cGMP Stimulation of Cystic Fibrosis Transmembrane Conductance Regulator Cl⁻ Channels Co-expressed with cGMP-dependent Protein Kinase Type II but Not Type Iβ*

In order to investigate the involvement of cGMP-dependent protein kinase (cGK) type II in cGMP-provoked intestinal Cl⁻ secretion, cGMP-dependent activation and phosphorylation of cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channels was analyzed after expression of cGK II or cGK Iβ in intact cells. An intestinal cell line which stably expresses CFTR (IEC-CF7) but contains no detectable endogenous cGK II was infected with a recombinant adenoviral vector containing the cGK II coding region (Ad-cGK II) resulting in co-expression of active cGK II. In these cells, CFTR was activated by membrane-permeant analogs of cGMP or by the cGMP-elevating hormone atrial natriuretic peptide and cAMP-dependent protein kinase are able to substitute for cGK II in this cGMP-regulated function.

In intestinal epithelium a cGMP-signaling pathway can activate cystic fibrosis transmembrane conductance regulator (CFTR) Cl⁻ channels, resulting in the net secretion of salt and water (1, 2). Guanylin and/or uroguanylin, small peptides derived from larger precursor proteins synthesized by intestinal epithelial cells, may function as the physiological activator of the cGMP-mediated signaling route in intestine by activating guanylyl cyclase C located in the apical membrane of enterocytes (2–4). Heat-stable enterotoxins secreted by various pathogenic strains of Escherichia coli mimic the action of guanylin and elicit a severe secretory diarrhea by hyperactivating guanylyl cyclase C (2–4).

Localization studies have suggested a key role for a recently cloned isotype of cGMP-dependent protein kinase (cGK), designated type II, as the mediator of the cGMP-provoked intestinal Cl⁻ secretion (5–7). Type II cGK is expressed predominantly in epithelial cells of the intestine (5–7), although it was also detected in kidney (6, 8, 9) and brain (6, 8, 10). In contrast, type I cGK, consisting of α and β isoforms, and shown to act as a key regulator of cardiovascular homeostasis (11, 12), is not expressed in enterocytes (7). Furthermore, purified endogenous pig cGK II, in contrast to bovine lung cGK I, was shown to activate CFTR in excised membrane patches (13). However, the mechanism of the apparent cGK type selectivity in activating CFTR was not clear, since both cGK I and cGK II could phosphorylate immunoprecipitated CFTR in vitro (13). An explanation for this discrepancy might be that cGK II but not cGK I selectively phosphorylates CFTR in a native environment. To examine this possibility we investigated activation and phosphorylation of CFTR in intact cells expressing either cGK II or cGK Iβ. Endogenous expression of cGK isoforms in intact cells also permitted use of native enzyme in these experiments, such that alterations, particularly of cGK II, that occur during purification could be avoided. Purification of the membrane-bound cGK II requires the use and subsequent removal of detergents, a procedure which potentially contributes to nonspecific (hydrophobic) interactions of this enzyme. Furthermore, purification results in partial proteolytic modification of cGK II and renders cGK II less sensitive to cGMP (5, 13).

For the analyses of the interactions of CFTR and cGK II or cGK Iβ in intact cells, we established a highly efficient co-expression system in which rat intestinal IEC-CF7 cells previously stably transfected with CFTR (14) were infected with recombinant adenoviral vectors containing the cDNA of cGK II or cGK Iβ. Here we report that co-expression with cGK II but not with cGK Iβ renders CFTR sensitive to activation by cGMP in intact cells. Furthermore, CFTR is shown to be a selective substrate for only cGK II-mediated phosphorylation under physiological conditions, providing a possible explanation for the present and previously (13) observed isotype-specific activation of CFTR by cGK II.

