Increased Diffusional Mobility of CFTR at the Plasma Membrane after Deletion of Its C-terminal PDZ Binding Motif*¶

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The cystic fibrosis transmembrane conductance regulator (CFTR) protein is a cAMP-regulated Cl⁻ channel expressed at the apical plasma membrane. It has been proposed that the C-terminal PDZ binding motif of CFTR is required for its apical membrane targeting and that PDZ-domain interactions may tether CFTR to the actin cytoskeleton via soluble proteins including EB50/NHERF1 and ezrin. We measured the diffusional mobility of human CFTR in the plasma membrane of Madin-Darby canine kidney cells by photobleaching of green fluorescent protein (GFP)-CFTR chimeras. After bleaching by a focused laser beam, GFP-CFTR fluorescence in the bleached membrane region recovered to ~90% of its initial level, indicating that nearly all of the CFTR was mobile. The GFP-CFTR diffusion coefficient (D) was 0.99 ± 0.09 × 10⁻⁹ cm²/s at 37 °C, similar to that of other membrane proteins. GFP-CFTR diffusion was not altered by protein kinase A or C activators but was blocked by paraformaldehyde and filipin. CFTR mutants lacking functional PDZ-binding domains (GFP-CFTRΔTRL and GFP-CFTRΔTRA) were also mobile with D significantly increased by ~60% compared with GFP-CFTR. However, GFP-CFTR, GFP-CFTRΔTRL, and GFP-CFTRΔTRA had similar mobilities (D ~12 × 10⁻¹⁰ cm²/s) at the endoplasmic reticulum in brefeldin A-treated cells. Agents that modulate the actin cytoskeleton (cytochalasin D and jasplakinolide) altered the plasma membrane mobility of CFTR but not CFTRΔTRL. EB50 (NHERF1), a PDZ domain-containing protein that interacts with the C terminus of CFTR, diffused freely in the cytoplasm with a diffusion coefficient of 0.9 ± 0.1 × 10⁻⁷ cm²/s. EB50 diffusion increased by ~2-fold after deletion of its ezrin-binding domain. These results indicate that wild-type CFTR is not tethered statically at the plasma membrane but that its diffusion is dependent on PDZ-domain interactions and an intact actin skeleton. PDZ-domain interactions of CFTR are thus dynamic and occur on a time scale of seconds or faster.

The cystic fibrosis transmembrane conductance regulator protein (CFTR) is a cAMP-regulated Cl⁻ channel that facilitates transepithelial Cl⁻ transport in the airways, intestine, pancreas, testis, and other tissues. The lethal genetic disease cystic fibrosis is caused by mutations in CFTR. There is evidence that CFTR may regulate directly or indirectly the activities of many membrane transport proteins including ENaC Na⁺ channels, outwardly rectifying Cl⁻ channels, HCO₃⁻ exchangers/cotransporters, ROMK K⁺ channels, AQP3 water channels, NHE3, and others (reviewed in Refs. 1 and 2). It has been proposed that many of these regulatory phenomena are mediated by protein complexes composed of CFTR, associated soluble and membrane-bound proteins, and the actin cytoskeleton (3–6). The formation of CFTR-containing complexes has been proposed to be governed by PDZ interactions. The last three amino acids at the C terminus of CFTR Thr-Arg-Leu comprise a PDZ binding motif that is highly conserved across species (7). PDZ interactions have been shown to be involved in supramolecular complexes and polarized expression of membrane proteins in a variety of epithelia and neurons (reviewed in Refs. 8–11).

Several soluble proteins have been reported to interact with the PDZ-binding domain of CFTR at the cell surface (see Fig. 1A) including EB50 (ERM-binding phosphoprotein 50; alternate name NHERF1, Na⁺/H⁺ exchanger-regulatory factor 1), CAP70 (PDZK1), and E3KARP (NHERF2) and in the Golgi (CAL; alternate names GOPC, FIG, PIST) (3, 6, 12–16). EB50 and E3KARP have been proposed to mediate associations between CFTR and the cytoskeleton via the actin-binding protein ezrin as well as with other membrane and soluble proteins (3, 6, 12, 13, 17, 18). Other regulatory proteins may also be associated with CFTR-containing protein complexes including ezrin, which is a protein kinase A-anchoring protein that binds protein kinase A (19), and receptor for activated C kinase 1 (20), which binds protein kinase C and interacts with EB50.

