FEBS Letters 440 (1998) 268–272 FEBS 21219

Role of CFTR and anion exchanger in bicarbonate fluxes in C127 cell lines

Teresa Mastrocola, Anna Maria Porcelli, Michela Rugolo*

Dipartimento di Biologia Ev.Sp., Università di Bologna, Via Irnerio 42, 40126 Bologna, Italy

Received 26 October 1998

Abstract C127 cell lines transfected with wtCFTR, ΔF508CFTR or vector were employed to determine HCO_3^- fluxes in the presence or absence of functional CFTR, using the pH-sensitive dye BCECF. Both cytosolic alkalinization and acidification were due to activity of anion exchanger and were similar in the three cell lines, indicating that expression of CFTR did not influence anion exchanger activity. In C127wt cells only, cAMP elevating agents significantly stimulated HCO_3^- fluxes, insensitive to the inhibitor of anion exchanger 4,4′-diisothiocyanate dihydrostilbene-2,2′-disulfonic acid, suggesting that activated CFTR directly mediates both HCO_3^- influx and efflux and therefore can contribute to intracellular and extracellular pH regulation.

Key words: Intracellular pH; HCO_3^- transport; Cystic fibrosis; Cystic fibrosis transmembrane conductance regulator; Anion exchanger

1. Introduction

Cystic fibrosis (CF) is a severe genetic disease caused by mutations in a single gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) [1]. CFTR is an epithelial Cl^- channel regulated by cAMP-dependent protein kinase A [2,3]. The most common mutation of CFTR is deletion of phenylalanine 508 (ΔF508). Proteins with this mutation remain incompletely processed in the endoplasmic reticulum and are not delivered at the plasma membrane [4]. It is not yet clear how a defect in Cl^- conductance could account for the numerous abnormalities of the CF phenotype. Recent studies suggest that CFTR acts also as a regulator of other proteins, such as Na^+ [5] and K^+ channels [6] and outwardly rectifying Cl^- channel [7,8].

Another abnormality consists in decreased HCO_3^- as well as Cl^- secretion in pancreatic ductal cells [9] and airway epithelia from CF patients [10,11]. By means of electrophysiological studies, the HCO_3^-/Cl^- permeability ratio of the CFTR was calculated to be 1:4 [12]. It is well known that the main mechanism for HCO_3^- transport across the plasma membrane is the anion exchanger [13]. The anion exchanger gene family includes the erythroid band 3 (AE1), its homologue expressed in epithelial cells (AE2) and that mainly expressed in brain neurons and heart (AE3) [14]. The anion exchangers share the electroneutrality of transport, the ion substrate specificity and the sensitivity to the disulfonic stilbene class of inhibitors [13]. Both HCO_3^- and Cl^- can be transported and the direction of transport is determined by gradients of the two anions [15]. Under physiological conditions, the inward-directed Cl^- gradient is larger than the inward-directed HCO_3^- gradient, and the anion exchanger exchanges intracellular HCO_3^- for extracellular Cl^-. When the intracellular pH (pH_i) is increased, the inward-directed HCO_3^- gradient is reduced or reversed, and this further favors efflux of HCO_3^-.

It seems therefore that both Cl^-/HCO_3^- exchanger and CFTR can mediate HCO_3^- transport. However, no data are available on the relative contribution of the two transport mechanisms on overall HCO_3^- fluxes and consequent pH_i regulation in normal and CF cells. Elgavish and Meezan [16] reported in a pancreatic ductal cell line derived from a CF patient that sulfate uptake, presumably via the anion exchanger, was 10-fold lower than that observed after transfection with wild type (wt) CFTR. Owing to this, expression of CFTR would be necessary to attain high capacity anion exchange, suggesting that CFTR might have a direct or an indirect effect on anion exchanger activity [16]. The aim of the present study was to investigate whether HCO_3^- influx and efflux were different in the absence or presence of functional CFTR. C127 mammary epithelial cell lines stably transfected with wtCFTR (C127wt), ΔF508CFTR (C127ΔF508) or vector (C127i) [17] have been employed. We report that HCO_3^- fluxes were mainly due to the anion exchanger activity, although a significant contribution by activated CFTR could be revealed in C127wt cells, when the activity of the anion exchanger was reduced.

