Cystic fibrosis (CF) is caused by inadequate CF transmembrane conductance regulator (CFTR) channel activity in the lung and intestines (1). CFTR channels are normally activated once they reach the cell surface, but the extent of this gating defect is unclear. Here, we describe potent activators of wild-type and ΔF508-CFTR channels that are structurally related to 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB), a negatively charged pore blocker that we show to have mixed agonistic activities (channel activation plus voltage-dependent pore block). These CFTR agonists include 1) an uncharged NPPB analog that stimulates channel opening at submicromolar concentrations without blocking the pore and 2) curcumin, a dietary compound recently reported to augment ΔF508-CFTR function in mice by an unknown mechanism. The uncharged NPPB analog enhanced the activities of wild-type and ΔF508-CFTR channels both in excised membrane patches and in intact epithelial monolayers. This compound increased the open probabilities of ΔF508-CFTR channels in excised membrane patches by 10–15-fold under conditions in which wild-type channels were already maximally active. Our results support the emerging view that CFTR channel activity is substantially reduced by the ΔF508 mutation and that effective CF therapies may require the use of channel openers to activate mutant CFTR channels at the cell surface.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfections—Baby hamster kidney (BHK) cells stably expressing human wild-type CFTR (BHK-CFTR cells) were provided by Dr. J. W. Hanrahan (McGill University). Human embryonic kidney (HEK) 293T cells were transiently transfected with wild-type or mutant CFTR cDNA using the Lipofectamine transfection kit (Invitrogen) following the manufacturer’s recommendations. Cells were cultured in Dulbecco’s modified Eagle’s medium (Mediatech) supplemented with 5 or 10% fetal bovine serum and 1 mM penicillin/streptomycin. CFTR expression in the transfected HEK-293T cells was verified by immunoblotting. The growth medium for the BHK-CFTR cells also contained 0.5 mM methotrexate to maintain selection for cells expressing ΔF508-CFTR or ΔF508-CFTR (where “R” is the regulatory domain) were grown for 1–2 days at 37 °C (low temperature-corrected) because these mutants are temperature-sensitive endoplasmic reticulum processing mutants that exhibit low surface expression when cells are cultured at 37 °C. Patch Clamp Analysis—Macroscopic and single channel currents were recorded in the excised inside-out configuration. Patch pipettes were pulled from Corning 8161 glass to tip resistances of 1.5–4.0 megohms (macroscopic recordings) or 15–18 megohms (single channel studies). CFTR channels were activated following patch excision by exposure of the cytoplasmic face of the patch to the catalytic subunit of PKA (110 units/ml; Promega) and MgATP (1.5 mM) unless indicated.
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otherwise. CFTR currents were recorded in symmetrical solutions each containing 140 mM N-methyl-D-glucamine chloride, 3 mM MgCl2, 1 mM EGTA, and 10 mM TES (pH 7.3). Macroscopic currents were evoked using a ramp protocol from +80 to –80 mV with a 10-s time period. Patches were held at –80 mV for single channel recordings. All patch clamp experiments were performed at 21–23 °C. Signals from macroscopic and single channel recording were filtered at 20 and 200 Hz, respectively. Data acquisition and analysis were performed using pCLAMP 9.1 software (Axon Instruments). Opening rates (openings/patch) were estimated from 3–5-min records by dividing the total number of events (openings plus closings) detected by the pCLAMP software by 2× the time period. Single channel opening rates and single channel open probabilities (Po) were calculated assuming that the number of channels/patch equals the maximum detected number of simultaneous openings. This analysis was limited to patches for which there were fewer than eight detectable open levels, i.e., the limit for the analysis software. Note that this bias the analysis to patches that are less responsive to activation by compounds. Curve fitting was performed using Microcal Origin software.

