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Cystic fibrosis gene expression is not correlated with rectifying Cl^- channels

(cDNA polymerase chain reaction/patch-clamp recording/cystic fibrosis transmembrane conductance regulator)

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ABSTRACT **Cystic fibrosis (CF) involves a profound reduction of Cl^- permeability in several exocrine tissues. A distinctive, outwardly rectifying, depolarization-induced Cl^- channel (ORDIC channel) has been proposed to account for the Cl^- conductance that is defective in CF. The recently identified CF gene is predicted to code for a 1480-amino acid integral membrane protein termed the CF transmembrane conductance regulator (CFTR). The CFTR shares sequence similarity with a superfamily of ATP-binding membrane transport proteins such as P-glycoprotein and STE6, but it also has features consistent with an ion channel function. It has been proposed that the CFTR might be an ORDIC channel. To determine if CFTR and ORDIC channel expression are correlated, we surveyed various cell lines for natural variation in CFTR and ORDIC channel expression. In four human epithelial cell lines (T84, CaCo2, PANC-1, and 9HTEo-/S) that encompass the full observed range of CFTR mRNA levels and ORDIC channel density we found no correlation.**

Cystic fibrosis (CF) is a recessive genetic disease that affects many exocrine tissues. The pathophysiology arises from blocked ducts and, in the lungs, susceptibility to chronic bacterial infections (1). The most consistent cellular abnormality is a marked reduction in Cl^- permeability, with consequent abnormalities in Cl^--dependent fluid secretion and salt absorption (2). More widespread defects have been documented, but their relation to the Cl^- impermeability and the operation of the CF gene product (CF transmembrane conductance regulator, CFTR) is unknown (2–4). Until recently, investigations of the ion channel basis for Cl^- impermeability have focused on a distinctive outwardly rectifying Cl^- channel widely observed in cells after patch excision and depolarization (5, 6), hence the acronym ORDIC channel for outwardly rectifying depolarization-induced Cl^- channel. Activation of ORDIC channels in cell-attached patches by agonists that elevate cAMP and in excised patches by exposure to conditions conducive to phosphorylation via cAMP-dependent protein kinase has been reported, with some groups also observing lack of activation in CF cells (7–17).

The CFTR is related to a superfamily of ATP-binding transport proteins such as P-glycoprotein and STE6, but it has some features consistent with a channel function (18). It is unknown how the CFTR regulates membrane Cl^- conductance. Two studies of CFTR-transfected cells came to opposite conclusions about the likelihood that CFTR is a channel, and they found different signatures for whole cell currents conferred by CFTR expression (19, 20). Comparison of CFTR and ORDIC channel expression among tissues can reveal if they can be dissociated. Studies of CFTR expression using blots of total RNA detected a 6.5-kilobase (kb) transcript in lung, colon, placenta, liver, parotid, pancreas, kidney, and intestine, but not brain or adrenal gland, and in primary cultures of nasal polyps and sweat gland cells, and a colonic cell line (T84), but not in cell lines of lymphoblasts or fibroblasts (18). A 6.5 kb-transcript has also been detected in HT-29 and CaCo2 colonic tumor cell lines (21, 22). Thus, CFTR mRNA is not rare in those tissues commonly associated with CF symptoms, and the levels of CFTR mRNA in the colonic tumor cell lines appear comparable to levels in native exocrine tissues. In contrast, many other epithelial cell lines have much lower CFTR mRNA levels (23).

The polymerase chain reaction (PCR) technique (24, 25) can detect levels of CFTR expression in which only a small proportion of cells contain transcripts [e.g., 1 transcript per 500–1000 cells (26)]. We have used PCR to detect CFTR expression in 20 cell lines and primary cultures (23) and have also tested each cell line for expression of ORDIC channels and cAMP-mediated conductance increases (ref. 27 and M.E.K., unpublished observations). Here we report results for 4 cell lines that encompass the full range of CFTR mRNA levels we observed.

