Defective regulation of gap junctional coupling in cystic fibrosis pancreatic duct cells

Marc Chanson, Isabelle Scerri, and Susanne Suter

Laboratory of Clinical Investigation III, Department of Pediatrics, University of Geneva, 1211 Geneva 14, Switzerland

Address correspondence to: Marc Chanson, Laboratory of Clinical Investigation III, Department of Pediatrics, University Hospital, PO Box 14, Micheli-du Crest 24, 1211 Geneva 14, Switzerland. Phone: 41-22-37-24-609; Fax: 41-22-37-24-088; E-mail: Marc.Chanson@hcuge.ch.

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The cystic fibrosis (CF) gene encodes a cAMP-gated Cl− channel (cystic fibrosis transmembrane conductance regulator [CFTR]) that mediates fluid transport across the luminal surfaces of a variety of epithelial cells. We have previously shown that gap junctional communication and Cl− secretion were concurrently regulated by cAMP in cells expressing CFTR. To determine whether intercellular communication and CFTR-dependent secretion are related, we have compared gap junctional coupling in a human pancreatic cell line harboring the ΔF508 mutation in CFTR and in the same cell line in which the defect was corrected by transfection with wild-type CFTR. Both cell lines expressed connexin45 (Cx45), as evidenced by RT-PCR, immunocytochemistry, and dual patch-clamp recording. Exposure to agents that elevate intracellular cAMP or specifically activate protein kinase A evoked Cl− currents and markedly increased junctional conductance of CFTR-expressing pairs, but not in the parental cells. The latter effect, which was caused by an increase in single-channel activity but not in unitary conductance of Cx45 channels, was not prevented by exposing CFTR-expressing cells to a Cl− channel blocker. We conclude that expression of functional CFTR restored the cAMP-dependent regulation of junctional conductance in CF cells. Direct intercellular communication coordinates multicellular activity in tissues that are major targets of CF manifestations. Consequently, defective regulation of gap junction channels may contribute to the altered functions of tissues affected in CF.


**Introduction**

Cystic fibrosis (CF), the most common autosomal recessive disease among Caucasians, is characterized by severely altered functions of adsorbing and secreting epithelia, including chronic suppurative lung disease, pancreatic fibrosis that usually leads to exocrine pancreatic failure, and high concentrations of electrolytes in sweat (1). Although the genetic defect responsible for CF is caused by mutations of the gene coding for cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-activated, nucleotide triphosphate-regulated Cl− channel in the apical membrane of epithelial cells (2–5), the pleiotropic manifestations of CF remain unclear.

Evidence is accumulating that failure of the apical Cl− channel is not solely responsible for the complex manifestations of the disease. Among the various roles of CFTR within the epithelial cells, it has been demonstrated that the anionic conductance also acts as a regulator of other membrane channels. Indeed, outward rectifying Cl− channels (ORCC) cannot be activated by cAMP in CF airway cells, an anomaly that can be corrected by expression of wild-type CFTR (6–8). Later studies have demonstrated that CFTR also modulates the CFTR-dependent regulation of amiloride-sensitive Na+ channels (ENaC) and activation of K+ channels in various epithelial cell types (9–12). More recently, it has been reported that regulation of intestinal HCO3− is impaired in CF mice (13, 14). The mechanisms by which CFTR coordinates the regulation of all of these independent ion transporters remain, however, a matter of debate.

Experimental approaches aimed at correcting the CF defect by expression of wild-type CFTR have revealed that correction of as few as 6–20% of CF cells is sufficient to restore their normal fluid transport properties (15, 16). It has been hypothesized that ionic coupling via gap junctions may serve as the mechanism for amplification of the functional effects of the corrected cells (15). Gap junctions, by mediating the intercellular diffusion of second messengers and small metabolites, indeed provide a pathway to coordinate multicellular activity. In keeping with this view, several studies have shown that agents that activate or inhibit CFTR activity in various epithelial cells also alter their capability for intercellular communication (17–19). Conversely, disruption of gap junctional communication has been associated with altered functions in tissues in which manifestations of CF occur (20–23). However, whether epithelial cells devoid of functional CFTR exhibit abnormal intercellular communication is not known.

