In cystic fibrosis (CF), dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel disrupts epithelial ion transport and perturbs the regulation of intracellular pH (pHi). CFTR modulates pHi through its role as an ion channel and by regulating transport proteins. However, it is unknown how CFTR senses pHi. Here, we investigate the direct effects of pHi on recombinant CFTR using excised membrane patches. By altering channel gating, acidic pHi increased the open probability (P_o) of wild-type CFTR, whereas alkaline pHi decreased P_o and inhibited Cl⁻ flow through the channel. Acidic pHi potentiated the MgATP dependence of wild-type CFTR by increasing MgATP affinity and enhancing channel activity, whereas alkaline pHi inhibited the MgATP dependence of wild-type CFTR by decreasing channel activity. Because these data suggest that pHi modulates the interaction of MgATP with the nucleotide-binding domains (NBDs) of CFTR, we examined the pHi dependence of site-directed mutations in the two ATP-binding sites of CFTR that are located at the NBD1:NBD2 dimer interface (site 1: K464A-, D572N-, and G1349D-CFTR; site 2: G551D-, K1250M-, and D1370N-CFTR). Site 2 mutants, but not site 1 mutants, perturbed both potentiation by acidic pHi and inhibition by alkaline pHi, suggesting that site 2 is a critical determinant of the pHi sensitivity of CFTR. The effects of pHi also suggest that site 2 might employ substrate-assisted catalysis to ensure that ATP hydrolysis follows NBD dimerization. We conclude that the CFTR Cl⁻ channel senses directly pHi. The direct regulation of CFTR by pHi has important implications for the regulation of epithelial ion transport.

The ATP-binding cassette (ABC)³ transporter cystic fibrosis transmembrane conductance regulator (CFTR) (1) is a multi-functional protein best known as a regulated Cl⁻ channel (2). CFTR is assembled from five domains: two membrane-spanning domains (MSDs) that form an anion-selective pore, two nucleotide-binding domains (NBDs) that bind and hydrolyze ATP to control channel gating, and a unique regulatory domain (RD), whose phosphorylation by PKA is critical for CFTR activation (2, 3). CFTR is principally expressed in the apical membrane of epithelia throughout the body where it plays a fundamental role in fluid and electrolyte movements (4). Malfunction of CFTR causes the common genetic disease cystic fibrosis (CF) (4).

Previous studies demonstrate that the regulation of intracellular pH (pHi) is defective in CF epithelial cells (e.g. Ref. 5). They also reveal that expression of recombinant CFTR in heterologous cells modulates pHi (e.g. Ref. 6). Analysis of the literature suggests that CFTR modulates pHi in three main ways. First, CFTR itself directly transports HCO₃⁻ ions with a modest permeability (P_HCO₃⁻/P_Cl⁻ ~0.26 (7)). Second, CFTR regulates the Na⁺/H⁺ exchanger isofor 3 (NHE3), which contributes to Na⁺−dependent HCO₃⁻ reabsorption in pancreatic duct epithelia. CFTR stabilizes NHE3 expression at the cell surface and inhibits NHE3 activity by a cAMP-dependent mechanism when pancreatic HCO₃⁻ secretion is stimulated (8). Of note, the regulation of NHE3 by CFTR involves the association of CFTR and NHE3 with the scaffolding protein EBP50 to form a macromolecular complex (8). Third, CFTR regulates the Cl⁻/HCO₃⁻ (anion) exchanger (AE), which plays a central role in pancreatic HCO₃⁻ secretion. CFTR regulation of AE requires the cell surface expression and cAMP-dependent phosphorylation of CFTR, but not its transport of anions (6). Interestingly, Ko et al. (9) demonstrated that CFTR and members of the SLC26 family of AEs coordinate their activities through the interaction of the, phosphorylated RD of CFTR with the STAS (sulfate transporter and antisigma-factor antagonist) domain of SLC26 transporters. Thus, CFTR modulates pHi through its roles as an ion channel and regulator of transport proteins.

A key unresolved question is how CFTR senses changes in pHi. As described above, CFTR might detect pHi changes indirectly through its interactions with NHE3 and SLC26 transporters. Consistent with this idea, Reddy et al. (10) demonstrated that pHi modulates indirectly the CFTR Cl⁻ conductance of sweat duct epithelia by altering the enzymatic interburst interval; MBD, mean burst duration; N, number of active channels; NBD, nucleotide-binding domain; NHE3, Na⁺/H⁺ exchanger isoform 3; PKA, protein kinase A; P_o, open probability; RD, regulatory domain; SAC, substrate-assisted catalysis; TAP, transporter associated with antigen processing; TES, Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.
activities of the protein kinases and phosphatases that control the phosphorylation status of CFTR. However, the ATPase activity of some ABC transporters (e.g. the transporter associated with antigen processing (TAP)) is pH-dependent (11), while the ATPase activity of an NBD1-RD-CFTR fusion protein is optimal at neutral pH (12). These data raise the possibility while the ATPase activity of an NBD1-RD-CFTR fusion protein

EXPERIMENTAL PROCEDURES

Cells and CFTR Expression—In this study we used mammalian cells heterologously expressing CFTR constructs. Where available, we used cells stably expressing CFTR constructs. These included (i) mouse mammary epithelial cells (C127 cells) expressing wild-type human CFTR, the CFTR variant ΔR1558Q (13) or the CF mutant G1349D (14), (ii) Fischer rat thyroid epithelial cells expressing the CF mutant G551D (15), and (iii) NIH-3T3 cells expressing the CFTR construct K1250M (16). To study the CFTR variants K464A, D572N, and D1370N, we employed the vaccinia virus/bacteriophage T7 hybrid expression system to transiently express CFTR variants in HeLa cells as described previously (17, 18). As a control, we verified that the single-channel behavior of wild-type human CFTR in different mammalian cells was equivalent (for further information, see supplemental “Results” and Figs. 1 and 2).

HeLa cells were purchased from the American Type Culture Collection (Manassas, VA). C127 cells expressing wild-type CFTR were a generous gift of Dr. C. R. O’Riordan (Genzyme, Framingham, MA), whereas Fischer rat thyroid cells were a generous gift of Drs. L. J. V. Galietta and O. Zegarra-Moran (Istituto Giannina Gaslini, Genova, Italy). Other cells and the vaccinia virus expression plasmid pTM1-CFTR4-expressing CFTR mutants were generous gifts of Professor M. J. Welsh (University of Iowa, Iowa City, IA). Cells were cultured and used as described (14–16, 19).

