Cystic Fibrosis-associated Mutations at Arginine 347 Alter the Pore Architecture of CFTR

EVIDENCE FOR DISRUPTION OF A SALT BRIDGE*

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Arginine 347 in the sixth transmembrane domain of cystic fibrosis transmembrane conductance regulator (CFTR) is a site of four cystic fibrosis-associated mutations. To better understand the function of Arg-347 and to learn how mutations at this site disrupt channel activity, we mutated Arg-347 to Asp, Cys, Glu, His, Leu, or Lys and examined single-channel function. Every Arg-347 mutation examined, except R347K, had a destabilizing effect on the pore, causing the channel to fluctuate between two conductance states. Chloride flow through the larger conductance state was similar to that of wild-type CFTR, suggesting that the residue at position 347 does not interact directly with permeating anions. We hypothesized that Arg-347 stabilizes the channel through an electrostatic interaction with an anionic residue in another transmembrane domain. To test this, we mutated anionic residues (Asp-924, Asp-993, and Glu-1104) to Arg in the context of either R347E or R347D mutations. Interestingly, the D924R mutation complemented R347D, yielding a channel that behaved like wild-type CFTR. These data suggest that Arg-347 plays an important structural role in CFTR, at least in part by forming a salt bridge with Asp-924; cystic fibrosis-associated mutations disrupt this interaction.

The cystic fibrosis transmembrane conductance regulator (CFTR) contains an anion-selective pore (1–6). Earlier work has shown that amino acid residues in the two membrane-spanning domains, MSD1 and MSD2, determine the properties of this pore and harbor a number of disease-associated mutations (7–12). Despite the importance of this region to CFTR function, an understanding of the pore and MSD structure is limited.

Studies of the effect of cystic fibrosis (CF)-associated mutations have been of value in identifying structurally and functionally important regions of CFTR. At least four CF-associated mutations have been identified at position 347 in M6: R347C, R347H, R347L, and R347T, suggesting that Arg-347 is important for CFTR structure and function (13–15).2 Early studies by Sheppard et al. (7) showed that mutation of Arg-347 to proline significantly decreased single-channel conductance with little effect on CFTR trafficking to the plasma membrane. Other work by Tabcharani et al. (8, 16) and Linsdell and Harrah (17) emphasized the importance of Arg-347 for anomalous mole-fraction behavior, iodide permeability, and voltage-dependent block by DIDS, in addition to single-channel conductance. Interestingly, mutation of Arg-347 to a histidine (R347H) produced a channel that displayed pH-dependent conductance and anomalous mole-fraction behavior (8). These studies suggested that Arg-347 may line the pore and that a positive charge at position 347 is sufficient for wild-type conductance. Because mutation of Arg-347 eliminated anomalous mole-fraction behavior, Arg-347 itself was proposed to be an anion binding site in the CFTR pore (8), and the presumed positive charge introduced upon protonation of His-347 was thought to facilitate interactions with permeating anions. An alternative interpretation is that Arg-347 may be important for maintenance of pore architecture without contributing directly to the permeation pathway. For example, mutation of this site could lead to a change in MSD conformation and loss of an anion-binding site(s) elsewhere; protonation of His-347 might then rescue the conformation of the R347H mutant.

As discussed by Perutz (18), charged residues within proteins reside in locations where they are either solvated or can interact with and be neutralized by oppositely charged residues. These electrostatic interactions mediate an important stabilizing effect, providing increased thermostability and resistance to denaturation. Based on these considerations, it is possible that Arg-347 may line the pore where it can interact with either water or permeating anions. However, it is also possible that Arg-347 may mediate a structural role in the MSDs; there are a number of negatively charged residues with which Arg-347 might interact. Both possibilities are consistent with the present data.

To better understand the role of Arg-347 in CFTR structure and function, we examined the effect of mutating Arg-347 to cysteine, aspartic acid, glutamic acid, lysine, and leucine on CFTR conductance. We examined the cytosolic pH (pHc)-dependent behavior of CFTR-R347H and that of the other residue 347 mutants both with (R347C, R347D, R347E, and R347K) and without (R347L) a pHc-titratable residue. The conductance of CFTR-R347H is pHc-dependent. Because the site of protona-

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2The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; M, transmembrane domain; MSD, membrane-spanning domain; Bis-Tris, bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane; MOPS, 3-(N-morpholino)propanesulfonic acid; TES, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; Tricine, N-tris(hydroxymethyl)methylylglycine; DIDS, 4,4′-dithiobis(3-nitroaniline); NMDG, N-methyl-D-glucamine; I-F, current-voltage relationship; pHi, cytosolic; bath pH; pHb, extracellular; pipette pH; Oi, little conductance state; Ob, big conductance state; tλ, lifetime of little conductance state; t∞, lifetime of big conductance state.

