Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2340–2344, March 1992
Medical Sciences

Characterization of the cystic fibrosis transmembrane conductance regulator in a colonocyte cell line

(T84 cell line/phosphopeptide mapping/Western blot/glycoprotein/immunohistochemistry)

JONATHAN A. COHN*†, ANGUS C. NAIRN‡, CHRISTOPHER R. MARINO§, OLA MELHUS¶, AND JOLANTA KOLE*

Departments of *Medicine and Cell Biology and of ¶Pathology, Duke University and Veterans Administration Medical Centers, Durham, NC 27710; ‡Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021; and §Department of Medicine, Yale University School of Medicine, New Haven, CT 06510

Communicated by Martin Rodbell, December 13, 1991 (received for review October 22, 1991)

ABSTRACT **An anti-peptide antibody raised to the C-terminal sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) was used to examine CFTR immunoreactivity in the T84 colonocyte cell line. Immunoblots of T84 cell lysates detected CFTR as a 170-kDa protein that appeared as a broad band or doublet in SDS/PAGE. This protein comigrated with the predominant immunoblot signal detected in human pancreas and colon. An equivalent protein was detected as a prominent substrate for protein kinase A and for protein kinase C in T84 cell immunoprecipitates with this antibody. The immunoprecipitated protein resembled the protein detected by immunoblot in that both proteins showed the same change in electrophoretic mobility after digestion by N-Glycanase. The precipitated protein was identified as CFTR by two criteria. First, the same protein was immunoprecipitated with an antibody to a different CFTR peptide, [Lys^{102}]CFTR-(102–116). Second, two-dimensional phosphopeptide mapping was used to compare the immunoprecipitated protein with a bacterially expressed protein known to contain most of the predicted protein kinase A phosphorylation sites in CFTR. Because the six most prominent peptides in each map were equivalent, these maps confirm that the precipitated protein is CFTR. By using these antibodies for immunofluorescence and immunoperoxidase staining, CFTR was localized to the apical region of T84 cells grown in tumors and in monolayers. Thus, T84 cells express CFTR at sufficient levels to permit identification and immunochemical studies of this protein in its endogenously occurring form.**

The ion-transport abnormality most closely associated with cystic fibrosis (CF) is a specific defect in the regulation of Cl^- channels that affects the activation of these channels by protein kinase A and by protein kinase C (1–7). When the CF gene was identified in late 1989 (8–10), the gene's protein product was named the CF transmembrane conductance regulator (CFTR) to indicate that the protein was expected to participate in Cl^- channel regulation. This expectation leads to two predictions about the properties of CFTR. First, CFTR should undergo phosphorylation by the protein kinases for which CF-specific defects in Cl^- channel regulation occur. Second, CFTR should occur at sites of protein kinase-regulated Cl^- channels in polarized epithelial cells.

To date, these predictions have been indirectly examined in a series of studies in transfected cells. These studies indicate that the CF ion-transport defect can be corrected by expression of the normal *CFTR* gene in CF cell lines (11–13), that many CF-causing mutations can affect the glycosylation and localization of CFTR (14, 15), that the expression of CFTR may be associated with a specific type of Cl^- channel (16–18), and that mutations at specific CFTR phosphorylation sites can affect the ability of this channel to be regulated by a protein kinase (19). Even though these findings have intriguing implications regarding the pathogenesis of CF, these studies are limited by uncertainties about the fidelity of CFTR processing and trafficking in transfected cells. To address these uncertainties, it is important to examine the properties of endogenously occurring forms of CFTR in untransfected cells.

This study will characterize endogenously expressed CFTR in the T84 colonocyte cell line. Based on electrophysiologic evidence of functional CFTR in T84 cells (20–23), this model promises to be useful for studying the physiologic processing and localization of this protein. Even though these cells contain large amounts of CFTR mRNA (9), only limited information exists concerning T84 cell CFTR immunoreactivity (11, 17, 24). In this study, CFTR will be detected by using two high-affinity, monospecific anti-peptide antibodies that we have used recently to localize CFTR in human pancreas and sweat gland (25, 26). These antibodies will be used to examine the localization and phosphorylation of CFTR in T84 cells.