**Ussing Chamber Experiments**—CFBE410 epithelial cells stably transduced with ΔF508-CFTR or transiently transfected with wild-type CFTR were cultured as electrically resistive monolayers and assayed in Ussing chambers as described (16). ΔF508-CFTR-expressing monolayers were grown at 27 °C for 2–3 days to enhance the surface expression of this temperature-sensitive mutant. A serosal-to-mucosal Cl (H9262) was added to block the residual CFTR current, we observed a large increase in current at positive voltages (i.e., voltages at which pore block by the negatively charged compound is less effective). The currents that are induced by NPPB at positive potentials are CFTR-mediated based on two criteria: (i) absence in membrane patches excised from CFTR cells (see Fig. 3B) and (ii) inhibition by higher doses of NPPB (Fig. 1, E and F) or by 200 μM glibenclamide, another CFTR blocker (17). (At lower doses, glibenclamide could partially mimic the stimulatory effect of NPPB (data not shown)). This stimulatory effect was specific in that CFTR currents were not induced by two other voltage-dependent blockers of the CFTR pore (10 mM SCN (18) and 0.5 mM diphenylamine carboxylate (13)) (data not shown) or by 400 μM 2-amino-4-phenylbutyric acid, a truncated derivative of NPPB (Fig. 1B; see Fig. 2A). The stimulation by NPPB was not due to a reducing agent-like effect of this compound on oxidized CFTR channels because the increase in current was rapidly reversed upon washout from the bath (Fig. 1A), unlike the case for dithiothreitol (12).

Based on the unexpected stimulatory effect of this pore blocker on glutathionylated channels, we determined whether NPPB could stimulate the activities of unmodified CFTR channels that are phosphorylated at low levels (i.e., under conditions of submaximum stimulation by PKA). Fig. 1C shows that NPPB also stimulated CFTR currents at depolarizing potentials when channels were first minimally phosphorylated by treating the patch with a low concentration of PKA (2 units/ml), followed by PKA inhibitory peptide (PKI) to inhibit further phosphorylation. Currents in both directions were increased by low micromolar concentrations of NPPB; at higher concentrations, a voltage-dependent block was observed. As expected, channels that were highly phosphorylated by continuous exposure to a high PKA concentration (110 units/ml; standard CFTR activation conditions) exhibited only a voltage-dependent block by NPPB (Fig. 1D). Thus, the relative enhancement of channel activity by NPPB is inversely related to the level of CFTR phosphorylation. We conclude that NPPB behaves as a mixed agonist toward thiolated or poorly phosphorylated CFTR channels, i.e., this compound stimulates partially active channels in addition to blocking the pore in a voltage-dependent fashion.

To determine whether NPPB activates CFTR by binding to the same (or different) site that causes a pore block, we tested its effects on a CFTR pore mutant (R347E) that is resistant to block by NPPB (19). Fig. 1E and F shows that NPPB stimulated the currents mediated by R347E-CFTR at positive potentials to a greater extent compared with wild-type CFTR at moderate levels of phosphorylation (high PKA concentration (110 units/ml), followed by PKI). Unlike the wild-type channel, the R347E-CFTR currents were stimulated by NPPB even at negative potentials. Thus, NPPB behaves more as a pure agonist for the R347E pore mutant, which implies that this compound stimulates channel opening by binding to a site that is distinct from the pore-blocking site.

**CFTR Agonists That Activate the Channel without Blocking the Pore**—The inhibitory effect of NPPB on CFTR currents presumably depends in part on the negative charge of this compound, as evidenced by the voltage dependence of the block. To explore this point further and to identify compounds that are pure CFTR agonists, we tested several neutral NPPB derivatives and related compounds for their effects on CFTR channel activity (see Fig. 2A). Two neutral NPPB derivatives had been synthesized earlier in a screen for photosystem II herbicides (20); a benzenesulfonamide derivative (NPPB-AM) and a benzenesulfonylamine derivative (NPPB-sulf). Neither of these compounds is an effective inhibitor of photosystem II (20). However, we discovered that NPPB-AM is a potent activator of CFTR channels (Fig. 2). In pilot experiments, we observed that 10 μM NPPB-AM effectively stimulated the currents mediated by moderately phosphorylated CFTR channels (Fig. 2, B–D) or glutathionylated channels (data not shown) in excised membrane patches. NPPB-sulf had only weak effects at 100 μM and was not studied further (data not shown). The stimulatory effect of NPPB-AM was rapid, stable, quickly reversible upon washout, and inhibited by the subsequent addition of 200 μM glibenclamide (Fig. 2, B and E; see Fig. 4A). The relative stimulation by NPPB-AM varied with the degree of CFTR phosphorylation (greatest effects at low levels of phosphorylation), as was observed for the parent compound, NPPB (Fig. 2, compare B and E). However, the stimulation by NPPB-AM was voltage-independent, with no evidence for CFTR inhibition at any holding potential. CFTR activation by either compound required the presence of both MgATP and at least a low concentration of PKA (2 units/ml) (data not shown). Like the parent compound, NPPB-AM had no effect on currents across membrane patches excised from CFTR cells (e.g., see Fig. 3B).