**EXPERIMENTAL PROCEDURES**

**Materials**—Protein A-Sepharose was from Pierce, 3-isobutyl-1-methylxanthine (IBMX), and rat atrial natriuretic peptide (ANP) from Sigma, cGMP analogs from Biolog (Bremen, Germany), and...
Protein kinase activity was determined by incubation of the samples (10 µg of protein) at 30°C for 4 min in 40 µl of 50 mM Tris-HCl, pH 7.4, 1 mM MgCl₂, 5 mM β-mercaptoethanol, 0.1 mM 3-isobutyl-1-methylxanthine, 0.2 µCi of [³²P]orthophosphate, 100 nM cGKI, 0.1 mg/ml leupeptin and 100 µg/ml aprotinin, and 0.1 mM 3-isobutyl-1-methylxanthine. The lysates were diluted 2-fold with a solution of 50 mM Tris-HCl, pH 7.4, 1 mM MgCl₂, 5 mM β-mercaptoethanol, 0.1 mM 3-isobutyl-1-methylxanthine, 25 mM Na-[β-glycerophosphate, 200 µg/ml protein kinase A inhibitor (PKI), 0.1 mg/ml of a cGK substrate peptide 2A3 (RRKVSQGE; see Ref. 18), 1 µCi of [³²P]ATP, 300 µM unlabeled ATP and cGMP or cGMP analogs as described (20). Immunoreactive proteins were detected after incubation with cGKI or cGK I antibody (1:3000) by the enhanced chemiluminescence method and quantitated by the Molecular Imaging System GS-363 (Bio-Rad) using standards of purified bovine lung cGKI. Rat intestinal cGKI was expressed per milligram of homogenate protein after correction for basal activity in the absence of cGMP. Inset, immunoblots of the homogenate (5 µg protein; hom), cyslot (cyt), and membrane (mem) fractions of cells infected with adenovirus containing cGK I (top) or cGK II (bottom). The blots were labeled with the respective antibodies against cGKI and II. In the lanes at right, 10 ng of pure cGKI (top) or rat intestinal brush borders containing 10 ng of cGK II (bottom) were loaded as standards (st). Shown are results of a typical experiment which was performed three times.

![Efflux Studies](image)

**FIG. 1. Adenovirus-mediated transfer of cGMP-dependent protein kinases (cGK) in IEC-CF7 cells.** Rat intestinal IEC-CF7 cells stably transfected with CFTR Cl⁻ channels were infected with 5 × 10⁶ particles/ml (approximately 10⁶ plaque-forming units/ml) of a replication-defective adenovirus containing the E4-04 and luciferase genes (mock; solid bar), cGK II (hatched bar), or cGK Iβ (open bar). Two days after infection, cells were harvested, homogenized, and separated into cytosol and membrane fractions. Phosphotransferase activity was determined with a cGK-selective substrate (2A3) in the presence or absence of 10 µM cGMP and was expressed per milligram of homogenate protein after correction for basal activity in the absence of cGMP. Inset, immunoblots of the homogenate (5 µg protein; hom), cyslot (cyt), and membrane (mem) fractions of cells infected with adenovirus containing cGK Iβ (top) or cGK II (bottom). The blots were labeled with the respective antibodies against cGKI and II. In the lanes at right, 10 ng of pure cGKI (top) or rat intestinal brush borders containing 10 ng of cGK II (bottom) were loaded as standards (st). Shown are results of a typical experiment which was performed three times.
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**FIG. 2.** cGMP increases iodide efflux in IEC-CF7 cells expressing cGK II but not cGK I\textbeta. Rat intestinal IEC-CF7 cells stably transfected with CFTR Cl\textsuperscript{−} channels were infected with replication deficient adenovirus (Ad) containing the cDNA of either luciferase (mock), cGK II, or cGK I\textbeta. Two days after infection, CFTR activity was monitored by measurements of fractional 125I\textsuperscript{2} efflux. A, time course of 125I\textsuperscript{2} efflux in Ad-cGK II-infected IEC-CF7 cells. At 5 min (arrow) 50 \muM 8-pCPT-cGMP (\(▲\)), 0.1 \muM ANP (\(●\)), 10 \muM forskolin (\(■\)), or vehicle (control; \(●\)) was added to the efflux medium. B, maximal increment in 125I\textsuperscript{2} efflux as determined 2 min after addition of forskolin (fors; open bars) and ANP (hatched bars) or 4 min after addition of cGMP analogs (cGMP; solid bars) to IEC-CF7 cells infected as shown. 125I\textsuperscript{2} efflux was corrected for basal efflux at the same time points determined in the absence of the agonists. Concentration of the agonists added was as in A, except that 20 \muM 8-Br-PET-cGMP was used to activate cGK I\textbeta. Data are means \pm S.E. of 3–6 experiments.