To address this question, we have studied basal and cAMP-stimulated Cl− and gap junctional currents in a human pancreatic cell line (CFPAC-1) harboring the ΔF508 mutation of CFTR and have compared responses in the same cell line transfected with wild-type CFTR cDNA (24, 25). We show in this study that expression of functional CFTR correlates with a reduction in the extent of cell-cell coupling and restores the normal cAMP-dependent regulation of gap junctional coupling.
Methods

Cell culture. SKHep1, T84, and CFPAC-1 cells were purchased from the American Type Culture Collection (Rockville, Maryland, USA); PLJ-CFTR cells, a clone of CFPAC-1 cells stably transfected with wild-type CFTR (25), were provided by M. Fanjul (Université Paul Sabatier, Toulouse, France) with the permission of R.A. Frizzell (University of Pittsburgh, Pittsburgh, Pennsylvania, USA). SKHep1 cells were maintained in DMEM, T84 cells were maintained in 1:1 (vol/vol) DMEM/F-12 medium, and CFPAC-1 and PLJ-CFTR cells were maintained in RPMI-1640 medium. All media were supplemented with 10% FCS, 30 U/mL penicillin, and 30 μg/mL streptomycin. PLJ-CFTR cells were continuously selected in the presence of 1 mg/mL neomycin, as reported previously (25).

RNA isolation and RT-PCR. Cellular mRNA was isolated from cell lines using oligo-dT columns (Pharmacia Biotech, Dübendorf, Switzerland), according to the manufacturer’s instructions. Reverse transcription was carried out using random hexamers, and the resulting cDNA was amplified by PCR using the following primer pairs: for connexin45 (Cx45): sense 5′-GGAG-CACGGTGGAACGACGAC and antisense 5′-CCGGTTGAACTTG-GAAGCCA (predicted size: 309 bp); for CFTR: sense (exon 3) 5′-AGAATGGGATAGAGAGCTGGCTTC and antisense (exon 5) 5′-TTTACAAATTGTTCAAGGTGTGGT (predicted size: 410 bp). After a 5-minute start at 94°C, amplification of CFTR cDNA was carried out for 35 cycles, each comprising 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C, using an UNOII PCR cycler (Biometra GmbH, Göttingen, Germany). After the last cycle, an elongation step of 5 minutes at 72°C was performed. To detect connexin mRNAs, 2 rounds of PCR amplification with 31 cycles were used (26). Amplified DNA fragments were separated in a 2% agarose gel and viewed after ethidium bromide staining. No products were amplified in the absence of reverse transcriptase (not shown).

Immunofluorescence. For immunofluorescent labeling, cell lines were cultured on glass coverslips and fixed for 2–3 minutes with methanol at -20°C. The coverslips were rinsed and incubated successively with 0.2% Triton X-100 for 1 hour, 0.5 M NH₄Cl for 15 minutes, and PBS supplemented with 2% BSA for another 30 minutes. Cells were then rinsed and incubated overnight with polyclonal antibodies (diluted 1:100) raised against Cx45. The Cx45 antibodies were provided by N.J. Severs (Imperial College School of Medicine at National Heart & Lung Institute, London, United Kingdom; ref. 27). After rinsing, the coverslips were incubated with secondary antibodies conjugated to FITC for 4 hours. Coverslips were examined using a Zeiss Axiohot photomicroscope (Carl Zeiss, Oberkochen, Germany) equipped for fluorescence, and immunostained cells were scanned with a high-sensitivity Photonic Science Coolview camera (Carl Zeiss) connected to a 486DX2/66 Intel PC. Images were captured using the software package Image Access 2.04 (Imagic, Zurich, Switzerland), processed using Adobe Photoshop 3.0.5 (Adobe Systems Inc., Mountain View, California, USA), and printed with a digital Pictography 4000 printer (Fuji Film, Tokyo, Japan).