2C. Ferec, personal communication.
tion may exist in one of two states, either protonated or deprotonated, we tested the hypothesis that CFTR-R347H may display two pH-dependent conductance states, which it did. If the protonatable site lines the pore, then the two ionization states of the protonatable residue might yield two distinct conductance states. Alternatively, if the protonatable site influences structure, then the two conductance states might represent two distinct conformational states of CFTR. Moreover, like CFTR-R347H, all the other residue 347 mutants, except R347K, displayed two pH-dependent conductance states over a similar pH range. The residue at position 347 did not influence current flow through either conductance state. These data suggested that residue 347 probably does not line the pore but likely stabilizes CFTR structure. To pursue this, we studied the effect of mutating residues elsewhere in the MSDs that might interact with Arg-347.

**EXPERIMENTAL PROCEDURES**

**Site-directed Mutagenesis and Transfection**—All mutants were constructed in the pTM-CFTR4 plasmid by the method of Kunkel (19). The mutagenesis was confirmed by restriction digestion of silently introduced restriction sites and by sequencing around the introduced mutation site. In vitro transcription and translation of each mutant was performed to confirm expression of full-length protein. Wild-type and mutant channels were expressed transiently in HeLa cells using the vaccinia virus/T7 bacteriophage hybrid expression system as described previously (20). Cells were routinely studied 12 to 24 h after infection-transfection.

**Patch-Clamp Technique**—Methods used for excised, inside-out patch clamp recordings were as described previously (21–23). Voltages were referenced to the extracellular side of the membrane. All studies were done at room temperature (22–24 °C) to facilitate kinetic analysis. The membrane potential was clamped at −120 mV unless otherwise indicated.

CFTR was activated by excising patches into a bath solution (pH 7.3; Tricine or TES) containing 1 mM ATP and 75 nM catalytic subunit of cAMP-dependent protein kinase (Promega Corp., Madison, WI). During the pH studies, cAMP-dependent protein kinase was removed, and bath (cytosolic side) ATP concentration was adjusted (usually to less than 0.05 mM ATP) to resolve single-channel bursting activity within multichannel macropatches. For experiments with excised, inside-out patches, the pipette (extracellular) solution contained (in mM): 140 N-methyl-D-glucamine (NMDG), 140 aspartic acid, 10 Bis-Tris, 5 CaCl2, 2 MgSO4, pH 6. The bath (intracellular) solution contained (in mM): 140 NMDG, 10 Bis-Tris, 3 MgCl2, 4 CsOH/1 EGTA, pH 6.5 (unless indicated) with HCl ([Ca2+]o < 10−8). We selected Bis-Tris as a buffer to avoid the blocking effect that Good’s type buffers such as MOPS have on CFTR (24). pH was adjusted at room temperature (22–24 °C) using a Metrohm Accumet meter fitted with a gel-filled combination electrode calibrated with pH 6.0 or 7.0 standardized buffer solutions, where appropriate (all pH analytical equipment was from Fisher Scientific, Pittsburgh, PA).

**Data Analysis**—Single-channel current amplitudes were determined from the peaks in all-points histograms. Single-channel conductances were derived from the slope of the single-channel linear I-V relationship. Single-channel data were filtered at 500 Hz using an 8-pole Bessel filter (902LPF, Frequency Devices, Haverhill, MA), digitized at 5000 Hz, and digitally filtered at 500 Hz. The resolution of the A/D converter was 0.0012 pA. Events lists were generated using a half-height transition protocol; transitions less than 1 ms were excluded. For events last generation, a prompt was positioned by eye at each current level representing the closed state, the little conductance state (O_L), and the big conductance state (O_B). The lifetimes of individual sojourns in O_L or O_B were collected, binned (10 bins per decade), and fitted with a one-component exponential using the maximum likelihood method. Wild-type CFTR enters a short-lived intraburst closed state; for simplicity we omitted this state in our analysis of residue 347 mutants since it is rare in wild-type CFTR in the absence of MOPS or other blocking buffers (approximately once every 213 ms within bursts of duration equaling 649 ms; n = 2 at pH 6.0, −120 mV) on the time-scale of transitions between the O_L and O_B state (24). Single-channel current variance analysis was done as described in the legend for Fig. 3 and in Ref. 25. Data acquisition and analysis were done using the pClamp software package (Axon Instruments Inc., Foster City, CA) and Excel 5.0 (Microsoft Corp., Redmond, WA).

