Ablation of Internalization Signals in the Carboxyl-terminal Tail of the Cystic Fibrosis Transmembrane Conductance Regulator Enhances Cell Surface Expression*

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The cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel that undergoes endocytosis through clathrin-coated pits. Previously, we demonstrated that Y1424A is important for CFTR endocytosis (Prince, L. S., Peter, K., Hatton, S. R., Zaliauxkiene, L., Cotlin, L. F., Clancy, J. P., Marchase, R. B., and Collawn, J. F. (1999) J. Biol. Chem. 274, 3602–3609). Here we show that a second substitution in the carboxyl-terminal tail of CFTR, I1427A, on Y1424A background more than doubles CFTR surface expression as monitored by surface biotinylation. Internalization assays indicate that enhanced surface expression of Y1424A,I1427A CFTR is caused by a 76% inhibition of endocytosis. Patch clamp recording of chloride channel activity revealed that there was a corresponding increase in chloride channel activity of Y1424A,I1427A CFTR, consistent with the elevated surface expression, and no change in CFTR channel properties. Y1412A showed an intermediate phenotype compared with the double mutation, both in terms of surface expression and chloride channel activity. Metabolic pulse-chase experiments demonstrated that the two mutations did not affect maturation efficiency or protein half-life. Taken together, our data show that there is an internalization signal in the COOH terminus of CFTR that consists of Tyr1424-X-X-Ile1427 where both the tyrosine and the isoleucine are essential residues. This signal regulates CFTR surface expression but not CFTR biogenesis, degradation, or chloride channel function.

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated chloride channel that resides at the apical surface of epithelial cells. Previous studies have demonstrated that CFTR is internalized from the cell surface (1–3) through clathrin-coated pits (2, 4). Furthermore, CFTR has been shown to interact with PDZ-domain-containing proteins at its COOH terminus (5, 6) and syntaxin 1A at its NH2 terminus (7, 8). How these interactions affect cell surface expression is not clear, but they imply that CFTR may exist in at least two cell surface pools, one tethered to the actin cytoskeleton and one associated with the endocytic pathway. Subcellular localization studies reveal that CFTR is found in the endosomes in epithelial cells (9), supporting the view that CFTR enters the endocytic pathway. Whether CFTR is constitutively recycled is not known.

In previous studies, our laboratory demonstrated that a key feature of CFTR endocytosis was the presence of a tyrosine residue at position 1424 in the COOH-terminal tail of CFTR. Because tyrosine-based signals have been proposed to consist of the motif YXXΦ where Φ is a large hydrophobic residue and X is any residue (10), we tested the hypotheses that the isoleucine residue at position 1427 is important for CFTR endocytosis and that ablation of this putative signal YXXI would increase the steady-state surface expression of CFTR. To this end, we performed an integrated series of biochemical and electrophysiological assays designed to study maturation efficiency, trafficking, and Cl− channel function of the wild-type and two COOH-terminal mutant CFTR proteins. We find that the substitution of Tyr1424 and Ile1427 with alanine residues results in a 2-fold increase in surface expression, whereas the single Y1424A mutation shows an intermediate phenotype. CFTR internalization assays revealed that the elevated surface expression was attributed to a dramatic decrease in endocytosis, suggesting that these residues are necessary for CFTR internalization. Because the chloride channel activity and relative surface expression of Y1424A and I1427A CFTR are elevated to a similar extent, we propose that these substitutions affect protein trafficking but not CFTR chloride channel function. To our knowledge, this is the first CFTR mutant that has enhanced rather than diminished activity at the cell surface because of attenuation of internalization.

**MATERIALS AND METHODS**

Construction of CFTR Mutants—CFTR (wild-type) was provided by the Gregory James Cystic Fibrosis Center Vector Core and Dr. Jeong Hong. The construction of the Y1424A mutant was described previously (3). For construction of the Y1424A,I1427A mutant, a BstXI-SgrAI fragment that coded for the COOH-terminal tail region of Y1424A CFTR was subcloned into pSK-Bluescript (Stratagene). A second-site mutation was prepared from the corresponding pSK-Bluescript vector containing the BstXI-SgrAI fragment from single-stranded DNA as described previously (11) by the method of Kunkel (12). Mutants were selected by sequencing and then subcloned into the BstXI-SgrAI site of pG7-1-CFTR. The mutations were verified by dideoxynucleotide se-
sequencing (13) using the Sequenase kit (U. S. Biochemical Corp.) according to the manufacturer’s directions.