In titration experiments, we observed detectable increases in CFTR currents at NPPB-AM concentrations as low as 125 nM, with EC50 ≅ 1 μM (Fig. 2, C and D). Thus, NPPB-AM behaves as a pure CFTR agonist over the nanomolar to low micromolar range.

The structure of NPPB-AM (two aromatic rings separated by a hydrocarbon spacer) is generally similar to that of a dietary compound that has attracted considerable interest in the CF field, viz., curcumin (a main ingredient in turmeric) (Fig. 2A). Egan et al. (21) reported that curcumin promotes the biosynthetic maturation and functional correction of the ΔF508-CFTR mutant in tissue culture cells and in mice. They proposed that the mechanism for this apparent effect is indirect and involves
FIG. 1. NPPB behaves as a mixed agonist toward thiolated or poorly phosphorylated CFTR channels. A, NPPB stimulates positive currents mediated by thiolated CFTR channels in an inside-out membrane patch excised from a BHK-CFTR cell. CFTR was activated with 110 units/ml PKA (high PKA) and 1.5 mM MgATP. Further phosphorylation was blocked by the addition of PKI (1.4 μg/ml). The moderate decrease in current following PKI addition is presumably due to the activity of membrane-bound phosphatases. Equimolar diamide (Dia)/GSH was added to promote channel thiolation (12). B, a derivative of NPPB that lacks the benzamide ring (2-amino-4-phenylbutyric acid (APB)) fails to stimulate
perturbations in Ca²⁺ pump activity and chaperone function in the endoplasmic reticulum. Whether curcumin promotes the maturation of the ΔF508-CFTR protein in the endoplasmic reticulum is controversial (e.g., see Ref. 22). However, because of its general similarity to NPPB-AM, we were intrigued by the possibility that curcumin could have a direct effect on CFTR gating, which might explain some of the functional correction that was reported by Egan et al. (21). Fig. 2F shows that curcumin (0.5–10 μM) also stimulated the currents mediated by wild-type CFTR in excised membrane patches. This stimulatory effect was voltage-independent, although it was less stable (i.e., time-dependent) than that observed for NPPB-AM. We show below that curcumin also markedly stimulated the activities of ΔF508-CFTR channels.

**NPPB-AM Increases the Single Channel Opening Rate**—Fig. 3 shows that the most potent agonist (NPPB-AM) primarily stimulated CFTR activity by increasing the channel opening rate independently of any effect on CFTR phosphorylation. Two observations indicate that NPPB-AM stimulated CFTR channel activity in excised membrane patches (for which channel number is presumably constant) without affecting CFTR phosphorylation. First, this compound and the parent compound (NPPB) reversibly stimulated CFTR currents following PKA washout or the addition of PKI (Figs. 1 and 2). Second, NPPB-AM markedly stimulated the currents mediated by a regulatory domain deletion mutant (ΔR-S660A-CFTR) (Fig. 3A) that exhibits low constitutive activity in the absence of PKA (23). NPPB-AM had no obvious effect on the MgATP sensitivity of CFTR activity as determined in MgATP titration experiments performed in the presence and absence of this compound (data not shown). However, NPPB-AM did markedly increase the opening rates and mean single channel Po values for partially phosphorylated CFTR channels (2 units/ml PKA) determined in “micropatch” experiments (i.e., patches containing fewer than eight channels each) (Fig. 3, C and D). We conclude that this compound stimulates the opening of individual CFTR channels possibly at a step downstream of nucleotide binding.

**NPPB-AM Markedly Stimulates the Opening of ΔF508-CFTR Channels under Conditions in Which Wild-type Channels Are Maximally Activated**—We next tested the effects of NPPB and derivatives on the two most common CF mutants: G551D-CFTR and ΔF508-CFTR. G551D-CFTR is a gating mutant (24) that, unlike ΔF508-CFTR, is trafficked to the cell surface with efficiency similar to that of wild-type CFTR. The G551D mutation maps to a region in NBD1 that likely plays a role in MgATP binding or the conformational coupling between ATP binding and the opening of the pore within the transmembrane domains (ATP-binding cassette transporter signature sequence) (25). Interestingly, the activity of this mutant was negligibly stimulated by 10 μM NPPB-AM (Fig. 3E), a dose that maximally stimulated the wild-type channel (Fig. 2). However, G551D-CFTR activity was markedly stimulated by high doses of the charged parent compound (NPPB), doses that were impossible to achieve for the less soluble uncharged derivative (Fig. 3E). In NPPB titration experiments, we observed an appreciable shift toward higher concentrations of NPPB for G551D-CFTR activation compared with wild-type channel activation (Fig. 3F). This result implies that the G551D mutation in NBD1 reduces the apparent affinity of NPPB (and presumably of NPPB-AM) for its activation site.