**RESULTS**

**pH-dependent Conductance of Residue 347 Mutants**—To determine whether R347H exhibits two discrete conductance states, we studied single channels in excised, inside-out patches. Fig. 1 shows a representative single-channel trace...
from R347H displaying two pH-dependent conductance states, O_L and O_B. Unexpectedly, all the other variants at residue 347, except R347K, showed two pH-dependent conductance states over a similar pH range. Even R347L, which does not have a titratable side chain, displayed this behavior (Fig. 1). The conservative substitution of Arg-347 by Lys (R347K) showed only a single conductance state and no pH dependence. Similar results were observed when NMDG-Cl was replaced with NaCl or when Bis-Tris was replaced with MES (n = 2 each, not shown), suggesting that this behavior is not due to block by a pH-dependent change in one of the pH-titratable bath reagents, NMDG or Bis-Tris. The pH of the pipette solution did influence the results (not shown). Also, there was no state-dependent bias in opening or closing. Channels opened into or closed from the O_L or O_B states as predicted based solely upon pH.

The all-points histograms (Fig. 1) illustrate qualitatively that as pH increased, each mutant except R347K spent an increased fraction of time in O_L and a correspondingly decreased fraction of time in O_B. Points in the histogram from the zero current state (C), which are largely a function of ATP concentration (26–28), are included only for purposes of clarity. Wild-type CFTR and R347K possess only one predominant conductance state and no pH dependence. Since R347L displayed two pH-dependent conductance states, O_L and O_B, we examined the I-V relationship and slope conductance of the mutants with slower pH-dependent kinetics, R347H and R347E, as well as R347K. Filtering obscured the true current amplitudes of the other pH-dependent mutants with more rapid kinetics. Fig. 2 shows that the I-V relationships and slope conductances for the O_L and O_B states were not significantly affected by the nature of the residue at position 347. The single-channel conductance at pH_6.0 of wild-type CFTR, R347K, and the O_B state of R347E and R347H were all very similar (in pS): 7.7 ± 0.4, 8.3 ± 0.6, 7.4 ± 0.4, and 6.9 ± 0.2, respectively (n = 3 for each). The single-channel conductance of the O_L states of R347E and R347H at pH_6.0 were also very similar (in pS): 1.5 ± 0.1 and 1.6 ± 0.1, respectively (n = 3 and 4 for each). These data suggest that the amino acid at residue 347 does not affect single-channel current amplitude, rather the predominant effect is on the lifetime of the O_L and O_B conductance states. The lack of effect of the specific residue at position 347 on the rate of Cl^- flow through the pore in either the O_L or O_B conductance state suggests that residue 347 does not interact directly with permeating anions.

There are at least two possible explanations for the pH-dependent behavior of residue 347 mutants. One explanation is that mutations at residue 347 reveal the effect of a protonatable residue in the permeation path that directly interacts with anions. In this case, the lifetime of the O_B and O_L state represent the lifetime of the protonated and deprotonated ionization states of this unknown site, respectively. A second explanation is that when Arg-347 is mutated, the membrane-spanning domains that form the pore fluctuate between two conformational states, O_L and O_B; protonation or deprotonation of some unknown site favors the O_B or O_L conformational states, respectively. The first explanation seems unlikely because the reciprocal lifetime of the O_L state which represents the "on" rate for the proton is very slow (e.g., it is 6 × 10^7 M^-1 s^-1 for R347E). This rate is ~200-fold slower than proton transfer onto an imidazole in free solution (29, 30) and ~10-fold slower than transfer onto an imidazole in the pore of ROMK1 (25). The slow rate, however, might be explained in part by residence of the protonatable site in a sterically and electrostatically shielded position. In addition, the site of protonation revealed by a residue 347 mutation would have to perfectly replace the lost anion-binding site incurred through the mutagenesis; this seems exceedingly fortuitous. This consideration convinced us to favor a model involving a conformational change in CFTR.