2. Materials and methods

2.1. Materials

BCECF-AM (Molecular Probes, Eugene, OR) was dissolved in dimethylsulfoxide, H_2DIDS (Molecular Probes, Eugene, OR) in water, forskolin and IBMX (Sigma, St. Louis, MO) in ethanol.

2.2. Cell culture

C127i cells (a cell line of mouse mammary epithelial origin) expressing CFTR or CFTRΔF508 were generated by calcium phosphate-mediated transfection with a bovine papilloma virus-based vector containing CFTR cDNA under control of the mouse metallothionein MT1 promoter and neomycin resistance gene under control of another copy of the MT1 promoter [17]. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FCS) (DMEM-FCS medium) and supplemented with 200 μg/ml geneticin (Gibco) and non-essential amino acids (Sigma). Since C127wt and C127ΔF508 were stably transfected and cloned, variation of CFTR expression is unlikely, as reported in [18]. The presence of

*Corresponding author. Fax: (39) (51) 242576.
E-mail: rugolo@kaiser.alma.unibo.it

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; ΔF508, deletion of Phe^{508}; wt, wild type; IBMX, 3-isobutyl-1-methylxanthine; BCECF-AM, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein tetraacetoxymethyl ester; pH_i, intracellular pH; H_2DIDS, 4,4′-diisothiocyanate dihydrostilbene-2,2′-disulfonic acid

PII: S0014-5793(98)01468-9

functional CFTR was regularly assayed by determining the cAMP-dependent stimulation of Cl^- channels with the Cl^- indicator dye 6-methoxy-*N*-(3-sulfopropyl)quinolinium (SPQ) [19]. The homogeneity of C127wt cell population was assayed with experiments of digital imaging in single SPQ-loaded cells: all cells ($n=16$) responded to cAMP elevation (not shown).

2.3. Fluorescence recording

HCO_3^- fluxes were evaluated by following changes of pH_i in cells loaded with 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein tetraacetoxymethyl ester (BCECF-AM). Trypsinized cells (4×10^6 cells/ml) were incubated for 30 min with DMEM-FCS medium (pH 7.4) containing 4 μM BCECF-AM at 37°C, in an incubator with a humidified atmosphere of 5% CO_2 in 95% air. Cells were then washed twice with a dye-free DMEM-FCS medium, resuspended in DMEM-FCS medium and maintained in ice and in the dark until use. BCECF leakage from the cells was negligible. BCECF fluorescence was measured in the cuvette compartment (20°C) of a Perkin-Elmer LS50B Luminescence Spectrometer, with excitation and emission wavelengths of 505 nm and 530 nm, respectively. Aliquots of BCECF-loaded cells (3×10^5 cells) were centrifuged for 10 s at 7000 rpm with an Eppendorf centrifuge, resuspended in solutions containing 25 mM $NaHCO_3$, as detailed below, and transferred to a cuvette plugged with a snugly fitted teflon holder. The holder had inlet and outlet ports to permit continuous gassing of cellular suspension with a 95% O_2-5% CO_2 mixture. All saline solutions containing $NaHCO_3$ were gassed before use. HCO_3^- influx was evaluated by determining intracellular alkalinization after removal of extracellular Cl^-, by incubating the cells with Cl^--free HCO_3^--buffered Na-gluconate solution, as described in [15,20]. HCO_3^- efflux was determined by intracellular acidification. Cells were first alkalinized by incubation with HCO_3^--buffered Na-gluconate solution for 6 min, then centrifuged and resuspended in HCO_3^--buffered NaCl solution. Calibration curves of pH_i against fluorescence were generated as described in [21,22] by lysing cells with 0.1% Triton X-100 at the end of each trace and titrating the suspension by the addition of small amounts of HCl or tris(hydroxymethyl)-aminomethane, while measuring the pH with a semimicrocombination pH electrode (Radiometer, Denmark). BCECF fluorescence was linear with pH over the range between pH 6.5 and 7.9; the coefficient of linear regression ranged between 0.85 and 0.99 in all the experiments. From the traces ($n=8$–14) of each experiment, the average change of fluorescence caused by 0.1 pH unit variation was determined. The value was 41 ± 2 ($n=15$), with a coefficient of variation of 5%. This calibration procedure was used in alternation with the classical one with nigericin in high K^+ medium, because it allowed calibration of the fluorescence signal at the end of each trace. The rate of change in pH_i (ΔpH_i/min) was measured by linear regression of traces within the time intervals defined in the legends of the tables. The extent of pH_i increase or decrease was determined as the difference between the initial value and that measured at steady state.