**Cell Culture and Transient Transfection of COS-7 Cells—** COS-7 cells were cultured as described previously (3) and transiently transfected using LipofectAMINE Plus reagent (Invitrogen) according to the manufacturer’s directions. The cells were incubated at 37 °C in a humidified incubator for 24–48 h before analysis.

**Immunoprecipitation of CFTR Proteins—** One 100-mm dish of transfected COS-7 cells was used 48 h post-transfection. CFTR was immunoprecipitated using a polyclonal antibody to nucleotide binding domain 1 and antibody A-agarose. One of the immunoprecipitated samples was then eluted from the beads using Laemmli sample buffer (without bromophenol blue), diluted in RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 8.0) 10-fold, and the biotinylated fraction was captured with avidin-Sepharose beads (Pierce). Both total CFTR, biotinylated CFTR, and phosphorylated CFTR were then in vitro phosphorylated using [γ-32P]ATP (PerkinElmer Life Sciences) and camp-dependent protein kinase (Promega) as described previously (3).

**Biotinylation of Surface CFTR—** Cell-surface biotinylation of glycoproteins and detection of CFTR were performed as described previously (1) with the following modifications. After biotinylation and lysis, samples were divided into two equal samples and immunoprecipitated with anti-CFTR nucleotide binding domain 1 antibody and protein A-agarose. One of the immunoprecipitated samples was then eluted from the beads using Laemmli sample buffer (without bromophenol blue), diluted in RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 8.0) 10-fold, and the biotinylated fraction was captured with avidin-Sepharose beads (Pierce). Both total CFTR, biotinylated CFTR, and phosphorylated CFTR were then in vitro phosphorylated using [γ-32P]ATP (PerkinElmer Life Sciences) and camp-dependent protein kinase (Promega) as described previously (3).

**Metabolic Pulse-Chase Assays—** One day post-transfection, COS-7 cells (one 35-mm dish/time point) were rinsed three times and incubated in methionine-free Dulbecco’s modified Eagle’s medium for 1 h and then pulsed in the same media containing 200 μCi/m A trans-[35S]methionine (ICN Biomedicals). Pulse-labeled cells were chased for 0, 4, 14, 18, or 24 h in complete media. At each time point, the cells were placed on ice and rinsed with cold phosphate-buffered saline, lysed in RIPA buffer, and incubated for an additional 30 min on ice. CFTR was immunoprecipitated from the post-nuclear supernatants and analyzed by SDS-PAGE and autoradiography (PhosphorImager, Amersham/Pharmacia). Calculation of the protein half-lives was performed as described by Straley et al. (1998) (14).

**Whole Cell Patch Clamp Assays—** Individual dishes of transfected COS-7 cells were used in electrophysiological recordings as described previously (15). One modification is that Pclamp 8.0 software was used in this study. COS-7 cells were transiently transfected with each of the CFTR constructs along with pGL1 (pGreen Lantern-1, a green fluorescence protein (GFP) plasmid). Under these conditions, >90% GFP and CFTR co-transfectants respond to cyclic AMP mixture (250 μM 8-Br-cAMP and chlorphenyl thio-cAMP plus 2 μM forskolin) treatment with an increase in whole cell Cl⁻ conductance. Background levels of cyclic AMP-induced Cl⁻ conductance were monitored in non-transfected cells in the same dish that lack GFP fluorescence, inmock-transfected cells, and in parental cells. In these whole-cell recordings, the bath (extracellular) solution contained 145 mM Tris-Cl, 1 mM CaCl₂, 1 mM MgCl₂, 5 mM glucose, 60 mM sucrose, and 5 mM HEPES, pH 7.45. The pipette (intracellular) solution contained 145 mM Tris-Cl, 5 mM HEPES, 100 mM CaCl₂ and MgCl₂ (chelated with 2 mM EGTA), and 5 mM Mg²⁺-ATP, pH 7.45. These solutions were designed to study the only current flowing through Cl⁻ channels because Cl⁻ is the only permeant ion in solution, to clamp intracellular Ca²⁺ at ~100 nM, and to prevent swelling-activated Cl⁻ currents with added sucrose in the bath solution.