**Dwell-time Analysis of O_L and O_B States**—We performed a dwell-time analysis of the lifetimes of the O_L and O_B states of R347E and R347H to enable more quantitative comparisons between them and to better understand their pHdependence. Both O_L and O_B dwell-time histograms were best fit by a single exponential function at all pH values examined. This suggests that the distribution between O_L and O_B state does not change markedly throughout the gating cycle; this can also be observed visually in Fig. 1. Fig. 3A shows that the reciprocal lifetime of the O_L state increased relativel y linearly with increasing proton concentration. This indicates that the rate of entry into the O_B state increased with increasing proton concentration. This linear, first order dependence on proton concentration suggests that protonation of only a single site limits the rate of movement from the O_L to the O_B state. The rate of exit from the O_B state decreased non-linearly with increasing proton concentrations. The kinetics and the equilibria between O_L and O_B were very similar for both mutants (Fig. 3A). The observable pK (0 mV) for the equilibrium between O_L and O_B of R347E and R347H were 6.4 and 6.3, respectively. The faster kinetics of R347D, R347C, and R347L made dwell-time analysis for these mutants less reliable. Therefore, to obtain an estimate of the approximate pK of these mutants, we examined changes in the variance of the open-state current with changes in pH (25). The current variance should go through a maximum when the pH equals the observable pK. Each mutant displayed an increase in current variance with increasing proton concentration (i.e. decreasing pH). Fig. 3B shows that R347C, R347D, and R347L did not reach a peak variance over the range of pH studied, suggesting that their apparent pK is less than 5.0–5.5.
Visual inspection of the tracings suggested that the mutants were largely in their O_L state over the range of pH employed such that we did not miss a peak in the variance. In this analysis, we assumed that the mutants would display minimal pH-independent movement between O_L and O_B states at very acidic pH, so that the variance should pass through a maximum as pH decreases. As a control for the variance analysis, we examined the R347E mutant on which we had also done dwell-time analysis (Fig. 3A); as expected, Fig. 3B shows that the variance of R347E goes through a maximum between pH 6.5 and 5.5.

**Voltage Dependence of O_L and O_B—**To learn more about the nature of the two conductance states of residue 347 mutants, we examined the voltage dependence of the O_L and O_B lifetimes. Fig. 4A shows qualitatively that both conductance states of R347E were voltage-dependent. Fig. 4B shows quantitatively that at pH 6.0 the O_L and O_B states for R347E and R347H were both influenced by the transmembrane voltage. The voltage dependence may derive from alterations in the distribution of protons near the protonatable site or from charged regions of CFTR moving through the voltage field. We assumed the Boltzmann distribution to quantify the effect of voltage on the equilibrium between O_L and O_B states such that

$$pK(mV) = pK(0 \text{ mV}) + \frac{zFE}{2.303RT}$$

(Eq. 1)

in which $\theta$ = electrical distance from the cytosolic surface, $z$ = valence, $F$ = Faraday’s constant, $E$ = transmembrane potential, $r$ = gas constant, $T$ = temperature, $pK$ = the negative log of the equilibrium constant between O_L and O_B conformations. The degree of voltage dependence was similar for both mutants despite the charge differences at residue 347 and yielded a $\theta$ of 0.25 and 0.21 for R347E and R347H, respectively. The voltage dependence was asymmetrically disposed between the rate of entry into the O_B state and the rate of exit from O_B (Fig. 4B). To quantify these differences we used the following:

$$\tau_{O_L}^{-1}(mV) = \frac{1}{\tau_{O_L}(0 \text{ mV})} \cdot \exp((1 - \delta)vFE/RT)$$

(Eq. 2)

$$\tau_{O_B}^{-1}(mV) = \frac{1}{\tau_{O_B}(0 \text{ mV})} \cdot \exp(-\delta)vFE/RT$$

(Eq. 3)

in which $\delta$ = symmetry factor which partitions voltage dependence (31, 32). The rate of exit from the O_B state ($\tau_{O_B}^{-1}$) was more voltage-dependent than the rate of exit from the O_L state ($\tau_{O_L}^{-1}$) for both mutants ($\delta = 0.8$ versus $1 - \delta = 0.2$ for R347E and $\delta = 0.7$ versus $1 - \delta = 0.3$ for R347H). This observation may be explained by the protonatable site moving through the voltage field; protonation and deprotonation of this site before and after the conformational change will affect the net charge migrating through the applied voltage. Since voltage dependence
arises from charge movement through a voltage field and since R347E and R347H displayed similar voltage dependences and carry different charges at position 347, residue 347 is likely moving through a transmembrane potential during interchange between O_L and O_B states.