MATERIALS AND METHODS

Cell Culture. T84, CaCo2, and PANC-1 cells were obtained from the American Type Culture Collection. 9HTEo- cells were originally transformed with origin-defective simian virus 40 as described (17). The 9HTEo-/S subclone was inadvertently selected from the parent cell line during a freeze–thaw procedure. These cells have been partially characterized (Y. Xia and J.J.W., unpublished results). For RNA isolation, cells were grown to confluency or near confluency in 75-cm^2 tissue culture flasks. T84 cells were grown in 50:50 Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 medium with 6% newborn calf serum (all from GIBCO). CaCo2 cells were grown in Eagle's minimal essential medium with Earle's balanced salt solution, 10% fetal bovine serum, and 1% nonessential amino acids; 9HTEo-/S cells were grown in the same medium without nonessential amino acids; and PANC-1 cells were grown in DMEM H21 with 10% fetal bovine serum and 2 mM glutamine (all from the University of California, San Francisco, Cell Culture Facility). For patch-clamp experiments, epithelial cells were grown on 35-mm tissue culture dishes coated with human placental collagen (Sigma). All cells were incubated at 37°C in an atmosphere of 5% CO_2/95% air.

RNA Extraction. RNA samples were derived from 5–7.5 × 10^6 cells. Total RNA was extracted by using guanidinium thiocyanate as described (28); yields (determined by measuring A_{260}) were between 100 and 600 μg of total RNA. Poly(A)$^+$ RNA was extracted from all cells by using the

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Abbreviations: CF, cystic fibrosis; ORDIC, outwardly rectifying depolarization-induced chloride; CFTR, CF transmembrane conductance regulator; DNDS, 4,4'-dinitrostilbene-2,2'-disulfonic acid.

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Fast-Track mRNA isolation kit (Invitrogen); yields were typically 15 μg of poly(A)$^+$ RNA.

Reverse Transcription. RNA [1–5 μg of total RNA or 1 μg of poly(A)$^+$ RNA] was reverse transcribed to cDNA in a 10-μl reaction volume containing 400 μM dNTP, 10 mM dithiothreitol, RNasin (Promega) at 1 unit/μl, 2 mM random hexamer, and reverse transcriptase (Seikagaku America) at 4.2 units/μl. RNA was heated to 65°C for 3 min and cooled on ice before being added to the reaction volume. Reverse transcription was allowed to proceed for 1 hr at 42°C. The reaction was stopped by heating at 95°C for 5 min.

Primers for CFTR. Three pairs of primers were synthesized for amplification of CFTR cDNA fragments. Boldface nucleotides indicate those present in the CFTR sequence; other nucleotides were added to provide restriction sites for future experiments. Primers 5/7, consisting of the sequences 5′-**ACTTTAAAGCTGTCAAGCCGTG**-3′ (sense) and 5′-**CTGTATTTTGTTTATTGCTCCAA**-3′ (antisense), were designed to generate a 627-base-pair (bp) fragment from the first base in exon 5 to the last base in exon 7. Primers 20/24 consisted of the sequences 5′-AAACTCGA**GGATCGATG**-**GTGTGTCTTGGGATTC**-3′ (sense) and 5′-AAACTG-**CAGCTAAAGCCTTGTATCTTGCACCTCTTC**-3′ (antisense) and generated a 658-bp sequence corresponding to the C terminus. Primers 10/15 consisted of the sequences 5′-AAACTC**GAGCCTTCAGAGGGTAAAATTAAGC**-3′ (sense) and 5′-AAAGGGCCCTGCAGATATCGTC**GACAT**-**AGGTGCTTGAAGAACAG**-3′ (antisense) and generated a 1488-bp sequence (nucleotides 1558–3017). The "efficiency" of the pairs of primers, based upon their ability to generate detectable signals from diluted samples or from cell lines with low levels of CFTR RNA, was 5/7 > 20/24 > 10/15.

PCR. The 10-μl reverse transcription reaction volume was used in a PCR reaction volume of 100 μl containing PCR buffer (10 mM Tris·HCl, pH 8.3/50 mM KCl/2.5 mM $MgCl_2$/0.001% gelatin), each dNTP (Pharmacia) at 250 μM, 2.5 units of *Taq* DNA polymerase (Perkin–Elmer/Cetus), and each primer at 1 μM. To prevent evaporation, 100 μl of mineral oil was layered on top of each sample. A thermocycler (Ericomp, San Diego) was set to the following cycle parameters: 0.5–1 min at 92–94°C for denaturation; 1 min at 55°C for primer annealing; and 1–2 min at 72°C for elongation. This cycle was repeated 30 times. In some experiments (see *Results*) a fresh portion of *Taq* DNA polymerase was added to the reaction tube and an additional 30 cycles were run. This generated clear signals in several transformed airway cell lines and keratinocyte cell lines that had previously appeared negative (C.L.W., unpublished results), but 9HTEo-/S cells remained negative after this treatment. (A nonmobile aggregate was formed in the reaction mixture from PANC-1 cells by this treatment. No other cell lines tested responded in this way and the problem has not been pursued.) Samples (5–10 μl) were electrophoresed in 1.5% agarose gels and visualized with ethidium bromide staining. A 123-bp ladder (Bethesda Research Laboratories) was used for size determination. Strict procedures were followed to reduce the possibility of contamination (e.g., see ref. 29).

