Enhanced osmotic water permeability has been observed in Xenopus oocytes expressing cystic fibrosis transmembrane conductance regulator (CFTR) protein. Subsequent studies have shown that CFTR activates an endogenous water permeability in oocytes, but that CFTR itself is not the water channel. Here, we show CFTR-dependent activation of endogenous water permeability in normal but not in cystic fibrosis human airway epithelial cells. Cell volume was measured by novel confocal x-z laser scanning microscopy. Glycerol uptake and antisense studies suggest CFTR-dependent regulation of aquaporin 3 (AQP3) water channels in airway epithelial cells. Regulatory interaction was confirmed by coexpression of CFTR and AQP3 cloned from human airways in Xenopus oocytes and of CFTR and rat AQP3 in Chinese hamster ovary cells. These findings indicate that CFTR is a regulator of AQP3 in airway epithelial cells.

The cystic fibrosis transmembrane conductance regulator (CFTR) protein is mutated and defective in cystic fibrosis (CF), a common lethal genetic disease. CFTR has been demonstrated to function in two ways: (i) as a protein kinase A-regulated Cl⁻ channel and (ii) as a regulator of other membrane conductances (1–3). Thus, CF may not only affect cAMP-dependent Cl⁻ conductance, but may also affect other membrane conductances normally regulated by wild-type CFTR. Such a defect in CFTR-dependent regulation was found for the epithelial Na⁺ conductance (1, 4). Thus, enhanced Na⁺ conductance was detected in the airways and intestinal epithelium of CF patients, which is very likely to contribute to enhanced absorption of electrolytes and water and to the altered mucociliary clearance as well as intestinal obstructions, respectively (5–7).

Apart from epithelial Na⁺ conductance, osmotic water permeability has been reported to be influenced by CFTR (8, 9). These studies were performed in Xenopus oocytes and indicated enhanced osmotic cell swelling after expression and cAMP-dependent activation of CFTR. Since water-injected control oocytes did not demonstrate such a cAMP-activated water permeability, the most likely explanation was that cAMP acted through activation of CFTR. In fact, it was suggested initially that water uses the same conductive pathway and moves together with Cl⁻ through the CFTR Cl⁻ channel. This pathway should therefore be formed by transmembrane domains (8). However, it was shown in a subsequent study (a) that both water and Cl⁻ ions moved independently when CFTR was activated by an increase in intracellular cAMP. Independence was demonstrated by showing selective inhibition of CFTR Cl⁻ conductance by glibenclamide and selective blockade of water permeability by mercurial compounds and phloretin (9). In addition, this water pathway activated by CFTR was permeable for glycerol, too. These results support the assumption that some sort of endogenous water and glycerol permeability must be present in Xenopus oocytes and that this is activated by CFTR.

So far, it was unclear whether CFTR-activated water permeability is unique to Xenopus oocytes or whether a similar interaction can also be observed in mammalian cells. This question was addressed in this study by measuring cell volume changes and radioactive glycerol uptake in human airway epithelial and Chinese hamster ovary (CHO) cells. We found CFTR-dependent activation of osmotic water permeability in normal respiratory cells and identified one member of the aquaporin family (AQP3) as the interacting partner. Since CFTR-dependent stimulation of osmotic water permeability is absent in airway cells derived from CF patients, we speculate that this has a pathophysiological impact on the lung disease in CF.

### EXPERIMENTAL PROCEDURES

#### Cell Culture—Human non-CF, CF, and CF airway epithelial cells transfected with 6REP-wtCFTR have been described in previous studies (10, 11) and were kindly provided by Dr. D. C. Gruener (University of California, San Francisco). CHO-K1 cells and CHO cells stably expressing wtCFTR (CHO-wtCFTR) or ΔF508-CFTR (CHO-ΔF508) were kindly provided by Dr. X.-B. Chang (Mayo Clinic, Scottsdale, AZ). Cells were cultured on tissue culture plastic dishes or glass coverslips and kept in an atmosphere of 5% CO₂ under conditions described elsewhere (10, 12). CHO cells were transfected with rat AQP3 cDNA (kindly provided by M. Echevarria, University of Sevilla, Sevilla, Spain) inserted into expression vector pZeoSV (Invitrogen) using standard techniques. Isolated colonies were expanded and assayed for AQP3 expression by measurement of glycerol uptake and detection of AQP3 mRNA (RT-PCR).