Fig. 4 shows that membrane-resident ΔF508-CFTR channels were dramatically stimulated by NPPB, NPPB-AM, and curcumin under conditions that maximally stimulated wild-type channels. HEK-293T cells transiently transfected with ΔF508-CFTR or CF bronchial epithelial cells stably transfected with ΔF508-CFTR (CFBE41o Δ cells) (16) were cultured at 27°C (temperature-corrected) to enhance the surface expression of this temperature-sensitive mutant (26). NPPB-AM was added after treating the patch for an extended time (5–10 min) with a high PKA concentration (110 units/ml) to exclude the possibility that ΔF508-CFTR channels had not achieved steady-state phosphorylation prior to adding the agonist (Fig. 4A) (11). NPPB-AM (10 μM) induced a 10–15-fold increase in ΔF508-CFTR macroscopic currents in excised patches in the presence of normally saturating concentrations of MgATP (1.5 mM) and PKA (110 units/ml) (Fig. 4, A and B). As observed for wild-type channel activity, NPPB-AM markedly stimulated ΔF508-CFTR channel activity when added in the absence of active kinase (e.g., after adding PKI) (Fig. 4B). In micropatch experiments, we observed that the stimulation of ΔF508-CFTR channel activity by NPPB-AM was due primarily to a large increase in the single channel opening rate (Fig. 4, C and D). (Note that this micropatch analysis is biased toward less responsive patches for which the number of simultaneous channel openings does not exceed the limit of the analysis software (see “Experimental Procedures”)). The 10–15-fold stimulation of ΔF508-CFTR macroscopic currents by NPPB-AM occurred under conditions in which the wild-type channel was nearly maximally active, e.g., the same dose of compound stimulated the activity of the wild-type channel by 1.2–1.5-fold in the absence of PKI (Fig. 4B). This implies that the Po values for ΔF508-CFTR channels were at least an order of magnitude lower under these conditions (because channel number is presumably constant in this recording configuration), a finding that is inconsistent with the view that ΔF508-CFTR channels exhibit near-normal activity when they reach the plasma membrane (8). The present uncertainty about the extent to which the ΔF508 mutation affects CFTR channel activity may be due in part to a simple technical issue, viz. the difficulty in estimating the mean single channel Po and opening rates for poorly active channels when the number of channels in the patch is unclear. Fig. 4E illustrates this point for a patch for which we detected no more than two simultaneous openings prior to adding NPPB-AM. Using this as the standard criterion for estimating the number of channels/patch, we calculated a single channel Po and opening rate/channel of 0.04 and 1.2/s, respectively. However, following the addition of NPPB-AM, as many as 12–15 simultaneous openings were observed (best guess assuming a unitary current of 0.5 pA/channel). Thus, it was clear that the number of channels in this patch had been underestimated and that, consequently, the mean single channel Po and opening rates had been grossly overestimated for the control conditions. We conclude that ΔF508-CFTR channel opening is markedly inhibited under these experimental conditions and argue that this gating defect is normally difficult to appreciate because of uncertainties in estimating the numbers of channels in a membrane patch.