cGMP-stimulated phosphotransferase activity (Fig. 1). Infection of IEC-CF7 cells with 5 \times 10\textsuperscript{9} particles of replication deficient adenovirus containing the cDNA of rat cGK II (Ad-cGK II) resulted in the expression of 0.5 \pm 0.2 \muM of cGK II/mg protein as assessed by immunoblotting (n = 4; Fig. 1), which is in the range of the endogenous cGK II content of isolated rat enterocytes determined by immunoblotting (0.2–0.4 \muM/mg protein; data not shown). Furthermore, only the full-length 86-kDa form of cGK II was observed in homogenates of Ad-cGK II-infected IEC-CF7 cells (Fig. 1), which is similar to cGK II in native rat intestinal brush border membranes (Fig. 1) (7). In contrast, a mixture of cGK II forms (86 kDa intact and 70 and 75 kDa proteolyzed forms) was present in the purified preparations of pig cGK II used in previous experiments demonstrating cGK II-mediated activation of CFTR in excised membrane patches (5, 12). Infection of IEC-CF7 cells with a similar dose of adenovirus vector containing the coding region of human cGK I\textbeta (Ad-cGK I\textbeta) produced a relatively high expression level of cGK I\textbeta (2.4 \pm 1 \muM/mg protein; detected by immunoblotting, n = 4) corresponding to a large increase in phosphotransferase activity exceeding the activity measured for cGK II by 5-fold (Fig. 1). In contrast to cGK II, which was associated primarily with the membrane fraction, cGK I\textbeta was cytosolic (Fig. 1). The observed subcellular localization of the recombinant cGKs after adenovirus-mediated transfer are in agreement with the membrane localization of endogenous cGK II (5, 7) and the soluble character of endogenous cGK I\textbeta (25). The selectivities of the recombinant cGKII and cGK I\textbeta expressed in the IEC-CF7 cells for membrane permeant cGMP analogs were similar to those previously determined for endogenous or recombinant forms of cGK II and I\textbeta (18, 22, 26, 27)\textsuperscript{2}, i.e. cGK II was preferentially activated by 8-pCPT-cGMP over 8-Br-PET-cGMP (\(K_a = 0.1\) and 1.7 \muM, respectively; data not shown), and cGK I\textbeta was more readily activated by 8-Br-PET-cGMP than by 8-pCPT-cGMP (\(K_a = 0.04\) and 0.9 \muM, respectively; data not shown). Taken together, these results show that both the amount and subcellular distribution of cGK isotype expression produced by adenovirus-mediated gene transfer in IEC-CF7 cells reflects that of endogenous cGK I and cGK II, allowing a meaningful comparison of the effects of recombinant cGK isotypes on CFTR in IEC-CF7 cells.

**Activation of CFTR by cGMP in cGK II-expressing Cells**—125I\textsuperscript{2} efflux measurements provide a simple assay for monitoring the activation of CFTR Cl\textsuperscript{−} channels (14, 23). The cAMP-elevating agent forskolin caused a large increase in 125I\textsuperscript{2} efflux from IEC-CF7 cells (Fig. 2) but not from CFTR-deficient wild-type or mock-transfected IEC-6 cells (14). In contrast, both the membrane-permeable cGMP analog 8-pCPT-cGMP and the cGMP-elevating hormone atrial natriuretic peptide (ANP) (28) were unable to mimic the forskolin-provoked increase in 125I\textsuperscript{2} efflux in either IEC-CF7 cells infected with control adenovirus containing luciferase cDNA (Fig. 2B, mock), or in noninfected IEC-CF7 cells (data not shown). These results indicate that cGMP was unable to provoke activation of CFTR via endogenous cAMP-dependent protein kinase (cAK) in cGK-deficient IEC-CF7 cells under the conditions tested. However, after infection of IEC-CF7 cells with 5 \times 10\textsuperscript{9} particles/ml Ad-cGK II, addition of 8-pCPT-cGMP caused a gradual increase in the 125I\textsuperscript{2} efflux rate, reaching a maximum after 4 min (14 \pm 4%/min above basal, n = 6; Fig. 2). A 5-fold lower dose of Ad-cGK II (10\textsuperscript{9} particles/ml) resulted in an approximately 5-fold lower expression of cGK II, and a 3–4-fold lower increment in 125I\textsuperscript{2} efflux rate (3.8 \pm 1.3%/min; n = 3) in response to 8-pCPT-cGMP (data not shown). Relatively high doses of Ad-cGK II (>2 \times 10\textsuperscript{10} particles/ml) were more effective than the standard dose of 5 \times 10\textsuperscript{9} particles/ml in facilitating the 8-pCPT-cGMP-provoked increase in 125I\textsuperscript{2} efflux but were also toxic for the IEC-CF7 cells as judged from their morphology and an increased rate of basal 125I\textsuperscript{2} efflux (data not shown).