CFTR-dependent Cl⁻ currents. CFTR-dependent Cl⁻ currents were measured using the whole-cell configuration of the patch-clamp technique. Cells were cultured on Petri dishes, transferred to the stage of an inverted TMD300 microscope (Nikon AG, Kusnacht, Switzerland), and attached to a three-line perfusion system. Patch electrodes (2–6 MΩ) were filled with a solution containing (in mM) 1 NaCl, 138 KCl, 2.9 CaCl₂, 5.5 EGTA, 3 MgATP, and 0.1 GTP, buffered to pH 7.4 with 10 mM HEPES-NaOH, and 2.5 glucose, buffered to pH 7.4 with 12.5 mM HEPES-NaOH; 50 mM sucrose was added to bath solutions to prevent swelling-activated Cl⁻ currents (8). Junction potentials were nulled immediately before seal formation. After the establishment of the whole-cell configuration, cells were held at -70 mV (the reversal potential for K⁺) and depolarized to +20 mV (the reversal potential for Cl⁻) for 200 milliseconds. A mixture of 500 μM 8-bromo-cAMP (8-Brc-AMP), 500 μM 8-(4-chlorophenylthio)-cAMP (CPT-cAMP), and 10 μM forskolin was then added to the external solution. Currents were stimulated and recorded routinely for up to 15 minutes. Thereafter, this bath solution was supplemented with...
200–500 μM diphenylamine carboxylic acid (DPC), a Cl− channel blocker (28). Forskolin and DPC were maintained as stock solutions in DMSO and 100% ethanol, respectively. These agents were added to the bath solution at the appropriate final concentrations. All currents and voltage signals were acquired at a 2-kHz sampling rate using Pulse software connected to an EPC-9 patch-clamp amplifier (Heka Elektronik, Lambrecht, Germany), and stored on the hard disk of a Power Macintosh computer (Apple Computer, Inc, Cupertino, California, USA). For off-line analysis of the data, the average membrane current recorded at ~70 mV was calculated for each sweep and expressed as a function of time. Final displays of the traces were generated using the IGOR software (WaveMetrics Inc., Lake Oswego, Oregon, USA).

**Cell-coupling measurements.** For dye coupling studies, 1 cell within a cluster was impaled with thin-tip microelectrodes filled with a 4% Lucifer yellow (Sigma Chemical Co., St. Louis, Missouri, USA) solution prepared in 150 mM LiCl and buffered to pH 7.2 with 10 mM HEPES-KOH. The tracer was allowed to fill the cells by simple diffusion for 3 minutes. At the end of the injection, the electrode was removed and the number of fluorescent cells was counted.

For electrical coupling studies, the dual whole-cell patch-clamp approach was applied on pairs of cells to monitor gap junctional conductance. Both cells of a pair were voltage clamped at a common holding potential of 0 mV using the EPC-9 amplifier and a PC-501A amplifier (Warner Instrument Corp., Hamden, Connecticut, USA). To measure junctional currents (Ij), transjunctional potential differences (Vj) were elicited by changing the holding potential of 1 member of a cell pair. I was defined as the current recorded in the cell kept at a 0 mV. Junctional conductance (gj) was then calculated by gj = Ij/Vj. Series resistance was not compensated and was less than 2% of the combined junctional and cell input resistances. Cell pairs exhibiting junctional currents that could be measured while applying Vj of 10 mV were defined as coupled. In the remainders, or in pairs in which g was pharmacologically reduced with the gap junction blocker halothane (29), gating of single gap junction channels could be detected with Vj of 35–85 mV. Digitized current traces were filtered at 0.1–1.0 kHz for analysis and display of single-channel currents that could be blocked by 2 successive applications of 200 μM DPC (n = 6), the cAMP cocktail (cAMP) had no effect on membrane currents of CFPAC-1 cells. Note a small leakage current that developed with time in both traces. Bars indicate the duration of the drugs’ superfusion. The bath solution is renewed every minute. Dashed lines indicate the zero current level.

**Figure 2**
Differential activation of Cl− currents by cAMP in CFPAC-1 and PLJ-CFTR cell pairs. Examples of membrane currents recorded from a CFPAC-1 (a) and PLJ-CFTR (b) cell pair. Whereas exposure of PLJ-CFTR cells to 10 μM forskolin, 500 μM 8-Br-cAMP, and 500 μM CPT-cAMP reduced inward currents that could be blocked by 2 successive applications of 200 μM DPC (n = 6), the cAMP cocktail (cAMP) had no effect on membrane currents of CFPAC-1 cells. Note a small leakage current that developed with time in both traces. Bars indicate the duration of the drugs’ superfusion. The bath solution is renewed every minute. Dashed lines indicate the zero current level.

