Cystic Fibrosis Transmembrane Conductance Regulator
Cl⁻ Channels with R Domain Deletions and Translocations Show
Phosphorylation-dependent and -independent Activity*

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Phosphorylation of the R domain regulates cystic fibrosis transmembrane conductance regulator Cl⁻ channel activity. Earlier studies suggested that the R domain controls activity via more than one mechanism; a phosphorylated R domain may stimulate activity, and an unphosphorylated R domain may prevent constitutive activity, i.e. opening with ATP alone. However, the mechanisms responsible for these two regulatory properties are not understood. In this study we asked whether the two effects are dependent on its position in the protein and whether smaller regions from the R domain mediate the effects. We found that several portions of the R domain conferred phosphorylation-stimulated activity. This was true whether the R domain sequences were present in their normal location or were translocated to the C terminus. We also found that some parts of the R domain could be deleted without inducing constitutive activity. However, when residues 760–783 were deleted, channels opened without phosphorylation. Translocation of the R domain to the C terminus did not prevent constitutive activity. These results suggest that different parts of the phosphorylated R domain can stimulate activity and that their location within the protein is not critical. In contrast, prevention of constitutive activity required a short specific sequence that could not be moved to the C terminus. These results are consistent with a recent model of an R domain composed primarily of random coil in which more than one phosphorylation site is capable of stimulating channel activity, and net activity reflects interactions between multiple sites in the R domain and the rest of the channel.

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The cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channel contains three cytosolic domains as follows: a regulatory R domain and two nucleotide-binding domains (NBDs). ATP binding and hydrolysis by the NBDs gate the channel, and phosphorylation of the R domain regulates activity (for reviews see Refs. 1 and 2). Although the boundaries of the R domain are not known precisely, several lines of evidence suggest that this domain extends from approximately residues 700 to 830 (3–6). Within this region, we found that the cAMP-dependent protein kinase (PKA) phosphorylates three serines (residues 737, 795, and 813) in intact cells to regulate channel activity (7). Picciotto and colleagues (8) found that these residues plus residue 700 were phosphorylated in vivo. Outside this region, Ser-660 is phosphorylated by PKA and contributes to channel regulation (7, 8). Studies of CFTR variants in which the phosphoserines have been mutated to alanine show that phosphorylation of multiple R domain serines contributes to regulation, although no one serine is required for activity (for reviews see Refs. 1 and 2). Further insight into R domain structure and function has come from studies in which the R domain has been deleted. Such studies have suggested that the R domain may have two functions as follows: the unphosphorylated R domain may prevent constitutive activity (i.e. opening in the presence of ATP alone), and the phosphorylated R domain may stimulate activity.

Speculation that the unphosphorylated R domain prevents constitutive activity has been based primarily on studies of CFTR in which residues 708–835 have been deleted. Expression of this variant generated a channel that was constitutively open; only ATP was required for activity, and PKA-dependent phosphorylation was not required (9, 10). Larger deletions that extended the N terminus of residue 708 failed to generate channels, likely because of a severe disruption of structure (3). There have been only two reports of a smaller deletion, and although the portion deleted was similar in the two studies, the results were different (11, 12).

The R domain can also stimulate channel activity. Addition of a phosphorylated recombinant R domain protein stimulated activity of CFTR in which residues 708–835 were deleted. Phosphoproteins consisting of residues 645–834, 590–858, and 708–831 each stimulated Cl⁻ current (6, 10, 13).

Thus it appears that the R domain may both prevent constitutive activity and, when phosphorylated, stimulate activity. However, it is not clear whether different portions of the R domain mediate these functions. To investigate these functions, we took two approaches; we deleted portions of the R domain, and we translocated portions of the R domain to the C terminus of the channel. We then asked how constitutive and stimulated activity were affected.

EXPERIMENTAL PROCEDURES

Chemicals and Solutions—The catalytic subunit of PKA was obtained from Promega (Madison, WI) and ATP from Sigma. Protein
phosphatase 2Ca was expressed and purified as described previously (14).