Control Experiments. To increase confidence that our primers were amplifying authentic CFTR, *Hin*dIII and *Eco*RI (New England Biolabs) were used to cut PCR products derived from T84 RNA, yielding fragments of the appropriate mobilities expected for authentic CFTR—i.e., *Hin*dIII cut the 658-bp band generated with primers 20/24 to yield 236- and 452-bp bands, and *Eco*RI cut the band generated with primers 10/15 to give bands consistent with predicted sizes of 675 and 813 bp (data not shown). Faint PCR products of slightly different sizes than the expected bands were seen in some gels, and these were not cut. To control for the quality of RNA samples, primers were designed to generate a 523-bp fragment (nucleotides 251–773 of the coding sequence) of human β-actin cDNA (30); sequences were 5′-CATCGAG-CACGGCATCGTCA-3′ (sense) and 5′-GTCAGGCAGC-TCGTAGCTCT-3′ (antisense). To estimate the sensitivity of the PCR procedure, T84 cDNA was diluted serially 10-, 100-, and 1000-fold in water prior to amplification.

Patch-Clamp Experiments. For outside-out patch recording, pipets (2–5 MΩ in solutions listed below) were formed from very soft glass (LA16, Dagan Corp., Minneapolis) and coated with Sylgard (Dow Corning). The standard bath solution was (in mM): 150 NaCl/2.5 $CaCl_2$/2.5 $MgCl_2$/10 Hepes, pH 7.3; the standard pipet solution was (in mM): 150 CsCl/5 $MgCl_2$/10 EGTA/10 Hepes, pH 7.3. After formation of outside-out patches, channel activity was surveyed briefly at several voltages. When no ORDIC channels were observed, the patches were progressively depolarized until ORDIC channels were induced or the patch was lost. A patch was said to contain no ORDIC channels when no channel was seen after the patch was depolarized to +125 mV for at least 100 sec. ORDIC channels were identified by their outward rectification, their sudden induction after variable periods of strong depolarization, and their distinctive kinetics (31). The Cl^- selectivity of the channels was confirmed in some patches by ion replacement, and, when tested, the putative ORDIC channels were invariably blocked by the Cl^- channel blocker 4,4′-dinitrostilbene-2,2′-disulfonic acid (DNDS) (Pfaltz & Bauer). DNDS was stored as a 10 mM stock solution in standard bath solution at −20°C. It was effective at 50 μM, but mM concentrations were often used to produce marked inhibition rapidly, with subsequent dilutions if the patch was maintained.

Currents were recorded via an Axopatch 1C (Axon Instruments, Burlingame, CA) amplifier, digitized (PCM-2, Medical Systems, Greenvale, NY), and stored on videotape. Currents were also stored on an IBM hard disk by using the program pClamp (Axon Instruments). All recordings were made at 21–23°C.

RESULTS

Detection of CFTR Message with PCR. When total cellular RNA was reverse-transcribed and subjected to 30 rounds of PCR amplification, T84 cells gave strong signals with all three sets of primers and CaCo2 cells gave similar strong signals with two sets of primers (primers 5/7 not tested), while no appropriate bands were detected in the PANC-1 and 9HTEo-/S cell lines. Fig. 1*A* shows the results for total RNA obtained with CFTR primers 20/24.