#### Cell Volume Measurements by Confocal Microscopy—Cells grown on coverslips were loaded with the fluorescent dye calcein/AM (10 μmol/liter for 30 min at room temperature), and repetitive x-z line scans at 488 nm excitation of one scanning line through three to six cells were performed using an LSM 410 apparatus (Zeiss, Germany) (13). A water immersion lens (Zeiss C-APo 63/1.2w) was used to avoid optical distortions due to refractive index mismatches. The z-line scan distance was set to result in square voxel; usually these were in z-steps of 0.33 μm. The size of the confocal pinhole was set to give a full-width half-maximum in the z-direction of 0.4 μm. The z-line scans were stacked in a two-dimensional image giving a x-z slide through the cells. A customized macro allowed the recording and storage of time series of x-z slides (usually 200–300 images). Cell area and the mean intensity of the cell

*This work was supported in part by Deutsche Forschungsgemeinschaft Grant Ku756/2-3, the Fritz Thyssen Stiftung, and Zentrum Klinische Forschung I. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be

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The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; wtCFTR, wild-type CFTR; CF, cystic fibrosis; CHO, Chinese hamster ovary; AQP, aquaporin; hAQP, human AQP; rAQP, rat AQP; RT-PCR, reverse transcription-polymerase chain reaction; IBMX, isobutylmethylxanthine.
area at the cutting z-line were analyzed with Metaflour software (Universal Imaging Corp., West Chester, PA). Initial area increase and intensity decrease induced by hypotonic bath solution (Hypo) resulted in an increase of cell area and a decrease in fluorescence intensity, as shown for a cell line scan were recorded from two cells. Cell area and fluorescence intensity are taken as independent quantitative measures of the cell volume. Cell swelling in response to a rapid change to hypotonic bath solution (Hypo) resulted in an increase of cell area and a decrease in fluorescence intensity, coded in false colors. B, initial and absolute area increase and intensity decrease induced by hypotonic bath solution were determined in the same cell under control conditions and 1 min after stimulation with IBMX (0.5 mmol/liter) and forskolin (10 μmol/liter). Initial change of area and intensity was calculated by linear regression of the first 30 s of recording in hypotonic solution.

**Fig. 1.** Cell volume measurements by confocal microscopy. A, non-CF airway epithelial cells were loaded with calcein/AM, and images from a confocal z-line scan were recorded from two cells. Cell area and fluorescence intensity are taken as independent quantitative measures of the cell volume. Cell swelling in response to a rapid change to hypotonic bath solution (Hypo) resulted in an increase of cell area and a decrease in fluorescence intensity, coded in false colors. B, initial and absolute area increase and intensity decrease induced by hypotonic bath solution were determined in the same cell under control conditions and 1 min after stimulation with IBMX (0.5 mmol/liter) and forskolin (10 μmol/liter). Initial change of area and intensity was calculated by linear regression of the first 30 s of recording in hypotonic solution.

**CFTR Activates AQP3**

**GACCAT-3′ (antisense) and 5′-ATGGGTCGACAGAAGGAGCTG-3′ (sense); AQP1, 5′-CTTCCTGAGACTCGTCG-3′ (antisense); and AQP5, 5′-GGAGCACCTCCTCTT-3′ (antisense) in serotonin-free culture medium supplemented with 2% Ultraser G (Life Technologies, Inc.) for 48 h, and subsequently, glycerol uptake was measured. Osmotic Water and Glycerol Permeability in Xenopus Oocytes—Isolation and microinjection of oocytes have been described in a previous report (15). Oocytes of identical batches were injected with 10–50 ng of cRNA (wild-type CFTR, G551D-CFTR, and hAQP3 cloned from human airway cells). Water-injected oocytes served as controls. The osmotic water permeability coefficient (Pf) was calculated from the rate of hypertonic volume increase, measured by gravimetric techniques at 22 °C (9). Hypotonicity was induced by omission of 120 mmol/liter mannitol from ND96 normotonic medium. Pf was calculated according to Ref. 16: $P_f = \frac{dV}{dt}$ (1/S), with S being the oocyte surface area, V being the volume, and $dV/dt$ being the osmolality gradient at zero time. The ratio $dV/dt$ was calculated from the weight change 1 min after exposure to hypotonic buffer. Glycerol permeability ($P_{gly}$) was calculated from the initial rate of $[^{14}C]$glycerol uptake per oocyte during incubation with $[^{14}C]$glycerol (2 μCi/ml) and 1 mmol/liter glycerol. Oocytes were lysed overnight in 100 g/liter SDS at room temperature, and radioactivity was counted.