2.4. Solutions

HCO_3^--buffered NaCl solution contained 115 mM NaCl, 3 mM KCl, 2 mM KH_2PO_4, 1 mM $MgSO_4$, 10 mM glucose, 1 mM $CaCl_2$ and 25 mM $NaHCO_3$ (pH 7.4). In Cl^--free HCO_3^--buffered Na-gluconate solution, NaCl and $CaCl_2$ were replaced with equal concentrations of Na-gluconate and Ca-gluconate.

2.5. Statistical evaluation

All values are expressed as mean ± S.D., with the number of experiments in parentheses, *P* is the level of significance in Student's *t*-test.

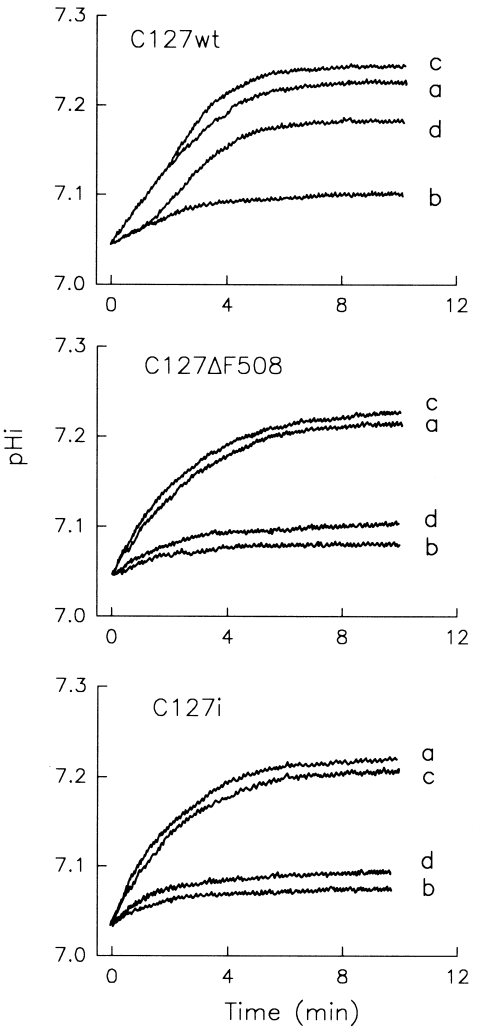

Fig. 1. Cytoplasmic alkalinization of C127 cells after extracellular Cl^- removal. BCECF loaded cells were incubated in Cl^--free HCO_3^- buffered medium containing Na-gluconate (traces a), plus 0.2 mM H_2DIDS (traces b), plus 10 μM forskolin and 0.1 mM IBMX (traces c), plus 0.2 mM H_2DIDS, 10 μM forskolin and 0.1 mM IBMX (traces d). Upper panel: C127wt cells; middle panel: ΔF508CFTR cells; lower panel: C127i cells. Traces are representative of 3–8 experiments.

3. Results

The values of pH_i determined in the three C127 cell lines incubated in HCO_3^--buffered NaCl solution, gassed with 95% O_2-5% CO_2 mixture, were not significantly different (7.03 ± 0.04, $n=8$, in C127wt, 7.02 ± 0.03, $n=5$, in C127ΔF508 and 7.05 ± 0.06, $n=7$, in C127i cells).

Table 1
Average rate of intracellular alkalinization (ΔpH/min) in C127 cell lines

Addition	C127wt	C127ΔF508	C127i
None	0.020 ± 0.004 ($n=8$)	0.018 ± 0.003 ($n=4$)	0.020 ± 0.003 ($n=4$)
Forsk+IBMX	0.035 ± 0.004 ($n=4$)	0.023 ± 0.003 ($n=3$)	0.015 ± 0.004 ($n=3$)
H_2DIDS	0.006 ± 0.001 ($n=3$)*§	0.004 ± 0.002 ($n=4$)*	0.005 ± 0.002 ($n=4$)*
Forsk+IBMX+H_2DIDS	0.028 ± 0.003 ($n=4$)§	0.005 ± 0.002 ($n=5$)	0.003 ± 0.001 ($n=4$)

BCECF-loaded cells were incubated in Cl^--free, HCO_3^--buffered Na-gluconate solution. Where indicated, 10 μM forskolin, 0.1 mM IBMX, 0.2 mM H_2DIDS was added. The basal rate of intracellular alkalinization, determined by linear regression of traces from 2 to 4 min, was not significantly different in the three cell lines. In each column, *significantly different from control (none): $P<0.02$; between values marked by §$P<0.0005$, according to Student's *t*-test.