METHODS

Generation of Antibodies. Peptides corresponding to two domains of the predicted structure of CFTR were synthesized, HPLC-purified, conjugated to thyroglobulin, and used to generate rabbit antisera as described (25, 26). One peptide, designated [Lys^{102}]CFTR-(102–116) and of sequence Lys-Gly-Arg-Ile-Ile-Ala-Ser-Tyr-Asp-Pro-Asp-Asn-Lys-Glu-Glu-NH_2, corresponds to a predicted extracellular domain of CFTR. The second peptide, designated CFTR-(1468–1480) and of sequence Lys-Glu-Glu-Thr-Glu-Glu-Glu-Val-Gln-Asp-Thr-Arg-Leu-OH, corresponds to the carboxyl-terminal end of the protein. Each antibody was purified by using an affinity column containing the appropriate peptide bound to epoxy-activated Sepharose (Pharmacia) and the resulting purified antibody preparations, designated α-102 and α-1468, were stored at final concentrations of $\approx$0.5 mg/ml in 50% glycerol at −20°C.

Immunoblot. Human tissue specimens and lysates of T84 cell monolayers were snap-frozen and stored in liquid nitrogen. Routinely, the specimens were homogenized in 5–10 volumes of 3% SDS with a glass/Teflon homogenizer preheated to 95°C, centrifuged to remove insoluble material, resolved by SDS/PAGE, and electrophoretically transferred as described (25). Immunoblots were performed with α-1468 (final dilution 1:100) and ^{125}I-labeled goat anti-rabbit IgG

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; α-1468 and α-102, monospecific rabbit antibodies raised against the synthetic CFTR peptides, CFTR-(1468–1480) and [Lys^{102}]CFTR-(102–116).

†To whom reprint requests should be addressed at: Box 3378, Duke University, Durham, NC 27710.

(1:1000 dilution; New England Nuclear) or with an enhanced chemiluminescence detection kit (Amersham) as indicated in the figure legends.

Immunocytochemistry. Cell staining was performed with T84 cell tumors grown in athymic mice (27). Athymic (*nu/nu*) mice were obtained from The Jackson Laboratory and were housed in facilities provided by the Duke Comprehensive Cancer Center. T84 cells (10^6) were injected at each of four sites, and tumors were harvested after approximately 10 weeks. Cryosections (2–4 μm) were fixed in acetone for 10 min, and immunofluorescence staining and peptide competition studies were performed as described (25). Immunoperoxidase staining was performed with biotinylated goat anti-rabbit IgG (1:200 dilution) and a Vectastain Elite ABC kit (Vector Laboratories) as described (26). For immunoperoxidase studies, specimens were counterstained with modified Harris hematoxylin. In additional studies, T84 cells were grown as monolayers on collagen-coated filters (28). Cell monolayers were fixed by using a pH shift/paraformaldehyde procedure (29) before sectioning.

Immunoprecipitation. T84 cell proteins were solubilized in precipitation buffer containing 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 0.1 mg of bovine serum albumin per ml, 25 mM Tris (pH 7.0), 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 1 mM benzamidine, and 5 μg of leupeptin per ml. Approximately 1 mg of protein (800 μl of cell lysate) was incubated with 1–5 μl of α-1468 or α-102 overnight at 4°C and then incubated for 1 h with 1 mg of protein A-agarose beads (Sigma). The beads were washed with precipitation buffer and then with phosphate-buffered saline before being incubated for 1 hr at 30°C with one of two protein kinases and 5 μCi (185 kBq) of [γ-^{32}P]ATP. Labeling with protein kinase A catalytic subunit was performed with 10 units of the kinase in a final volume of 60 μl containing 50 mM Tris (pH 7.5), 1 mM EDTA, 10 mM $MgCl_2$, and 1.2 μg of bovine serum albumin (1 unit of kinase activity transfers 1 pmol of phosphate per min). Labeling with protein kinase C was performed with 10 units of this kinase in 60 μl containing 120 mM Hepes, 1 mM EDTA, 5 mM $MgCl_2$, 2 mM $CaCl_2$, 1 μM phorbol myristate acetate, and 0.1 μg of phosphatidylserine per μl. The phosphorylation reactions were terminated by adding 500 μl of precipitation buffer, and the beads were washed with this buffer. Proteins were then solubilized with 2% SDS and resolved by SDS/PAGE. To minimize antibody-independent binding of proteins to the protein A-agarose beads, cell lysates were routinely cleared by preincubation with the beads.