**Single Channel Patch Clamp—** Assays of single channel recordings were obtained from membrane patches in both the cell-attached and inside-out configurations. Recording pipettes were constructed from borosilicate glass capillaries (Warner Instrument Corporation, Hamden, CT) using a Narishige PC-10 microelectrode puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan) and were fire-polished with a Narishige microforge. The pipettes were partially filled with an 80% potassium solution and had tip resistances of 5–10 megohms. Experiments were performed at room temperature (20–22 °C). Currents were recorded at 50–60 mV (negative to pipette potential) using an Axopatch 200B patch clamp amplifier (Axon Instruments, Union City, CA) low pass-filtered at 1000 Hz (LPF-5, Warner Instruments), sampled every 100 μs with a Digidata 1321A interface (Axon Instruments), and stored onto the computer hard disk using Pclamp 8 software (Axon Instruments). A brief protocol of stepping the holding potential from −100 to +100 mV and back to −100 mV served to inactivate a contaminating voltage-dependent Cl⁻ channel (probably CIC-2) that was hyperpolarization-activated but inactivated perma-

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**FIG. 1.** Surface expression levels of wild-type and mutant CFTR in COS-7 cells. The levels of expression of wild-type (Wt), Y1424A, and Y1424A, I1427A CFTR were analyzed in COS-7 cells 48 h after transfection. Cells were lysed in RIPA buffer, and CFTR was immunoprecipitated using an anti-nucleotide binding domain 1 polyclonal antibody. total CFTR from 50% of the lysate. Biotinylated CFTR was eluted from the antibody-protein A beads and reprecipitated using avidin-Sepharose beads. Biotinylated, CFTR from 50% of the lysate. Mock transfected cells were used as a negative control (lanes 1 and 5). Immunoprecipitated (Total) and reprecipitated (Biotinylated) CFTR were in vitro phosphorylated with protein kinase A and [γ-32P]ATP and analyzed by SDS-PAGE and autoradiography. A representative gel of 10 is shown (top panel). The relative amounts of wild-type (lanes 2 and 6), Y1424A (lanes 3 and 7), and Y1424A, I1427A CFTR (lanes 4 and 8) are shown. The averages ± S.E. were calculated from the phosphorimaging analysis from 10 independent experiments, *, p < 0.02; +, p < 0.001 (compared with wild-type CFTR (lower panel)).

**RESULTS**

**Mutations in the Carboxyl-terminal Tail of CFTR Increase Surface Expression—** Our hypothesis in these experiments is that if both tyrosine 1424 and isoleucine 1427 are important for CFTR internalization, complete disruption of these residues should increase CFTR surface expression. Because little is known concerning the nature of CFTR endocytosis and recycling or how these processes affect CFTR function, we constructed a double substitution COOH-terminal mutant in which both tyrosine 1424 and the isoleucine 1427 were changed (Fig. 1). First, we determined the effects of these substitutions on CFTR surface expression by comparing the percentage of wild-type and mutant CFTR at the cell surface using a surface biotinylation assay. COS-7 cells expressing wild-type, Y1424A, and Y1424A, I1427A CFTR were surface-biotinylated and lysed in RIPA buffer (see “Materials and Methods”). Total CFTR was measured following immunoprecipitation from 50% of the lysate detected by in vivo phosphorylation (Iγ-PATP and protein kinase A) and analyzed by SDS-PAGE and autoradiography (Fig. 1, top panel, total CFTR). CFTR was also immunoprecipitated from the other half of the lysate. This fraction was then eluted from the protein-A beads, reprecipi-
tated using monomeric avidin-Sepharose (to separate biotinylated CFTR), and detected as described above for the total CFTR (Fig. 1, top panel). The percentage CFTR at the cell surface was markedly increased for Y1424A,I1427A CFTR compared with both wild-type (108% increase, n = 10, p < 0.001) and Y1424A CFTR (59% increase, n = 10, p < 0.001) (Fig. 1, bottom panel). The surface biotinylation data indicated that modification of residues Tyr 1424 and Ile1427 increased the steady-state surface expression of CFTR. The potential mechanisms that could account for these differences include changes in 1) maturation efficiency, 2) protein half-life, or 3) internalization and/or recycling rates.

**Mutations at Tyr1424 and Ile1427 Do Not Alter CFTR Maturation Efficiency or Protein Half-life**—To test the effects of these mutations on maturation efficiency and protein half-life, we performed metabolic pulse-chase experiments on COS-7 cells expressing wild-type, Y1424A, and Y1424A,I1427A CFTR. CFTR half-lives were measured 24 h post-transfection. The results in Fig. 2 show that the half-lives for wild-type (Wt), Y1424A, and Y1424A,I1427A CFTR were 10.3 ± 2.3, 11.3 ± 2.6, and 11.3 ± 1.5 h (mean ± S.D.). This finding indicated that the elevated surface expression of the mutants was not attributed to enhanced protein half-life.