For patch clamp studies, the pipette (external) solution contained (in mM) the following: 140 N-methyl-D-glucamine, 2 MgCl$_2$, 5 CaCl$_2$, 10 L-aspartic acid, and 10 HEPES, pH 7.3, with HCl (Cl$^-$ concentration, 50 mM). The bath (cytosolic) solution contained (in mM) the following: 140 N-methyl-D-glucamine, 3 MgCl$_2$, 1 Cs-EGTA, and 10 HEPES, pH 7.3, with HCl (Cl$^-$ concentration, 140 mM).

Mutagenesis and Transfection—CFTR deletion mutants were constructed in the pTM1-CFTR/S660A plasmid (in which Ser-660 is mutated to alanine) as described previously (15). To make ΔR-C(709–835), ΔR-C(709–759), and ΔR-C(760–835), a unique MluI site was inserted in the plasmid encoding CFTR-ΔR/S660A using the method of Kunkel (16). The inserts encoding R domain fragments (709–835, 709–759, and 760–835) were generated by polymerase chain reaction from wild-type CFTR with flanking MluI sites. These fragments were then inserted into the MluI site in CFTR-ΔR/S660A, preserving the C-terminal amino acid sequence DTRL. Constructs were verified by restriction digest, sequencing through insertions, and by in vitro transcription and translation assays. The amino acid sequences of the variants are shown schematically in Fig. 1. All of the variants contained the S660A mutation and an intact Ser-700.

We transiently expressed wild-type and mutant CFTR in HeLa cells using the vaccinia virus/T7 hybrid expression system described previously (15). Cells were studied 4–24 h after transfection depending on the level of expression desired.

Immunoprecipitation and Phosphorylation of CFTR—CFTR was immunoprecipitated from soluble lysates of HeLa cells with an antibody that recognizes the C-terminal amino acids (1477–1480). The antibody-CFTR complex was labeled by phosphorylation with $\gamma^32$PATP and the catalytic subunit of cAMP-dependent protein kinase (PKA). Protein was analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography, as described previously (17).

Electrophysiologic Methods—The methods for excised inside-out patch clamp recordings are similar to those described previously (18). For patch clamp studies, the pipette (external) solution contained (in mM) the following: 140 N-methyl-D-glucamine, 2 MgCl$_2$, 5 CaCl$_2$, 10 L-aspartic acid, and 10 HEPES, pH 7.3, with HCl (Cl$^-$ concentration, 50 mM). The bath (cytosolic) solution contained (in mM) the following: 140 N-methyl-D-glucamine, 3 MgCl$_2$, 1 Cs-EGTA, and 10 HEPES, pH 7.3, with HCl (Cl$^-$ concentration, 140 mM).

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Electrophysiologic Methods—The methods for excised inside-out patch clamp recordings are similar to those described previously (18).

Wild-type and mutant CFTR channels were phosphorylated with 1 mM PKA, and 75 mM ATP was added to the bath solution. Bath was maintained at 35–37 °C by a temperature-controlled microscope stage (Brook Industries, Lake Villa, IL). Pipette resistance was 4–10 MΩ, and seal resistance was 2–25 GΩ. An Axopatch 200-A amplifier (Axon Instruments, Foster City, CA) was used for voltage clamping and current amplification. The pClamp 6.0.3 software package (Axon Instruments) was used for data acquisition and analysis. Data were recorded on digital audiotape (DTR-1203, Biological Science Instruments, Molecular Kinetics, Fullman, WA).

When recording from patches containing multiple channels, data were filtered at 1 kHz using a variable 8-pole Bessel filter (Frequency Devices, Haverhill, MA) and digitized at 2 kHz. Membrane voltage was maintained at 35–37 °C by a temperature-controlled microscope stage (Brook Industries, Lake Villa, IL). Pipette resistance was 4–10 MΩ, and seal resistance was 2–25 GΩ. An Axopatch 200-A amplifier (Axon Instruments, Foster City, CA) was used for voltage clamping and current amplification. The pClamp 6.0.3 software package (Axon Instruments) was used for data acquisition and analysis. Data were recorded on digital audiotape (DTR-1203, Biological Science Instruments, Molecular Kinetics, Fullman, WA).