Phosphopeptide Maps. Two-dimensional thin-layer phosphopeptide mapping was performed by using a modification of the procedure of Hunter and Sefton (30). Briefly, gel pieces containing labeled proteins were lyophilized, suspended in 50 mM NH_4HCO_3 (pH 8.0), and digested for 20 h at 37°C with 50 μg of L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (TPCK-trypsin) per ml. Phosphopeptides were resolved by electrophoresis at 400 V for 90 min in 10% acetic acid/1% pyridine, pH 3.5, followed by chromatography in 15:10:12:3 (vol/vol) pyridine/1-butanol/water/acetic acid on 20 × 20 cm cellulose sheets (Eastman Kodak). ^{32}P-labeled phosphopeptides were detected by autoradiography. Phosphopeptides derived from the immunoprecipitated protein were compared with phosphopeptides contained in CF-2, a bacterially expressed protein designed to include the CFTR sequence from amino acid 645 to amino acid 835, a sequence including most of the CFTR R domain (31, 32). Prior to phosphopeptide mapping, purified CF-2 was labeled by using protein kinase A catalytic subunit (in the presence of [γ-^{32}P]ATP) and digested with TPCK-trypsin as above.

Other Methods and Reagents. T84 cell membrane proteins were prepared and digested with N-Glycanase (N-glycosidase F, Boehringer Mannheim) precisely as described (16).

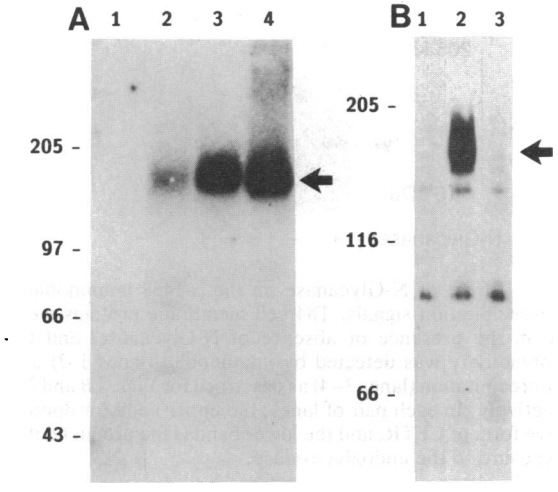

FIG. 1. Immunoblot analysis with α-1468 to detect CFTR immunoreactivity in T84 cells and in human tissues. (*A*) Tissue survey. Solubilized cell protein (40 μg) was loaded from the following tissues: brain (lane 1), colon (lane 2), T84 cells (lane 3), and pancreas (lane 4). (*B*) T84 cell CFTR immunoreactivity detected without primary antibody (lane 1), with α-1468 (lane 2), and with α-1468 and a competing peptide (lane 3). Immunoreactive proteins were detected by using ^{125}I-labeled goat anti-rabbit IgG (*A*) or enhanced chemiluminescence (*B*). The arrows point to a protein of 155–170 kDa. The positions of molecular mass markers are indicated in kDa.

Protein assays were performed with kits obtained from Bio-Rad and from Pierce. Protein kinase A catalytic subunit was obtained from T. Gettys (Duke University) or from Sigma. Protein kinase C was obtained from R. M. Bell (Duke University). Autoradiographs were exposed at −80°C by using Lightning Plus intensifying screens.

RESULTS

The only form of CFTR immunoreactivity detected in T84 cells by Western blot was a protein of 155–170 kDa (Fig. 1). This protein comigrated with the predominant signal detected

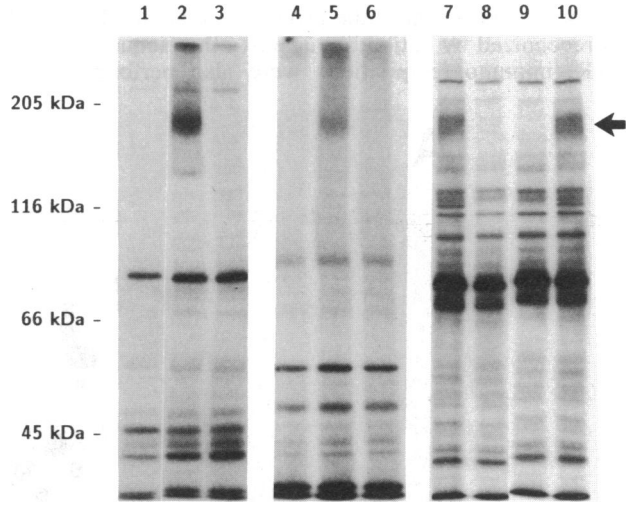

FIG. 2. Immunoprecipitation of protein kinase substrates from T84 cell lysates by α-1468 and α-102. Precipitated proteins were incubated with [γ-^{32}P]ATP and either protein kinase A (lanes 1–3 and 7–10) or protein kinase C (lanes 4–6). Labeled proteins were resolved by SDS/PAGE and detected by autoradiography. α-1468 was used to precipitate proteins in lanes 2, 3, 5, and 6, and α-102 was used for lanes 7, 9, and 10. Lanes 1, 4, and 8 contained controls without antibody. Peptide competition was tested by using 1 μM CFTR-(1468–1480) in lanes 3, 6, and 10 or 1 μM [Lys^{102}]CFTR-(102–116) for lane 9. The arrow points to a protein of 155–170 kDa. Positions of molecular mass markers are indicated.