When amplification began with cDNA reverse-transcribed from poly(A)$^+$ RNA, which should give about a 10-fold enrichment of target sequences (32), the T84 and CaCo2 band intensity was not enhanced, indicating that amplification of CFTR message from these cell lines had already reached plateau with total RNA as the starting material (see also Fig. 2*B*). With poly(A)$^+$ RNA, 30 cycles of amplification was sufficient to detect faint bands with all sets of primers for the PANC-1 cells, but the 9HTEo-/S cells remained negative (Fig. 1 *C* and *D*). For comparison, we found that this amplification protocol used with the 20/24 primers was sufficient to detect the low levels of CFTR mRNA that are present in the Jurkat line of lymphocytes (23). In a related study with several transformed human airway cell lines having low CFTR expression, we found that signals could be obtained in otherwise negative cell lines when 30 cycles of amplification with the more efficient 5/7 primers was followed by the addition of fresh *Taq* DNA polymerase and another round of 30 cycles, for a total of 60 cycles. However, the 9HTEo-/S cells remained negative even with this procedure, with only nonspecific bands being generated (data not shown). PCR results are summarized in Table 1.

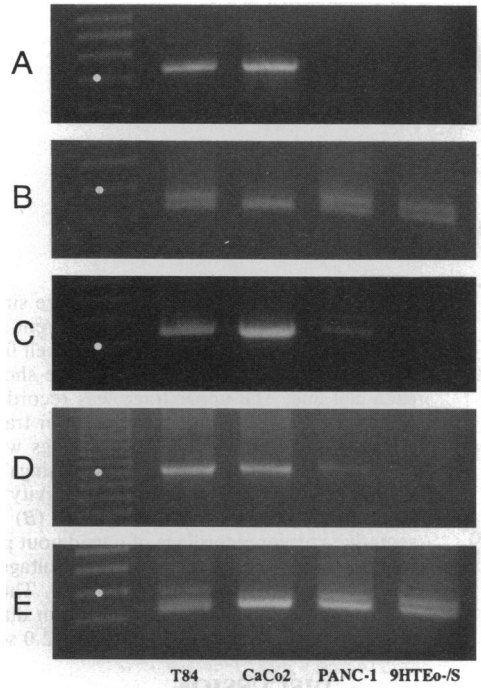

FIG. 1. Expression of CFTR mRNA in four cell lines assessed with PCR, gel electrophoresis, and ethidium bromide staining. Leftmost lane: 123-bp ladder size markers. White dot indicates a 1476-bp marker in *D*, a 615-bp marker in all other gels. (*A*) Thirty cycles of amplification of cDNA from total RNA using CFTR primers 20/24. (*B*) Identical amplification conditions, used with primers to β-actin. (*C*) Thirty cycles of amplification beginning with cDNA reverse-transcribed from poly(A)$^+$ RNA and CFTR primers 20/24. (*D*) Same as *C*, but now with CFTR primers 10/15. (*E*) Identical amplification conditions as *D*, but with primers to β-actin.

The integrity of the reverse-transcribed samples was confirmed by amplifying a β-actin sequence from both total and poly(A)$^+$ RNA. As shown in Fig. 1 *B* and *E*, all four cell lines gave positive signals for both total and poly(A)$^+$ RNA samples. Additional amplification and the enhanced resolution possible with Southern blotting would likely reveal some level of CFTR expression in the 9HTEo-/S cell line (cf. ref. 26), but for the purposes of this paper it is sufficient to rank the cells ordinally with regard to CFTR mRNA levels, thus T84 ≈ CaCo2 > PANC-1 > 9HTEo-/S, and to estimate the total range of mRNA levels.

Two methods were used to estimate the range and sensitivity of our PCR protocols. When diluted samples of reverse-transcribed T84 poly(A)$^+$ RNA were run for 30 cycles, appropriate bands were still detected at 1000-fold dilutions (Fig. 2*A*). As an additional method of estimating colonic cell mRNA levels, aliquots were removed from the colonic cell reaction mixture after 15, 20, 25, 30, and 35 cycles. As shown in Fig. 2*B*, a clear band was seen in both colonic tumor cell lines after 20 cycles of amplification and the process reached the plateau stage by 25 cycles. In the original gels, faint bands of the appropriate size could also be seen after 15 cycles. Taken together, these results show that the four cell lines we surveyed express an extremely wide range of CFTR mRNA levels, with the colonic cells having, as a conservative estimate, 1000 times more CFTR mRNA than the 9HTEo-/S cells.