When recording from patches containing multiple channels, data were filtered at 1 kHz using a variable 8-pole Bessel filter (Frequency Devices, Haverhill, MA) and digitized at 2 kHz. Membrane voltage was held at +40 mV, referenced to the external surface of the membrane patch. Data points during time course experiments are mean current values during 10-s sweeps. Average current for an intervention was determined as the average of the last 2–5 min of that intervention. For single channel analysis, data were filtered at 1 kHz using a variable 8-pole Bessel filter, digitized at 5 kHz, and digitally filtered at 500 Hz. Open state probability ($P_o$) was determined from patches containing 1–3 channels, and the number of channels in a patch was determined by the greatest number of simultaneously open channels observed during the entire experiment.

Statistical Analysis—Values are presented as means ± S.E. An unpaired Student’s t test and analysis of variance were used for assessment of statistical significance. Values were considered statistically significant at $p < 0.05$.

RESULTS

Effect of Deleting Portions of the R Domain—In the presence of ATP, wild-type CFTR generates little current until it is phosphorylated with PKA (Fig. 2A) (1, 2). Earlier studies showed that when residues 708–835 were deleted, channels were constitutively active, producing current without phosphorylation in the presence of ATP alone (9). Subsequent addition of PKA further increased current. As observed previously (15), when Ser-660 was mutated to alanine in that construct, Δ708–835 (see Fig. 1), the channel functioned in a constitutive, ATP-dependent, and PKA-independent manner (Fig. 2C). A full-length CFTR with only the Ser-660 mutated (CFTR-S660A) generated no constitutive current but was stimulated by PKA (Fig. 2B). Thus, Ser-660 is neither required to keep the unphosphorylated channel closed nor required for phosphorylation-dependent stimulation. Therefore, to avoid confounding effects due to phosphorylation of Ser-660, all of the constructs described here contained the S660A mutation (Fig. 1). For brevity, we do not include the S660A designation in the names of the channel variants. Δ708–835 also retains Ser-700. One study reported that Ser-700 is phosphorylated in vivo (8), although another did not (7). Wilkinson et al. (19) showed a small but significant increase in the concentration of 3-isobutyl-1-methylxanthine required for half-maximal activation of channels with an S700A mutation expressed in Xenopus oocytes. Nevertheless, when residues 708–835 were deleted, the presence of Ser-700 seemed to have little functional effect (3, 13), suggesting that it plays a minor role in regulating the variants we studied.

To identify portions of the R domain that may prevent constitutive activity and that may contribute to phosphorylation-dependent stimulation, we deleted portions of the R domain.

\[ \begin{align*}
\text{Wild-type} & \\
\Delta S660A & \\
\Delta 708-835 & \\
\Delta 708-759 & \\
\Delta 760-835 & \\
\Delta 760-783 & \\
\Delta 784-835 & \\
\Delta 708-759/\Delta 768A & \\
\Delta R-C709-835 & \\
\Delta R-C709-759 & \\
\Delta R-C760-835 & \\
\Delta R-C709-835/\Delta 835 & \\
\end{align*} \]

FIG. 1. Schematic diagram of CFTR variants. Dashed line indicates deleted sequence. All variants had Ser-660 mutated to alanine. Sites of serines at positions 660, 700, 737, 795, and 813 are indicated. The variants with portions of the R domain translocated to the C terminus retained the DTRL sequence (residues 1477–1480) at the C terminus.
Deleting the first half of the R domain (Δ708–759) produced channels that were closed until PKA addition (Fig. 2D), whereas deleting the second half (Δ760–835) generated channels with constitutive activity (Fig. 2E). These data suggested that a region between residues 759 and 836 prevented constitutive activity. Therefore, we further subdivided this region. Fig. 2, F and G, shows that prior to PKA addition, Δ760–783 channels were open in the presence of ATP alone, and Δ784–835 channels were closed. Thus, whenever residues 760–783 were deleted (Δ708–835, Δ760–835, and Δ760–783), the channels were constitutively active, and when these residues were present (CFTR-S660A, Δ708–759, and Δ784–835) the channels were closed. These results suggest that this portion of the R domain contains sequences that prevent constitutive activity.

Because a consensus site for PKA-dependent phosphorylation, Ser-768, lies within residues 760–783, we wondered if Ser-768 might be important in preventing constitutive activity. Phosphorylation of Ser-768 has not been detected in cells stimulated with cAMP agonists (7, 8). However, a CFTR-S768A mutant expressed in Xenopus oocytes showed half-maximal stimulation with a lower concentration of 3-isobutyl-1-methylxanthine than wild-type CFTR, raising the possibility that Ser-768 might have an inhibitory function (19). Therefore, we mutated Ser-768 to alanine in the Δ708–759 and Δ784–835 constructs (Fig. 1). Neither of these channels showed significant constitutive activity (Figs. 2H and 3). These data suggest that although residues between 760 and 783 were important in keeping the unphosphorylated channel closed, this function was not dependent on the presence of Ser-768.