Electrophysiology—CFTR Cl− channels were recorded in excised inside-out membrane patches using an Axopatch 200A patch-clamp amplifier and pCLAMP software (both from MDS Analytical Technologies, Union City, CA) as described (20).

The pipette (extracellular) solution contained 140 mM N-methyl-D-glucamine, 140 mM aspartic acid, 5 mM CaCl2, 2 mM MgSO4, and 10 mM TES, pH 7.3, with Tris ([Cl−], 10 mM). The control bath (intracellular) solution contained 140 mM N-methyl-D-glucamine, 3 mM MgCl2, 1 mM CsEGTA, 5 mM Tris, and 5 mM Bis-Tris, pH 7.3, with HCl ([Cl−], 147 mM; free [Ca2+], <10−8 M) and was maintained at 37 °C; voltage was −50 mV. To ensure that the Cl− concentration of different intracellular solutions was identical, all solutions were first titrated to pH 7.3 with HCl before we added either H2SO4 to titrate pH to acidic values or Tris/NaOH to titrate to alkaline values. By using Bis-Tris (pKα 6.5) and Tris (Trizma base; pKα 8.1) instead of TES (pKα 7.5) as the biological buffer, we buffered intracellular solutions over a wide range of pH values (pH, 5.8–8.8).

After excision of inside-out membrane patches, we added PKA (75 nM) and ATP (1 mM) to the intracellular solution within 5 min of patch excision to activate CFTR. Once CFTR Cl− channels were fully activated, we varied the pH, value and/or [ATP] of the intracellular solution depending on the experimental protocol. Because MgATP controls CFTR channel gating (e.g. Refs. 21 and 22), we maintained the [MgATP] constant in different pH solutions by adding different amounts of Na2ATP (for further information, see supplemental “Experimental Procedures” and Table 1). To minimize the rundown of CFTR Cl− channels in excised membrane patches, we added PKA to all intracellular solutions. However, for studies of some CFTR constructs and wild-type CFTR in the absence of Mg2+ ions, it was also necessary to increase the [ATP] to 1 or 3 mM, respectively, to sustain channel activity.

To investigate how pH modulates CFTR Cl− currents, we used membrane patches containing large numbers of active channels. For all other studies, we used membrane patches containing 5–5 active channels. We determined the number of channels in a membrane patch from the maximum number of simultaneous channel openings observed during the course of an experiment. To minimize errors when counting the number of active channels, we employed the strategies described by Cai et al. (14). For further information, see supplemental “Experimental Procedures.” In all experiments, intracellular solutions titrated to different pH values were compared with the average of pre- and postintervention control periods titrated to pH 7.3, which contained the same concentrations of MgATP (or Mg2+-free ATP) and PKA.

To investigate the effects of voltage on the CFTR Cl− channel at different pH i, we bathed membrane patches in symmetrical Cl−–rich solutions (extracellular pH (pH e) 7.3; pH i, 7.3, 8.3, 8.8, or 6.3). From a holding voltage of −50 mV, we stepped voltage from −120 to +80 mV in 20-mV increments of 30 s. At each test voltage, we measured the single-channel current amplitude (i) and open probability (P o) of CFTR (see below). We calculated the OH− ion concentration ([OH−]) of different intracellular solutions directly from pH i values (pH i, 7.3; [OH−] = 0.2 μM; pH i, 8.3, [OH−] = 2 μM; pH i, 8.8, [OH−] = 6.3 μM).

CFTR Cl− currents were initially recorded on digital audi-tape using a digital tape recorder (Biologic Scientific Instruments, model DTR-1204; Intracel Ltd., Royston, UK) at a bandwidth of 10 kHz. On playback, records were filtered with an eight-pole Bessel filter (model 902LF2; Frequency Devices, Inc., Ottawa, IL) at a corner frequency of 500 Hz and acquired using a Digidata 1200 interface (MDS Analytical Technologies) and pCLAMP at sampling rates of 2.5 kHz (macroscopic currents) or 5 kHz (single-channel currents). For the purpose of illustration, current records were filtered at 500 Hz and digitized at 1 kHz.

To analyze the effects of pH i on CFTR Cl− currents, we determined the average CFTR Cl− current (I CFTR) at a specific pH i by averaging all the data points collected at that pH i and subtracting basal currents recorded in the absence of ATP and PKA. To plot the relationship between pH i or [H+], and CFTR Cl− current, current values at different pH i or [H+] were ex-
pressed as a percentage of the control CFTR Cl\(^-\) current recorded at pH\(_i\) 7.3. To measure \(i\), Gaussian distributions were fit to current amplitude histograms.

For \(NP_\circ\), \(P_\circ\), and burst analyses, lists of open and closed times were created using a half-amplitude crossing criterion for event detection as described under supplemental "Experimental Procedures." Burst analysis was performed as described by Carson et al. (16) using membrane patches that contained a single active channel and a \(t_b\) (the time that separates interburst closures from intraburst closures) determined from analyses of closed time histograms (supplemental Table 2). The mean interburst interval (IBI or \(T_{\text{IBI}}\)) was calculated using Equation 1,

\[
P_o = \frac{T_b}{(T_{\text{MBD}} + T_{\text{IBI}})}
\]

(Eq. 1)

where \(T_b\) = (mean burst duration) \(\times\) (open probability within a burst). Mean burst duration (MBD or \(T_{\text{MBD}}\)) and open probability within a burst (\(P_o(\text{burst})\)) were determined directly from experimental data using pCLAMP software. \(P_o\) was calculated from open and closed times as described (23). However, for G551D and G1349D, because the number of active channels in a membrane patch was unknown, we measured \(NP_o\) instead of \(P_o\).

To calculated the apparent voltage-dependent dissociation constant (\(K_o\)) for OH\(^-\) ion inhibition of CFTR and estimate the location of the binding site for OH\(^-\) ions within the CFTR pore, we employed the methods described by Sheppard and Robinson (20). For further information, see supplemental "Experimental Procedures."

**Reagents**—With the exception of PKA purified from bovine heart (Promega, Southampton, UK), chemicals were purchased from Sigma-Aldrich (Gillingham, UK). Stock solutions of ATP were prepared immediately before each experiment. When studying CFTR channel gating at ATP = 10 mM, we added MgSO\(_4\) (7 mM) to the intracellular solution. For nominally Mg\(^{2+}\)-free intracellular solutions, we omitted MgCl\(_2\) (3 mM) from the intracellular solution, titrated to pH 7.3 with HCl, and added EDTA (1 mM) to buffer free [Mg\(^{2+}\)] to <6 mM (22) before adjusting pH\(_i\) to 6.3 with H\(_2\)SO\(_4\) and to pH 7.3 and 8.3 with Tris.