Fig. 1, upper panel, trace a, shows the pH_i elevation in C127wt cells incubated in a Cl^--free HCO_3^--buffered medium containing gluconate. pH_i increased gradually with time and reached a steady-state value within 6 min (0.19 ± 0.06, ΔpH_i unit, $n = 8$). In the presence of 0.2 mM H_2DIDS, a specific inhibitor of the anion exchanger, only a small increase in pH_i was observed (0.03 ± 0.01 ΔpH_i, $n = 3$, shown in trace b). This finding indicates that intracellular alkalinization is mainly due to the activity of the anion exchanger, which mediates influx of extracellular HCO_3^- in exchange for internal Cl^-. Addition of 10 μM forskolin plus 0.1 mM IBMX, known to cause an elevation of cAMP concentration, did not significantly affect the extent of pH_i elevation in comparison with control (0.21 ± 0.02 ΔpH_i, $n = 4$, trace c). However, addition of cAMP elevating agents in the presence of H_2DIDS caused a significant increase of pH_i, after a lag time of approximately 2 min (0.10 ± 0.01 ΔpH_i, $n = 4$, trace d, significantly different from H_2DIDS alone, $P < 0.01$). Experiments in C127wt cells loaded with the Cl^--sensitive dye SPQ showed that stimulation of Cl^- efflux by cAMP elevating agents was apparent after a lag time of approximately 2 min (result not shown). The data illustrated in Fig. 1, upper panel, suggest that under conditions leading to inhibition of the anion exchanger, a significant HCO_3^- influx is mediated by activated CFTR.

The middle and lower panels of Fig. 1 report the results of similar experiments performed in C127ΔF508 and C127i cells, showing that in both lines the extent of alkalinization was similar (0.12 ± 0.04, $n = 4$, and 0.13 ± 0.06, $n = 4$, ΔpH_i, respectively, not significantly different from C127wt cells). The extent of pH_i increase was strongly inhibited by H_2DIDS (0.04 ± 0.02, $n = 4$, in C127ΔF508 cells, and 0.03 ± 0.02, $n = 4$, ΔpH_i, in C127i cells, shown in traces b) and was not affected by addition of cAMP elevating agents, either in the absence or in the presence of H_2DIDS (traces c and d, respectively). It seems therefore that cells lacking CFTR or expressing mutated CFTR do not increase pH_i in the presence of cAMP elevating agents, even when the activity of the anion exchanger was blocked.

Table 1 reports that the rates of pH_i elevation in the three cell lines, incubated in Cl^--free HCO_3^--buffered medium containing gluconate, were similar. Only in C127wt cells did cAMP-elevating agents cause a significant increase of the rate of alkalinization both in the absence and in the presence of H_2DIDS.

Fig. 2 shows acidification of C127 cells after an alkaline load. When C127wt cells, incubated first in a Cl^--free HCO_3^--buffered medium containing gluconate, were transferred to a HCO_3^--buffered medium containing Cl^-, pH_i slowly decreased, reaching a steady state within approximately