Constitutive activity and the response to PKA from multiple patches are shown in Fig. 3. With the exception of Δ708–835, PKA stimulated all the variants. Because each of these other channels retains at least a portion of the R domain and at least one of the in vivo consensus phosphorylation sites, these data suggest that phosphorylation of any one or two of these serines can stimulate activity.

Phosphorylation-dependent Regulation of ΔR-C(709–835)—To understand better how portions of the R domain determine constitutive and phosphorylation-stimulated activity, we moved residues 709–835 from their normal location to the C terminus, producing ΔR-C(709–835) (Fig. 1). Fig. 4A shows that this protein was phosphorylated by PKA. In the presence of ATP alone, ΔR-C(709–835) was constitutively ac-
Data are currents from patches containing multiple channels. Shaded bars indicate current in presence of ATP (1 mM), and black bars indicate current in presence of ATP (1 mM) and PKA (75 mM). Asterisk indicates significant difference compared with current in the presence of ATP alone. Previous studies showed that protein phosphatase 2Ca dephosphorylates and inactivates wild-type CFTR (14, 20). Consistent with phosphorylation-dependent regulation by the C-terminal R domain, addition of protein phosphatase 2Ca reduced current to prestimulation values (Fig. 4, B and C).

We also studied patches containing 1–3 channels. Fig. 5A shows that AR-C(709–835) was constitutively active and phosphorylation further increased activity. The Pn of this unphosphorylated channel was similar to that of unphosphorylated Δ708–835 (Fig. 5B). These results suggest that an R domain translocated to the C terminus does not prevent constitutive activity. However, because PKA increased Pn (Fig. 5, A and B), the translocated, phosphorylated R domain retained its stimulatory function.

Effects of Smaller Portions of the R Domain Translocated to the C Terminus—Several studies have suggested that various portions of the R domain might differentially influence activity. For example, mutation of the various phosphoserines produces qualitatively and quantitatively different functional effects (13, 19, 21). Therefore, we attached smaller portions of the R domain to the C terminus (Fig. 1); this also reduced the number of phosphoserines. Fig. 6A shows that AR-C(760–835) was constitutively active, that PKA increased current, and that dephosphorylation with PP2Ca decreased current. AR-C(709–759) also showed constitutive and phosphorylation-stimulated activity (Fig. 6B). Thus both the first and second halves of the R domain retained PKA-dependent stimulatory activity. However, all three channels with R domain translocations were constitutively active (Fig. 7). These results suggest that, when translocated, the R domain loses the ability to prevent constitutive activity.

Fig. 7 shows that all three C-terminal variants were regulated by PKA-dependent phosphorylation. These results suggest that residues 709–759 and residues 760–835 both contributed to PKA-induced current in AR-C(709–835). As an additional test that it was serines 737, 795, and 813 that were responsible for PKA stimulation of AR-C(709–835), we mutated them to alanine (ΔR-C(709–835)/S3A, Fig. 1). Mutation of these three serines abolished PKA-dependent stimulation (Fig. 7).

Current-Voltage Relationship of ΔR-C(709–835)—Phosphorylation will change the charge of the R domain. If the C-terminal R domain is influenced by the electrical field across the membrane, then voltage might affect the activity of ΔR-C(709–835) channels. Like wild-type CFTR (22) and Δ708–835 (15), the current-voltage relationship of ΔR-C(709–835) was linear, both when unphosphorylated and phosphorylated (Fig. 8). These results suggest that the function of the C-terminal R domain is not affected by the membrane electrical field.

**DISCUSSION**

Previous studies have suggested that in the unphosphorylated state, the R domain may prevent constitutive activity, and when phosphorylated, it may stimulate channel activity. In the Introduction, we reviewed some of the evidence that supports these conclusions. In this study, we investigated these functions by deleting portions of the R domain and by translocating portions of the R domain to the C terminus.

