G551D CFTR channel behavior differed from that of the ΔF508 channels in several important respects. First, thermal deactivation was more rapid, although less complete, than that seen with ΔF508 CFTR. Second, following a 37 °C thermal challenge, the conductance due to G551D channels recovered almost fully (85%), in contrast to that seen with ΔF508 channels, which
although variable, was on average 43% of the original conductance. Most importantly, however, G551D CFTR channels appeared to be protected from thermal instability at 37 °C by CFTR potentiators, including VX-770. Furthermore, potentiators also provoked an increase in conductance due to G551D channels at 37 °C, following thermal deactivation, a condition more like that in vivo. These results provide a mechanistic basis for the differing efficacy of VX-770 in patients carrying G551D and ΔF508 CFTR and have important implications for the design of assays for small molecule screening.

**MATERIALS AND METHODS**

**Mutagenesis and in Vitro Transcription.** CFTR mutants were generated using a site-directed mutagenesis method similar to those reported previously. The sequences in the region of the mutation were confirmed by direct DNA sequencing. CFTR cRNAs for *Xenopus* oocyte injection were synthesized using the mMessage mMachne T7 Ultra transcription kit (Ambion).

**Preparation and Microinjection of Oocytes.** *Xenopus laevis* oocytes were prepared using methods previously described in detail. Briefly, oocytes were defolliculated by mechanical agitation (1–2 h) in a Ca²⁺-free solution containing 82.5 mM NaCl, 2 mM KCl, 1 mM MgCl₂, and 5 mM HEPES (pH 7.5) with 0.2 Wünsch units/mL Liberase Blendzyme 3 (Roche Molecular Biochemicals, Indianapolis, IN) and maintained in a modified Barth’s solution containing 88 mM NaCl, 1 mM KCl, 0.82 mM MgSO₄, 0.33 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 2.4 mM NaHCO₃, 10 mM HEPES-Hemi-Na, and 250 mg/L Amikacin with 150 mg/L Gentamicin (pH 7.5). Stage V to VI oocytes were injected with CFTR cRNA plus cRNA encoding the human β₂-adrenergic receptor. The CFTR RNA concentration was adjusted so that the maximal steady-state-stimulated conductance was less than 200 μS (~12.5–25 ng/oocyte).

**Whole-Cell Recordings.** Individual oocytes were continuously perfused with Frog Ringer’s solution, which contained 98 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, and 5 mM HEPES-Hemi-Na (pH 7.4). CFTR channels were activated using 10 μM isoproterenol (a β₂-adrenergic agonist) and 1 mM IBMX (a phosphodiesterase inhibitor) as the stimulating cocktail (Isop+IBMX) to increase the level of intracellular cAMP. The data were acquired using an Oocyte 725 amplifier (Warner) and the pClamp 8 data acquisition program (Molecular Devices, Sunnyvale, CA). Oocytes were maintained under the open circuit condition, and the membrane potential was periodically ramped from −120 to 60 mV over 1.8 s. Data are reported as means ± the standard error of the mean, and a t test was performed where appropriate.

**Temperature Control.** The bath temperature was controlled using a Dual Automatic Temperature Controller (CL-200) and an in-line solution heater/cooler (SC-20) (Warner Instruments, Hamden, CT) and monitored in real time using a thermo probe positioned near the oocytes. The temperature was digitized and recorded using a USB Data Acquisition Device (DI-158, DATAQ Instruments, Inc., Akron, OH). All experiments were begun at room temperature (22–23 °C) unless otherwise specified.

**Reagents.** Isobutylmethyl xanthine (IBMX) and isoproterenol (Isop) were purchased from Sigma (St. Louis, MO). The following compounds were kindly provided by R. Bridges (Rosalind Franklin University, Chicago, IL) and The Cystic Fibrosis Foundation (CFF): 4-[4-oxo-2-thioxo-3-(3-trifluoromethylphenyl)thiazolidin-5-ylidenemethyl]-benzoic acid (CFTRinh-172 or CF172), 2-[2-(1H-indol-3-ylacetyl)-methylamino]-N-(4-isopropylphenyl)-2-phenyl-acetamide (PG-01 or P2), and 4-methyl-2-(5-phenyl-1H-pyrazol-3-yl)-phenol (VRT-S32 or P1). Genistin was purchased from TCI America (Portland, OR). N-(2,4-Di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (VX-770 or Iva-col) was purchased from Sellce Chemicals (Houston, TX).