**Statistics**—Results are expressed as means ± S.E. of \(n\) observations. To compare sets of data, we used Student’s \(t\) test. Differences were considered statistically significant when \(p < 0.05\).

**RESULTS**

**Multiple Effects of pH\(_i\) on CFTR Activity**—To investigate whether pH\(_i\) regulates directly the CFTR Cl\(^-\) channel, we studied CFTR Cl\(^-\) currents in membrane patches excised from C127 cells expressing wild-type human CFTR. After CFTR activation by PKA-dependent phosphorylation at pH 7.3, we varied pH\(_i\) over the range pH\(_i\) 5.8 to 8.8 and recorded CFTR Cl\(^-\) currents while keeping the MgATP and PKA concentrations constant (for further information, see supplemental “Experimental Procedures” and Table 1. Fig. 1A demonstrates that changing pH\(_i\) altered the magnitude of CFTR Cl\(^-\) current in a readily reversible manner. At acidic pH\(_i\), CFTR Cl\(^-\) currents were enhanced robustly at pH\(_i\) 6.8 and 6.3, but decreased at pH\(_i\) 5.8 (Fig. 1B). By contrast, at alkaline pH\(_i\), CFTR Cl\(^-\) currents decreased steadily (Fig. 1B). To examine the pH\(_i\) sensitivity of CFTR, we explored the relationship between CFTR Cl\(^-\) current and the proton concentration ([H\(^+\)]) of pH\(_i\) solutions. Over the pH\(_i\) range pH\(_i\) 6.3–8.3, the data were best fit by a hyperbolic function with a half-maximal stimulatory concentration (\(K_s\) at pH\(_i\) 7.06 (Fig. 1C). We interpret these data to suggest that CFTR activity is regulated by pH\(_i\) and that this regulation involves the protonation of CFTR.

In principle, pH\(_i\) might alter CFTR Cl\(^-\) current in one or more of three ways: (i) by regulating the number of active channels (\(N\)), (ii) by modulating current flow through open channels, and (iii) by controlling channel gating and, hence, \(P_\circ\). To understand how pH\(_i\) regulates CFTR, we investigated the effects of pH\(_i\) on the single-channel activity of CFTR. Fig. 2 shows representative recordings of a single wild-type CFTR Cl\(^-\) channel at different pH\(_i\). Visual inspection of these records...
CFTR Senses Directly $pH_i$

suggests that varying $pH$ had striking effects on CFTR channel gating, little effect on single-channel current amplitude ($i$), and none on $N$. However, when we measured $i$, we found that $i$ was unaltered at acidic $pH$, but diminished progressively at alkaline $pH_i$ (Fig. 3A), suggesting that $OH^-$ ions inhibit the CFTR $Cl^-$ channel.

The pattern of gating of wild-type CFTR at $pH_i 7.3$ is characterized by bursts of channel openings interrupted by brief flickery closures and separated by longer closures between bursts (e.g. Ref. 24 and Fig. 2, third trace from the top). To quantify the $pH_i$ dependence of channel gating, we measured $P_o$ and performed an analysis of bursts. Over the $pH_i$ range $pH_i 6.3–8.3$, $P_o$ decreased steadily as $pH_i$ increased (Fig. 3B). However, between $pH_i 6.3$ and $5.8$, $P_o$ fell sharply, whereas between $pH_i 8.3$ and $8.8$, it rose, returning to a value similar to that of $pH_i 7.8$ (Fig. 3B). At acidic $pH_i$, $MBD$ increased dramatically, whereas at alkaline $pH_i$, $MBD$ decreased (Fig. 3C). By contrast, Fig. 3D demonstrates that the relationship between $pH_i$ and $IBI$ is complex. At acidic $pH_i$, IBI decreased at $pH_i 6.3$, but increased markedly at $pH_i 5.8$, whereas at alkaline $pH_i$, IBI increased at $pH_i 8.3$, but was unchanged at $pH_i 8.8$ (Fig. 3D). Thus, $pH_i$ has intricate effects on CFTR channel gating. However, the effects of $pH_i$ on the single-channel behavior of CFTR account for the $pH_i$ dependence of CFTR $Cl^-$ currents (supplemental Table 3). Based on the control experiments described under supplemental “Results”, we suggest that $pH_i$ regulates directly the CFTR $Cl^-$ channel.

$OH^-$ ions Occlude the CFTR Pore—Fig. 3A demonstrates that the $i$ of CFTR diminishes progressively with alkalinization of the intracellular solution, suggesting that $OH^-$ ions might inhibit the CFTR $Cl^-$ channel by acting as an open-channel blocker (25). To test this hypothesis, we investigated the effects of membrane voltage on the $i$ of wild-type CFTR at alkaline $pH_i$. We reasoned that negative voltages would drive $OH^-$ ions from the intracellular solution into the CFTR $Cl^-$ channel where they might bind, occlude the channel pore, and block $Cl^-$ permeation. Conversely, positive voltages would drive $Cl^-$ ions into the CFTR $Cl^-$ channel from the extracellular solution, flushing blocking $OH^-$ ions from the channel pore and relieving inhibition.

Fig. 4A shows representative recordings of a single CFTR $Cl^-$ channel at $–80$ and $+80 mV$, Fig. 4B shows the single-channel current-voltage ($I-V$) relationship of wild-type CFTR at $pH_i 7.3$ and $8.3$, and Fig. 4C shows the voltage dependence of the fraction of $i$ inhibited at $pH_i 8.3$. Fig. 4, A–C, demonstrates that at negative voltages there was a small, but significant, decrease in the $i$ of wild-type CFTR at $pH_i 8.3$ compared with that at $pH_i 7.3$. By contrast, at positive voltages the inhibition of $i$ was completely relieved (Fig. 4, A–C) (similar results were observed at $pH_i 8.8$ over the voltage range $–80$ to $+80 mV$ (data not shown)). Block of $i$ at negative voltages, but relief at positive voltages suggests that $OH^-$ ions occlude the intracellular vestibule of the CFTR pore.