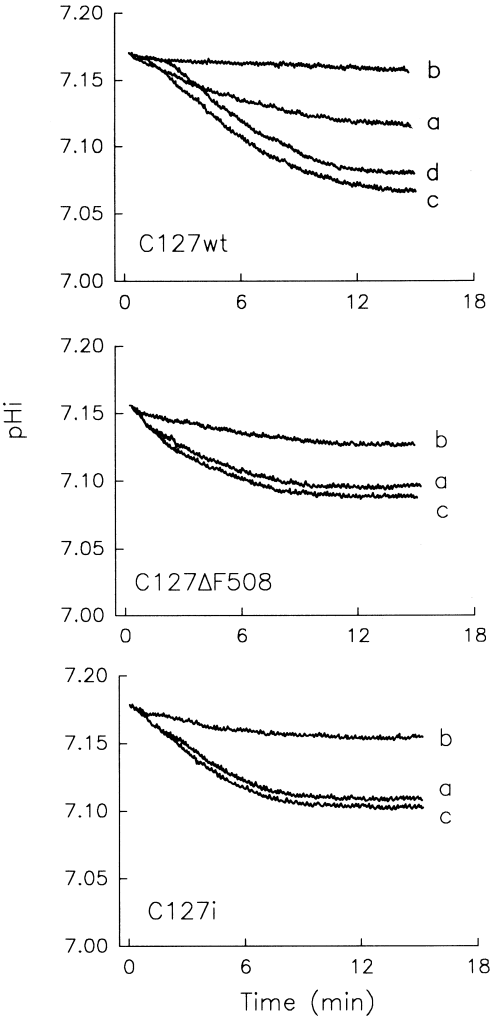

Fig. 2. Cytoplasmic acidification in C127 cells after an alkaline load. BCECF-loaded cells were incubated for 6 min in HCO_3^--buffered Na-gluconate medium, then centrifuged and resuspended in HCO_3^--buffered NaCl medium (traces a), plus 0.2 mM H_2DIDS (traces b), plus 10 μM forskolin and 0.1 mM IBMX (traces c), plus 0.2 mM H_2DIDS, 10 μM forskolin and 0.1 mM IBMX (traces d). Upper panel: C127wt cells; middle panel: ΔF508CFTR cells; lower panel: C127i cells. Traces are representative of 3–7 experiments.

8–10 min (Fig. 2, upper panel, trace a). At steady state, the extent of acidification was 0.098 ± 0.041 ΔpH unit ($n = 6$). In the presence of H_2DIDS, no pH_i recovery was determined (trace b). Addition of forskolin plus IBMX significantly stimulated the extent of pH_i acidification both in the absence and in the presence of H2DIDS (0.16 ± 0.03, $n = 6$, and

Table 2
Average rate of pH_i decrease (ΔpH/min) after an alkaline load

Addition	C127wt	C127ΔF508	C127i
None	0.008 ± 0.002 ($n = 6$)	0.009 ± 0.001 ($n = 3$)	0.009 ± 0.002 ($n = 5$)
Forsk+IBMX	0.017 ± 0.003 ($n = 8$)*	0.010 ± 0.003 ($n = 3$)	0.008 ± 0.002 ($n = 5$)
H_2DIDS	0.002 ± 0.001 ($n = 5$)*§	0.002 ± 0.002 ($n = 4$)*	0.003 ± 0.001 ($n = 5$)*
Forsk+IBMX+H_2DIDS	0.014 ± 0.002 ($n = 6$)§	0.004 ± 0.002 ($n = 4$)	0.004 ± 0.002 ($n = 4$)

BCECF-loaded cells were incubated for 6 min in Cl^--free, HCO_3^--buffered Na-gluconate solution, centrifuged and resuspended in HCO_3^--buffered NaCl solution. Where indicated, 10 μM forskolin, 0.1 mM IBMX, 0.2 mM H_2DIDS was added. The basal rate of acidification, determined by linear regression of traces from 2 to 6 min, was not significantly different in the three cell lines. In each column, *significantly different from control (none): $P < 0.01$; between values marked with §$P < 0.001$, according to Student's t-test.

0.15 ± 0.03, $n = 6$, ΔpH unit, significantly different from control, $P < 0.03$) (traces c and d of Fig. 2, upper panel). Table 2 reports that cAMP elevating agents also significantly increased the rates of pH_i acidification both in the absence and in the presence of H_2DIDS in C127wt cells.

The other two panels of Fig. 2 show the behavior of C127ΔF508 and C127i cells. As shown in traces a, the extent of pH_i acidification was similar in the two cell lines (0.072 ± 0.014, $n = 3$, ΔpH unit in C127ΔF508 cells and 0.065 ± 0.020, $n = 4$, ΔpH unit in C127i cells) and not significantly different from that obtained in C127wt cells. In the presence of H_2DIDS, pH_i acidification was negligible (traces b). Addition of forskolin plus IBMX significantly increased neither the extent nor the rate of pH_i recovery both in the absence (traces c) and in the presence of H_2DIDS (not shown). The values of these rates are shown in Table 2. It is noteworthy that the initial value of traces shown in Fig. 2 was always slightly lower (approximately 0.05 pH unit) than that obtained at the end of the alkalinization treatment, shown in Fig. 1. Furthermore, in no cell lines did pH_i return to the initial pH_i value, but a steady state was reached at approximately 0.05 pH unit higher than the initial value. The reason for this behavior is not known, but could be related to either the different anion composition of the two solutions or the slightly different treatment of cells in the two experiments.