**Figure 3**
Differential effects of cAMP on junctional conductance of CFPAC-1 and PLJ-CFTR cell pairs. Examples of junctional conductances evaluated from a CFPAC-1 (a) and a PLJ-CFTR cell pair (b). Whereas exposure of PLJ-CFTR cells to 10 μM forskolin, 500 μM 8Br-cAMP, and 500 μM CPT-cAMP increased their electrical coupling in a reversible manner, the cAMP cocktail (cAMP) was without effect on junctional conductance of the CFPAC-1 cell pair. Bars indicate the duration of drugs superfusion. Dashed lines indicate the zero junctional conductance level.
Effect of cAMP on CFTR-dependent Cl– currents in CF and CFTR-corrected cells. cAMP-dependent Cl– currents were searched for in single CFPAC-1 cells under whole-cell patch-clamp conditions. As expected, exposure of CFPAC-1 cells to agents that elevate the intracellular cAMP concentration, a mixture of 500 μM 8-Br-cAMP, 500 μM CPT-cAMP, and 10 μM forskolin (hereafter referred to as the cAMP cocktail), did not evoke Cl– currents (Figure 2a) in 87.5% of the cells studied (Table 1). Only in 1 CFPAC-1 cell was a small cAMP-activated inward current observed. The expression of wild-type CFTR in CFPAC-1 cells has been shown to restore cAMP-dependent activation of Cl– currents (10, 25). As shown in Figure 2b and Table 1, the cAMP cocktail evoked inward currents in 6 (67%) of 9 PLJ-CFTR cells studied. These currents were ascertained to be carried by Cl–, as they were blocked by DPC (Figure 2b), a Cl– channel blocker (28).

Effects of cAMP on gap junctional coupling in CF and CFTR-corrected cells. The strength of intercellular communication in CFPAC-1 and PLJ-CFTR cells was first examined by injection of Lucifer yellow (mol wt = 443), a negatively charged fluorescent dye. In most cases, however, the tracer remained restricted to the injected cells, indicating absence of dye coupling. Exposure of the cells to agents that elevate intracellular cAMP did not change the extent of dye coupling (Table 2). Similar results were obtained with positively charged tracers of smaller radius, such as propidium iodide (mol wt = 414; n = 4) and neurobiotin (mol wt = 287; n = 4) (data not shown).

Despite the absence of dye coupling, electrical coupling could be detected in about 100 pairs of CFPAC-1 and PLJ-CFTR cells. Although the magnitude of gap junctional conductance (gj) was variable in both cell lines, gj remained stable throughout the recordings (from 5 to 45 minutes) and could be blocked in a reversible manner by halothane (n = 5). To evaluate the effects of elevation of intracellular cAMP on electrical coupling, pairs of CFPAC-1 cells were exposed to the cAMP cocktail while monitoring gj as a function of time. As shown in Figure 3a and Table 1, no changes in gj were observed in 89% of the CFPAC-1 cell pairs studied. In contrast, the cAMP cocktail markedly increased gj in 8 of 12 PLJ-CFTR pairs studied (Figure 3b and Table 1). The cAMP-induced changes in gj, which represent an increase...
of 43 ± 9% (n = 8) of the initial junctional conductance, were fully reversible after removal of the cAMP cocktail from the superfusing solution (Figure 3b).

A limitation of superfusing membrane-permeant cAMP analogues is that their effective intracellular concentration cannot be controlled, and nonspecific effects on other signaling pathways thus cannot be ruled out. To specifically activate PKA, 100 μM S₃-cAMPS was directly added to the intracellular solution. In these experiments, gᵢ was evaluated 2–3 minutes after establishment of the dual whole-cell recording to allow diffusion of the PKA agonists into the cytosol (Figure 4). Under control conditions, CFPAC-1 cell pairs showed variable gᵢ values with a median at 1,820 pS, whereas lower conductances were measured in PLJ-CFTR cell pairs (median: 195 pS). In the presence of S₃-cAMPS in the intracellular solution, no change in the distribution of gᵢ values was observed in CFPAC-1 pairs (median: 1,690 pS). In contrast, PLJ-CFTR cell pairs showed increased (P < 0.05) gᵢ values with a median of 2,230 pS. These observations strongly suggest that cAMP regulates gap junctional communication in PLJ-CFTR but not in parental CFPAC-1 cells. The effect of extracellular ATP (a potent autacoid agonist) on electrical coupling was also studied. No changes of gᵢ (n = 7) were observed in CFPAC-1 and PLJ-CFTR cell pairs monitored in the presence of 10 μM ATP (data not shown).