To determine the potency with which $OH^-$ ions block the CFTR pore, we calculated the apparent voltage-dependent dissociation constant ($K_{s}$) for $OH^-$ ion inhibition of wild-type
CFTR Senses Directly $pH_i$

**FIGURE 4.** Voltage-dependent inhibition of current flow through the CFTR pore by OH$^-$ ions. *A*, representative recordings show the effects of alkalinizing the intracellular solution to pH 8.3 on the activity of a single wild-type CFTR Cl$^-$ channel at $-80$ mV (top) and $+80$ mV (bottom). The recording conditions were identical to those used in Figs. 1 and 2 with the exception that the membrane patch was bathed in symmetrical 147 mM Cl$^-$ solutions. Dotted lines indicate where the channel is closed, and downward deflections at $-80$ mV and upward deflections at $+80$ mV correspond to channel openings. The 1-s portions indicated by bars are shown on an expanded time scale below each 5-s recording. *B*, single-channel current-voltage (I-V) relationships of wild-type CFTR at pH 7.3 (white circles) and pH 8.3 (gray circles) using the conditions described in *A*. C, voltage dependence of the fraction of $i$ inhibited at pH 8.3. *D*, relationship between the voltage-dependent dissociation constant ($K_o$) and voltage at alkaline pH (pH 8.3 and pH 8.8). $E$, voltage dependence of $P_o$ at pH 6.3, pH 7.3, and pH 8.3. Data are means $\pm$ S.E. (*B, C, and E, n = 6; D, n = 3–9, except $-120$ mV, where n = 2). In *B* and *D*, the continuous lines are the fits of first-order regression functions to the data. In *B* and *E*, the asterisks indicate values that are significantly different from the control ($p < 0.05$). Error bars are smaller than symbol size except where shown.

CFTR. Fig. 4D demonstrates that apparent $K_o(V)$ values for CFTR inhibition by OH$^-$ ions are weakly voltage-dependent. The data also indicate that the potency of CFTR inhibition by OH$^-$ ions (apparent $K_o(0 \text{ mV}) = 54 \pm 6 \mu$M; $n = 9$) approaches that of the best-studied CFTR blocker, the sulfonylurea glibenclamide ($K_o(0 \text{ mV}) = 37 \mu$M) (20).

To estimate the location of the binding site for OH$^-$ ions, $z' = 0.23 \pm 0.03$ ($n = 9$), measured from the inside of the membrane over the voltage range $-120$ to $-40$ mV. These data suggest that the penetration of OH$^-$ ions into the transmembrane electric field from the intracellular side is $23\%$, similar to that of SCN$^-$ ions (20%) (27). Thus, at alkaline pH$_i$, OH$^-$ ions cause open-channel block of the CFTR Cl$^-$ channel by occupying a superficial site within the intracellular vestibule of the CFTR pore.

In contrast to the effects of pH$_i$ on $i$ (Figs. 3A and 4, *B* and *C*), the pH$_i$ dependence of $P_o$ was voltage-independent (Fig. 4E). At all voltages tested, the $P_o$ of wild-type CFTR was diminished at pH 8.3, but augmented at pH 6.3. The data also suggest that inhibition of CFTR Cl$^-$ current at alkaline pH$_i$ results predominantly from a diminution of $P_o$ rather than a decrease in $i$ (supplemental Table 3).

$pH_i$ Modulates the Function of the NBDs and RD—CFTR channel gating is controlled by the NBDs and RD (2, 3). Therefore, we reasoned that pH$_i$ might modulate CFTR activity by altering the function of these domains. To test this hypothesis, we adopted two strategies; first, we employed the CFTR construct $\Delta R$-S660A that deletes a large part of the RD and is not regulated by PKA-dependent phosphorylation (13) to explore separately how pH$_i$ influences the function of the RD and NBDs. Second, we investigated the effects of pH$_i$ on the ATP dependence of CFTR channel gating. For these experiments, we studied CFTR potentiation at pH 6.3 and CFTR inhibition at pH 8.3.

**FIGURE 5.** Acidic pH$_i$ potentiates the activity of $\Delta R$-S660A-CFTR. *A*, representative recordings show the effects of pH$_i$ on the activity of a single $\Delta R$-S660A-CFTR Cl$^-$ channel. Dotted lines indicate where the channel is closed, and downward deflections correspond to channel openings. B, C, and D, effects of pH$_i$ on the $P_o$, MBD, and IBI of $\Delta R$-S660A (columns) and wild-type CFTR (circles). Data are means $\pm$ S.E. (*B, n = 6 for all data; *C and *D, $\Delta R$-S660A-CFTR, n = 3; wild-type-CFTR, n $\geq$ 6). Asterisks indicate $\Delta R$-S660A-CFTR values that are significantly different from the pH 7.3 control ($p < 0.05$).
**CFTR Senses Directly pH**

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**A**

- **WT**
  - pH 6.3
  - pH 7.3
  - pH 8.3

**B**

- **WT**
  - pH 6.3
  - pH 7.3
  - pH 8.3

**C**

- **WT**
  - pH 6.3
  - pH 7.3
  - pH 8.3

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FIGURE 6. pH modulates the MgATP dependence of CFTR channel gating.

A, relationship between [MgATP] and $P_o$ of wild-type CFTR at the indicated pH values. The continuous lines are Michaelis-Menten fits to mean data. B and C, effects of pH on the $P_o$ of wild-type (WT), D572N, and D1370N-CFTR in the presence of ATP (3 mM in B or 1 mM in C). In B, wild-type CFTR data were acquired in the presence (circles) and absence (columns) of MgATP (3 mM), whereas in C, wild-type, D572N, and D1370N-CFTR data were acquired in the continuous presence of MgATP (3 mM). All data are means ± S.E. ($n = 5–7$); asterisks indicate values that are significantly different from the control value ($p < 0.05$).

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because its MBD was reduced 0.4-fold, while its IBI was prolonged 9-fold (Fig. 5, B–D). Acidifying to pH 6.3 potentiated the $P_o$ of wild-type CFTR 0.4-fold, but that of Δ-R-S660A-CFTR 3.3-fold (Fig. 5B). Fig. 5, C and D, demonstrates that the marked potentiation of Δ-R-S660A-CFTR $P_o$ at pH 6.3 was primarily caused by a 0.9-fold decrease in IBI, but it was also enhanced by a 1.5-fold increase of MBD. Alkalining to pH 8.3 decreased the $P_o$ of wild-type CFTR 0.3-fold, but was without effect on the $P_o$ of Δ-R-S660A-CFTR (Fig. 5B). An explanation for this striking difference between wild-type and Δ-R-S660A-CFTR is shown in Fig. 5D. Between pH 7.3 and 8.3, the IBI of wild-type CFTR increased 0.3-fold, whereas that of Δ-R-S660A-CFTR decreased 0.5-fold (Fig. 5D). Fig. 5D also reveals that the relationship between IBI and pH is linear for wild-type CFTR, but bell-shaped for Δ-R-S660A-CFTR over the pH range 6.3–8.3.