4. Discussion

It is well known that both Cl^-/HCO_3^- exchanger and CFTR can mediated HCO_3^- transport. In this study, ion gradients have been manipulated to evaluate the relative contribution of the two transport mechanisms to both HCO_3^- influx and efflux in normal and CF cells. Removal of extracellular Cl^- resulted in cytoplasmic alkalinization due to influx of extracellular HCO_3^-, which was mediated mainly by the anion exchanger, since it was almost abolished in the presence of H_2DIDS, and was similar in the three C127 cell lines. When alkalinized C127 cells were incubated in HCO_3^--buffered NaCl medium, a significant acidification, due to HCO_3^- efflux, was observed. The Cl^-/HCO_3^- exchanger also mediated this acidification, which was similar in the three cell lines, because H_2DIDS nearly completely prevented this pH_i recovery. These results clearly indicate that the activity of the anion exchanger, assessed by both HCO_3^- influx and efflux, was not significantly different in the absence or presence of functional CFTR. It seems therefore that no functional relationship occurs between the two anion transport mechanisms. These findings are in disagreement with data previously reported [16], where the activity of the anion exchanger was measured by following sulfate fluxes. It is possible that the anion exchanger exhibits different affinity and efficacy of transport for HCO_3^- and sulfate, however, it has to be noticed that in vivo the physiological substrate of anion exchanger is HCO_3^-.

Addition of cAMP elevating agents to C127wt cells increased the rate of alkalinization but not the extent of pH_i elevation; conversely, in the presence of H_2DIDS, a significant increase of both parameters was observed. It is noteworthy that H_2DIDS was shown to have no effect on anion transport through CFTR [18,23]. In the other two cell lines, cAMP elevation failed to stimulate HCO_3^- entry. These results clearly indicate that activated CFTR allowed the influx of HCO_3^-, which was very evident when the anion exchanger was blocked, and less relevant in the presence of functional anion exchanger.

Furthermore, we show that cAMP increasing agents induced a significant stimulation of both the extent and rate of pH_i recovery after alkaline load in C127wt cells but not in C127ΔF508 and C127i cells. It has been previously reported that an increase in cAMP level can modulate the anion exchanger activity, although conflicting results have been reported [24–26]. In C127 cell lines not expressing a functional CFTR, forskolin plus IBMX significantly stimulated neither HCO_3^- influx nor efflux (shown in Figs. 1 and 2), and, furthermore, the cAMP-activated pH_i recovery in C127wt cells was not reduced by H_2DIDS, clearly indicating a direct role for CFTR in mediating HCO_3^- efflux. Since H_2DIDS alone nearly completely inhibited acidification (shown in Fig. 2, upper panel), it is apparent that in the presence of forskolin plus IBMX, HCO_3^- fluxes are forced to flow through CFTR, in a H_2DIDS-insensitive mechanism.

All together, the results presented in this study indicate that a significant stimulation of HCO_3^- fluxes occurs through CFTR under conditions leading to elevation of cAMP. The loss of HCO_3^- would tend, on one hand, to lower intracellular pH, and on the other, to increase extracellular pH in normal cells. Recently, CFTR-mediated acidification has been reported to accompany apoptosis, resulting in endonuclease activation and cleavage of DNA. In contrast, C127ΔF508 cells, which failed to acidify, did not show nuclear condensation or the typical DNA fragmentation [27]. Accordingly, the failure of cells expressing mutated CFTR to undergo apoptosis has been suggested to contribute to the pathogenesis of CF [27].

The alkalinization of extracellular fluid might also be important to pulmonary defense mechanisms [10]. In this respect, it has been reported that common CF pathogens, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, were killed when added to the apical surface of normal airway epithelia, whereas they multiply on CF epithelia [28]. It is possible to speculate that different fluid pH might be involved in pathogen proliferation, which is one of the clinical hallmarks of CF.