In the same series of experiments, we also compared the amount of immaturely glycosylated CFTR (Band B) at 0 time with the amount of maturely glycosylated CFTR (Band C) at 4 h (top panel). The average maturation efficiency for wild-type (Wt), Y1424A, and Y1424A,I1427A CFTR were 32, 31, and 31%, respectively (bottom right panel). This finding demonstrated that elevated surface expression of Y1424A,I1427A CFTR was not because of alterations in maturation efficiency.

**Tyrosine 1424 and Isoleucine 1427 Are Necessary for CFTR Endocytosis**—To test whether elevated surface expression was

![Figure 2](image_url)

**FIG. 2.** Point mutations in the CFTR COOH terminus do not affect protein stability or maturation efficiency. The protein turnover and processing of CFTR and CFTR mutants were monitored in COS-7 cells 24 h after transfection in metabolic pulse-chase experiments. After a 1-h pulse and the indicated chase time periods, the cells were lysed in RIPA buffer and CFTR or CFTR mutants were immunoprecipitated and analyzed as described under "Materials and Methods." Mock (M) transfected cells were used as a negative control. The top panel shows a representative gel. Bands B and C of CFTR indicated on the left. The average half-lives and maturation efficiencies from four independent experiments shown below demonstrate that the half-lives (left panel) and maturation efficiencies (right panel) are not affected by these two CFTR substitutions.

![Figure 3](image_url)

**FIG. 3.** Comparisons of the internalization rates of CFTR and CFTR mutants. COS-7 cells transfected with wild-type, Y1424A, or Y1424A,I1427A CFTR were analyzed 48 h post-transfection. Wild-type or mutant CFTR was biotinylated using a two-step surface periodate/LC-hydrazide biotinylation procedure. At zero time, both steps were conducted at 4 °C to label the entire pool of CFTR. Internalization was monitored by a loss of biotinylated of the cell surface pool by including a 37 °C incubation period (Time (min)) between periodate and biotin LC-hydrazide treatments. Biotinylated CFTR and total CFTR were detected as shown in Fig. 1. The percentage of wild-type, Y1424A, and Y1424A,I1427A CFTR internalized after 2.5 min was 34, 18, and 8 respectively. 1 of 8 representative experiments is shown. In the bottom panel, the percentage of CFTR internalized at each time point was calculated based on phosphorimaging analysis (averages from eight experiments: *, p < 0.05 compared with Wt; +, p < 0.001 compared with Wt).
attributed to alterations in the internalization rate of CFTR, we performed internalization assays on COS-7 cells expressing wild-type, Y1424A, and Y1424A,I1427A CFTR. Using a warm-up period between periodate and the biotin LC-hydrazide treatments (0 or 2.5 min), we monitored the loss of the surface pool of CFTR (see “Materials and Methods”). During this warm-up period, previously oxidized carbohydrate residues are internalized and therefore do not react with the membrane-impermeant biotin LC-hydrazide (1). A representative internalization assay for each of the constructs is shown in Fig. 3, top panel. A summary of eight assays is shown in the lower panel. For wild-type CFTR, 34% of the surface pool was internalized in 2.5 min. For Y1424A and Y1424A,I1427A CFTR, internalization dropped to 21 and 8%, respectively, during the same time period. These results demonstrate that CFTR endocytosis is inhibited by 76% when these two residues are modified.

The Y1424A and Y1424A,I1427A CFTR Have Normal Chloride Channel Properties—Because the biochemical data suggested that a specific motif in the CFTR COOH terminus dramatically affected endocytosis and because point mutations in the NH2 terminus lead to both disruption of binding to docking machinery and changes in CFTR ion channel function, we tested whether the mutation of Tyr1424 and Ile1427 affected chloride channel function. Whole cell patch clamp recordings were performed to assess the total population of CFTR Cl− channels in the plasma membrane of transfected COS-7 cells. Tris-Cl-containing solutions were used in bath (extracellular) and pipette (intracellular) solutions so that Cl− was the only major permeant ionic species in the recordings. GFP was also expressed together with the CFTR-bearing vectors to detect cells that were successfully transfected prior to recording. Cells that did not express GFP served as internal controls. Three sets of transiently transfected COS-7 cells were examined in parallel with the above biotinylation experiments (Table I). In agreement with the surface biotinylation assays, CFTR whole cell Cl− currents in Y1424A CFTR and Y1424A,I1427A CFTR-transfected cells were elevated compared with wild-type CFTR-expressing cells (Table I), suggesting that the elevated Cl− channel activity was the result of the elevated surface expression of CFTR. Typical whole cell current traces after stimulation with cAMP agonist mixture for wild-type and mutant CFTR are shown in Fig. 4A. Fig. 4B shows wild-type CFTR Cl− current-voltage relationships demonstrating insensitivity of the currents to DIDS (100 μM) and inhibition of the currents by glibenclamide (100 μM). These pharmacological properties are consistent with wild-type CFTR (16). The time and voltage independence of the currents and the linear I−V relationship are also consistent with CFTR chloride channel activity. Fig. 4, C and D, show the Y1424A and Y1424A,I1427A Cl− current-voltage relationships, respectively, and indicate that although the sensitivities to DIDS and glibenclamide remain similar to wild-type (Fig. 4B), the total current is elevated in the single and double mutants. Whereas the representative I−V plots show a variability in sensitivity to glibenclamide, inhibition with this Cl− channel-blocking drug was only partial ranging from 50 to 90% for both wild-type and mutant currents.