PDZ interactions are involved in a diverse set of processes in different cellular systems (8–10). For CFTR, PDZ interactions were shown initially to be important for apical membrane polarization in kidney and airway epithelial cells (12, 13). More recent studies have suggested that multiple sequences within the C terminus of CFTR may govern polarization in a cell type-specific manner (21–23). The C terminus of CFTR has also been demonstrated to affect CFTR stability. C-terminal deletions reduce plasma membrane CFTR stability without affecting biogenesis, and sequences within the C terminus alter lysosomal and proteasomal-dependent CFTR degradation (24).

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Interactions between CFTR and CAL has been observed for other membrane proteins such as β2-adrenergic regulation of the Na+/H+ exchanger type 3 by NHERF/EBP50 (29). Finally, recent studies of CFTR (30) and other membrane proteins (31–34) suggest that PDZ-binding sequences may also function as endocytic recycling motifs, which maintain polarized plasma membrane expression.

The purpose of this study was to test the hypothesis that CFTR is immobilized (or its mobility slowed) at the cell surface by PDZ-domain interactions. Measurements were done using fluorescence recovery after photobleaching on green fluorescent protein (GFP)-CFTR chimeras (N terminus of CFTR labeled) expressed in MDCK epithelial cells. Our laboratory has applied photobleaching methods extensively to study the mobility of GFP-labeled proteins in living cells (reviewed in Ref. 35), including a recent analysis of ΔF508-CFTR diffusion in the endoplasmic reticulum (ER) (36). We found that, contrary to initial predictions, CFTR diffusion in the cell plasma membrane was unrestricted and fairly rapid, but that CFTR membrane diffusion was increased significantly by deletion of its tri-amino acid C terminus PDZ-binding domain (CFTR-ΔTRL) or by mutagenesis of the C-terminal leucine to alanine (CFTR-ΔTRA). Moreover, CFTR diffusion was dependent on an intact/functional actin cytoskeleton. Our results provide evidence that wild-type CFTR is not tethered statically at the plasma membrane but that its diffusion is dependent on PDZ-domain interactions. PDZ-domain interactions of CFTR are thus dynamic and occur on a time scale of seconds or faster.

EXPERIMENTAL PROCEDURES

DNA Constructs, Cell Culture, and Transfection—Complementary DNA encoding human wild-type CFTR was fused downstream of GFP as described previously (12, 13). This construct was mutated to generate GAP-CFTR-ΔTRL and GAP-CFTR-ΔTRA, constructs with the C-terminal three amino acids deleted and the C-terminal leucine mutated to alanine, respectively. Complementary DNA encoding murine EBP50 was polymerase chain reaction-amplified and fused downstream of GFP in the vector pEGFP-C3 (Clontech) as a HindIII-BamHI fragment. To generate a fusion between GFP and EBP50 lacking its C-terminal ezrin-binding domain (37), EBP50 cDNA was polymerase chain reaction-amplified as an MscI-EcoRI fragment with primers designed to remove the C-terminal 30 amino acids and insert a new stop codon. The amplified fragment was ligated into pEGFP-C3 as a Scal-EcoRI fragment. For expression of GFP in cytoplasm, the GFP-coding region (from plasmid pEGFP, Clontech) was ligated into pcDNA3.1 (Invitrogen) as a HindIII-EcoRI fragment.

For photobleaching experiments, cells were grown on 18-mm-diameter glass coverslips in 12-well plates in a 5% CO2, 95% air incubator at 37 °C. MDCK cells stably expressing GFP chimeras of wild-type CFTR and CFTR-ΔTRL were generated as described previously (12, 13) and cultured in minimum Eagle’s media containing 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 150 μg/ml G418. Image-photobleaching experiments on stably transfected MDCK cell lines were performed at 2 days after plating with the medium supplemented with 5 mM sodium butyrate for 18–24 h prior to experiments. COS7 cells were transfected using LipofectAMINE for studies of EBP50 and ER-retained CFTT. EBP50 mobility measurements were done in COS7 and MDCK cells at 1–2 days after transfection. For measurements of CFTR mobility at the ER, COS7 cells were used 2 days after transfection and treated with brefeldin A (5 μg/ml) at 16 h prior to experiments.