**Compounds and Statistics—**All chemicals were used at the highest grade of purity available. Data are given as means ± S.E. (n), where n refers to the number of experiments. Paired and unpaired t tests were used for statistics with a p value of <0.05, indicating statistical significance.

**RESULTS**

**Water and Glycerol Permeability Is Increased by cAMP in Normal Airway Cells, but Not in Cells Derived from CF Patients—**Water permeability in airway epithelial cells was assessed by measuring osmotically induced cell swelling using the novel microscopic techniques described above (13). Rapid change to hypertonic bath solution resulted in an increase in cell area and a decrease in fluorescence intensity, as shown for a non-CF cell in Fig. 1. Linear regression was fitted to the data points obtained during the first 30 s after exposure to the hypertonic bath solution (Fig. 1B). Stimulation with IBMX and forskolin augmented the area increase and the fluorescence pixel (calcein concentration) decrease elicited by hypertonic bath solution. These measured volume changes necessarily underestimate the real rate of hypertonic cell swelling and the effects of CFTR on osmotic water permeability because the cells activate their volume regulatory decrease mechanisms, which will be even augmented when CFTR Cl− conductance is activated (9). The summary shown in Fig. 2A indicates that cell swelling was significantly enhanced after activation of CFTR (solid bars). Such a CAMP-dependent increase in hypertonic cell swelling was not observed in airway epithelial cells derived
from CF patients (Fig. 2B).

Previous studies have demonstrated that osmotic water permeability activated by CFTR in Xenopus oocytes is also permeable for glycerol. Therefore, we measured [$^{14}$C]glycerol uptake in airway epithelial cells that expressed endogenous wild-type CFTR (non-CF), mutant CFTR (CF), or exogenous wild-type CFTR (CF-wtCFTR) (Fig. 3). After stimulation of non-CF cells with IBMX and forskolin, [$^{14}$C]glycerol uptake (closed circles) was enhanced when compared with control uptake (open circles). Such an activation of [$^{14}$C]glycerol uptake by cAMP was not observed in CF cells. Here, [$^{14}$C]glycerol uptake was even attenuated when the cAMP-dependent pathway was activated.

However, expression of exogenous wild-type CFTR in CF cells (CF-wtCFTR) restored the effects of IBMX and forskolin. Pretreatment of the cells with HgCl$_2$ (5 μmol/liter, 15 min) completely inhibited the effect of cAMP on [$^{14}$C]glycerol uptake in non-CF cells as well as in CF cells expressing exogenous CFTR (closed squares). At this concentration, CFTR CI$^-$ currents were not affected (data not shown). These results suggest, in agreement with what has been observed in Xenopus oocytes (9), that, also in human airway epithelial cells, a glycerol-permeable water channel is activated by CFTR.

**AQP3 Is Responsible for CFTR-dependent Glycerol Uptake**

Water permeability in mammals is facilitated in many cells by specific water channels, so-called aquaporins (17). Expression of various types (AQP1, AQP3, AQP4, and AQP5) in non-CF and CF airway epithelial cells was performed by RT-PCR. No expression of AQP4 could be detected in airway epithelial cells. However, expression of AQP1, AQP3, and AQP5 was found (data not shown). Message for AQP3 was detected in freshly isolated airway epithelial cells from nasal polyps as well as in various cultured CF and non-CF airway epithelial cells (Fig. 4). Since AQP3, in contrast to AQP1 or AQP5, has been described to transport glycerol (18, 19), these results suggest that AQP3 is the water pathway responsible for CFTR-dependent water and glycerol uptake. This was further supported by staining of AQP3 in these cells. Immunocytochemical localization of AQP3 demonstrated abundant expression of this protein throughout the plasma membrane (Fig. 4A). AQP3 was also immunolocalized in CHO cells expressing AQP3 and in CF airway cells, but not in control CHO cells or in the absence of the primary antibody (Fig. 4A).