**RESULTS**

**G551D Channels Exhibit Thermal Instability.** Figure 1A shows the results of a representative experiment in which an oocyte expressing G551D CFTR channels was exposed to an increase in temperature from 22 to 37 °C for 10 min. The results of three such experiments are summarized in Figure 1B. The effect of the thermal challenge was qualitatively similar to that we previously reported for ΔF508 channels expressed in *Xenopus* oocytes, a rapid, transient increase in conductance followed by a profound reduction, and partial recovery after the bath temperature was returned to 22 °C. However, there was a distinct, quantitative difference between the thermal deactivation of G551D CFTR and that we previously reported for ΔF508 CFTR. The rate of thermal deactivation was more rapid for the G551D channels [t½ = 0.3 min (±0.04; n = 3) vs 3.7 min (±0.3; n = 11) for ΔF508 channels] (P < 0.05). More striking, however, was the greater extent of recovery following the thermal challenge, 85 ± 15% (n = 3) for G551D and 43 ± 15% (n = 3) for ΔF508 (P < 0.05). The relatively rapid and nearly complete recovery of the G551D channels was reminiscent of that seen with ΔF508 channels when the phenylalanine deletion was combined with a nearby, second-site suppressor mutation like I539T (ΔF508/1539T CFTR). Upon comparison to contemporaneous experiments with ΔF508 CFTR channels (see Figures 3 and 4), it also appeared that the extent of thermal deactivation was lower for G551D channels. The conductance remaining after thermal deactivation, expressed as a percent of the initial, activated steady state conductance...
conductance at 22 °C, averaged 40 ± 8.1% (n = 7) for G551D and 7 ± 1.4% (n = 6) for ΔF508. Taken together, these results suggested that G551D CFTR channels, despite their rapid, thermal deactivation, are less severely impacted by the elevated temperature than ΔF508 channels.

**CFTR Potentiators Protect G551D CFTR Channels but Not ΔF508 CFTR Channels from Thermal Deactivation.** The unexpected finding of thermal instability of G551D channel function prompted us to compare the effects of CFTR potentiators on the two constructs during a 37 °C thermal challenge. In a previous report, we examined the effect of several potentiators obtained from CFF, VRT-532 (P1), PG-01 (P2), and Genistein, on the thermal stability of ΔF508 CFTR channel function. Although each of these compounds increased conductance due to ΔF508 CFTR channels at 22 °C, none of them protected ΔF508 CFTR channels from thermal deactivation at 37 °C. In fact, one of the potentiators (P2) actually exacerbated both the rate and extent of thermal deactivation at 37 °C. As illustrated in Figure 2, however,
similar experiments employing oocytes expressing G551D channels produced dramatically different results.

Panels A–E of Figure 2 compare representative experiments in which we tested the effects of four CFTR potentiators, VX-770, P1, P2, and Genistein, on the thermal stability of conductance due to G551D CFTR channels. As summarized in Figure 2F via comparison of the conductance near the termination of the thermal pulse with that seen prior to exposure to a potentiator, all four potentiators protected G551D CFTR channels from thermal deactivation, albeit to varying extents. It can be seen that, at a predetermined maximal dose, VX-770 (10 μM) and P2 (10 μM) were the most potent as protectors, whereas P1 and Genistein (50 μM) was less so. Also, note that the steady state-activated conductance due to G551D CFTR channels exhibited substantial variation from oocyte to oocyte.