Fluorescence Recovery after Photobleaching—Cultured cells were mounted in a custom-built chamber designed to fit a PMDI-2 microscope (Harvard Apparatus) and bathed in phosphate-buffered saline supplemented with 6 mM glucose and 1 mM pyruvate. Chamber and objective lens temperatures were maintained at 37 °C by a TC-201A temperature regulator (Harvard Apparatus) and a lens-thermoregulator (Biotracs Inc., Butler, PA), respectively. Photobleaching measurements with image detection were done on a Leitz upright microscope with cooled CCD camera detector (Photometrics) to record serial full-field epifluorescence images. Cells were viewed from above using an oil immersion objective (1.4 numerical aperture). The first-order acousto-optic modulator as described previously (36). The first-order diffracted beam was directed onto cells by a 510-nm dichroic mirror and ×60 oil immersion objective (numerical aperture 1.4) using an inverted epifluorescence microscope. Emitted fluorescence was filtered by serial 530 ± 15-nm bandpass and 515-nm-long pass filters and detected by a photomultiplier whose gain was gated off during the bleach pulse. For photobleaching experiments, 5–15 cells/coverslip on 3–10 coverslips were studied.

Cell Treatments—Cell treatments were performed at 37 °C for specified times prior to photobleaching experiments. Treatment included paraformaldehyde (4%, 30 min) for protein immobilization, filipin (10 μM, 10 min) for membrane cholesterol binding, forskolin (5 μM, 15 min) for protein kinase A activation, and phorbol 12-myristate 13-acetate (600 ng/ml, 15 min), for protein kinase C activation. The actin cytoskeleton was modulated using cytochalasin D (2 μM, 15 min) or jasplakinolide (5 μM, 15 min). Agents for cell treatment were included in the cell-bathing solution during experiments (with the exception of paraformaldehyde).

Analysis of Photobleaching Recovery Data—For photobleaching with image detection, background-subtracted fluorescence was computed for a region of interest consisting of a bleached region of membrane in which background fluorescence was determined using a region outside of the region of interest. The region of interest-integrated fluorescence values were normalized to unity for prebleach fluorescence and corrected for bleaching during acquisition of serial images (correction generally <2% per image acquisition). Absolute diffusion coefficients (D) were computed from the time course of recovery using the analytical solution to the one-dimensional diffusion equation with appropriate initial and boundary conditions as described for the measurements of GFP-aquaporin diffusion in cell plasma membranes (38). Photobleaching data was analyzed as described previously (36).

RESULTS

Photobleaching of GFP-CFTR chimeras was done to measure CFTR diffusion in the plasma membrane and to investigate whether PDZ-domain interactions immobilize or otherwise restrict CFTR mobility. Fig. IA shows a schematic of GFP-labeled CFTR along with putative interactions of its C-terminal PDZ-binding domain with PDZ-binding proteins, ezrin and the actin cytoskeleton. Experiments were done on MDCK cells stably expressing GFP-labeled CFTR or CFTR-ΔTRL, which lacks the three amino acid C-terminal PDZ-binding motif. Also, MDCK cells transiently expressing GFP-labeled CFTR-ΔTRA (CFTR with the C-terminal Leu-to-Ala mutation) were studied. Although fluorescence staining was somewhat heterogeneous in
all of the cell lines, most cells showed a prominent membrane pattern of GFP fluorescence with additional variable fluorescence in organellar structures including the endoplasmic reticulum and Golgi.

