State-dependent Regulation of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gating by a High Affinity Fe$^{3+}$ Bridge between the Regulatory Domain and Cytoplasmic Loop 3* [+]

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The unique regulatory (R) domain differentiates the human CFTR channel from other ATP-binding cassette transporters and exerts multiple effects on channel function. However, the underlying mechanisms are unclear. Here, an intracellular and exerts multiple effects on channel function. However, the underlying mechanisms are unclear. Here, an intracellular high affinity (2.3 × 10$^{-19}$ M) Fe$^{3+}$ bridge is reported as a novel approach to regulating channel gating. It inhibited CFTR activity by primarily reducing an open probability and an opening rate, and inhibition was reversed by EDTA and phenanthroline. His-950, His-954, Cys-832, His-775, and Asp-836 were found essential for inhibition and phosphorylated Ser-768 may enhance Fe$^{3+}$ binding. More importantly, inhibition by Fe$^{3+}$ was state-dependent. Sensitivity to Fe$^{3+}$ was reduced when the channel was locked in an open state by AMP-PNP. Similarly, a K978C mutation from cytoplasmic loop 3 (CL3), which promotes ATP-independent channel opening, greatly weakened inhibition by Fe$^{3+}$ no matter whether NBD2 was present or not. Therefore, although ATP binding-inducedimerization of NBD1-NBD2 is required for channel gating, regulation of CFTR activity by Fe$^{3+}$ may involve an interaction between the R domain and CL3. These findings may support proximity of the R domain to the cytoplasmic loops. They also suggest that Fe$^{3+}$ homeostasis may play a critical role in regulating pathophysiological CFTR activity because dysregulation of this protein causes cystic fibrosis, secretary diarrhea, and infertility.

The cysic fibrosis transmembrane conductance regulator (CFTR) is a member of the human C subfamily of ATP-bind-
PKA does regulate a NBD1-NBD2 interaction (12) and that PKA can regulate ATP-independent gating in CFTR constructs with G551D (20) or without NBD2 (Δ1198) (21, 22). Finally, PKA can also regulate the NEM effect, which potentiates channel activity by modifying Cys-832 (23). Therefore, phosphorylation of the R domain by PKA is a key physiological regulator. Most phosphorylation sites including Ser-700, Ser-795, Ser-813, and Ser-660 stimulate channel activity but Ser-737 and Ser-768 are inhibitory sites. Substitutions of these two residues with alanines increase channel activity (18, 19, 24, 25). In addition, removal of a segment 760–783 or these two residues with alanines increase channel activity (18, 19, 24, 25). In addition, removal of a segment 760–783 or these two residues with alanines increase channel activity (18, 19, 24, 25).

Observations may support this electron microscopy structural model and may underlie some inhibition mechanisms of the R domain and the NBD1 (11). However, direct biochemical and electrophysiological evidence in the native whole protein is still missing. Moreover, the use of isolated CFTR fragments may lead to an interdomain interaction, which might not be present in the native channel. In fact, recent electron microscopy studies demonstrated that the R domain is mainly close to the cytoplasmic loops (16). However, an exact regulation mechanism of the R domain is still poorly understood because of low resolution. In this study, an intracellular high affinity Fe$^{3+}$ bridge was employed to infer a spatial relationship or a relative orientation between the R domain and CL3 because a high affinity metal bridge involves at least several amino acids in proteins (30). The interaction between the R domain and CL3 was further confirmed by thiol-specific disulfide bond cross-linking. State-dependent regulation by Fe$^{3+}$ and effects of Fe$^{3+}$ on channel gating were also investigated. Resulting observations may support this electron microscopy structural model and may underlie some inhibition mechanisms of the R domain.

**EXPERIMENTAL PROCEDURES**

**Molecular Biology**—The human wild type CFTR (hCFTR) was subcloned into the pCDNA3 mammalian expression vector (Invitrogen). A Cys-free construct without all 18 cysteines was subcloned into the pCDNA3 mammalian expression vector. State-dependent regulation by Fe$^{3+}$ and effects of Fe$^{3+}$ on channel gating were also investigated. Resulting observations may support this electron microscopy structural model and may underlie some inhibition mechanisms of the R domain. A Cys-free construct without all 18 cysteines was subcloned into the pCDNA3 mammalian expression vector (Invitrogen). A Cys-free construct without all 18 cysteines was subcloned into the pCDNA3 mammalian expression vector.