We interpret the failure of pH 8.3 to attenuate Δ-R-S660A-CFTR channel gating to suggest that alkaline pH, might inhibit CFTR channel gating, at least in part, by modulating RD function. However, the robust potentiation of Δ-R-S660A-CFTR channel gating at pH 6.3 argues that acidic pH likely enhances CFTR channel gating by acting on sites distinct from the RD.

To investigate whether pH influences NBD function, we studied the ATP dependence of CFTR channel gating. Fig. 6A shows the relationship between [MgATP] and $P_o$ for wild-type CFTR at pH 6.3, 7.3, and 8.3. At each pH, tested, as the [MgATP] increased, $P_o$ values enlarged. However, altering pH influenced both the MgATP sensitivity and channel activity of wild-type CFTR (Fig. 6A). These differences are best illustrated by considering values of $K_D$ (the [MgATP] required for half-maximal $P_o$, which describes the apparent affinity of CFTR for MgATP) and $P_o$ max (the maximum $P_o$) determined from Michaelis-Menten fits to the mean data (pH 6.3: $K_D = 36 ± 5$ μM, $P_o$ max = 0.72 ± 0.01, $r^2 = 0.99$; pH 7.3: $K_D = 90 ± 14$ μM, $P_o$ max = 0.61 ± 0.02, $r^2 = 0.99$; pH 8.3: $K_D = 100 ± 24$ μM, $P_o$ max = 0.49 ± 0.02, $r^2 = 0.97$; Fig. 6A). Two conclusions are apparent from these data. First, acidifying pH enhances markedly the apparent MgATP affinity of CFTR, whereas alkalining pH, has little effect. Second, acidifying pH, augments channel activity, whereas alkalining pH, inhibits CFTR.

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The Role of Mg$^{2+}$ Ions in Determining the pH Sensitivity of the NBDs—Mg$^{2+}$ ions are a pre-requisite for the hydrolysis of ATP that drives CFTR channel closure (21, 22). To learn whether pH, modulates CFTR channel gating by affecting the interaction of Mg$^{2+}$ ions with the NBDs, we examined the single-channel activity of CFTR in the absence of Mg$^{2+}$ ions at different pHs (Figs. 6B and supplemental Fig. 3A). At pH 7.3 removal of Mg$^{2+}$ ions attenuated markedly the $P_o$ of CFTR (Fig. 6B). Nevertheless, acidification to pH 6.3 enhanced strongly $P_o$, whereas alkalining to pH 8.3 was without effect (Fig. 6B). These data suggest that the presence of Mg$^{2+}$ ions is critical for the inhibition of CFTR channel gating at alkaline pH. By contrast, the potentiation of CFTR channel gating at pH 6.3 is both dependent on, and independent of, Mg$^{2+}$ ions.

To understand better the role of Mg$^{2+}$ ions in determining the pH sensitivity of the NBDs, we studied Asp-572 and Asp-1370, the Walker B aspartates that coordinate Mg$^{2+}$ ions at site 2. This suggests that pH influences NBD function, we studied the ATP dependence of CFTR channel gating. Fig. 6A shows the relationship between [MgATP] and $P_o$ for wild-type CFTR at pH 6.3, 7.3, and 8.3. At each pH, tested, as the [MgATP] increased, $P_o$ values enlarged. However, altering pH influenced both the MgATP sensitivity and channel activity of wild-type CFTR (Fig. 6A). These differences are best illustrated by considering values of $K_D$ (the [MgATP] required for half-maximal $P_o$, which describes the apparent affinity of CFTR for MgATP) and $P_o$ max (the maximum $P_o$) determined from Michaelis-Menten fits to the mean data (pH 6.3: $K_D = 36 ± 5$ μM, $P_o$ max = 0.72 ± 0.01, $r^2 = 0.99$; pH 7.3: $K_D = 90 ± 14$ μM, $P_o$ max = 0.61 ± 0.02, $r^2 = 0.99$; pH 8.3: $K_D = 100 ± 24$ μM, $P_o$ max = 0.49 ± 0.02, $r^2 = 0.97$; Fig. 6A). Two conclusions are apparent from these data. First, acidifying pH enhances markedly the apparent MgATP affinity of CFTR, whereas alkalining pH, has little effect. Second, acidifying pH, augments channel activity, whereas alkalining pH, inhibits CFTR.
ATP Binding Site 2 Is a Crucial Determinant of the pH$_i$ Sensitivity of CFTR Channel Gating—Structural (29–32) and functional (16, 21) studies suggest that the phosphate groups of ATP are key determinants of ATP binding to sites 1 and 2. The Walker A lysine residues Lys-464 (site 1) and Lys-1250 (site 2), which bind the γ-phosphate of ATP, are highly conserved and critical for ATP binding and hydrolysis (16, 21, 29). Moreover, the LSGGQ motifs contain the highly conserved glycine residues Gly-551 (site 2) and Gly-1349 (site 1) that clamp the phosphate tail of ATP during ATP-driven NBD dimerization (29, 30, 32). Of note, the CF mutations G551D and G1349D perturb severely CFTR channel gating (14, 33). Using CFTR constructs bearing site-directed mutations, we examined the roles of the residues Lys-464, Lys-1250, Gly-551, and Gly-1349 in determining the pH$_i$ sensitivity of CFTR channel gating.

Consistent with previous studies (14, 33), G1349D-CFTR and especially G551D-CFTR attenuated strongly CFTR channel gating at pH 7.3, with brief, poorly resolved channel openings separated by very long-lasting closures (Fig. 7, A and C). Fig. 7, A–D, demonstrates that the gating behavior and, hence, $P_o$, of G551D- and G1349D-CFTR were unaffected by pH$_i$. Because tight dimerization of the NBDs is a prerequisite for channel opening (28) and because G551D and G1349D perturb severely channel gating (14, 33), we speculate that the effects of pH$_i$ on CFTR channel gating are dependent on the formation of an NBD1:NBD2 dimer.