**NPPB-AM Enhances CFTR Activity in Epithelial Monolayers**—Fig. 5 shows that NPPB-AM also potently stimulated wild-type and ΔF508-CFTR channels in intact epithelial cell
FIG. 2. CFTR channel activation by an uncharged NPPB analog. A, structures of test compounds. APB, 2-amino-4-phenylbutyric acid. B, NPPB-AM stimulates currents in both directions for moderately phosphorylated wild-type channels in excised BHK-CFTR patches. Results are representative of four experiments. Also see mean HEK-293T data in Fig. 4B. glib, glibenclamide. C, NPPB-AM titration for a representative patch. D, mean titration data fit to the Michaelis-Menten function (EC_{50} = 0.96 ± 0.19 μM). The conditions were as described for B and C. Data were normalized to the peak current at 10 μM NPPB-AM. The lowest dose tested was 125 nM, which increased the current by ~20%. The results are means ± S.E. (n = 8). E, NPPB-AM has a greater relative stimulatory effect on poorly phosphorylated CFTR channels. F, curcumin also stimulates CFTR currents, although less potently and less stably than NPPB-AM. The results are representative of six experiments.
Fig. 3. NPPB-AM increases the rate of CFTR channel opening without affecting the phosphorylation state. A, NPPB-AM and NPPB markedly stimulate ΔR-S660A-CFTR currents minus PKA across an excised HEK-293T patch. Note the scale change at the asterisk. The results were repeated three times. B, neither NPPB-AM nor NPPB affects currents across a patch excised from an untransfected (CFTR+/H11002) HEK-293T cell. C, effects of NPPB-AM (10 μM) and glibenclamide (glib; 300 μM) on CFTR channel activity in a BHK-CFTR micropatch. The holding potential was −80 mV. Channels were first activated by 2 units/ml PKA, followed by PKI. D, mean data showing that NPPB-AM stimulates the opening rates of poorly phosphorylated CFTR channels. The conditions were as described for C. The data are means ± S.E. (n = 7). Opening rates represent the total number of openings/s/patch. The “best guess” estimates of the mean single channel opening rates and single channel $P_o$ values for the pre- and post-NPPB-AM conditions (assuming that N is the maximum number of simultaneous openings after NPPB-AM addition) were as follows: 0.26 ± 0.09 (pre) and 1.07 ± 0.29 (post) openings/s/channel and $P_o = 0.06 ± 0.01$ (pre) and 0.26 ± 0.06 (post) (means ± S.E., n = 7). E, NPPB-AM weakly activates G551D-CFTR, whereas high doses of NPPB markedly stimulate this mutant at high PKA concentrations in excised HEK-293T patches. Note the scale change at the asterisk. See mean data in F. F, NPPB stimulates wild-type CFTR currents (WT; ○) at lower doses than G551D-CFTR currents (●) in excised HEK-293T patches. Wild-type channels were activated with low PKA concentration. The conditions for G551D-CFTR were as described for E. Shown are mean data at ±80 mV obtained from four and seven experiments for the wild-type and G551D-CFTR channels, respectively. Data were normalized to the peak current induced by NPPB at each voltage.
FIG. 4. NPPB-AM and NPPB markedly stimulate ΔF508-CFTR channels. A, stimulation of a ΔF508-CFTR macroscopic current across an excised HEK-293T patch. High PKA, 110 units/ml. B, mean data showing that NPPB-AM much more greatly stimulates ΔF508-CFTR currents than wild-type currents (WT) in excised patches exposed to a high PKA concentration. Error bars indicate the means ± S.E. of five to eight experiments. All results were obtained from wild-type CFTR- or ΔF508-CFTR-transfected HEK-293T cells with the exception of the gray bar (CFBE41o− cells stably transfected with ΔF508-CFTR). ΔF508-CFTR-expressing cells were “low temperature-corrected” as described under “Experimental Procedures.” C, ΔF508-CFTR channel activation by NPPB-AM in an excised HEK-293T micropatch containing sufficiently few channels to resolve unitary currents. Other conditions were as follows: high PKA concentration, 1.5 mM MgATP, and −80 mV. glib, glibenclamide. D, effects of NPPB-AM on the mean opening rates and \( P_o \) for ΔF508-CFTR in excised membrane patches (n = four HEK-293T patches). Opening rates represent the total number of openings/s/patch. The best guess estimates of the mean single channel opening rates and single channel \( P_o \) values for the pre- and post-NPPB-AM conditions (assuming that \( N \) is the maximum number of simultaneous openings after NPPB-AM addition) were as follows: 0.20 ± 0.11 (pre) and 0.87 ± 0.31 (post) openings/s/channel and \( P_o = 0.05 \pm 0.03 \) (pre) and 0.25 ± 0.07 (post) (mean ± S.E., n = 4). E, a representative excised HEK-293T micropatch showing the dramatic activation of ΔF508-CFTR channels by NPPB-AM and illustrating the difficulty in estimating channel number under control conditions for this mutant. The conditions were as described for C.
Opening CFTR Channels with Blocker Analogs

We have identified potent CFTR agonists based on the discovery that a pore blocker (NPPB) has mixed agonistic activity. The uncharged NPPB derivative (NPPB-AM) behaves as a pure CFTR agonist that affects CFTR gating by increasing the channel opening rate. NPPB-AM has several features that make it a suitable lead compound for developing therapeutically useful compounds, including: (i) specificity, (ii) simple chemistry, (iii) cell permeability, and (iv) ability to activate ΔF508-CFTR channels. Although we have not established that curcumin stimulates CFTR by the same mechanism, the striking (albeit transient) effect of this dietary compound on ΔF508-CFTR channel activity might explain in part its previously reported positive effect in ΔF508-CFTR mice (21). This observation appears to be in general agreement with the results of Berger et al. (28), who recently reported that curcumin can activate CFTR channels in membrane patches excised from transfected HeLa cells.