**Cell Culture and Transfection**—HEK-293T cells were transiently transfected with wild type or mutant CFTR cDNAs using the Lipofectamine transfection kit (Invitrogen). Cells were cultured in Dulbecco’s modified Eagle’s medium (Mediatech) supplemented with 10% fetal bovine serum and 1 mM penicillin/streptomycin. For patch clamp recordings, all cells were grown on plastic coverslips and used 1–4 days post-seeding. To improve expression of a Cys-free CFTR-based construct, V510A was inserted (31), and cells expressing the construct were grown for 1–2 days at 24 °C and then for 2–5 h at 37 °C before measurements.

**Patch Clamp Analysis**—All channels expressed in HEK-293T cells were recorded in an inside-out configuration (Axon Instruments, Foster City, CA) for intracellular application of reagents to a cytoplasmic face. CFTR currents were recorded in symmetrical solutions each containing the following: 140 mM N-methyl-d-glucamine chloride, 3 mM MgCl$_2$, 1 mM EGTA, and 10 mM TES (pH 7.3). Resulting resistance of a borosilicate patch pipette was 1–2 megohms in the bath solution. Macroscopic currents were evoked using a ramp protocol from +80 to −80 mV and filtered at 200 Hz. Single-channel recordings at −60 mV were filtered at 20 Hz. In experiments designed to increase $P_{o}$ of a CFTR channel, 2.7 mM AMP-PNP was added to an intracellular solution containing 1.5 mM MgATP and 100 units/ml PKA. When 500 $\mu$M Fe$^{3+}$ was added to the bath solution containing 1 mM EGTA (its stability constant for Fe$^{3+}$ is $\sim 10^{20}$ M$^{-1}$ at 20 °C), the free Fe$^{3+}$ concentration was $5 \times 10^{-19}$ M. For 500 $\mu$M Fe$^{3+}$, free Fe$^{3+}$ concentration was $5 \times 10^{-13}$ M in the presence of 1 mM EGTA (its stability constant for Fe$^{3+}$ is $\sim 10^{11.8}$ M$^{-1}$ at 20 °C). EDTA was chosen for its high stability constant for Fe$^{3+}$ ($10^{25.1}$ M$^{-1}$ at 20 °C) or for Fe$^{3+}$ ($10^{14.3}$ M$^{-1}$ at 20 °C). Alternatively, phenanthroline (its stability constant for Fe$^{3+}$ is $10^{4.1}$ M$^{-1}$ at 20 °C) or DTT was used to reverse inhibition of the current by Fe$^{3+}$. All experiments were carried out at room temperature (20 ± 1 °C). Data were acquired and analyzed using pCLAMP10.2 software (Axon Instruments). A Student’s $t$ test was used to test statistical significance. Curve fitting was made using Microcal Origin software. Data are shown as mean ± S.E.

**RESULTS**

**Internal Fe$^{3+}$ Inhibits hCFTR Activity**—The hCFTR channel transfected in HEK-293T cells was activated by ATP (1.5 mM) and PKA (24 units/ml). Further phosphorylation was blocked by a protein kinase inhibitory peptide (PKI) to make sure that the subsequent reagents targeted not PKA but the CFTR channel. In response to addition of Fe$^{3+}$ to the cytoplasmic side of an inside-out patch, ~60% of both outward and inward Cl$^{-}$ currents were inhibited quickly and inhibition was reversed by 5 mM EDTA (Fig. 1A). Finally, the inward current was blocked by a voltage-dependent blocker glibenclamide (200 $\mu$M), whereas both outward and inward currents were blocked by a CFTR-specific inhibitor CFTRinh172 (10 $\mu$M). In contrast, only 25% of the current of the hCFTR channel was inhibited by internal Fe$^{3+}$ (Fig. 1B, C). It is worth mentioning that reversal by EDTA was not due to removal of Mg$^{2+}$ because phenanthroline (5 mM), a Fe$^{3+}$-specific chelator, partially reversed inhibition by Fe$^{3+}$ (Fig. 1D and E). More importantly, a D1370N mutant, which removes the Mg$^{2+}$ binding site (32), was also inhibited by Fe$^{3+}$ (Fig. 1C), and inhibition was reversed by 5 mM EDTA (Fig. 1F). However, unlike the WT channel, the D1370N mutant exhibited less inhibition by Fe$^{3+}$ but more reversal of inhibition by EDTA. This difference is reasonable because washout of Mg$^{2+}$ has been reported to decrease channel activity (32). On the other hand, it may suggest that endogenous Fe$^{3+}$ in the HEK-293T cell may have been partially bound to the CFTR channel. Supporting this possibility, Fig. 1F demonstrates that...
inhibition by Fe$^{3+}$ was dose-dependent. An apparent affinity was very high ($K_{0.5} = 2.3 \times 10^{-19} M$). It is comparable with that found in the *Haemophilus influenzae* Fe$^{3+}$-binding protein (33).