**VX-770 Did Not Protect ΔF508 CFTR from Thermal Deactivation.** Our previous study produced no evidence that potentiators could protect ΔF508 CFTR channels from thermal instability.7 VX-770 was not tested in this earlier study, however, as the compound was not available to us. Panels A and C of Figure 3 show the results of representative experiments that compared thermal deactivation of conductance due to ΔF508 channels but also increased conductance beyond that seen at 22 °C. VX-770 did not protect ΔF508 CFTR from thermal deactivation. (A) Following activation of conductance via an increase in intracellular cAMP concentration (see Materials and Methods), an oocyte expressing G551D CFTR was warmed to 37 °C (gray bar and circles) in the presence of VX-770. The CF172-insensitive conductance was subtracted from the final analysis. (D) Summary of conductance due to ΔF508 CFTR channels at 37 °C before and after stimulation by 10 μM VX-770. The CF172-insensitive conductance was subtracted from the final analysis. (D) Summary of conductance due to ΔF508 CFTR channels at 37 °C before and after stimulation by 10 μM VX-770.

Figure 4. VX-770 stimulated G551D but not ΔF508 CFTR channels at 37 °C. (A) Following activation of conductance via an increase in intracellular cAMP concentration (see Materials and Methods), an oocyte expressing G551D CFTR was warmed to 37 °C (gray bar and circles). The oocyte was then exposed to 10 μM VX770 and subsequently to 10 μM CF172 at 37 °C. (B) Following stimulation, an oocyte expressing ΔF508 CFTR was warmed to 37 °C (gray bar and circles) and then exposed to 10 μM VX770 at 37 °C. (C) Summary of the conductance due to G551D CFTR at 37 °C before and after stimulation by 10 μM VX770. (D) Summary of conductance due to ΔF508 CFTR channels at 37 °C before and after stimulation by 10 μM VX770.

experiments in which we investigated the efficacy of the most potent potentiator, VX-770, in oocytes expressing G551D CFTR channels when the drug was applied following the completion of thermal deactivation at 37 °C, to better mimic the in vivo condition. Exposure to VX-770 induced a robust increase in the conductance due to G551D channels at 37 °C (Figure 4A,C). Because of the small sample size and the large variance, this robust increase did not reach statistical significance, although the stimulatory effect of VX770 was unambiguous in each case. Comparison of the data summarized in Figure 4 with that in Figure 2 suggests that VX-770, when applied at 37 °C, not only “protected” the G551D CFTR channels but also increased conductance beyond that seen at 22 °C. Consistent with our previous report, however, neither VX-770 nor any of the other potentiators previously tested [P1, P2, and Genistein (not shown)] produced significant stimulation of ΔF508 channels when applied at 37 °C (Figure 4C,D). Figure 4D summarizes the efficacy of VX-770 in G551D and ΔF508 CFTR channels at 37 °C.

Potentiator Efficacy following Thermal Deactivation of CFTR Channels. The results described above point to an important difference in the effect of CFTR potentiators like VX-770 on G551D and ΔF508 CFTR channels that may have a counterpart in the efficacy of VX-770 in patients carrying these mutations. In a patient’s first encounter with the drug, however, any G551D CFTR channels would presumably be partially deactivated, raising the question of the efficacy of potentiators in channels previously inactivated by exposure to 37 °C. Panels A and B of Figure 4 show the results of representative
DISCUSSION

The introduction of a CF therapeutic that directly targets the CFTR chloride channel was an important validation of the promise of high-throughput screening and should invigorate the search for a new generation of compounds that target the more common mutation, ΔF508. The results presented here have implications for such future drug discovery efforts. G551D channels, like ΔF508 channels, exhibit thermal instability. It would seem appropriate, therefore, to add assays of the thermal stability of channel function to those currently used to screen chemical libraries for efficacious compounds; that is, screening should include assays for “protection” from thermal instability, and compound efficacy should be compared at 37 °C. Yu et al.6 reported that, in membrane patches detached from FRT cells at room temperature, Ivacaftor (VX-770) was effective in increasing the open probability of a variety of CFTR channel constructs bearing mutations that adversely affected channel gating, consistent with a previous report of an increase in the open probability of ΔF508 CFTR channels under similar conditions.16 However, in FRT cell layers assayed in Ussing chambers at 37 °C, the efficacy of Ivacaftor on conductance due to ΔF508 CFTR channels was minimal, whereas a robust activation of conductance due to G551D channels was detected, despite a minimal conductance prior to exposure to Ivacaftor.6 It seems clear that CFTR potentiators, compounds selected for their ability to increase CFTR channel open probability, can, among other things, stabilize the activated state of G551D CFTR channels at 37 °C, an effect that may provide clues about the mode of action of these compounds and ultimately their binding sites.