Furthermore, normal respiratory epithelial cells expressing endogenous CFTR (non-CF) and CF epithelial cells expressing exogenous wild-type CFTR (CF-wtCFTR) were incubated with AQP3 sense and antisense oligonucleotides, respectively. After 48 h of incubation, CFTR-dependent, i.e. forskolin/IBMX-induced, glycerol uptake was abolished in non-CF and CF-wtCFTR cells. No such effects on forskolin/IBMX-activated glycerol uptake were observed in cells incubated with sense oligonucleotides for AQP3 or antisense oligonucleotides for AQP1 and AQP5 (Fig. 5), suggesting that AQP3 is responsible for glycerol uptake.

**Fig. 2. Activation of CFTR enhances water permeability in non-CF but not CF airway epithelial cells.** A. shows a summary of cell volume measurements in non-CF cells. Stimulation by IBMX and forskolin (black bars) significantly enhanced initial area increase and initial fluorescence decrease in cells compared with control conditions before and after stimulation (n = 18). CFTR-activated water permeability led to an increase in the absolute area, whereas fluorescence intensity was decreased (n = 18). B. In CF epithelial cells, stimulation by IBMX and forskolin had no effect on cell volume change (n = 12). Data are means ± S.E. Asterisks indicate significant difference from control (p < 0.05).

**Fig. 3. Activation of CFTR increases glycerol permeability.** [$^{14}$C]Glycerol uptake was measured in the initial 3 min after exposure (open circles) to non-CF and CF airway epithelial cells and in transfected CF cells (CF-wtCFTR). Stimulation of non-CF cells with IBMX and forskolin increased [$^{14}$C]glycerol uptake, whereas a decrease in [$^{14}$C]glycerol uptake was observed for CF cells (closed circles) (n = 3 each). cAMP-dependent inhibition of glycerol uptake in CF cells is currently poorly understood and is due to another CFTR-independent mechanism. Transfection with wtCFTR (CF-wtCFTR) restored the effects of IBMX and forskolin on [$^{14}$C]glycerol uptake in CF cells (n = 6). Pretreatment of non-CF and CF-wtCFTR cells with HgCl$_2$ (5 μmol/liter, 15 min) abolished forskolin/IBMX-induced [$^{14}$C]glycerol uptake (closed squares). Data are means ± S.E. Asterisks indicate significant difference from control (p < 0.05).
CFTR Is Required for Activation of AQP3 by cAMP in Xenopus Oocytes—Human AQP3 was cloned from human airway epithelial cells used here by RT-PCR. The obtained nucleotide coding sequence was identical to the sequence published in a previous report (20). Expression of the cloned hAQP3 cDNA in Xenopus oocytes induced an enhanced osmotic glycerol permeability coefficient ($P_{\text{gly}}$) (9) ($11.0 \pm 1.2 \times 10^{-6} \text{ cm/s}; n = 13$) compared with control oocytes ($1.7 \pm 1.7 \times 10^{-6} \text{ cm/s}; n = 11$). When CFTR and hAQP3 were coinjected into Xenopus oocytes, enhanced basal $P_{\text{gly}}$ was further augmented by stimulation of the oocytes with IBMX (Fig. 6C). No increase was observed when hAQP3 was expressed alone (data not shown). Moreover, in hAQP3/CFTR-coexpressing oocytes, activation of CFTR by IBMX in the presence of an isotonic bath solution induced initial cell shrinkage due to $\text{Cl}^-$ exit from the oocytes (Fig. 6A). Subsequent exposure to an extracellular hypotonic solution in the continued presence of IBMX augmented cell swelling and increased the osmotic water permeability coefficient ($P_f$) significantly, indicating activation of hAQP3 water channels by CFTR (Fig. 6, A and C). As described for the experiments performed in airway cells, the detected volume changes will even underestimate the impact of CFTR on osmotic water permeability because of the volume regulatory decrease that is augmented by activation of CFTR $\text{Cl}^-$ conductance (9). A mutation in the first nucleotide-binding fold of CFTR (G551D-CFTR) abolished the effects of IBMX on $P_f$ (Fig. 6B), confirming impaired regulation of hAQP3 by mutant forms of CFTR.