An advantage of this study is that we were able to examine multiple CFTR variants under well defined conditions, i.e., in excised inside-out patches of membrane with addition of ATP and PKA to the cytosolic surface. Moreover, for the variants in which portions of the R domain were translocated to the C terminus, the stoichiometry of channels to R domains was fixed.
Conversely, a limitation of our study is that the number of channels in a patch was not defined, and for only a few constructs did we measure $P_o$. This limits our ability to compare activity between the various constructs. However, our interpretation does not depend on such an analysis.

**Prevention of Constitutive Activity by a Portion of the R Domain**—Earlier studies showed that deleting residues 708–835 generated a constitutively active channel (9, 10, 15). Here we show that the N- and C-terminal portions of the R domain (residues 708–759 or residues 784–835) could be deleted without producing constitutive activity. However, residues 760–783 were critical for preventing constitutive activity; when they were deleted, channels opened without phosphorylation. These results suggest that a relatively small and specific portion of the R domain may keep the unphosphorylated channel closed.

It is interesting that this small region did not contain a serine that is phosphorylated in vivo (7, 8). Although Ser-768 is contained within this region, mutating it to alanine in either $\Delta R$708–759 or $\Delta R$784–835 was not sufficient to either produce constitutive activity or to prevent phosphorylation-stimulated activity.

Two other studies each examined the effect of a single short deletion. Vankeerberghen et al. (11) studied channels in whole-cell patches and found that deletion of residues 780–830 generated channels that were closed under basal conditions but opened when phosphorylated. This result is similar to our findings with $\Delta R$784–835. In contrast, Xie et al. (12) studied channels in planar lipid bilayers and reported that deleting residues 817–838 caused channels to be open without phosphorylation. Despite the presence of serines 660, 737, 795, and 813, they found that phosphorylation did not stimulate activity of this channel. The reason for the differences between these studies is not clear, but they may relate to differences in methodology (whole-cell and excised patches of membrane versus planar bilayers) or to differences in the constructs studied (the construct studied by Xie et al. (12) deleted three residues, 836–838, that were not deleted in the other two studies).

**FIG. 5.** Single-channel analysis of CFTR with the R domain translocated to the C terminus. A, recording from an excised membrane patch containing two $\Delta R$-C(709–835) channels. Dotted line indicates closed state (C) and downward deflection indicates channel opening. The upper panel shows activity in the presence of ATP (1 mM); lower panel shows activity after addition of ATP and PKA (75 nM). Data were obtained at -80 mV. Single channel conductance for the variants was: wild-type 11.5 ± 0.3 pS, $\Delta R$708–835 11.3 ± 0.3 pS, and $\Delta R$-C(709–835) 11.2 ± 0.3 pS (n = at least 3 for each). There was no significant difference between these values. B, open state probability ($P_o$) of CFTR-S660A, $\Delta R$708–835, and $\Delta R$-C(709–835), before and after addition of PKA. Data are mean ± S.E., n = 4. Asterisk indicates significant difference compared with the presence of ATP alone. Cross indicates significant difference in $P_o$ obtained with ATP alone compared with CFTR-S660A (p < 0.05).

**FIG. 6.** Current recordings from membrane patches containing $\Delta R$-C(760–835) channels (A) and $\Delta R$-C(709–759) channels (B). ATP (1 mM), PKA (75 nM), and PP2Ca (0.1 unit/ml) were present during time indicated by bars.

**FIG. 7.** Current from CFTR with portions of the R domain translocated to the C terminus. Data are from membrane patches containing multiple CFTR channels. Data were obtained in the presence of ATP alone (shaded bars) or in the presence of ATP plus PKA (black bars). Data are mean ± S.E., n = 7–10 for each variant. Asterisk indicates significant increase following addition of PKA (p < 0.05). There was no significant difference between these values.

**FIG. 8.** Current-voltage relationship of $\Delta R$-C(709–835). Data were obtained in the presence of ATP alone (■) and after phosphorylation with PKA (○) (n = 3).
Because the data described above suggested that a portion of the R domain prevents constitutive activity, it seemed surprising that when we translocated residues 709–835 or shorter portions of the R domain to the C terminus, constitutive activity persisted. This was the case even though the translocated C-terminal R domain constructs were able to stimulate activity, suggesting that they had access to the rest of the channel. These data are also consistent with earlier findings that adding exogenous unphosphorylated portions of the R domain (residues 645–834, 590–858, or 708–831) did not close the Δ708–835 channel (6, 10, 13).