Fig. 7, E and G, show the hallmark effects of mutating the Walker A lysines in NBD1 and NBD2 on CFTR channel gating (16, 21). At pH$_i$ 7.3, bursts of K464A-CFTR channel openings were separated by prolonged channel closures, whereas dramatically prolonged bursts of K1250M-CFTR channel openings were separated by very long-lived channel closures (Figs. 7, E and G, and 8). Although $P_o$ values of K464A-CFTR were less than those of wild-type CFTR at all pH$_i$ values tested, the effects of acidic and alkaline pH$_i$ on K464A- and wild-type CFTR were similar (Fig. 7, E and F). This suggests that K464A-CFTR does not change the pH$_i$ sensitivity of CFTR. By contrast, the pH$_i$ sensitivity of K1250M-CFTR differed strikingly from that of wild-type CFTR. At pH$_i$ 6.3, the $P_o$ of K1250M-CFTR was reduced because MBD was decreased 0.7-fold, whereas IBI was unchanged (Figs. 7, G and H, and 8). At pH$_i$ 8.3, the $P_o$ of K1250M-CFTR was increased, albeit slightly, as a result of a small increase in MBD and no change in IBI (Figs. 7H and 8). Fig. 7H also reveals that the $P_o$ of K1250M-CFTR was similar to that of wild-type CFTR at pH$_i$ 8.3, but greatly diminished at pH$_i$ 7.3 and 6.3. Thus, the pH$_i$ sensitivity of K1250M-CFTR is the converse of that of wild-type CFTR.

Taken together, our data argue that ATP binding site 2 is a crucial determinant of the pH$_i$ sensitivity of the CFTR Cl$^-$ channel. They also suggest that MgATP binding at site 1 might be important for mediating the inhibitory effects of alkaline pH$_i$ and cooperating with site 2 to drive channel gating.

**DISCUSSION**

In this study we investigated whether pH$_i$ regulates directly CFTR activity and the responsible mechanisms. Our data demonstrate that acidic pH$_i$ stimulates CFTR Cl$^-$ currents by potentiating channel gating, whereas alkaline pH$_i$ attenuates CFTR Cl$^-$ currents predominantly by inhibiting channel gating, but also by obstructing Cl$^-$ flow through the CFTR pore. Using CFTR constructs, we demonstrate that ATP-binding site 2 is a primary determinant of the pH$_i$ sensitivity of CFTR. Our data suggest that the mechanism by which pH$_i$ regulates CFTR is distinct from other ion channels. Instead of protons modifying channel gating by interacting with residues within (e.g. Kir1.1 (35)) or close to the intracellular vestibule (e.g. Kir1.1 (35)) of the channel pore, in CFTR protons modulate channel gating by altering the function of ATP-binding site 2 in the NBDs.

**Molecular Mechanisms of CFTR Regulation by pH$_i$**—Our data demonstrate that pH$_i$ has multiple effects on the CFTR Cl$^-$ channel. They also reveal that pH$_i$ modulates CFTR function by altering the activity of each of the domains from which CFTR is assembled. In Fig. 9 we summarize the molecular mechanisms by which pH$_i$ regulates current flow through
the CFTR pore and the control of channel gating by the NBDs and RD.

OH− ions inhibit CFTR function in several ways. First, OH− ions are open-channel blockers of the CFTR pore that disrupt CI− flow by interacting with a superficial site within the intracellular vestibule of the CFTR pore. Second, studies of the CFTR construct ΔR-S660A suggest that OH− ions might inhibit CFTR channel gating, at least in part, by modulating RD function. Third, OH− ions inhibit CFTR function by regulating the control of channel gating by ATP-driven NBD dimerization (3, 21). The decrease in channel activity at alkaline pH argues that OH− ions might destabilize ATP binding at sites 1 and 2, promoting dissociation of the NBD dimer and channel closure. However, our data also suggest that the binding of Mg2+ ions at site 1 is essential for CFTR inhibition by OH− ions.

In contrast to the effects of OH− ions, H+ ions mediate their effects on CFTR predominantly by modulating the control of channel gating at the NBDs. Our studies argue that ATP-binding site 2 is a crucial determinant of the pHi sensitivity of CFTR. The data suggest that H+ ions enhance the affinity with which MgATP binds at site 2 and stabilize ATP binding at this site with the result that the frequency of channel openings is increased and their duration prolonged. Thus, pHi regulates directly CFTR function. Importantly, this regulation occurs within the physiological pHi range (36), consistent with previous work demonstrating a significant role for CFTR in the regulation of pHi (e.g. Ref. 5, 6, 37, and 38).

Open-channel Block of the CFTR Pore by OH− Ions—At negative voltages OH− ions caused a small reduction of the i of CFTR. Given the low concentration of OH− ions in our test solutions (see “Experimental Procedures”), the potency of OH− ion inhibition of CFTR is surprisingly strong, exceeding that of many open-channel blockers of CFTR (e.g. diphenylamine-2-carboxylate and flufenamic acid (39)). Interestingly, the location of the OH− ion-binding site within the transmembrane electric field (23%) is similar to that of SCN− ions (20%) (27). These data argue that OH− and SCN− ions interact with a superficial site(s) within the intracellular vestibule of the CFTR pore (Fig. 9). The shallow penetrance of OH− and SCN− ions into the CFTR pore contrasts with that of large organic anions (e.g. glibenclamide (20)), which penetrate deep into the intracellular vestibule to interact with a site located halfway across the electric field of the membrane from the inside. Site-directed mutagenesis of Lys-95, located toward the extracellular end of the first transmembrane segment, reveals that the positive charge at Lys-95 is essential for channel block by glibenclamide and four other open-channel blockers of CFTR with unrelated chemical structures (40). However, studies of the kinetics of channel block (41–43) demonstrate that the interaction of open-channel blockers with CFTR is complex and likely to involve multiple binding sites within the intracellular vestibule of the CFTR pore. One of these binding sites might overlap with that of OH− ions.

Intricate Effects of pHi on CFTR Channel Gating—Our data reveal that pHi has complex effects on CFTR channel gating. Over the pHi range 6.3 to 8.3, the Po of wild-type CFTR decreases steadily because of reciprocal changes in IBI and MBD. This suggests that between pHi 6.3 and 8.3, the effects of pHi on MBD and IBI are coordinated. By contrast, our data suggest that in strongly acidic and alkaline intracellular solutions, MBD values are stable, whereas those of IBI appear unstable.

At pHi 5.8 the IBI of wild-type CFTR was prolonged strikingly, indicating that channel opening is very difficult at strongly acidic pHi. At pHi 5.8, MgATP2− is partially protonated at the γ-phosphate to become MgHATP− (44). This suggests that protonated ATP at pHi 5.8 might interfere with the formation of H-bonds between CFTR and the γ-phosphate of ATP, attenuating ATP binding and impeding channel opening. However, we speculate that protonation of phosphate groups in other parts of the CFTR gating pathway or on the RD at strongly acidic pHi might also contribute to this result.