8-pCPT-cGMP was unable to stimulate 125I\textsuperscript{2} efflux in CFTR-deficient IEC-6 cells infected with Ad-cGK II (data not shown), further strengthening the concept that the CFTR Cl\textsuperscript{−} channel is the mediator of cGMP/cGK II-enhanced 125I\textsuperscript{2} efflux in IEC-CF7 cells. Accordingly, whole-cell patch clamp analysis of Ad-cGK II-infected IEC-CF7 cells stimulated with 8-pCPT-cGMP revealed rapid induction of a linear Cl\textsuperscript{−} current that was in-

\[2\] A. B. Vaandrager, unpublished observations.
distinguishable from forskolin-provoked currents (Fig. 3) (24).

This 8-pCPT-cGMP-triggered anion current was observed in 8 of 9 IEC-CF7 cells infected with Ad-cGK II. In contrast, only in 1 of 6 mock-infected cells was a small increase observed, indicating that cGK II expression was a prerequisite for CFTR Cl$^{-}$ channel activation.

In excised membrane patches, the activation of CFTR Cl$^{-}$ channels by purified pig cGK II was relatively slow in comparison to their activation by cAK (13). In Ad-cGK II-infected IEC-CF7 cells 8-pCPT-cGMP caused a similar sluggish activation of CFTR (Fig. 2A). As ANP was shown previously to induce a prompt rise in intracellular cGMP in IEC cells by activating endogenous guanylyl cyclases (28), the time course of ANP activation of $^{125}$I efflux was compared with the response to the cGMP analog. As shown in Fig. 2A, the rate of ANP-stimulated $^{125}$I efflux was similar to that observed with the cAMP-elevating agonist forskolin, suggesting that cGK II and cAK are able to activate CFTR in intact cells with similar kinetics and that the lag phase in activation by 8-pCPT-cGMP is due to a relatively slow permeation of the analog across the IEC-CF7 cell membrane. These results also imply that the delayed CFTR channel opening in response to purified cGK II observed in excised patches (13) is apparently an in vitro artifact and may represent the lag time needed for re-anchoring of the solubilized enzyme to the membrane.

In agreement with our previous in vitro observations in excised membrane patches (13), cGMP activation of CFTR in intact IEC-CF7 cells was specifically mediated by type II cGK, since neither 8-Br-PET-cGMP nor ANP provoked an increase in $^{125}$I efflux in Ad-cGK I$\beta$-infected cells (Fig. 2B). However, these cells showed a normal forskolin response, excluding any deleterious effect of cGK I$\beta$ expression on CFTR activation (Fig. 2B). Furthermore, the ANP-provoked increase in cGMP did not differ between Ad-cGK II and Ad-cGK I$\beta$-infected IEC-CF7 cells (data not shown). Since infection of IEC-CF7 cells with equivalent doses of adenovirus caused a 5-fold higher expression of cGK I$\beta$ than cGK II, and this dose of Ad-cGK I$\beta$ did not stimulate $^{125}$I efflux, whereas even a 5-fold lower dose of Ad-cGK II still did, we conclude that cGK II is at least 25-fold more effective than cGK I$\beta$ in activating CFTR Cl$^{-}$ channels. However, since both cGK II and cGK I were found to phosphorylate immunopurified CFTR and a cloned regulatory domain fragment of CFTR (CF-2) in vitro with similar kinetics (13), we next investigated the in situ phosphorylation of CFTR in cGK II and cGK I$\beta$-expressing IEC-CF7 cells.