Only CHO Cells Coexpressing Both CFTR and Rat AQP3 Demonstrate cAMP-activated $[^{14}\text{C}]$Glycerol Uptake—The above results obtained in Xenopus oocytes have been verified in a mammalian expression system by coexpressing rAQP3 and wtCFTR or $\Delta F508$-CFTR, respectively, in CHO cells. $[^{14}\text{C}]$Glycerol uptake was measured before and after stimulation with forskolin and IBMX. As shown in Fig. 7, control cells or cells expressing only wtCFTR or $\Delta F508$-CFTR demonstrated base-line $[^{14}\text{C}]$glycerol uptake, which was not influenced by stimulation with forskolin and IBMX. Control, $\Delta F508$-CFTR, and wtCFTR-expressing CHO cells were transfected with rAQP3, and expression was detected by RT-PCR analysis (data not shown). Additional expression of rAQP3 enhanced base-line...
activation of CFTR Cl−, osmotic swelling, cells were allowed to recover in isotonic bath and were then exposed to forskolin/IBMX, which led to cell shrinkage due to measured by gravimetric techniques (9). Hypotonic (Hypo) volume changes in water-injected oocytes were independent of the presence of IBMX (open circles; n = 9). Hypotonic cell swelling was augmented by IBMX in AQP3/CFTR-coexpressing oocytes (closed circles; n = 8). After initial osmotic swelling, cells were allowed to recover in isotonic bath and were then exposed to forskolin/IBMX, which led to cell shrinkage due to activation of CFTR Cl− conductance and Cl− exit. B, the calculated osmotic water permeability coefficient (Pf) in Xenopus oocytes was larger after stimulation with IBMX due to CFTR-dependent activation of the endogenous water channel in Xenopus oocytes (9). Pf was increased in AQP3/CFTR-coexpressing oocytes and was further enhanced by IBMX, whereas oocytes coexpressing AQP3 and G551D-CFTR did not show an increase in Pf upon stimulation by IBMX. C, the osmotic glycerol permeability coefficient (Pf,g) (9) was enhanced by IBMX in CFTR-expressing oocytes. In hAQP3/wtCFTR-coexpressing oocytes, base-line Pf,g was enhanced and was further increased by IBMX. Data are means ± S.E. (n). Asterisks indicate significant difference from control (p < 0.05).

**FIG. 6.** wtCFTR activates human AQP3 in *Xenopus* oocytes. A, shown is the volume increase in oocytes under hypotonic conditions as measured by gravimetric techniques (9). Hypotonic (Hypo) volume changes in water-injected oocytes were independent of the presence of IBMX (open circles; n = 9). Hypotonic cell swelling was augmented by IBMX in AQP3/CFTR-coexpressing oocytes (closed circles; n = 8). After initial osmotic swelling, cells were allowed to recover in isotonic bath and were then exposed to forskolin/IBMX, which led to cell shrinkage due to activation of CFTR Cl− conductance and Cl− exit. B, the calculated osmotic water permeability coefficient (Pf) in Xenopus oocytes was larger after stimulation with IBMX due to CFTR-dependent activation of the endogenous water channel in Xenopus oocytes (9). Pf was increased in AQP3/CFTR-coexpressing oocytes and was further enhanced by IBMX, whereas oocytes coexpressing AQP3 and G551D-CFTR did not show an increase in Pf upon stimulation by IBMX. C, the osmotic glycerol permeability coefficient (Pf,g) (9) was enhanced by IBMX in CFTR-expressing oocytes. In hAQP3/wtCFTR-coexpressing oocytes, base-line Pf,g was enhanced and was further increased by IBMX. Data are means ± S.E. (n). Asterisks indicate significant difference from control (p < 0.05).

**FIG. 7.** wtCFTR activates rAQP3 in CHO cells. The variable endogenous [14C]glycerol uptake in CHO cells (control) and in CHO cells expressing either wtCFTR or ΔF508-CFTR remained unchanged upon stimulation by IBMX and forskolin. Additional expression of rAQP3 resulted in a significant increase in [14C]glycerol uptake in all three cell lines. When stimulated by IBMX and forskolin, only cells coexpressing wtCFTR and rAQP3 demonstrated a further increase in glycerol uptake, indicating the requirement of both wtCFTR and rAQP3. The CFTR-dependent increase in [14C]glycerol uptake was inhibited by HgCl2 (5 μmol/liter). Data are means ± S.E. (n). Asterisks indicate significant difference from control (p < 0.05).

glycerol uptake in all three cell lines to differences. Control and ΔF508-CFTR-expressing cells did not further enhance glycerol uptake upon exposure to IBMX and forskolin, whereas it was significantly increased in wtCFTR-expressing cells (Fig. 7). Both the basal [14C]glycerol uptake caused by expression of rAQP3 and the uptake caused by activation of wtCFTR were inhibited by HgCl2 (5 μmol/liter). These experiments clearly indicate CFTR-dependent activation of hAQP3 when both proteins are expressed heterologously in CHO cells.