The Phenotype of R347D Is Suppressed by the D924R Mutation—The data suggest that Arg-347 and Lys-347 may stabilize the structure of the pore; in their absence, the channel “flickers” between two conductance states. Arginine and lysine residues through electrostatic interactions with anionic residues are important for the structure of membrane-spanning domains in other proteins such as the Lac permease and the inward-rectified K⁺ channel, IRK1 (33–35). As discussed by Perutz (18), salt bridges within proteins are an important structural feature that confers thermostability and resistance to denaturation. We hypothesized that Arg-347 may mediate a stabilizing influence by contributing to a salt bridge within the MSDs. There are multiple glutamates and aspartates within transmembrane (M) regions with which Arg-347 or Lys-347 might interact: Glu-92 (M1), Glu-873 (M7), Asp-924 (M8), Asp-993 (M9), and Glu-1104 (M11). To identify the Arg-347 interaction partner, we replaced Arg-347 with an anionic residue (R347E or R347D) and introduced an arginine residue in the place of candidate partners in a salt bridge. We studied the conductance properties of the following double mutants: R347D/D924R, R347D/D993R, and R347E/E1104R. The R347D/D993R and R347E/E1104R mutants each had two conductance states with pH₃-dependent behavior (Fig. 5). For R347D/D993R the increased entry into the O_B state was apparent as a shoulder on the amplitude histogram at pH₃ 5.5. Accordingly, for R347D/D993R and R347E/E1104R the current variance in the open state increased with decreasing pH₃ (Fig. 5B). Qualitatively, the lifetimes of the O_L and O_B conductance states in the R347D/D993R and R347E/E1104R were similar to that of the R347D and R347E mutants, respectively. The amplitude of the O_L state was larger for both of these double mutants as compared with the single mutants (Figs. 1B and 5B). We also observed an infrequent, additional small conductance state in the R347E/E1104R mutant (see amplitude histogram in Fig. 5A); this is likely due to the E1104R mutation itself.

In contrast to the other double mutants, the R347D/D924R mutant did not display the pH₃-dependent flicker found in the R347D single mutant (Fig. 5, A and B), and there was no effect of pH on open-channel variance (Fig. 5B). The single-channel conductance was similar to that of wild-type CFTR (6.1 ± 0.1 pS; n = 3; Fig. 2). These data suggest that the D924R mutation compensates for or rescues the phenotype of the R347D mutation. This result predicts that the D924R mutation alone (with Arg at position 347) would generate an unstable channel with at least two open conductance states. Fig. 6 shows that the D924R single mutant displayed multiple (≈3) conductance states that appeared to be pH₃-independent.

**DISCUSSION**

Function of Arg-347 in CFTR—Previous work from our and Hanrahan’s laboratories (7, 8, 17) has led to the speculation that Arg-347 may be an anion-binding site in the CFTR pore. However, in contrast to earlier interpretations, our current data show that residue 347 does not influence permeation properties via a direct interaction with permeating anions. Instead they suggest that Arg-347 may be more important for maintenance of pore architecture.

We found that mutation of residue 347 to glutamate, aspartate, cysteine, histidine, or leucine all produced channels with two distinct conductance states, O_L and O_ip, pH₃, and voltage influenced the movement between these two states over a similar range for all mutants. Additionally, the single-channel slope conductances of R347H and R347E were the same in both O_B and O_L states. The O_B state for each had the same conductance as wild-type CFTR. The average calculated pKₐ for a glutamate and a histidine within a protein are 4.0 and 6.9 (36), respectively. If His-347 or Glu-347 line the permeation pathway, at pH₃ 6.0 (bath solution) or pH₃ 6.5 (pipette solution) His-347 is predicted to be protonated and to have at least a partial positive charge, and Glu-347 should be fully deprotonated and have a negative charge. Since the charge or the structure of the residue at position 347 failed to affect single-channel conductance, it seems unlikely to be either an anion-binding site or to be positioned such that it interacts sterically or electrostatically with permeating anions. Thus, mutation of Arg-347 may decrease single-channel conductance and anomalous mole-fraction behavior by disrupting pore architecture and the function of some other anion-binding sites(s).