**Effects of cAMP on single gap junction channels in CF and CFTR-corrected cells.** To examine the effects of cAMP on single gap junction channel activity, large driving forces were applied to cell pairs exhibiting low gᵢ or to pairs in which gᵢ was reduced with halothane. As shown in Figure 5a, the activity of single gap junction channels was discriminated from that of other channels as steplike changes of opposite polarities but identical amplitudes recorded simultaneously in both current traces. The single-channel conductances (γᵢ) of these unitary events were measured, and frequency histograms were constructed. As shown in Figure 5b (top), no difference was observed in the distribution of γᵢ values between CFPAC-1 and PLJ-CFTR cell pairs. Both cell lines expressed a single population of gap junction channels that could be described by Gaussian relations with peak values at 30.8 ± 0.1 pS for CFPAC-1 cells and 33.2 ± 0.1 pS for PLJ-CFTR cells. To determine whether these distributions were changed by an elevation in intracellular cAMP, γᵢ activity was studied in cell pairs dialyzed with S₃-cAMPS. Figure 5b (bottom) shows that PKA agonists did not change the distribution of γᵢ values measured in either CFPAC-1 or PLJ-CFTR cell pairs. Indeed peak values of 31.1 ± 0.3 pS and 29.1 ± 0.1 pS, which are not different from control values, were respectively measured in both cell lines.

In an attempt to explore the mechanisms that underlie the increase in sensitivity to cAMP of gᵢ, we studied the effect of the cAMP cocktail in PLJ-CFTR cell pairs exposed to the Cl⁻ channel blocker DPC. Although DPC (200–500 μM) reduced gᵢ by 49 ± 9% (n = 10), this effect was apparently unrelated to the expression of CFTR, as it could be observed in both CFPAC-1 and PLJ-CFTR cells. In the continuous presence of DPC, the cAMP cocktail was still able to increase gᵢ by 31 ± 8% in 3 of 6 PLJ-CFTR cell pairs. In addition, the cAMP cocktail markedly increased the activity of single gap junction channels in 4 other pairs. As shown in Figure 6, few channel openings could be recorded in a PLJ-CFTR cell pair exposed to 500 μM DPC. When the cAMP cocktail was added to the superfusing solution, single-channel activity increased, as indicated by frequent current transitions from the closed state.
exhibited Cl⁻ currents in response to cAMP (25). In symmetrical Cl⁻ conditions, we found that 67% of the PLJ-CFTR cell population showed inward currents in response to cAMP that could be inhibited by the Cl⁻ channel blocker DPC. The functional expression of CFTR in CFPAC-1 cells has also been associated with cAMP-dependent restoration of membrane recycling and activation of K⁺ currents (10, 31). Therefore, the CFPAC-1/PLJ-CFTR cells appear to represent a valid model to study the relationship between CFTR expression and modulation of gap junction–mediated intercellular communication.

Gap junction channels are composed of 12 subunits called connexins, which are members of a large family of homologous proteins (32, 33). Each gap junction channel type is characterized by intrinsic properties and differential sensitivities to modulation by intracellular signaling pathways (21, 26, 34, 35). Although modulation of cell-cell communication at the transcriptional and translational levels by cAMP has been extensively reported, some studies have documented the short-term modulation of junctional coupling during PKA activation (21, 34–37). Here we have demonstrated that cAMP increased gap junctional conductance within minutes in pairs of cells expressing wild-type CFTR. This effect, which was induced by agents that either elevate intracellular cAMP or activate PKA, was not observed in cells harboring the ΔF508 mutation in CFTR. The increase in junctional conductance was observed in 67% of the PLJ-CFTR cell pairs studied, a fraction of the cell population that is similar to that developing Cl⁻ currents in response to cAMP. The possibility that differential connexin expression underlies this difference in the cAMP effect is unlikely, as only Cx45 could be detected by RT-PCR and immunolabeling in both cell lines. Our results suggest, therefore, that expression of a functional CFTR protein in CFPAC-1 cells is associated with recovery of the cAMP-dependent regulation of Cx45-mediated gap junctional coupling.