 Trafficking and Thermal Stability of Channel Function. The finding that ΔF508 CFTR channels, rescued by a low temperature at the surface of Xenopus oocytes7 or mammalian cells,8 exhibit thermally induced decreases in open probability raised the question of whether the thermal instability of ΔF508 CFTR channel function is directly related to the well-established temperature sensitivity of ΔF508 CFTR trafficking.17-23 The simplest model, perhaps, would hold that the decrease in open probability that is evident at 37 °C is a manifestation of the same temperature-dependent unfolding process that, within the cell, triggers a quality control mechanism that leads ultimately to the intracellular degradation of the misfolded protein. Our results, however, seem to argue against this simple model. Clearly, G551D CFTR channels, which are trafficked normally or nearly normally, nevertheless exhibit severe thermal instability of channel function. On the other hand, the difference between G551D and ΔF508 CFTR could simply be one of degree. It may be that the G551D CFTR channels, although thermally unstable, may not proceed as far along the unfolding pathway at 37 °C as do the ΔF508 channels. G551D CFTR channels would, thereby, escape intracellular degradation but nevertheless fail to contribute adequately to chloride conductance due to the thermal instability of channel function that seems likely to reduce channel open probability at 37 °C.

AUTHOR INFORMATION

Corresponding Author

*Address: 3181 SW Sam Jackson Park Rd, HRCNS, Portland, OR 97239. E-mail: liuxu@ohsu.edu. Phone: (503) 346-3543. Fax: (503) 494-6251.

Present Address

X.L.: Knight Cardiovascular Institute, Oregon Health & Science University, Portland, OR 97239.

Funding

Supported by The Cystic Fibrosis Foundation [Dawson08G0 (D.C.D.)] and the National Institutes of Health [DK045880 (D.C.D.)].

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We gratefully acknowledge the gift of CFTR potentiators and inhibitors from Drs. Alan Verkman and Robert Bridges and CFF, without which this work would have been impossible.

ABBREVIATIONS

CFTR, cystic fibrosis transmembrane conductance regulator; IBMX, isobutylmethyl xanthine; Isop, isoproterenol; P1 (VRTS32), 4-methyl-2-(5-phenyl-1H-pyrazol-3-yl)phenol; P2 (PG-01), 2-[(2-1H-indol-3-ylacetyl)methylamino]-N-(4-isopropylphenyl)-2-phenyl-acetamide; VX-770 (Ivacaftor), N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide; CF172 (CFTRinh-172), 4-[4-oxo-2-thioxo-3-(3-trifluoromethylphenyl)thiazolidin-5-ylidenemethyl]-benzoic acid.

REFERENCES

(1) Accurso, F. J., Rowe, S. M., Clancy, J. P., Boyle, M. P., Dunitz, J. M., Durie, P. R., Sagel, S. D., Hornick, D. B., Konstan, M. W., Donaldson, S. H., Moss, R. B., Pilewski, J. M., Rubenstein, R. C., Ulander, A. Z., Attken, M. L., Freedman, S. D., Rose, L. M., Mayer-Hamblett, N., Dong, Q., Zha, J., Stone, A. J., Olson, E. R., Ordonez, C. L., Campbell, P. W., Ashlock, M. A., and Ramsey, B. W. (2010) Effect of VX-770 on persons with cystic fibrosis and the G551D-CFTR mutation. N. Engl. J. Med. 363, 1991–2003.

(2) Ramsey, B. W., Davies, J., McElvaney, N. G., Tullis, E., Bell, S. C., Drevenek, P., Griese, M., McKone, E. F., Wainwright, C. E., Konstan, M. W., Moss, R., Ratjen, F., Sermet-Gaudelus, I., Rowe, S. M., Dong, Q., Rodriguez, S., Yen, K., Ordonez, C., and Elborn, J. S. (2011) A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N. Engl. J. Med. 365, 1663–1672.

(3) Harrison, M. J., Murphy, D. M., and Plant, B. J. (2013) Ivacaftor in a G551D homozygote with cystic fibrosis. N. Engl. J. Med. 369, 1280–1282.