Single channel biophysical properties of wild-type, Y1424A, and Y1424A,I1427A CFTR were also assessed. Before the recording of CFTR Cl− channel properties under cAMP-stimulated conditions was undertaken, voltage steps between −100 and +100 mV were necessary to inactivate a pseudo-channel with similar Cl− conductance as CFTR. The properties of this channel were not inconsistent with ClC-2, known to be expressed in COS-7 cells (17). Representative recordings of wild-type, Y1424A, and Y1424A,I1427A CFTR at 50–60 mV (negative to pipette potential) are shown in Fig. 4E. Single channel conductance for all three constructs was 7–8 picoSiemens for stretches of the recordings where a subset of the channels could be analyzed. Biophysical analysis of single channel kinetics was not possible, because each patch obtained from a positively transfected cell had at least 10 channels. We could never obtain patches with a single channel. Furthermore, a base line without output openings was not observed. Nevertheless, the whole cell and single channel recordings together show that the difference in Cl− channel activity is attributed to elevated surface expression without a significant change in CFTR chloride channel properties among wild-type, Y1424A, and Y1424A,I1427A CFTR.

**DISCUSSION**

The data presented here demonstrate that two key residues in the COOH-terminal tail dramatically regulate the steady-state distribution of CFTR between the cell surface and intracellular sites. This is the first demonstration of a CFTR mutant whose activity is actually enhanced relative to wild-type CFTR. We established this observation using both surface biotinylation and patch clamp measurements.

In examining the mechanism for the elevated surface expression of CFTR, we first showed that total expression levels of wild-type, Y1424A, and Y1424A,I1427A were the same. We next demonstrated that maturation efficiency and protein half-life were unaffected, suggesting that a primary alteration caused by these substitutions involved changes in distribution

| Transient transfection | Non-green | cAMP-activated chloride currenta** | Green |
|------------------------|-----------|----------------------------------|-------|
|                        | Control   | Wild type                        | Y1424A | Y1424A/I1427A |
|                        |           | pA at +100 mV                    |       |
| Set 1                  | 255 ± 36 (13) | 1070 ± 95 (5) | 1650 ± 200 (5) | 2125 ± 195 (5) |
| (Fold-difference)      | 1.0       | 1.54                             | 1.99   |
| Set 2                  | 201 ± 68 (3) | 738 ± 52 (5) | 986 ± 24 (5) | 1451 ± 35 (5) |
| (Fold-difference)      | 1.0       | 1.34                             | 1.97   |
| Set 3                  | 393 ± 140 (4) | 1193 ± 55 (7) | 1767 ± 164 (7) | 3424 ± 205 (6) |
| (Fold-difference)      | 1.0       | 1.48                             | 2.87   |
| Fold-difference average| 1.45 ± 0.06** | 2.28 ± 0.30**                   |       |

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* Three sets of transiently transfected COS-7 cells were analyzed in parallel with the protein biochemistry. Data in terms of cAMP-activated currents at +100 mV are shown for each CFTR construct as well as GFP-negative controls as are the fold-differences in the currents, treating wild type CFTR as 1.0. The fold-difference average is the mean ± S.E. of the three sets of data pooled. The asterisk reflects p < 0.05 by analysis of variance, whereas the † reflects p < 0.01.

** Mean ± S.E.

† Number of experimental points.
between the intracellular and cell surface compartments. This alteration could result from decreased internalization or increased recycling or both. Moreover, we showed that Y1424A,I1427A CFTR was internalized much more slowly than the native protein (76% inhibition at 2.5 min) with an internalization rate of \( \text{rate} \) min.