Interestingly, at pHi 8.8 the IBI of wild-type CFTR was attenuated with the result that the Po of wild-type CFTR at pHi 8.8 enlarged to become equivalent to that at pHi 7.8. Because negative surface charge increases with alkalinization of the intracellular solution, the diminution of IBI at pHi 8.8 might represent a change in RD conformation or the interaction of the RD with other domains of CFTR (Fig. 9). For example, manipulation of the surface charge on the RD might mimic the effects of RD phosphorylation and disrupt interactions between the RD and NBDs leading to channel activation (13, 45, 46). Alternatively, increased negative surface charge at alkaline pHi might alter the balance between stimulatory and inhibitory RD phosphorylation sites to favor channel activation (47).
FIGURE 9. Multiple mechanisms of CFTR regulation by pH. The simplified models show an open CFTR Cl− channel with a phosphorylated RD and NBDs assembled to form a head-to-tail dimer with ATP molecules bound at ATP binding sites 1 and 2. Each ATP binding site is formed by the Walker A and B motifs of one NBD and the LSGGQ motif of the other NBD. The locations of the site-directed mutations examined in this study are shown. The models show the direct effects of H+ and OH− ions on the function of the individual domains of CFTR at alkaline, neutral, and acidic pH. Single-channel recordings are shown beneath each model. For the purpose of illustration, the recordings have been inverted, and upward deflections correspond to channel openings. MSD, membrane-spanning domain; P, phosphorylation of the RD; P2, inorganic phosphate. In and Out denote the intra- and extracellular sides of the membrane, respectively, whereas C and O indicate the closed and open-channel states, respectively. The long arrows represent the regulation of CFTR channel gating by ATP-driven NBD dimerization; arrow thickness and symbols indicate reaction speed (thick (+), fast (potenti- ation); thin (−), slow (inhibition)). Double-headed arrows denote interactions between the NBDs and RD and cross-talk between ATP binding sites 1 and 2. Short arrows within the CFTR pore denote Cl− ion flow; arrow thickness and symbols indicate ease of flow (thick, unobstructed; thin (−), obstructed). See “Discussion” and Refs. 2, 3, and 25 for further information.

least in part, by modulating RD function. The reason why ΔR-S660A-CFTR is not inhibited at pH 8.3 is that its prolonged IBI is attenuated, not extended, at this pHi value. Further studies are required to understand the underlying mechanisms. However, it is interesting to note that for both ΔR-S660A-CFTR and the ATP-binding site 1 mutant D572N-CFTR, the relationship between pHi and IBI is bell-shaped, not linear, between pHi 6.3 and 8.3 (Figs. 5D and 8B). We interpret these data to suggest that the pHi dependence of IBI reflects complex interactions between the RD and NBDs.

Use of the ATP-driven NBD Dimerization Model of CFTR Channel Gating to Explain the Effects of pHi—Like other ABC transporters, the two NBDs of CFTR likely form a head-to-tail dimer that sandwiches ATP molecules within two binding sites (termed site 1 and site 2) located at the NBD1:NBD2 interface (3, 21). In CFTR, site 1 has no ATPase activity, but site 2 cyclically hydrolyzes ATP to drive channel gating (3, 21). In this asymmetric gating model, ATP-induced dimerization at site 1 likely occurs before that at site 2, which in turn precedes CFTR opening (48).

Structural studies of ABC transporters (29–32) demonstrate that within the ATP-binding sites the cis NBD anchors the phosphate tail of an ATP molecule by a network of hydrogen bonds (H-bonds) and hydrogen-mediated salt bridges from the Walker A motif. By contrast, in the trans NBD, only H-bonds from the LSGGQ motif clamp the γ-phosphate of ATP. These data suggest that perturbation of the LSGGQ motif might have a greater impact on NBD dimerization than disruption of the Walker A motif. Accordingly, the Walker A lysine mutant K1250A-CFTR is an ATP-dependent channel with moderate Po (16), whereas the LSGGQ motif mutant G551D-CFTR is an ATP-independent channel with extremely low Po (14, 33). Thus, the loss of pHi sensitivity by G551D- and G1349D-CFTR suggests that correct alignment of the NBD1:NBD2 dimer is required for the potentiation of CFTR channel gating by acidic pHi and inhibition by alkaline pHi.

Based on several lines of evidence, we suggest that ATP-binding site 2 is a crucial determinant of the pHi sensitivity of CFTR. First, acidifying pHi enhances markedly the apparent MgATP affinity of CFTR, whereas alkalinizing pHi has little effect. Vergani et al. (49) argue that Ke values obtained from the [MgATP] dependence of the opening rate of CFTR are a reasonable estimate of the Ke value for ATP binding to site 2. Moreover, using a simple kinetic model of CFTR channel gating, Winter et al. (24) demonstrated that the opening rate of CFTR, not its closing rate, is ATP-dependent. These considerations suggest that the pHi sensitivity of the apparent MgATP affinity of CFTR likely reflect the effects of H+ ions on the interaction of MgATP with site 2.

Second, our studies of CFTR channel gating in the absence of Mg2+ ions demonstrate that Mg2+ ions are critical for channel inhibition at alkaline pHi and modulate channel potentiation at acidic pHi. Because MgATP binding at both ATP binding sites is a prerequisite for channel opening (50), these data suggest that pHi has effects on both ATP binding sites. However, Mg2+ ions are also a prerequisite for the hydrolysis of ATP that closes the channel (50). This argues that the potentiation of channel gating at pHi 6.3 is evidence for H+ ions mediating their effects, at least in part, via site 2.

Third, H+ ions potentiate the gating behavior of CFTR constructs bearing site-directed mutations in ATP-binding site 1 (K464A- and D572N-CFTR). By contrast, H+ ions are either without effect (D1370N-CFTR) or inhibit (K1250M-CFTR) the gating behavior of site-directed mutations in ATP-binding site 2. As discussed above, the failure of H+ ions to potentiate G551D- (site 2) and G1349D-CFTR (site 1) is likely a consequence of the profound disruption of NBD dimerization and, hence, channel gating by these constructs. Taken together, the simplest interpretation of our data is that ATP-binding site 2 is
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a crucial determinant of the pH$_i$ sensitivity of the CFTR Cl$^-$ channel (Fig. 9). This suggests that H-bond formation at site 2 enhances channel activity by accelerating NBD dimerization and stabilizing the ATP bound conformation of the NBD1-NBD2 dimer. Consistent with this idea, acidic pH enhanced greatly the formation of an engineered TAP1 homodimer (11).