(4) Polenakovic, H. M., and Sanville, B. (2013) The use of ivacaftor in an adult with severe lung disease due to cystic fibrosis (ΔF508/G551D). J. Cystic Fibrosis 12, 530–531.

(5) Flume, P. A., Liou, T. G., Borowitz, D. S., Li, H., Yen, K., Ordonez, C. L., and Geller, D. E. (2012) Ivacaftor in subjects with cystic fibrosis who are homozygous for the F508del-CFTR mutation. Chest 142, 718–724.

(6) Yu, H., Burton, B., Huang, C. J., Worley, J., Cao, D., Johnson, J. P., Jr., Urrutia, A., Joubran, J., Seepersaud, S., Suskky, K., Hoffman, B. J., and Van Goor, F. (2012) Ivacaftor potentiation of multiple CFTR channels with gating mutations. J. Cystic Fibrosis 11, 237–245.

(7) Liu, X., O’Donnell, N., Landstrom, A., Skach, W. R., and Dawson, D. C. (2012) Thermal Instability of ΔF508 Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Channel Function: Protection by Single Suppressor Mutations and Inhibiting Channel Activity. Biochemistry 51, s124–s124.

(8) Wang, W., Okeyo, G. O., Tao, B., Hong, J. S., and Kirk, K. L. (2011) Thermally Unstable Gating of the Most Common Cystic Fibrosis Mutant Channel (ΔF508): “Rescue” by Suppressor Mutations in Nucleotide Binding Domain 1 and 2 by Constitutive Mutations in the Cystolic Loops. J. Biol. Chem. 286, 41937–41948.

5617

dx.doi.org/10.1021/bi501007v | Biochemistry 2014, 53, 5613–5618
Regulatory insertion removal restores maturation, stability and function of ΔF508 CFTR. *J. Biol. Chem.* 260, 1941–210.
(10) Aleksandrov, A. A., Kota, P., Cui, L., Jensen, T., Reyes, S., He, L., Gentzsch, M., Dokholyan, N. V., and Riordan, J. R. (2012) Alloteric Modulation Balances Thermodynamic Stability and Restores Function of ΔF508 CFTR. *J. Mol. Biol.* 419, 41–60.

(11) Smith, S. S., Liu, X., Zhang, Z. R., Sun, F., Kriewall, T. E., McCarty, N. A., and Dawson, D. C. (2001) CFTR. Covalent and noncovalent modification suggests a role for fixed charges in anion conduction. *J. Gen. Physiol.* 118, 407–432.

(12) Liu, X., Alexander, C., Serrano, J., Borg, E., and Dawson, D. C. (2006) Variable reactivity of an engineered cysteine at position 338 in cystic fibrosis transmembrane conductance regulator reflects different chemical states of the thiol. *J. Biol. Chem.* 281, 8275–8285.

(13) Serrano, J. R., Liu, X., Borg, E. R., Alexander, C. S., Shaw, C. F., III, and Dawson, D. C. (2006) CFTR: Ligand Exchange between a Permeant Anion (\([\text{Au(CN)}_2]^{3−}\)) and an Engineered Cysteine (T338C) Blocks the Pore. *Biophys. J.* 91, 1737–1748.

(14) Al-Nakkash, L., and Hwang, T. C. (1999) Activation of wild-type and ΔF508-CFTR by phosphodiesterase inhibitors through cAMP-dependent and -independent mechanisms. *Pfluegers Arch.* 437, 553–561.

(15) Liu, S., Veilleux, A., Zhang, L., Young, A., Kwok, E., Laliberte, F., Chung, C., Tota, M. R., Dube, D., Friesen, R. W., and Huang, Z. (2005) Dynamic activation of cystic fibrosis transmembrane conductance regulator by type 3 and type 4D phosphodiesterase inhibitors. *J. Pharmacol. Exp. Ther.* 314, 864–854.

(16) Van Goor, F., Hadida, S., Grootenhuis, P. D., Burton, B., Cao, D., Neuberger, T., Turnbull, A., Singh, A., Joubran, J., Hazlewood, A., Zhou, J., McCurney, J., Arumugam, V., Decke, C., Yang, J., Young, C., Olson, E. R., Wine, J. J., Frizzell, R. A., Ashlock, M., and Negulescu, P. (2009) Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18825–18830.