GFP-CFTR mobility was measured by fluorescence recovery after photobleaching in which a segment of membrane was bleached with an 5-µm-diameter circular spot produced by a focused argon laser beam at 488-nm wavelength. Fluorescence images were acquired before and at specific times after brief (100 ms) photobleaching to define the kinetics of diffusion of unbleached GFP-CFTR into the bleached (darkened) region. Representative sequences of images are shown for wild-type CFTR (Fig. 1B, top), CFTR-TRL (middle), and CFTR-TRA (bottom). The circular bleached region contained en face membrane as well as membrane at the cell edge seen as a thin white band of fluorescence. Recovery of fluorescence from the edge to center of the bleached region (indicated by the white circle in prebleach images of series in Fig. 1B) was observed for all of the chimeras with qualitatively near-complete fluorescence recovery after several minutes. However, the kinetics of fluorescence recovery were different. The recovery for GFP-CFTR appeared to be >50% complete in 1–1.2 min, whereas recovery for GFP-CFTR-TRL and CFTR-TRA was >50% complete in 0.5–0.75 min.

Quantitative image analysis was used to determine the kinetics of fluorescence recovery after photobleaching. Averaged recovery curves are given in Fig. 2A (and summarized in Fig. 5) from an analysis of 15–16 sets of images for the stable cell lines (wild-type and TRL-CFTR) and 6 sets of images for TRA-CFTR. Computed half-times (t½) for fluorescence recovery in the membrane were 65 ± 4 s for GFP-CFTR, 42 ± 2 s for GFP-CFTR-TRL, and 41 ± 4 s for GFP-CFTR-TRA. The percentage fluorescence recoveries, generally interpreted as the fraction of mobile GFP-labeled molecules, were 89 ± 2% for GFP-CFTR, 91 ± 2% for GFP-CFTR-TRL, and 93 ± 3% for GFP-CFTR-TRA, indicating that these proteins were nearly fully mobile in the plasma membrane. From bleach spot size and t½ values, diffusion coefficients (D) for GFP-CFTR, GFP-CFTR-TRL, and GFP-CFTR-TRA were (in × 10⁻⁹ cm²/s) 0.99 ± 0.07, 1.55 ± 0.07, and 1.6 ± 0.1, respectively. These values are in the range found for other membrane proteins (see “Discussion”).

After confirming that two PDZ mutants of CFTR had similar diffusive characteristics, further studies were performed upon the stably transfected cell lines expressing GFP-CFTR and GFP-CFTR-TRL. Control experiments were done to verify that fluorescence recovery was produced by GFP diffusion rather than diffusion-independent phenomena including photophysical processes such as triplet state relaxation (35). Photobleaching of paraxialdehyde-fixed cells in which membrane proteins are immobilized showed no significant fluorescence recovery (Fig. 2B, top). Similar results were found for diffusion of GFP-CFTR-TRA (data not shown). Fluorescence recoveries at 5 min after photobleaching were 3 ± 2% and 4 ± 2% for GFP-CFTR and GFP-CFTR-TRL, respectively (eight series of images on different cells analyzed). Treatment of cells with the cholesterol-binding agent filipin also produced near-complete immobilization of GFP-CFTR (Fig. 2B, bottom). Fluorescence recoveries at 5 min were <6% for GFP-CFTR and GFP-CFTR-TRL (eight series of images on different cells analyzed). Trafficking of CFTR (39) could also in principle contribute to recovery of the plasma membrane fluorescence signal in these experiments. However, biochemical characterization of the cell lines used here demonstrated that <5% of plasma membrane CFTR is internalized and recycled during the course of photobleaching experiments (30), indicating a negligible contribution of endocytic trafficking to the fluorescence recovery of GFP-CFTR in the plasma membrane.

Possible modulation of GFP-CFTR mobility by protein kinases A and C was investigated. Protein kinase A, which activates CFTR by phosphorylation of the R-domain, has been demonstrated to associate with ezrin and may be a component of the CFTR-containing protein complex (5, 19, 40). PDZ interactions with CFTR have also been demonstrated to effect CFTR phosphorylation by protein kinase C (20, 27, 40). Protein kinase A stimulation by forskolin (5 µM, 15 min, 37 °C) had no significant effect on GFP-CFTR mobility (Fig. 3A, top), nor did protein kinase C stimulation by a phorbol ester (phorbol 12-myristate 13-acetate, 0.2 µM, 15 min, 37 °C, Fig. 3A, bottom). Fig. 3B gives averaged recovery curves (12–13 cells analyzed) with the data summarized in Fig. 5.