Table 1. Estimates of CFTR mRNA and ORDIC channel density in four cell lines

Cells	CFTR mRNA: Total RNA	CFTR mRNA: Poly(A)$^+$ RNA	CFTR mRNA: PCR 2 × 30	ORDIC channel density: No. of patches	ORDIC channel density: Patches with ORDIC channels	ORDIC channel density: Mean no. channels per patch
T84	++	++	++	47	39 (83%)	1.57
CaCo2	++	++	++	13	2 (15%)	0.15
PANC-1	−	+	NT	11	11 (100%)	1.83
9HTEo-/S	−	−	−	8	7 (88%)	0.89

++, Maximal band intensity (signal at plateau); +, band intensity not maximal; −, no band detected; NT, not tested.

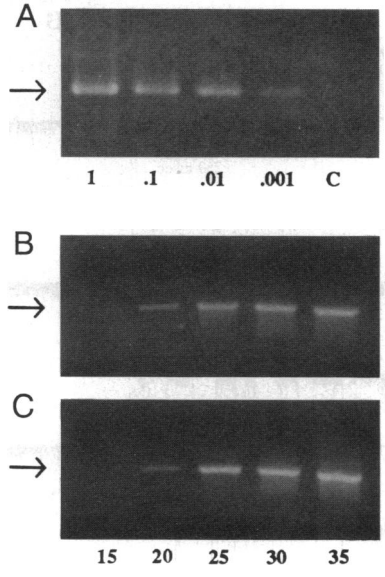

FIG. 2. (*A*) Dilution experiments with CFTR in T84 cells. Lane 1 is the PCR product derived from 1 μg of T84 poly(A)$^+$ RNA. In lanes 2–4, the cDNA in the PCR reaction mixture was progressively diluted with water 10-fold (lane 2), 100-fold (lane 3), and 1000-fold (lane 4). Lane 5 is a control with no cDNA in the reaction mixture. (*B*) Variation in signal as a function of number of cycles of amplification. Lanes 1–5 contain cDNA reverse-transcribed from 1 μg of poly(A)$^+$ RNA from T84 cells and subjected to 15, 20, 25, 30, and 35 cycles, respectively. (*C*) Same as *B* except poly(A)$^+$ RNA was obtained from CaCo2 cells. In the original gels, very faint bands were visible after 15 cycles.

Single-Channel Recordings of ORDIC Channels. Each cell line was assessed for ORDIC channels, using identical protocols. ORDIC channels were detected in all four cell types tested. For each of the cell lines, Fig. 3*A* shows representative current recordings from outside-out patches held at +50 mV and −50 mV clamp potential. The channels were silent until the patch had been depolarized—usually strongly (+100 mV or greater). The ORDIC channels in the different cell lines had similar conductances, rectification, and kinetics. The similarity among the ORDIC channels is reinforced by comparing the open-channel currents in response to ramp voltages from −100 mV to +100 mV for representative channels from each of the four cell lines (Fig. 3*B*). The properties of these channels in T84 cells and PANC-1 cells have been described in some detail (33–35). Since the channel has not previously been described in 9HTEo-/S cells, we used ion substitutions to establish that it is Cl^- selective (Fig. 4*A*) and blockable by DNDS (Fig. 4*B*). Similar experiments were not done on the channel in CaCo2 cells because of its low density (see below).

Estimates of ORDIC Channel Density. The probability of seeing at least one ORDIC channel in any patch ranged from 15% to 100% in the different cell lines (Table 1). When ranked by the percent of active patches, the order obtained was PANC-1 (100%) ≈ 9HTEo-/S (88%) ≈ T84 (83%) > CaCo2 (15%). This is a common way of estimating channel density, but since it does not discriminate between patches with single or multiple ORDIC channels, it can lead to distortions if