(17) Denning, G. M., Anderson, M. P., Amara, J. F., Marshall, J., Smith, A. E., and Welsh, M. J. (1992) Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. *Nature* 358, 761–764 (see comments).

(18) Lukacs, G. L., Chang, X. B., Bear, C., Karter, N., Mohamed, A., Riordan, J. R., and Grinstein, S. (1993) The ΔF508 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.

(19) Sharma, M., Benharouga, M., Hu, W., and Lukacs, G. L. (2001) Conformational and temperature-sensitive stability defects of the ΔF508 cystic fibrosis transmembrane conductance regulator in post-endoplasmic reticulum compartments. *J. Biol. Chem.* 276, 8942–8950.

(20) Gentzsch, M., Chang, X. B., Cui, L., Wu, Y., Ozols, V. V., Choudhary, A., Pagano, R. E., and Riordan, J. R. (2004) Endocytic trafficking routes of wild type and ΔF508 cystic fibrosis transmembrane conductance regulator. *Mol. Biol. Cell* 15, 2684–2696.

(21) Swiatecka-Urban, A., Brown, A., Moreau-Marquis, S., Renuka, J., Coutermarch, B., Barnaby, R., Karlson, K. H., Flotte, T. R., Fukuda, M., Langford, G. M., and Stanton, B. A. (2005) The short apical membrane half-life of rescued ΔF508-cystic fibrosis transmembrane conductance regulator (CFTR) results from accelerated endocytosis of ΔF508-CFTR in polarized human airway epithelial cells. *J. Biol. Chem.* 280, 36762–36772.

(22) Obiyoneda, T., Barriere, H., Bagdany, M., Rabe, W. M., Du, K., Kohfeld, J., Young, J. C., and Lukacs, G. L. (2010) Peripheral protein quality control removes unfolded CFTR from the plasma membrane. *Science* 329, 805–810.

(23) Wang, X., Koulov, A., Kellner, W., Riordan, J., and Balch, W. (2008) Chemical and biological folding contribute to temperature-sensitive ΔF508 CFTR trafficking. *Traffic* 9, 1878–1893.

(24) Wang, Y., Liu, J., Loizidou, A., Bugeja, L. A.; Warner, R.; Hawley, B. R.; Cai, Z.; Toye, A. M.; Sheppard, D. N.; Li, H. CFTR potentiators partially restore channel function to A561E, a cystic fibrosis mutant with a similar mechanism of dysfunction as F508del-CFTR. *Br. J. Pharmacol.*, accepted. DOI: doi:10.1111/bph.12791.

(25) Cai, Z., Taddei, A., and Sheppard, D. N. (2006) Differential sensitivity of the cystic fibrosis (CF)-associated mutants G551D and G1349D to potentiators of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl− channel. *J. Biol. Chem.* 281, 1970–1977.

(26) Yang, I. C. H., Cheng, T. H., Wang, F., Price, E. M., and Hwang, T. C. (1997) Modulation of CFTR chloride channels by calycinin A and genistein. *Am. J. Physiol.* 272, C142–C155.

(27) Pedemonte, N., Sonawane, N. D., Taddei, A., Hu, J., Zegarra-Moran, O., Suen, Y. F., Robins, L. I., Dicus, C. W., Willenbring, D., Nantz, M. H., Kurth, M. J., Galietta, L. J., and Verkman, A. S. (2005) Phenylglycine and sulfonamide correctors of defective ΔF508 and G551D cystic fibrosis transmembrane conductance regulator chloride-channel gating. *Mol. Pharmacol.* 67, 1797–1807.

(28) Van Goor, F., Coni, K., Cao, D., Gonzalez, J., Hadida, S., Hazlewood, A., Joubran, J., Knap, T., Makings, L. R., Miller, M., Neuberger, T., Olson, E., Panchenko, V., Rader, J., Singh, A., Stack, J. H., Tung, R., Grootenhuis, P. D., and Negulescu, P. (2006) Rescue of ΔF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am. J. Physiol.* 290, L1117–L1130.