It has been reported that an unphosphorylated R domain protein (residues 590–858) inhibits activity of wild-type CFTR studied in lipid bilayers (23). However, the significance of this observation is unclear based on the results of two different types of studies. First, an isolated unphosphorylated R domain protein encompassing residues 645–834 did not reduce activity of wild-type CFTR studied in excised membrane patches (13). Second, as indicated above, addition of unphosphorylated R domain protein (either residues 645–834, 590–858, or 708–831) did not reduce activity of CFTR in which residues 708–835 were deleted (6, 10, 13). Perhaps an explanation for the ability of residues 590–858 to inhibit wild-type CFTR (23) is that portions of the protein corresponding to NBD1 may have inhibited activity. Further work is needed to understand the mechanism.

It is possible that R domain deletions might lead to a constitutively active channel by inducing nonspecific structural changes. This might explain why an R domain translocated to the C terminus did not close the unphosphorylated channel. However, nonspecific structural changes seem unlikely given our finding that much of the domain can be deleted without causing the channel to open. The observation that the region responsible for keeping the channel closed may be fairly small (involving residues 760–783) suggests a more specific mechanism. Future studies focused on this small region of the R domain may help elucidate the molecular mechanism.

Phosphorylation-dependent Stimulation by Portions of the R Domain—Our data extend the earlier observations that exogenous addition of phosphorylated R domain proteins, either residues 645–834, 590–858, or 708–831 (6, 10, 13), stimulated channel activity. Here we found that the R domain retained its capacity for phosphorylation-stimulated activity when it was moved from its normal location to the C terminus. This ability to translocate a domain that regulates activity from one place in a channel protein to another has also been observed in the CIC2 channel (24). We also found that when we split the R domain into halves (residues 709–759 and 760–835) and attached them to the C terminus, PKA continued to stimulate the channel. These data indicate that the R domain contains more than one region that can stimulate activity; no one specific phosphoserine or unique sequence is required. It is also interesting that the regions responsible for phosphorylation-mediated stimulation may be different from those that prevent constitutive activity, perhaps suggesting different mechanisms. This suggests that the mechanisms that prevent constitutive activity may differ from those by which phosphorylation stimulates activity.

A Model of R Domain Structure—We recently showed that the R domain is a primarily random coil in solution (6). Our current work is consistent with the conclusion that the R domain does not form a region with a highly ordered tertiary structure. We found that it was not necessary to add the entire R domain to stimulate activity; portions of the R domain (residues 709–759 and 760–835) attached to the C terminus stimulated activity on addition of PKA. Moreover, portions of the R domain (residues 708–759, 708–783, and 760–835) remaining in our deletion mutants retained the capacity to mediate phosphorylation-stimulated activity. If the R domain had a well ordered tertiary structure that was required for activity, it seems likely that such alterations would disrupt its stimulatory activity. We speculate that short regions of sequence around the phosphoserines interact with and stimulate the rest of the channel. In addition, it is possible that sequences within the R domain associate with the rest of CFTR and in so doing adopt local regions of ordered tertiary structure. Recent data also suggest that an interaction between the R domain (residues 708–835) and the N terminus of CFTR has a stimulatory effect (25). It is possible that some or all of the variants we constructed retain such an interaction. However, if this interaction is required for stimulation, the data suggest that the N terminus can interact with multiple regions within the R domain.

A domain that is predominantly random coil is also consistent with our data on constitutive activity. We found that despite deletions of large portions of the R domain, the unphosphorylated channels remained closed. Only when residues 760–783 were missing did unphosphorylated channels open. These observations, pointing to a small region within a larger domain, can be explained by an R domain that exhibits considerable flexibility, although they do not exclude an R domain that folds into a well ordered tertiary structure.

These data and the observation that the R domain is composed predominantly of random coil are both consistent with a model in which net phosphorylation-induced channel activity reflects the integrated effect of interactions with multiple sites in the R domain. In this way, graded phosphorylation could generate graded Cl− channel activity to control precisely transepithelial Cl− transport. Further understanding these interactions and identification of specific R domain binding sites in CFTR may yield additional insights into the complex regulation of CFTR.

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