NPPB-AM and NPPB are capable of stimulating CFTR opening without affecting channel phosphorylation by PKA, i.e., these compounds enhance wild-type or ΔR-S660A-CFTR activity in the absence of active kinase. However, the relative degree of activation is inversely related to the degree of prior phosphorylation (greater relative stimulation of poorly phosphorylated channels). This probably explains why the activating effect of NPPB was not described previously. NPPB exhibits only a voltage-dependent block of CFTR channels under standard activation conditions (high PKA concentration) (see Fig. 1D). The inverse relationship between activation by these compounds and the apparent degree of phosphorylation may be due simply to the fact that highly phosphorylated wild-type channels are nearly maximally active already (i.e., single channel P0 ≈ 0.3–0.5) (2, 3, 29). In this regard, the ΔR-S660A-CFTR construct used here (23) has much lower channel activity than highly phosphorylated wild-type channels (P0 estimated at <0.05) (29), although this activity is not dependent on PKA. Presumably, this accounts for the robust stimulation of ΔR-CFTR channel activity by NPPB and NPPB-AM observed in this study.

Our findings indicate that the ΔF508 mutation has a much greater effect on CFTR channel activity than initially appreciated. The literature on this point is confusing, with reports of no gating defect for ΔF508-CFTR (8), a modest gating defect at a high PKA concentration (9, 11), and a substantial gating defect (10). Wang et al. (11) reported that ΔF508-CFTR channels in membrane patches excised from NIH3T3 cells activate more slowly than wild-type channels when exposed to PKA, but that the gating of these channels is near-normal after steady-state activation is achieved. In our experiments, we used a high PKA concentration and waited sufficiently long times (5–10 min) to achieve steady-state activation prior to adding compounds. Our results indicate that, even under these conditions, ΔF508-CFTR channels have at least 10-fold less activity (due in part to reduced opening rates) compared with wild-type channels at normally saturating MgATP and PKA levels. This profound defect became apparent only after ΔF508-CFTR channel opening was stimulated by NPPB-AM, when it became clear that the numbers of ΔF508-CFTR channels under control conditions had been substantially underestimated. We obtained similar results for CFTR channels that were expressed in HEK-293T cells or in CBE410− epithelial cells; thus, this difference between wild-type and ΔF508-CFTR channel activities appears to be unrelated to cell background.

The reduced channel activity seen with NPPB-AM and NPPB-AM is due in large part to reduced opening rates compared with wild-type channels at normally saturating MgATP and PKA levels. This profound defect became apparent only after ΔF508-CFTR channel opening was stimulated by NPPB-AM, when it became clear that the numbers of ΔF508-CFTR channels under control conditions had been substantially underestimated. We obtained similar results for CFTR channels that were expressed in HEK-293T cells or in CBE410− epithelial cells; thus, this difference between wild-type and ΔF508-CFTR channel activities appears to be unrelated to cell background.

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channel activity of the ΔF508 mutant could be related to altered phosphorylation levels as argued by Wang et al. (11). Although our experiments were performed under conditions in which the channels were exposed to normally supersaturating concentrations of PKA for extended time periods, it is possible that ΔF508-CFTR channels are less phosphorylated compared with wild-type channels even under these conditions. Alternatively, the reduced ΔF508-CFTR channel activity could be due to a defect in ATP-dependent gating that is unrelated to altered phosphorylation, a defect that has been difficult to quantify because of the uncertainty in estimating the numbers of mutant channels in membrane patches.

A substantial defect in ΔF508-CFTR channel activity has two broad implications. (i) This could exacerbate the severity of the disease for ΔF508 patients especially if these mutant channels can reach the surfaces of a subset of epithelial cell types as argued by some (7), and (ii) therapies that target only the biosynthetic processing defect of this mutant may be ineffective. Our results support the emerging view (14) that the treatment of most CF patients may require combination therapies that include CFTR channel openers.

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