**DISCUSSION**

The present data show compelling evidence for a novel type of CFTR-dependent regulation of yet another epithelial membrane. They demonstrate that CFTR, when stimulated by protein kinase A, activates a water permeability in respiratory epithelial cells. These results suggest coupling between Cl− transport as performed by the CFTR Cl− channel and water transport, thereby adjusting water permeability to electrolyte transport in the airway epithelium. In that sense, this mechanism would help to secure proper hydration of the surface fluid as well as mucociliary clearance (21, 22).

Several different types of aquaporins are expressed in the airways, including AQP1, AQP3, AQP4, and AQP5 (23). AQP expression in the lung shows a complex pattern suggesting functional specialization of the different AQPs, which seems to be required in the postnatal period as well as in the adult lung (23). AQP3 is expressed primarily in basolateral membranes of acinar cells forming the submucosal glands (24). These glands are regarded as the predominant site for electrolyte secretion (24–26). On the other hand, AQP3 is expressed abundantly in nonpolarized basal cells of the trachea and in basolateral membranes of nasal surface epithelial cells (23, 24) involved in reabsorption of electrolytes (6, 21). In this study, expression of AQP3 and its CFTR-dependent regulation were detected in cultured airway epithelial cells, which demonstrate features of cell differentiation such as expression of amiloride-sensitive Na+ currents (10, 27). However, expression of AQP3 does not seem to be limited to the basolateral membranes of these cells, thereby reflecting the properties of basal cells rather than those of surface cells. Within the respiratory tract, CFTR is expressed predominantly in apical membranes of submucosal gland cells and, to a lesser degree, also in those of surface epithelial cells. CFTR Cl− currents can also be detected in basal cells (28), but there is no evidence for CFTR expression in basolateral membranes of either cell type (29). This suggests that, except for the basal cells, CFTR and AQP3 are expressed on the different poles of airway cells. We may therefore currently only speculate in what cell type of the highly differentiated respiratory tract the CFTR-AQP3 interaction actually takes place. Further studies have to clarify whether AQP3 contributes to both airway absorption and secretion and how it participates in formation of the airway surface liquid, which is
essential for proper mucociliary clearance. CFTR-dependent activation of AQP3 does not take place in airway cells carrying the CF defect or in cells overexpressing both AQP3 and mutant forms of CFTR. We may therefore speculate that regulation of AQP3 by CFTR is impaired in the respiratory tract of CF patients, which may contribute to the imbalance between secretion and absorption detected in CF airways (6, 26, 30).

Activation of AQP3 by CFTR is yet another example for CFTR-dependent regulation of other epithelial membrane conductances. Several other ion conductances have been reported to be regulated by CFTR, like the epithelial Na\(^+\) conductance and intermediate conductance Cl\(^-\) channel (outwardly rectified Cl\(^-\) channel and intermediate conductance outwardly rectifying chloride channel) (1, 4, 31). Furthermore, the affinity of K\(^+\) channels like Kir6.1 and ROMK2 for the inhibitory compound glibenclamide is enhanced by CFTR (32). The mechanisms of this regulation are currently under examination (3). They are probably examined in most detail for CFTR-dependent inhibition of the epithelial Na\(^+\) conductance and intermediate conductance Cl\(^-\) channel, probably examined in most detail for CFTR-dependent inhibition of the epithelial Na\(^+\) conductance, which was shown only recently to take place in the native respiratory tissue (7). CFTR-dependent regulation of epithelial Na\(^+\) conductance depends on the presence of Cl\(^-\) ions and the existence of an intact first nucleotide-binding domain of CFTR. It takes place even in isolated membrane patches, suggesting a rather direct interaction with other membrane proteins, thereby emphasizing the new example to the growing list of CFTR-dependent interactions with other membrane proteins, thereby emphasizing the function of CFTR as a conductance regulator. The results may also help to understand the complex pattern of the common disease cystic fibrosis.

Acknowledgments—We gratefully acknowledge the expert technical assistance of H. Schauer. We thank Drs. X.-B. Chang and D. C. Gruenert for providing the various CHO cell lines and the airway epithelial cells, respectively. We thank S. Ricken for technical advice. We are also grateful to Prof. Dr. A. Schmitt-Graeff and B. Weinhold for support in the immunocytocchemistry study.

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