Phosphorylation of CFTR in IEC-CF7 Cells—As shown in Fig. 4, 8-pCPT-cGMP caused an almost 3-fold increase in $^{32}$P labeling of CFTR in Ad-cGK II-infected IEC-CF7 cells. In contrast, 8-Br-PET-cGMP had no effect on CFTR phosphorylation in Ad-cGK I$\beta$-infected cells, despite the fact that CFTR phosphorylation in response to forskolin was similar in both cGK I$\beta$ and cGK II-expressing cells. Therefore, the increase in CFTR phosphoryl content paralleled the activation of CFTR-mediated $^{125}$I efflux observed upon cGMP application. This suggests
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In conclusion, our demonstration that cGK II expression rendered CFTR sensitive to modulation by cGMP in cells which did not previously display a cGMP-inducible Cl\textsuperscript{−} conductance indicates that cGK II is a key mediator of cGMP-provoked activation of CFTR in, e.g., intestinal epithelial cells where both proteins are co-localized (7). This conclusion is also corroborated by the previously observed correlation between the presence of cGK II and detection of cGMP-induced CFTR-mediated Cl\textsuperscript{−} secretion in different intestinal segments (7). Furthermore, the role of cGK II in mediating cGMP-provoked activation of CFTR activation cannot be mimicked by cGK I or cAK in our cell system. In particular, the ascribed role of cAK in the cGMP-induced activation of CFTR in several cell lines (29–33) may therefore not be valid but restricted to cells either expressing a type III cGMP-inhibited phosphodiesterase to elevate cAMP (29) or cells in which very high levels of cGMP can be attained to cross-activate cAK (30–32). The intestinal cell lines T84 and Caco-2 used to demonstrate the latter mechanism appear nevertheless to be unsuitable models for studying physiological mechanisms, since they do not contain detectable levels of cGK II or cGK I (7). Furthermore, we have shown (7) that the major localization of cGK I in smooth muscle cells of the villus lamina propria, in contrast to the epithelial brush border where cGK II is located, makes cGK I an unlikely endogenous mediator of cGMP effects on CFTR. The particularly intriguing aspect of our present results is the observation that even when present cGK I cannot substitute for cGK II in phosphorylating native CFTR but, more importantly, may be a clue to mechanisms of subcellular compartmentalization of functions.

**REFERENCES**

1. Field, M., Rao, M. C., and Chang, E. B. (1989) *N. Engl. J. Med.* 321, 800−806, 879−883
2. Vaandrager, A. B., and De Jonge, H. R. (1994) *Adv. Pharmacol.* 26, 253−283
3. Forte, L. R., and Currie, M. G. (1995) *FASEB J.* 9, 643−650
4. Schulz, S., Green, C. K., Yuan, P. S. T., and Garbers, D. L. (1990) *Cell* 63, 941−948
5. De Jonge, H. R. (1981) *Adv. Cyclic Nucleotide Res.* 14, 315−333
6. Jarchau, T., Häusler, C., Markert, T., Pohler, D., Vendekerkhove, J., De Jonge, H. R., Lohmann, S. M., and Walter, U. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91, 9426−9430
7. Markert, T., Vaandrager, A. B., Gambaryan, S., Pohler, D. Häusler, C., Walter, U., De Jonge, H. R., Jarchau, T., and Lohmann, S. M. (1995) *J. Clin. Invest.* 96, 822−830
8. Uhlér, M. D. (1993) *J. Biol. Chem.* 268, 13586−13591
9. Gambaryan, S. Häusler, C., Markert, T., Pohler, D., Jarchau, T., Walter, U., Haase, W., Kurtz, A., and Lohmann, S. M. (1996) *J. Clin. Invest.* 98, 462−470
10. El-Husseini, A, Bladen, C., and Vincent, S. R. (1995) *J. Neurochem.* 64, 2814−2817
11. Butt, E., Geiger, J., Jarchau, T., Lohmann, S. M., and Walter, U. (1993)