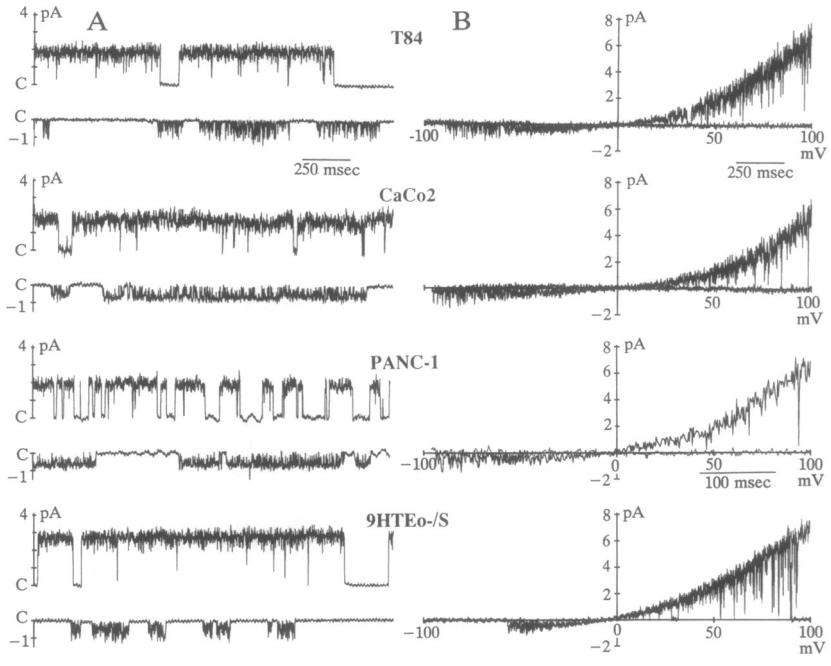

FIG. 3. ORDIC channel currents are similar in the four cell lines: Representative current recordings of outside-out patches from each cell line. (*A*) Two 1.8-sec continuous recordings are shown for each cell line. The upper trace was recorded at a clamp potential of 50 mV and the lower trace at a clamp potential of −50 mV. Recordings were selected to show several openings and closings at each voltage; considerable variability in activity is present for the channel in all cell lines. (*B*) Representative current recordings of outside-out patches from each cell line in response to a voltage ramp that went from −100 mV to +100 mV. The ramp shown for PANC-1 cells was 0.5 sec in duration; ramps shown for other cell lines were 2.0 sec.

channels are not distributed randomly. Since we commonly observed multiple ORDIC channels in PANC-1 and T84 cells, we also ranked the cells by the mean number of ORDIC channels per patch. The rank order for channel density estimated in this way (channels per patch) was T84 (1.9) ≈ PANC-1 (1.8) > 9HTEo-/S (0.9) > CaCo2 (0.2). Results are summarized in Table 1.

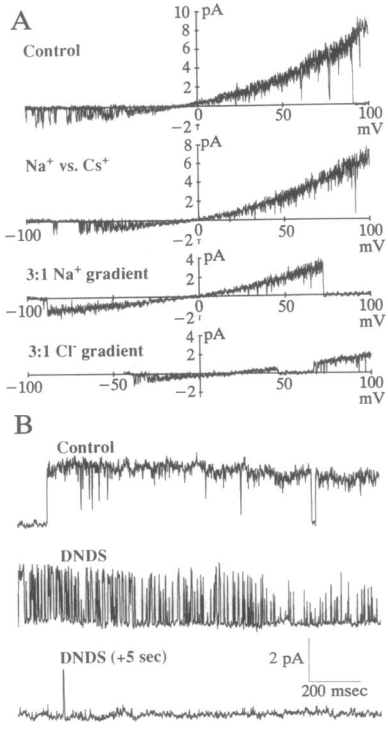

FIG. 4. Cl^- selectivity and DNDS sensitivity of the ORDIC channel in 9HTEo-/S cells. (*A*) Each trace is the current recording obtained in an outside-out patch from a 9HTEo-/S cell, in control solutions (top trace), with substitution of Cs^+ for Na^+ in the pipet (second trace), with a 3:1 Na^+ gradient (bath contained ⅓ normal Na^+) (third trace), and with a 3:1 Cl^- gradient (bath contained ⅓ normal Cl^-) (bottom trace). (*B*) DNDS block of channel in outside-out patch. Determination of k_i for DNDS inhibition of the channels was not attempted; instead a high concentration of DNDS was used to establish certain block prior to losing the patch.