Possible CFTR-actin interactions involving the CFTR PDZ-binding domain were investigated using the actin cytoskeletal-modulating agents cytochalasin D and jasplakinolide (4, averaged data summarized in Fig. 5). Cytochalasin D disrupts the actin cytoskeleton by binding to the barbed end of filamentous actin, inhibiting the addition of actin monomers without affecting depolymerization at the pointed end. Incubation conditions for cytochalasin D were optimized for disruption of the actin skeleton with little effect on cell shape or integrity, as confirmed by rhodamine-phalloidin staining of filamentous actin in fixed, permeabilized cells. Untreated cells (untransfected and stably transfected) showed characteristic stress fi-
fluorescence recovery was 77% in CFTR-β-lactoglobulin-expressing cells, and GFP-CFTR-ΔTRL (open circles) and GFP-CFTR-ΔTRA (open triangles) in transfected MDCK cells. Serial images (as in Fig. 1B) were analyzed as described under “Experimental Procedures.” Each point is the mean ± S.E. (errors smaller than circles in some cases) from images measured on 15–16 different cells (*, p < 0.01 for ΔTRL and ΔTRA-CFTR in all instances). B, photobleaching of MDCK cells expressing GFP-CFTR after paraformaldehyde fixation (4% in phosphate-buffered saline for 30 min, top) and filipin incubation (10 μM for 10 min, bottom). Image series were obtained as in Fig. 1B (representative of eight cells). Scale bar, 5 μm.

The cell-permeable, actin-stabilizing cell agent jasplakinolide was also used to investigate CFTR interactions with the cytoskeleton. Jasplakinolide inhibits actin-filament disassembly and increases the proportion of polymerized actin in cells. The jasplakinolide treatment conditions used here (5 μM, 15 min) should greatly reduce actin mobility (41, 42). Photobleaching of GFP-CFTR showed slowed fluorescence recovery and a greater immobile fraction, whereas treatment of GFP-CFTR-ΔTRL-expressing cells showed no significant effect on diffusion compared with untreated GFP-CFTR-ΔTRL-expressing cells (data not shown). Quantitative analysis of image sets (Fig. 4B, left, open triangles) gave a slowed diffusion of GFP-CFTR; the percentage fluorescence recovery was 78 ± 3%. As for cytochalasin D, the diffusion of ΔTRL-CFTR was not affected by jasplakinolide (Fig. 4B, right, open triangles).

We next measured the diffusive properties of EBP50, a PDZ domain-containing protein that interacts with the C terminus of CFTR (3, 6, 12) and the N terminus of ezrin (37). GFP-EBP50 was observed throughout the cytoplasm of transfected MDCK cells and also concentrated at the membrane of some cells (Fig. 6A, inset middle). This pattern is consistent with reports that EBP50 is present in both soluble and membrane-associated pools in a variety of cell lines (3, 43).

Comparative photobleaching experiments performed in MDCK cells expressing (unconjugated) GFP in the cytoplasm (Fig. 6A, top) showed nearly complete GFP mobility (100% recovery indicated by dashed line in Fig. 6) with a D of 3.3 ± 0.2 × 10⁻⁷ cm²/s (n = 6 coverslips), similar to that measured in other cell types (see Ref. 44 and references therein). The diffusion of GFP-EBP50 was also nearly complete (96 ± 1%) with D = 0.91 ± 0.08 × 10⁻⁷ cm²/s (n = 10 coverslips), slowed ~4-fold compared with unconjugated GFP (Fig. 6A, middle, note different time scale). To investigate whether transient interactions with ezrin reduced the diffusion of EBP50, a mutant protein lacking its ezrin-binding domain (GFP-EBP50−) was generated. GFP-EBP50− was localized exclusively to the cytoplasm of transfected MDCK cells (Fig. 6A, inset bottom). EBP50− diffusion (Fig. 6A, bottom) was significantly increased...
(−2-fold) compared with EBP50 (D = 1.9 ± 0.1 × 10⁻⁷ cm²/s, n = 7 coverslips, p < 0.005), suggesting that transient interactions with ezrin slow the diffusion of EBP50 (see Fig. 6C for data summary).