Several observations suggest that the only internalization signal in CFTR is the Tyr 1424-\( \text{X} \)-\( \text{X} \)-Ile1427 motif in the COOH-terminal tail. First, the ablation of the only endocytosis signal in the transferrin receptor YTRF resulted in a similar loss of internalization activity (11). Furthermore, the rate of endocytosis of the \( \text{YTTRF}^{20} \rightarrow \text{ARTA}^{23} \) mutant was the same as a transferrin receptor containing only a 4-amino acid cytoplasmic tail, indicating that this motif and more specifically these two residues were the only residues in the 61-amino acid cytoplasmic tail of the transferrin receptor that were necessary for endocytosis. Second, the internalization rate of Y1424A,I1427A CFTR is comparable with the rate of bulk flow lipid uptake via the endocytic pathway (\( \text{rate} \% \text{ min.} \)) (18), suggesting that the residual internalization activity observed in these studies reflects nonspecific uptake through clathrin-coated pits. Considering that clathrin-coated pits constitute \( \text{rate} \% \) of the cell surface (18), our findings suggest that the double mutant has completely lost the ability to concentrate in these surface domains. This result has particular significance given the increasing evidence that CFTR enters the endocytic pathway via clathrin-coated pits (2, 4, 19).

The signal identified here, \( \text{YXXI} \), appears to function only as an internalization signal and not a “down-regulation” signal for conferring CFTR degradation. If \( \text{YXXI} \) was important to mediate CFTR degradation, metabolic pulse-chase experiments would have revealed an extended half-life when the signal was inactivated. Our studies indicate that CFTR lacking \( \text{YXXI} \) is stabilized at the cell surface because endocytosis of this mutant

![FIG. 4. Chloride channel activity of the CFTR COOH-terminal mutants is normal but expression of the mutants is elevated. Table I shows the complete summary of the whole-cell patch clamp data. Panel A showed typical whole-cell current records. Typical whole-cell I-V plots for wild-type CFTR (panel B), Y1424A (panel C), and Y1424A,I1427A (panel D) showing cyclic AMP-stimulated chloride currents in the absence of blockers (squares), presence of DIDS (upward triangles), and presence of glibenclamide (inverted triangles). A non-green cell showing background cyclic AMP-stimulated chloride currents is also shown in each plot (circles). A linear I-V relationship and time- and voltage-independent kinetics are hallmarks of CFTR channels and were similar in nature between wild type (WT) and the mutants. Panel E shows representative single channel current traces for WT and the mutants. Although these segments of recordings show more channels and more “wave-like” cooperative gating in the mutants versus the wild type, N or number of channels per patch could not be calculated because a quiet 0-channel base line was never reserved in patches that contained CFTR channels.](image-url)
is severely compromised. This also suggests that CFTR participates in the membrane-recycling pathway. This idea is consistent with previously reported immunolocalization studies that have shown that CFTR co-localizes with rab4, a component of recycling endosomes (9). The reasons why CFTR would be part of this pathway are unclear, but it may be to regulate the amount of functional chloride channels at the cell surface in the same manner as aquaporins and glucose transporters are regulated (20–25).

The specific residues identified by these studies, YXXI, that are important for CFTR endocytosis are conserved in the ten COOH-terminal tail sequences spanning from Xenopus to human (3). The tyrosine residue is conserved among all species with the exception of the dogfish, which has a phenylalanine residue. The isoleucine residue is conserved in 7 of 10 sequences with a very conservative leucine residue substitution in the other three, indicating that this motif, YXX(I/L), is highly conserved in the sequences identified to date. Both FXXXL (dogfish) and YXX(I/L) conform to the YXXΦ motif common to internalization signals, where X is any amino acid and Φ is a hydrophobic residue (10).

The identification of the YXXI signal is also consistent with recent studies that a region that includes this sequence interacts with the endocytic clathrin adaptor complex AP-2 using plasmam resonance analysis (19). Together, their study (19) and ours support the view that CFTR endocytosis occurs through clathrin-coated pits. Our study shows that two residues in the COOH-terminal tail, tyrosine 1424 and isoleucine 1427, regulate the steady-state distribution of CFTR between the plasma membrane and intracellular sites. This raises the important and testable hypotheses that the Y1424.I1427 signal controls CFTR entry into clathrin-coated pit regions at the apical membrane and that ablation of this signal abrogates one type of microdomain targeting in polarized epithelial cells.

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