DISCUSSION

Estimates of CFTR Expression and ORDIC Channel Density. Our experiments (see also ref. 23) demonstrate a wide range of CFTR mRNA levels in epithelial cell lines. The PCR technique is extremely sensitive and nonlinear, and, as used here, it dramatically compresses measurements. In spite of that, we established that the range of mRNA levels in these cells is at least three orders of magnitude, on the basis of the gain expected when poly(A)$^+$ RNA rather than total RNA is used, estimates of the additional gains with various numbers of PCR cycles, and inferences from the results of poly(A)$^+$ RNA dilution experiments. T84 and CaCo2 CFTR mRNA levels were not distinguishable, PANC-1 mRNA was about 1–5% of T84 levels, while our inability to detect a signal after 60 cycles of amplification of mRNA from 9HTEo-/S cells (with refreshment of the *Taq* DNA polymerase after 30 cycles) suggested that CFTR mRNA in 9HTEo-/S cells was less than 1% of that in PANC-1 cells. On the basis of these considerations and the reported estimates of CFTR message levels in T84 cells (18), the 9HTEo-/S cells probably express CFTR at levels considerably below 1 copy per cell. Quantitative PCR (e.g., ref. 36) will be needed to establish the full range of mRNA levels in these cells, but for our present purpose such measures are unnecessary, especially since the linkage between CFTR mRNA and CFTR protein levels is not known. Thus, cells with lower levels of mRNA might translate and process more efficiently.

In contrast to the wide range of mRNA levels, ORDIC channel density varied only 10-fold. As is clear from Table 1, estimates of ORDIC channel density and estimates of CFTR mRNA levels were not correlated. This lack of correlation can be extended to nonepithelial cells, since ORDIC channels, once thought to be relatively specific for Cl^- secretory epithelia (5), now appear to be more widespread, occurring in such disparate cells as neuroglia (37), fibroblasts (38), lymphoblasts (15), and COS cells (27). These nonepithelial cells have very low levels of CFTR message (18, 23). Thus, CFTR and ORDIC channel proteins appear to be different molecules that are not obligatorily linked.

Reevaluation of Evidence Implicating the ORDIC Channel as the "CF Channel." The hypothesis that the CFTR is an ORDIC channel arose because ORDIC channels have been implicated in cAMP-mediated Cl^- conductance increases (e.g., refs. 7–15, 17, and 38) and were reported to be defec-

tively gated in intact CF cells (8, 9, 14) and in isolated patches (10–14). However, the role of ORDIC channels in CF-affected Cl^- conductances remains controversial (4, 6, 39–42). Reasons for questioning the hypothesis that the ORDIC channel is the CF channel fall into five general categories.

(*i*) In cell-attached recordings from several kinds of epithelia known to be affected in CF, ORDIC channels are encountered only rarely after stimulation (6, 7, 40, 41), and the few apparent instances of activation could result from inadvertent excision (6), since controls to eliminate such a possibility, such as reversibility of activation, have not been reported.

(*ii*) ORDIC channel activity observed in excised patches exposed to phosphorylating conditions is difficult to interpret. Activity begins after a long and variable latency, and it is not reversible. Since ORDIC channels can be activated by many conditions (42, 43), attributing the activity to a specific antecedent manipulation is difficult. Perhaps for these reasons, activation has not been observed by all investigators (42, 44).

(*iii*) A mismatch exists between the properties of ORDIC channels and CF-affected Cl^- conductances and whole-cell currents with regard to anion selectivity, susceptibility to blockers, and rectification (6, 42, 45).

(*iv*) Alternative roles have been suggested for ORDIC channels, which appear similar to channels that are activated by cell swelling (46–48), and which also might play a role in Ca^{2+}-mediated Cl^- secretion (9, 12, 13).

(*v*) Finally, different Cl^- channels have been identified that might constitute the CF-affected Cl^- permeability pathway. Linear, small conductance Cl^- channels are candidates for mediating cAMP-dependent Cl^- secretion in pancreatic duct cells from rats and humans (40, 49, 50), in T84 cells (42), and in dog tracheal cells (41). The properties of these channels are more consistent with the macroscopic Cl^- conductances that are affected in CF (45), and activation is reversible (40, 42, 49, 50). The smaller size of these channels and their tendency to disappear upon excision or prolonged periods of seal formation may have caused them to be overlooked.

In conclusion, our results dissociate ORDIC channel expression from CFTR mRNA expression. It now seems highly unlikely that the CFTR is either an ORDIC channel or a direct regulator of ORDIC channels; the possible identity of the CFTR with the linear Cl^- channels just mentioned remains to be assessed. ORDIC channels appear to be ubiquitous and may be involved in swelling-induced or Ca^{2+}-induced Cl^- currents. Prior experiments implicating ORDIC channels as sole or major components of cAMP-mediated Cl^- secretion need to be reevaluated in light of these findings. Single-channel assays of CFTR expression systems should be able to establish if CFTR expression is correlated with any particular Cl^- channel.

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