**Fig. 4.** cGMP promotes phosphorylation of CFTR in IEC-CF7 cells expressing cGK II but not cGK I. Rat intestinal IEC-CF7 cells stably transfected with CFTR Cl\textsuperscript{−} channels were infected with replication-deficient adenovirus containing the cDNA of either cGK II or I (5 × 10\textsuperscript{3} particles/ml). Two days after infection, cells were metabolically labeled with inorganic 32P for 1 h and subsequently incubated for 20 min with vehicle (−, none), 10 μM forskolin (forsk), or either 50 μM 8-pCPT-cGMP in the case of cGK II (cGMP) or 8 μM 8-Br-cGMP in the case of cGK I (cGMP). Subsequently CFTR was immunoprecipitated and separated by 6% SDS-polyacrylamide gel electrophoresis. A, autoradiograph showing 32P-phosphorylated CFTR (30-day exposure). The position of CFTR as determined with in vitro 32P-phosphorylated CFTR (see Refs. 13 and 14) is indicated with an arrowhead. The 220-kDa protein in lane 5 is nonspecific, as it does not comigrate with CFTR and was not observed in other experiments. B, amount of 32P incorporated into CFTR was quantitated by a phospho-imager and expressed relative to the basal 32P incorporation into CFTR in Ad-cGK II-infected IEC-CF7 cells (cfGK II, none). Data are means ± S.E. of three experiments.
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12. Vaandrager, A. B., and De Jonge, H. R. (1996) Mol. Cell. Biochem. 157, 23–30
13. French, P. J., Bijman, J., Edixhoven, M., Vaandrager, A. B., Scholte, B. J., Lohmann, S. M., Nairn, A. C., and de Jonge, H. R. (1995) J. Biol. Chem. 270, 26626–26631
14. Bijman, J., Dalemans, W., Kansen, M., Keulemans, J., Verbeek, E., Hoogeveen, A., de Jonge, H. R., Wilke, M., dreyer, D., Lecoeq, J.-P., Pavirani, A., and Scholte, B. J. (1995) Am. J. Physiol. 264, L229–L235
15. Picciotto, M. R., Cohn, J. A., Bertuzzi, G., Greengard, P., and Nairn, A. C. (1992) J. Biol. Chem. 267, 12742–12752
16. Poller, W., Schneider-Rasp, S., Liebert, U., Merklein, F., Thalheimer, P., Haack, A., Schwaab, R., Schmitt, C., and Brackmann, H.-H. (1996) Gene Therapy 3, 521–530
17. Sandberg, M., Natarajan, V., Ronander, I., Kalderon, D., Walter, U., Lohmann, S. M., and Jahnson, T. (1989) FEBS Lett. 255, 321–329
18. Meinecke, M., Geiger, J., Butt, E., Sandberg, M., Jahnson, T., Chakraborty, T., Walter, U., Jarchau, T., and Lohmann, S. M. (1994) Mol. Pharmacol. 46, 283–290
19. Bett, A. J., Haddara, W., Prevec, L., and Graham, F. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8802–8806
20. Vaandrager, A. B., Ehert, E. M. E., Jarchau, T., Lohmann, S. M., and de Jonge, H. R. (1996) J. Biol. Chem. 271, 7025–7029
21. Vaandrager, A. B., Schulz, S., De Jonge, H. R., and Garbers, D. L. (1993) J. Biol. Chem. 268, 2174–2179
22. Pohler, D., Butt, E., Meissner, J., Muller, S., Lohe, M., Walter, U., Lohmann, S. M., and Jarchau, T. (1995) FEBS Lett. 374, 419–425
23. Vaandrager, A. B., Bajnath, R., Groot, J. A., Bot, A. G. M., and De Jonge, H. R. (1991) Am. J. Physiol. 261, G958–G965
24. Sheppard, D. N., and Welsh, M. J. (1992) J. Gen. Physiol. 100, 573–591
25. Wolfe, L., Corbin, J. D., and Francis, S. H. (1989) J. Biol. Chem. 264, 7734–7741
26. Sekhar, K. R., Hatchett, R. J., Shabb, J. B., Wolfe, L., Francis, S. H., Wills, J. N., Justorff, B., Butt, E., Chakinala, M. M., and Corbin, J. D. (1992) Mol. Pharmacol. 42, 103–108
27. Gamm, D. M., Francis, S. H., Angelotti, T. P., Corbin, J. D., and Uhler, M. D. (1995) J. Biol. Chem. 270, 27380–27388
28. Vaandrager, A. B., Bot, A. G. M., De Vente, J., and De Jonge, H. R. (1992) Gastroenterology 102, 1161–1169
29. Kelley, T. J., Al-Nakkash, L., and Drumm, M. L. (1995) Pediatr. Pulmonol. Suppl. 12, 184 (abstr.)
30. Forte, L. R., Thorne, P. K., Eber, S. L., Krause, W. J., Freeman, R. H., Francis, S. H., and Corbin, J. D. (1992) Am. J. Physiol. 263, C607–C615
31. Tien, X.-Y., Brasitus, T. A., Kaetzel, M. A., Dedman, J. R., and Nelson, D. J. (1994) J. Biol. Chem. 269, 51–54
32. Chao, A. C., de Sauvage, F. J., Dong, Y.-J., Wagner, J. A., Goeddel, D. V., and Gardner, P. (1994) EMBO J. 13, 1065–1072
33. Dong, Y.-J., Chao, A. C., Konyama, K., Hsu, Y.-P., Bocian, R. C., Moss, R. B., and Gardner, P. (1993) EMBO J. 12, 2700–2707