**State-dependent Inhibition by Fe$^{3+}$** — It is of particular interest to determine whether inhibition by Fe$^{3+}$ can be varied by changing the gating state of the hCFTR channel or not. Because AMP-PNP is a nonhydrolyzable ATP analog that increases an open probability by stably binding to the NBDs, it was used to determine state dependence in this case. Fig. 2A shows that the hCFTR channel was activated by ATP (1.5 mM) and PKA (100 units/ml) but inhibited by PKI. 2.7 mM AMP-PNP clearly potentiated channel activity primarily by increasing the open probability. It is very interesting that only slight and slow inhibition by subsequent Fe$^{3+}$ and weak reversal of Fe$^{3+}$ inhibition by EDTA were found. This observation suggests that inhibition by Fe$^{3+}$ was state-dependent. The CFTR channel could be locked in a closed state by internal Fe$^{3+}$ binding. Once the channel was open, Fe$^{3+}$ could not exert any effect on hCFTR activity. Supporting this notion, a K978C mutant from CL3 was also insensitive to Fe$^{3+}$ for a limited time because of a high open probability of 0.84 (17).

![Figure 1](image-url)
closely coupled to CL3. Once K978C reduced the activation energy for channel opening, Fe$^{3+}$ had no influence on channel activity. Thus, CL3 may play a critical role in modulating the Fe$^{3+}$ effect.

Identification of Fe$^{3+}$ Ligands—Usually, most common Fe$^{3+}$ ligands in proteins are the imidazole side chain of histidine, the sulfhydryl side chain of cysteine and the acidic side chains of glutamate and aspartate. The presence of histidine or cysteine residues is essential for a high affinity Fe$^{3+}$ binding site (33). Among 18 cysteines, 22 histidines and many glutamates and aspartates at the intracellular side of the hCFTR channel, Cys-832 is a well known target for the NEM effect (23). It is of special interest to examine whether Cys-832 acts as a Fe$^{3+}$ ligand to inhibit channel activity. Previous studies demonstrated that strong binding of a transition metal to a cysteine in a protein could protect the cysteine from thiolspecific modification (34–36). Therefore, if Cys-832 is a ligand for Fe$^{3+}$ binding, excess Fe$^{3+}$ is expected to protect Cys-832 from NEM modification. Fig. 3 confirms this hypothesis. NEM doubled hCFTR activity (Fig. 3A), but C832A clearly stopped potentiation (Fig. 3B). This observation was consistent with the previous report (23). More importantly, this NEM effect was suppressed by pretreatment of Fe$^{3+}$ (Fig. 3, C and D). Subsequent application of EDTA clearly accelerated reversal of inhibition by Fe$^{3+}$ because exposure of Cys-832 to NEM could also increase channel activity together with EDTA (Fig. 3C). Thus, Cys-832 may be a Fe$^{3+}$ ligand. Fig. 3, E and F, further supports this proposal because C832A weakened inhibition by Fe$^{3+}$. This weak binding affinity seen with C832A was not due to a high open probability because the C832A mutant channel was further activated by curcumin dramatically (Fig. 3E). Thus, the R domain is involved in Fe$^{3+}$ binding. Because deletion of Cys-832-containing NEG2 increases channel activity without PKA (26), neighboring residues of Cys-832 were also investigated as candidates of Fe$^{3+}$ ligands (Fig. 4A). Fig. 4, B and E, indicate that only D836A dramatically prevented inhibition by Fe$^{3+}$, whereas E822A, E826A, D828A, E831A, and D835A did not. Similarly, activity of the D836A mutant was also potentiated by curcumin dramatically (Fig. 4B). Therefore, Asp-836 may be another Fe$^{3+}$ ligand. In addition, H667R, H775T, and H784Q but not Cys-832 and Asp-836 are found in the R domain of the mouse CFTR channel (Fig. 4A), which was found insensitive to Fe$^{3+}$ (Fig. 4C). It is interesting to determine whether these histidine residues are also involved in Fe$^{3+}$ binding, although mouse CFTR gating is different from hCFTR gating. Fig. 4E demonstrates that a CFTR construct with a His/Cys-free R domain was insensitive to Fe$^{3+}$. Further investigations indicate that only H775A suppressed inhibition by Fe$^{3+}$ and activity of the H775A mutant was also further potentiated by curcumin (data not shown). Accordingly, His-775 is proposed as the third Fe$^{3+}$ ligand in the R domain. Finally, the recent structural model suggested that the R domain is located near cytoplasmic loops (16) and His-950, an outward-facing residue of CL3, is found to be mutated to Arg in the mouse CFTR channel (Fig. 4A), it is fitting to ask whether His-950 is a Fe$^{3+}$ ligand. Fig. 4, D and E, confirm this possibility because both H950A and H950R profoundly reduced inhibition by Fe$^{3+}$, and their channel activity was also greatly potentiated by curcumin. Consistent with involvement of CL3, another neighboring mutant H954A from CL3 was also amelioratory to Fe$^{3+}$ (Fig. 4E), and its activity was increased by curcumin dramatically (data not shown). This result further supports the proposal that CL3 may serve an important role in regulating the Fe$^{3+}$ effect. Taken together, it is proposed that His-950 and His-954 from CL3, and Cys-832, Asp-836, and His-775 from the R domain be responsible for inhibition by Fe$^{3+}$. In support of this proposal, H950A/H954A and D836A/C832A/H774A completely prevented Fe$^{3+}$ inhibition, which was reversed by EDTA (Fig. 4E).