We observed that the average strength of electrical coupling was higher between CFPAC-1 cells compared with that measured in PLJ-CFTR cells. The possibility that both cell lines express different basal levels of Cx45 cannot be ruled out. Alternatively, wild-type CFTR may depress basal cell-cell coupling. In this context, the rapidly reversible effect of cAMP on gₜ suggests that inoperative gap junction channels are present in or near the junctional membranes of PLJ-CFTR cells and that CAMP allows their activation. An increase in cell-cell coupling might reflect a change in their unitary conductances or a change in their activity. Previous studies have shown that unitary gap junction channel conductance can be regulated by phosphorylating treatments. Although Cx45 is a phosphoprotein (30), exposure to agents leading to PKA activation had no effect on the level of phosphorylation or unitary conductances of Cx45 channels expressed in SKHep1 and HeLa cells (26, 38). Although the unitary gap junctional conductances recorded in PLJ-CFTR cells were not affected, single-channel activity was markedly increased in the presence of CAMP. This suggests that the increase of electrical coupling in PLJ-CFTR cells may be caused by a change in the open probability of the Cx45 channels or by a change in the rate of assembly/degrada-

**Figure 6**
Effects of cAMP on gap junction channel activity in a PLJ-CFTR cell pair. Traces represent junctional currents recorded under stationary conditions at a transjunctional potential of 55 mV. Four successive sweeps per experimental condition are shown. In the presence of 500 μM DPC, the channels remained closed almost all the time, with brief opening events (upward current deflections) being only occasionally detected. Addition of the cAMP cocktail (DPC + cAMP) increased the number of current transitions to the first and second levels of channel openings. Single-channel activity was again decreased when cAMP was omitted from the superfusing solution. Multiple levels of channel activity were rapidly detected as soon as DPC was washed out (Reversibility). Dashed lines represent the zero junctional current level.

Discussion
Our results describe the strength of gap junctional communication in a CF cell line and in the same cell line in which the defect was corrected by functional expression of CFTR. Whereas no Cl⁻ currents and no changes in gₜ were observed in CF cells exposed to agents elevating intracellular cAMP concentration, the expression of CFTR was found to restore cAMP-dependent activation of both non-junctional and junctional conductances.

The CFPAC-1 cells, which derive from a pancreatic duct adenocarcinoma of a patient with CF, express low levels of ΔF508 CFTR mRNA and thereby failed to generate Cl⁻ currents in response to cAMP (24). In contrast, PLJ-CFTR cells, a clone of CFPAC-1 cells transfected with a retroviral vector containing the wild-type CFTR cDNA,
tion of connexons at gap junctional membranes. In any case, the latter effect appeared to depend on the presence of functional CFTR in the cell membrane.

The links between the expression of a CFTR protein and the modulation of gap junction channels are not known. A large body of evidence indicates that CFTR acts as a regulator of transcellular ion transport in epithelial cells (6–14). For example, CFTR has been shown to upregulate the activity of ORCC channels and to downregulate that of ENaC channels (6, 39). Hypotheses trying to explain the mechanisms by which CFTR regulates the activity of other ion channels include direct protein-protein interactions and/or the transport of ATP out of the cells, which would in turn activate purinergic membrane receptors (9, 40–42). Direct interactions between CFTR and gap junction channels appear unlikely because of their differential localization in the epithelial membrane. The possibility of an autocrine mechanism was also ruled out because exposure of CFPAC-1 and PLJ-CFTR cells to ATP did not change their extent of electrical coupling. Since blockade of ion fluxes through CFTR channels with DPC did not prevent the modulation of g\textsubscript{i} by cAMP, it is conceivable that CFTR may associate with an intracellular factor that is necessary for basal and/or CAMP-dependent regulation of gap junction channels. Gap junctions are dynamic structures with short half-lives. Increasing evidence indicates that modifications of connexins, such as phosphorylation, dephosphorylation, and ubiquitination, may facilitate their incorporation to and/or retrieval from the membrane (43–47). Whether abnormal targeting of connexins for assembly/degradation is responsible for the differential regulation of gap junctional communication by CF cells remains to be investigated.

The pathophysiological consequences of CFTR as a regulator of intercellular communication in epithelial cells are unclear. Numerous studies have documented that perturbation of gap junctional communication in tissues known to be sites of CF manifestations is associated with the dysfunction of epithelial cells. For example, blockade of gap junctional communication alters the synchronized ciliary beating of tracheal cells and enhances the basal release of digestive enzymes by pancreatic acinar cells (23, 48). The CFPAC-1/PLJ-CFTR lines are known to have retained typical features of the native cells from which they derived (24, 25). Our finding that expression of CFTR is necessary for regulation of junctional conductance strongly suggests that cell-cell coupling is involved in the coordination of fluid secretion by the pancreatic duct epithelium. Consequently, the defective regulation of intercellular communication in human CF duct cells may contribute to certain clinical manifestations of the disease. Future studies should determine whether defect in gap junctional coupling regulation is a common feature in CF epithelia.

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