Acknowledgements: We thank the Genzyme Corporation, Framingham, MA, for the gift of the cell lines. This work was partially supported by a grant from Progetto Interdipartimentale 'Sonde Fluorescenti', University of Bologna.

References

[1] Riordan, J.R., Rommens, J.M., Kerem, B.S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.L., Drumm, M.L., Iannuzzi, M.C., Collins, F.S. and Tsui, L.-C. (1989) Science 245, 1066–1073.
[2] Tabcharani, J.A., Chang, X.-B., Riordan, J.R. and Hanrahan, J.W. (1991) Nature 352, 628–631.
[3] Anderson, M.P., Rich, D.P., Gregory, R.J., Smith, A.E. and Welsh, M.J. (1991) Science 251, 679–682.
[4] Welsh, M.J. and Smith, A.E. (1993) Cell 73, 1251–1254.
[5] Stutts, M.J., Canessa, C.M., Olsen, J.C., Hamrick, M., Cohn, J.A., Rossier, B.C. and Boucher, R.C. (1995) Science 269, 847–850.
[6] Mall, M., Kunzelmann, K., Hipper, A., Busch, A.E. and Greger, R. (1996) Pflügers Arch. 432, 516–522.
[7] Gabriel, S.E., Clarke, L.L., Boucher, R.C. and Stutts, M.J. (1993) Nature 363, 263–266.
[8] Schweibert, E.M., Egan, M.E., Hwang, T.-H., Fulmer, S.B., Al-

len, S.S., Cutting, G.R. and Guggino, W.B. (1995) Cell 81, 1063–1073.
[9] Kopelman, H., Corey, M., Gaskin, K., Durie, P., Weizman, Z. and Forstner, G. (1988) Gastroenterology 95, 349–355.
[10] Smith, J.J. and Welsh, M.J. (1992) J. Clin. Invest. 89, 1148–1153.
[11] Smith, J.J. and Welsh, M.J. (1993) J. Clin. Invest. 91, 1590–1597.
[12] Poulsen, J.H., Fischer, H., Illek, B. and Machen, T.E. (1994) Proc. Natl. Acad. Sci. USA 91, 5340–5344.
[13] Jennings, M.J. (1989) Annu. Rev. Biophys. Chem. 18, 397–430.
[14] Kopito, R.R. (1990) Int. Rev. Cytol. 123, 177–199.
[15] Tønnessen, T.I., Ludt, J., Sandvig, K. and Olsnes, S. (1989) J. Cell. Physiol. 132, 182–191.
[16] Elgavish, A. and Meezan, E. (1992) Am. J. Physiol. 263, C176–C186.
[17] Denning, G.M., Anderson, M.P., Amara, J.F., Marshall, J., Smith, A.E. and Welsh, M.J. (1992) Nature 358, 761–764.
[18] De Checchi, M.C., Tamanini, A., Berton, G. and Cabrini, G. (1993) J. Biol. Chem. 268, 11321–11325.
[19] Verkman, A.S. (1990) Am. J. Physiol. 259, C375–C388.
[20] Muallem, S. and Loessberg, P.A. (1990) J. Biol. Chem. 265, 12806–12812.
[21] Mariott, I. and Mason, M.J. (1996) J. Biol. Chem. 271, 26732–26738.
[22] Rink, T.J., Tsien, R.Y. and Pozzan, T. (1982) J. Cell Biol. 95, 189–196.
[23] Hawk, C., Krouse, M.E., Xia, Y., Gruenert, D.C. and Wine, J.J. (1992) Am. J. Physiol. 263, L692–L707.
[24] Green, J. and Kleeman, C.R. (1992) Am. J. Physiol. 262, C111–C121.
[25] Reuss, L. (1987) J. Gen. Physiol. 90, 173–196.
[26] Desilets, M., Puceat, M. and Vassort, G. (1994) Circ. Res. 75, 862–869.
[27] Gottlieb, R.A. and Dosanjh, A. (1996) Proc. Natl. Acad. Sci. USA 93, 3587–3591.
[28] Smith, J.J., Travis, S.M., Greenberg, E.P. and Welsh, M.J. (1996) Cell 85, 229–236.