Disulfide Bonding across the R Domain and CL3 Inhibits Channel Activity—To test a hypothesis that a Fe$^{3+}$ bridge between the R domain and CL3 inhibits channel activity, a sulfhydryl-specific cross-linking strategy was employed to determine whether disulfide bond cross-linking of the R domain to CL3 also inhibits channel activity as the putative metal bridge does. Fig. 5A shows that internal diamide (10 μM) suppressed ~30% of channel activity of a H950C/S832C/V510A construct, and suppression was partially reversed by
DTT (2 mM). In sharp contrast, channel activity of constructs with a single cysteine inserted at positions 950 and 832 was not affected by either diamide or DTT (Fig. 5, B and C). These observations suggest that disulfide bond cross-linking between H950C and S832C should inhibit channel activity. Similarly, disulfide bond cross-linking of H950C or H954C to S832C, H775C, or D836C also inhibited channel activity, whereas single cysteine mutants were not affected by diamide (Fig. 5F and supplemental Fig. S1). However, both diamide and DTT failed to exert any effect on a H954C/D828C mutant possibly because of a poor orientation or a long distance between these two engineered cysteines (Fig. 5F and supplemental Fig. S1). Additionally, small inhibition of the current by diamide (Fig. 5F) was not due to a background noise induced by a low current amplitude in the Cys-free constructs because a WT CFTR-based H954C mutant, which contains Cys-832 in the R domain, also exhibited a clear inhibition of the current by diamide (Fig. 5D). In contrast, either diamide or DTT had no effect on the WT hCFTR construct, which has Cys-832 only in the R domain (Fig. 5E). Taken together, these observations support the idea that Fe$^{3+}$ inhibited channel activity primarily by bridging the R domain to CL3.

**Phosphorylated Ser-768 Enhanced Fe$^{3+}$ Binding**—It is well known that Ser-768 and Ser-737 are two inhibitory PKA phosphorylation sites (25). However, the underlying mechanism is unclear. Although Fig. 4E indicates that both S768A and S737A mutants were much inhibited by Fe$^{3+}$, these sites may not be excluded as Fe$^{3+}$ ligands. To further test whether phosphorylated Ser-768 or Ser-737 is involved in Fe$^{3+}$ binding, reversal of Fe$^{3+}$ inhibition by DTT was determined to evaluate a Fe$^{3+}$ binding affinity. Fig. 6A demonstrates that DTT failed to reverse inhibition of the WT CFTR current by Fe$^{3+}$, suggesting that DTT is a weak Fe$^{3+}$ chelator in this case. However, DTT partially reversed Fe$^{3+}$ inhibition of S768A channel activity (Fig. 6B). This result suggests that dephosphorylation of Ser-768 may weaken the Fe$^{3+}$ binding affinity. In other words, phosphorylated Ser-768 may enhance the Fe$^{3+}$ binding affinity. Supporting this argument, DTT did
not reverse Fe$^{3+}$ inhibition found in S768D, which is equivalent to phosphorylated Ser-768 (Fig. 6, C and D). This finding is reasonable because an affinity of a protein for Fe$^{3+}$ has been found to be anion-dependent, and phosphate clearly increases the Fe$^{3+}$ binding affinity of the bacterial (Neisseria) transferrin ferric binding protein by up to 4.2 $\times$ 10$^{18}$ (37). Therefore, phosphorylated Ser-768 is also proposed as a ligand for Fe$^{3+}$ binding.