Pharmacological modulation of the actin cytoskeleton on the diffusion of EBP50 was investigated (Fig. 6B). The diffusion of GFP-EBP50 was slowed by cytochalasin D (Fig. 6B, top) and jasplakinolide (Fig. 6B, middle) by ∼1.6-fold (cytochalasin D D = 0.59 ± 0.03 × 10⁻⁷ cm²/s, n = 6 coverslips; jasplakinolide D = 0.56 ± 0.08 × 10⁻⁷ cm²/s, n = 4 coverslips; p < 0.01 for both agents) with an increased immobile fraction (cytochalasin D 88 ± 1% mobile, n = 6; jasplakinolide 90 ± 2% mobile, n = 4; p < 0.01 for both agents). However, cytochalasin D (Fig. 6B, bottom) and jasplakinolide (not shown) did not effect the diffusion of GFP-CFTR under control conditions.

Finally, the diffusion of CFTR and the PDZ-binding mutants CFTR-ΔTRL and CFTR-ΔTRA were measured in an alternative cellular compartment to test the hypothesis that their diffusive properties at the plasma membrane differed because of plasma membrane-specific interactions. CFTR, CFTR-ΔTRL, and CFTR-ΔTRA diffusion were measured in the ER of brefeldin-treated COS7 cells (MDCK cells were not used because of their brefeldin A resistance). Transient transfection with GFP chimeras and brefeldin A treatment gave a characteristic ER localization pattern (Fig. 7A, insets). Spot photobleaching showed comparable diffusion of the GFP-labeled CFTRs (Fig. 7A).

Analysis of the recovery curves, as performed for GFP-labeled CFTR and ΔF508-CFTR in the ER (36), yielded t₁/₂ values of 3.5 s (n = 3–4 coverslips) with no significant differences in D (−12 × 10⁻¹⁰ cm²/s) for the three groups (Fig. 7B). The diffusive properties of EBP50 and GBP50− were also measured in COS7 cells, which express EBP50 natively (17), to verify that this protein has similar diffusive characteristics when compared with MDCK cells. Recovery curves for cytoplasmic GFP, GBP-EGBP50, and GBP-EGBP50− expressed in COS7 cells are shown in Fig. 7C (top) and summarized in Fig. 7C (bottom, n = 4–7 coverslips). The diffusive properties of these proteins were not significantly different from those in MDCK cells.

**DISCUSSION**

A central conclusion of this study is that CFTR is freely mobile at the cell surface over a distance scale of microns and a time scale of seconds. Thus, static tethering of CFTR to the actin skeleton through its C-terminal PDZ-binding domain or elsewhere does not occur. Such tethering interactions must therefore be dynamic and occur over a time scale of seconds or faster. We conclude formally that at least one of the proposed interacting proteins linking CFTR with the actin skeleton must have sufficiently rapid on-off binding rates to permit apparently unhindered CFTR lateral mobility in the plane of the membrane. The ~60% increase in CFTR-diffusive mobility after deletion/mutagenesis of its C-terminal PDZ binding domain (CFTR-ΔTRL and CFTR-ΔTRA) provides in vivo biophysical evidence supporting the involvement of the terminal three
amino acids in protein-protein interactions. The influence of actin skeletal modulation by cytochalasin D and jasplakinolide on the mobility of wild type but not H9004 TRL-CFTR provides further evidence for involvement of actin in CFTR interactions. It was shown previously that disruption of the actin cytoskeleton with either cytochalasin D or jasplakinolide resulted in a substantial reduction in the mobility of GFP-tagged actin (41, 42).

To examine the network of CFTR C-terminal interactions, we investigated the mobility of EBP50, a PDZ domain-binding protein that links the C terminus of CFTR to ezrin and actin (3, 12, 13, 37). Our results indicate that the diffusion of EBP50 in cytoplasm is rapid and complete but slowed by transient interaction with the actin-based cytoskeleton, indirectly through binding with ezrin (-2-fold). Furthermore, the diffusion of membrane-associated EBP-50 was unrestricted and quite rapid, indicating that EBP50 diffusion is not rate-limiting for CFTR diffusion. In accord with the observed slowing of wild-type CFTR by cytoskeletal disruption, both cytochalasin D and jasplakinolide reduced the mobility of actin-associating EBP50 but not the ezrin-binding domain-deficient mutant EBP50-.