Mechanism of ATP Hydrolysis at Site 2 — A common feature of the ATP binding sites of the ABC transporters CFTR and TAP is their asymmetric ATPase activity (11, 29). In an engineered human TAP1 homodimer, the ATP-binding site with ATPase activity contains a catalytic dyad composed of a histidine residue in the H-loop and a glutamic acid residue distal to the Walker B motif (11, 31). However, instead of hydrolyzing ATP by general base catalysis, this dyad employs substrate-assisted catalysis (SAC) for this reaction (11, 31). In SAC, the interaction of the substrate, ATP, with catalytic water via its γ-phosphate is stabilized by an H-bond between the dyad histidine and the γ-phosphate of ATP (31). A key advantage of SAC over general base catalysis is that it prevents ATP hydrolysis occurring before NBD dimerization (31). In this way so-called “futile ATP hydrolysis” by ABC transporters is avoided.

It is intriguing to consider whether CFTR hydrolyzes ATP at site 2 by SAC. In support of this idea, site 2 but not site 1 contains a catalytic dyad like that found in human TAP1 (11, 29). Although the duration of bursts of channel openings are not determined solely by the ATPase activity of the NBDs (31), the effects of pH$_i$ on the MBD of CFTR (Fig. 3C) suggest that alkaline pH$_i$ might enhance ATP hydrolysis at site 2, whereas acidic pH$_i$ has the converse effect. This raises the interesting possibility that the pH$_i$ sensitivity of the ATPase activity at site 2 of CFTR might be similar to the ATP-binding site of an engineered TAP1 homodimer that uses SAC to hydrolyze ATP (11). Finally, in SAC, OH$^-$ ion attack of the γ-phosphate is the rate-limiting step for ATP hydrolysis (11, 31). Taken together, the data suggest that SAC might provide an efficient way to couple NBD dimerization and ATP hydrolysis in the CFTR Cl$^-$ channel. Future studies should explore this possibility.

The pH$_i$ Sensitivity of CFTR Reveals Cross-talk between ATP Binding Sites 1 and 2 — An interesting aspect of our data is the pH$_i$ sensitivity of K1250M-CFTR, which is the reverse of that of wild-type CFTR. This observation suggests that there might be hidden minor pH$_i$ effects, masked by the major effects of pH$_i$ on wild-type CFTR. If K1250M-CFTR severely disrupts the function of site 2 (16), these hidden minor pH$_i$ effects might originate from site 1. Our data also reveal that D572N-CFTR has a very unusual response to pH$_i$: exaggerated potentiation of channel gating at acidic pH$_i$ and potentiation, not inhibition, at alkaline pH$_i$. In ABC transporters, the Walker B motif is located adjacent to the highly conserved D-loop motif (30, 31). Structural studies of ABC transporters (30, 31) suggest that in CFTR the D-loop of NBD1 (site 1) might (i) interact with the Walker A motif of NBD2 (site 2) and (ii) sense the functional state of site 1 via the H-loop motif of NBD1. In support of this idea, the effects of acidic and alkaline pH$_i$ on D572N-CFTR are reminiscent of the enhanced activity of P574H-CFTR, a CF mutant affecting a residue in the Walker B-D-loop region of NBD1 (52). Thus, the D-loop might mediate cross-talk between the ATP binding sites of CFTR and influence the pH$_i$ sensitivity of CFTR.

Identification of pH$_i$-sensitive Amino Acids in CFTR — The changes in CFTR channel gating at pH$_i$ 6.3 and 8.3 suggest that histidine (pK$_a$ ~ 6.1) and cysteine (pK$_a$ ~ 8.3) might serve as pH$_i$-titratable amino acid residues. Examination of the NBD1-NBD2 dimer identifies a number of histidine and cysteine residues located close to the ATP binding sites. Site 1 contains two histidine residues in its LSGGQ motif (His-1348 and His-1350) and a cysteine residue Cys-491 (1, 29). Because Cys-491 is located spatially close to the Walker B aspartate, Asp-572, of site 1 (29), it might modulate the role of Asp-572 in CFTR channel gating by sensing alkaline pH$_i$. Interestingly, the H-loop of site 2 contains histidine (His-1402) and cysteine (Cys-1400) residues that are lacking in the H-loop of site 1 (29). This raises the interesting possibility that Cys-1400 and His-1402 might act as a pH$_i$ sensor for site 2. However, the potential roles of other histidine and cysteine residues in the control of CFTR channel gating should not be overlooked. For example, the sulf-hydryl-modifying reagent N-ethylmaleimide rapidly and irreversibly potentiates CFTR channel gating by covalently modifying Cys-832 in the RD (53). Moreover, analyzes of the pH$_i$ dependence of other ion channels (e.g. Ref. 35) suggest that other amino acid residues might act as pH$_i$ sensors for CFTR channel gating.

Physiological Significance — Reddy et al. (10) demonstrated that pH$_i$ regulates indirectly the CFTR Cl$^-$ conductance of sweat duct epithelia by altering the activity of protein kinases and phosphatases that control RD phosphorylation. By contrast, our data reveal that pH$_i$ regulates directly CFTR channel gating. Taken together, these data suggest that CFTR activity is regulated by pH$_i$ via two different control mechanisms (Fig. 10). First, at the cell membrane localized pH$_i$ fluctuations are
detected directly by CFTR, leading to prompt changes in its behavior, coordinated with alterations in the activity of NHE3 and SLC26 transporters. Second, when there are global changes in pH, the effects of pH on the enzymes controlling the phosphorylation status of CFTR regulate CFTR indirectly.

Fig. 10 also suggests how the direct and indirect mechanisms of CFTR regulation by pH might be integrated in the control of HCO₃⁻ secretion by epithelial cells. HCO₃⁻ secretion is driven by the coordinated activity of CFTR and electrogenic SLC26 transporters at the apical membrane (9, 11). CFTR channel gating and, hence, CI⁻ secretion (Fig. 10). Because CFTR activity is reciprocally coupled to that of SLC26 transporters (9), our data suggest that the two transporters form a positive feedback loop to promote HCO₃⁻ secretion (Fig. 10). To break this positive feedback loop, a further CFTR control mechanism is required. This is provided by pH regulating indirectly RD phosphorylation by controlling enzymatic activity through a negative feedback loop (Fig. 10).

The two-loop model of CFTR regulation by pH (Fig. 10) provides a mechanism to control the magnitude and duration of epithelial HCO₃⁻ secretion. This model might also be exploited to understand better how CF causes aberrant HCO₃⁻ secretion (37, 38), abnormal pH regulation (5, 6), and defects in acidic pH-induced apoptosis (54) and bacterial killing (55).

Thus, by investigating the effects of pH on CFTR, we understand better its mechanism of action, role in fluid and electrolyte transport, and malfunction in CF.

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