Unlike S768A, S737A exhibited a weak DTT effect (Fig. 6 D), suggesting that phosphorylated Ser-737 may not participate in Fe$^{3+}$ binding.

**Single Channel Recordings**—To address whether Fe$^{3+}$-induced inhibition changes gating kinetics, a three-channel recording of the hCFTR was done. Fig. 7, A and B, demonstrate that Fe$^{3+}$ clearly decreased an open probability and an opening rate. Moreover, the decrease was reversed by 5 mM EDTA. However, the single-channel conductance was not altered greatly. In contrast, the open probability and the single channel conductance were a little higher for the H775A mutant than for the WT channel (Fig. 7C). Upon application of Fe$^{3+}$, channel gating was not affected (Fig. 7C). However, the open probability was a little decreased by 5 mM EDTA (Fig. 7D), suggesting that removal of Mg$^{2+}$ may be involved (32). Taken together, channel gating of CFTR was regulated by internal Fe$^{3+}$. In agreement with state-dependent inhibition by Fe$^{3+}$, a closed state could be locked by internal Fe$^{3+}$. 

**DISCUSSION**

This study indicates an intracellular high affinity Fe$^{3+}$ bridge between the R domain and CL3, which inhibits activity of the hCFTR channel by primarily reducing the open probability. This finding may support the recent structural model on which the R domain is close to cytoplasmic loops regulating channel activity (16). It is exciting that Fe$^{3+}$ inhibited channel activity in a state-dependent manner. Further investigations suggest that this inhibitory Fe$^{3+}$ bridge may underlie some inhibition mechanisms of the R domain. This study also motivated me to consider a pathophysiological role of Fe$^{3+}$ homeostasis in rescuing cystic fibrosis patients.

**Role of CL3**—Generally, activation of CFTR is triggered by ATP-dependent dimerization of NBD1-NBD2 (12), which is coupled to a change in TMDs from inward to outward facing via CLs (5, 13, 14, 16). Thus, CLs may function as a key regulatory switch to modulate normal CFTR activity. Supporting this notion, our recent studies demonstrated that constitutive mutations at 190 of CL1 and 978 of CL3 drive channel activation without ATP and NBD2 but still need phosphorylation of the R domain for optimal activity (17). What is more, allosteric modulation of CFTR activity by the R domain may involve putative gating rearrangements of CLs (17).
C, also suggest that CL3 may be important for channel regulation by Fe$^{3+}$. Moreover, the recent electron microscopy structural model also indicated that the R domain is close to CLs (16). However, it is unknown how the R domain interacts with CLs. In this study, the Fe$^{3+}$ bridge between the R domain and CL3 is first proposed to regulate channel gating. As shown in Fig. 8A, His-950 and His-954 from CL3 may form an Fe$^{3+}$ binding site together with His-775, Cys-832, and Asp-836 from the R domain no matter whether the R domain is phosphorylated or not. However, phosphoserine Ser-768 may enhance the Fe$^{3+}$ binding affinity (37). As suggested in Fig. 8B, an interaction between CL1 and CL3 may drive channel opening by stabilizing the outward-facing conformation of TMD1 and TMD2. Fe$^{3+}$ binding at the R-CL3 interface may weaken the CL1-CL3 interaction and thus stop the channel from opening. However, removal of Fe$^{3+}$ by EDTA may promote channel opening primarily by enhancing the CL1-CL3 interaction (Fig. 8). Once a K978C- or AMP-PNP-induced decrease in the activation energy overcomes an energy barrier caused by the interfacial Fe$^{3+}$ bridge, channel activity will no longer be modulated by the metal bridge (Fig. 8B). Therefore, state-dependent regulation by Fe$^{3+}$ may be physiologically significant.