The mobility of ezrin was investigated previously in epithelial cells (42). Ezrin diffused rapidly in cytoplasmic regions as a small non-interacting protein ($D = 3 \times 10^{-7}$ cm$^2$/s), whereas in microvilli its mobility was reduced with a recovery $t_{1/2}$ of 1–2 min for diffusion over ~4 μm. Thus proteins that interact with the C terminus of CFTR are in general quite mobile.

The values of $D$ for CFTR and CFTR-ΔTRL/ΔTRA (1.0–1.6 $\times 10^{-10}$ cm$^2$/s) are in the range determined for other plasma...
membrane proteins. For example, $D$ values are in the range of $0.5–1 \times 10^{-10}$ cm$^2$/s for aquaporin water channels (38), Kv1.4 K$^+$ channels (45), and the vasopressin receptor (46). Measured $D$ values for $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionate glutamate receptor in neurons are 5.4 and $45 \times 10^{-10}$ cm$^2$/s for the $\gamma$-amino butyric acid, type A receptor (48), and $40–120 \times 10^{-10}$ cm$^2$/s for the $\beta_2$-adrenergic receptor (49). The $D$ for wild type and $\Delta F 508$-CFTR at the ER was $12 \times 10^{-10}$ cm$^2$/s (36), $12$-fold greater than CFTR in the plasma membrane. Interestingly, the diffusion of $\Delta T R I /\Delta T R A$-CFTR was similar to that of wild-type CFTR at the ER.

Reduction of CFTR diffusion in the plasma membrane probably represents decreased membrane fluidity because of the relatively high cholesterol content of the plasma membrane compared with the ER membrane. Transient interactions through ezrin, which has reduced mobility in the plasma membrane compared with cytosol (42), could also account in part for the slowed mobility of CFTR at the plasma membrane compared with the ER.

Recent data (8, 50) suggest that the consequence of PDZ interactions on membrane protein diffusion may be protein-specific. Examples include proteins from the postsynaptic density (PSD) of neurons, which contain a high density of scaffold proteins with multiple PDZ domains. Kv1.4, a neuronal K$^+$ channel, $35\%$ mobile when expressed in human embryonic kidney 293 cells and $15\%$ mobile when co-expressed with PSD-95, a prominent PDZ domain protein of the PSD that interacts with Kv1.4 (45). The mobility of Kv1.4 was $70\%$ after mutation or deletion of its PDZ-binding domain, suggesting that PDZ interactions could statically tether channel proteins at the plasma membrane. Mobility of $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionate glutamate receptors, which associate with multiple PDZ domain proteins, including GRIP1, GRIP2, PICK1, and PSD-95 (through stargazin) is domain-specific (47). In extra-synaptic regions (away from the PSD), $90\%$ of receptors were highly mobile ($D = 45 \times 10^{-10}$ cm$^2$/s). In the PSD only, $50\%$ of the receptors diffused ($D = 5.4 \times 10^{-10}$ cm$^2$/s), which increased to $80\%$ for freshly excytosed receptors or at low calcium concentration. It should be noted that PSD-95 was essentially immobile in neurons (51), contrasting with the dynamics of EBP50 as found here. The consequences of PDZ interactions on the mobility of membrane proteins thus depend on the identity and dynamics of PDZ domain-interacting proteins.

Neither activation of protein kinase A nor protein kinase C influences the plasma membrane mobility of CFTR. Although possible association or dissociation of proteins with CFTR may occur during stimulation (5, 17, 27, 28), these processes do not alter CFTR diffusion.

In summary, the data here support the conclusion that significant interactions occur between the CFTR C-terminal PDZ-binding domain and the actin cytoskeleton, probably through intermediate cytoplasmic proteins, but that such interactions are dynamic so as to allow unrestricted CFTR diffusion over micron distances and seconds time scales.

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