The O_B and O_L Conductance States—All the mutants except R347K showed two pH-dependent conductance states. The equilibria between O_B and O_L states were similar despite the
nature of the mutation at residue 347 with values of pK ranging from −5–7.3 Because the values of pK were roughly similar, the data suggest that the structure, titratability, and pK of residue 347 do not markedly influence the distribution between O\textsubscript{b} and O\textsubscript{h} states. The linear dependence on proton concentration for O\textsubscript{h} entry suggests that a single protonatable site largely determines entry into this state.

The O\textsubscript{b} and O\textsubscript{h} states may represent two protonation states of the CFTR molecule or two distinct conformational states that are influenced by protonation. For the reasons discussed above, we favor the latter possibility, that alternating residence in two discrete conformations is responsible for the two conductance states. How might this occur? We hypothesize that Arg-347 forms a salt bridge with another negatively charged residue in CFTR. Mutation of Arg-347 would leave a negatively charged residue unpaired within the membrane. Charged molecules within a low dielectric constant are unstable (37). Therefore, the protein might reorient to solvate the charge, i.e. enter the O\textsubscript{h} state. Subsequent protonation of that site would neutralize its charge and allow the protein to reorient back to its native conformation, i.e. return to the O\textsubscript{b} state.

This model suggests that the residue(s) with which Arg-347 interacts may be the protonatable site. Several observations are consistent with this hypothesis. First, we found that a positively charged lysine, which can support a salt bridge, was able to supplant arginine. However, the other residues tested were not able to substitute for arginine. Perhaps histidine was not able to replace arginine because its side chain was not long enough or it may have an anomalously acidic pK\textsubscript{a} within the low dielectric constant of the membrane.

Second, and more importantly, we found that a second-site complementary mutation at position 924 (D924R) largely eliminated the pH\textsubscript{c}-dependent flickering phenotype of the R347D mutation and restored current amplitude to near wild-type values. We also recognize the possibility that Arg-347 may interact with additional yet untested residues, e.g. Glu-873 in M7.

Third, a salt bridge between Arg-347 and Asp-924 should be disrupted by mutation of Asp-924. As predicted, we found that the D924R mutant displayed erratic flickering, pH\textsubscript{c}-independent behavior. Presumably, this mutation also generates an unstable channel. The pH\textsubscript{c} independence of D924R is also consistent with the hypothesis that Asp-924 is the site of protonation in the residue 347 mutants. Interestingly, mutation of a glutamate in the putative salt bridge in the P-loop of the IRK1 channel leads to a single-channel flickering phenotype reminiscent of residue 347 mutations (35).

**Tertiary Structure of the Membrane-spanning Domains—At the simplest level, the data suggest that both MSDs functionally interact in a manner that influences permeation. The studies of R347D/D924R are consistent with a salt bridge between Arg-347 and Asp-924 and thus an interaction between M6 and M8. This further suggests that mutations in MSD2 may alter the phenotype of mutations in MSD1. Consistent with this, mutation of D993R and E1104R in MSD2 increased the relative amplitude of the O\textsubscript{b} conductance state in the context of Arg-347 mutations.

The Arg-347 residue is targeted by several CF-associated mutations, R347C, R347H, R347L, and R347P (13–15). Our data suggest that CF-associated as well as other mutations at residue 347 affect CFTR similarly. They disrupt pore architecture by disrupting an interaction between Arg-347 and another residue(s), one possibly being Asp-924 in M8. These data highlight the importance of residue 347 for CFTR function and may explain in part why this residue is targeted by multiple CF-associated mutations.

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3 The shift toward a lower pK for mutants with more rapid kinetics (Fig. 1 and 3F) may be a filtering artifact; from analysis of the R347E mutant and the R347H mutant, we know that the O\textsubscript{b} state tends to be shorter than the O\textsubscript{h} state at the applied transmembrane voltage and is therefore more vulnerable to filtering. We presume that this relationship is preserved throughout all the mutants.
Mutations at Residue 347 of CFTR

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