Possible Mechanisms of NEM Effect—Cotton and Welsh (23) found that modification of Cys-832 with NEM doubles channel activity. Furthermore, effects of N-substitution on maleimide-mediated stimulation of the hCFTR are different. The larger the N-group, the stronger the effect on channel activity (23). However, the underlying mechanism is obscure. The finding of the Fe$^{3+}$ binding site at the R-CL3 interface may account for the NEM effect. As shown in Fig. 8A, Fe$^{3+}$ may be bound to the R-CL3 interface of the hCFTR in a cell (supplemental Fig. S2). When an inside-out patch is exposed to an EGTA-containing bath solution, fractional Fe$^{3+}$ may be released from CFTR channels, and thus the thiol group of Cys-832 could be accessible to NEM. Once Cys-832 is modified by NEM, the equilibrium in Fig. 8A will be rapidly shifted to a Fe$^{3+}$-free state as EDTA does (supplemental Fig. S2) and thus, an open probability increases. On the other hand, excess Fe$^{3+}$...
keeps the R-CL3 interface in a Fe$^{3+}$/H11001-bound state so that the thiol group of Cys-832 may be protected against NEM modification (Fig. 3C). It is reasonable that different N-substituted maleimides exert various stimulatory effects. Even if Cys-832 is modified by a small N-substituted maleimide, His-775 and Asp-836 from the R domain could still form an inhibitory Fe$^{3+}$/H11001 site with His-950 and His-954 from CL3, which will continue to inhibit channel activity. However, a large N-substituted maleimide could completely destroy the metal bridge between the R domain and CL3.

FIGURE 6. Phosphorylated Ser-768 enhances Fe$^{3+}$ binding. Macroscopic currents across inside-out membrane patches excised from transfected HEK-293T cells expressing the hCFTR (A), S768A (B), and S768D (C) constructs. DTT was added after channel activity was inhibited by Fe$^{3+}$. The arrows indicate the final concentrations. D, fractional reversal of Fe$^{3+}$ inhibition by DTT for four CFTR constructs.

FIGURE 7. Effects of Fe$^{3+}$ on hCFTR channel gating. Unitary currents from the WT hCFTR (A) and the H775 mutant (C). The control current was obtained with ATP (1.5 mM) and PKA (24 units/ml) followed by PKI (1.4 μg/ml). EDTA was added after the patch was treated with Fe$^{3+}$. Membrane potential was −60 mV. c, closed. B and D, open state probability ($P_o$) for the WT hCFTR (B) and the H775A mutant (D) (n = 3–5). *, p < 0.000005 compared with the control.
Inhibition Mechanisms of R Domain—Although R domain phosphorylation is necessary for full CFTR activation, the R domain also inhibits channel activity by phosphorylated Ser-768 (24, 25), NEG2 (amino acids 817–838) (26) and the segment (amino acids 760–783) (27–28). However, the underlying mechanisms are still unknown. The intracellular Fe$^{3+}$/H$^{11001}$ bridge may be responsible for some inhibition mechanisms of the R domain because Fe$^{3+}$ inhibition involves both the R domain and CL3. NEG2 contains two Fe$^{3+}$ ligands Cys-832 and Asp-836, whereas the segment (amino acids 760–783) includes two other Fe$^{3+}$ ligands His-775 and phosphorylated Ser-768 (Fig. 8A). Deletion of either segment from the R domain may weaken the metal binding affinity and thus lower the activation energy for channel opening (Fig. 8B). Similarly, an exogenous NEG2 peptide with high helical tendency may compete off endogenous NEG2 and thus stimulate channel activity by weakening metal binding (Fig. 8A) (26). Of course, other mechanisms are also possible. For example, an electrostatic attraction between Asp-836 and Lys-946 (or Lys-951) could also inhibit channel activity. Alternatively, formation of an H-bond between the R domain and CL3 could also inhibit channel activity because both inhibitory segments of the R domain and CL3 have several hydrophilic residues, which can function as H-bonding donors or acceptors (Fig. 4A).

Pathophysiological Significance in Cystic Fibrosis—Cystic fibrosis is caused by mutations in the gene encoding CFTR. In this case, D836Y is found in cystic fibrosis patients. Thus, this study may have some implications for cystic fibrosis patients with correct protein expression in the cell membrane because Fe$^{3+}$ could regulate CFTR activity in a state-dependent manner. Therefore, Fe$^{3+}$ homeostasis may serve as a critical factor in the treatment of cystic fibrosis. Increased understanding of pathophysiological significance of Fe$^{3+}$ homeostasis may help us design novel pharmacologic approaches.
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