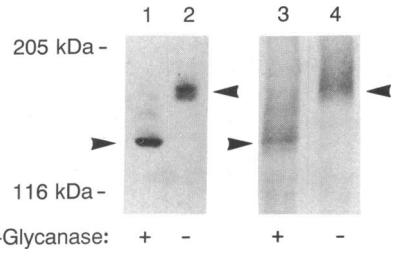

FIG. 3. Effect of N-Glycanase on the α-1468 immunoblot and immunoprecipitation signals. T84 cell membrane proteins were incubated in the presence or absence of N-Glycanase, and CFTR immunoreactivity was detected by immunoblot (lanes 1–2) and by immunoprecipitation (lanes 3–4) as described for Figs. 1*B* and 2, lane 2, respectively. In each pair of lanes, the upper band (or doublet) is the native form of CFTR, and the lower band is the product detected after exposure to the endoglycosidase.

in human pancreas and colon, and this protein was apparently absent in brain. The immunoblot signal was detected either as a broad band or as a doublet (see below), and the signal was specific in that it did not occur in the absence of α-1468 and was eliminated when blots were performed with α-1468 in the presence of competing peptide.

Immunoprecipitations were performed to further characterize the protein recognized by α-1468. This antibody specifically precipitated a protein kinase substrate of 155–170 kDa from T84 cell lysates (Fig. 2). Fig. 2 presents data from three experiments in which immunoprecipitates were cleared by using different amounts of protein A-agarose and were then labeled by using [γ-^{32}P]ATP with either protein kinase A (lanes 1–3 and lanes 7–10) or protein kinase C (lanes 4–6). When control precipitations were performed without primary antibody, there were several nonspecifically precipitated proteins detected by the phosphorylation reaction (lanes 1, 4, and 8). By contrast, when precipitations were performed in the presence of α-1468, a prominent band at 155–170 kDa was phosphorylated (lanes 2 and 5, indicated by arrow); labeling of this band did not occur in control precipitations performed with α-1468 and a competing peptide (lanes 3 and 6).

To determine whether the protein precipitated by α-1468 was recognized by antibodies against other sequences from CFTR, immunoprecipitations were also performed with α-102. The most prominent protein kinase A substrate recognized by this antibody was also a protein of 155–170 kDa appearing in this example as a doublet (Fig. 2, lanes 7–10). Precipitation of this protein by α-102 was also specific in that the appropriate peptide did compete (lane 9), while an unrelated peptide did not (lane 10).

In additional studies, the immunoreactive protein recognized by α-1468 was also examined with respect to its susceptibility to digestion by N-Glycanase. When solubilized T84 cell proteins were incubated with this endoglycosidase, the predominant immunoblot signal changed from a broad band or doublet at 155–170 kDa to a sharper band at roughly 130 kDa (Fig. 3, lanes 1 and 2). When these proteins were detected by immunoprecipitation followed by labeling with protein kinase A, a similar change in electrophoretic mobility was observed (Fig. 3, lanes 3 and 4).

To further characterize the immunoprecipitated protein, the two-dimensional tryptic phosphopeptide map of the precipitated T84 cell protein was compared to the map of CF-2, a bacterially expressed protein containing most of the CFTR regulatory domain (Fig. 4). These maps demonstrate that all six labeled phosphopeptides detected in digests of the precipitated protein correspond to phosphopeptides contained in CF-2. Because these six phosphopeptides have been purified from phosphorylated CF-2 and shown to contain authentic CFTR sequences (32), these findings unambiguously identify the precipitated protein as the native form of CFTR in T84 cells. Moreover, because no additional peptides were detected in digests of the immunoprecipitated protein, these findings suggest that CFTR is the only phosphoprotein contributing to the broad band or doublet detected in immunoprecipitates at 155–170 kDa.

The localization of CFTR in T84 cells grown as a tumor in an athymic mouse is shown in Fig. 5. The morphology of this tumor is consistent with earlier reports that T84 cells grow to form well-differentiated adenocarcinomas under these conditions (27). Within the T84 cell tumor, α-1468 staining was most prominent in areas where the cells formed glandular structures. Staining within these cells was polarized and was limited to the apical (i.e., lumenal) region. Equivalent patterns were observed with both immunofluorescence and immunoperoxidase staining, and this staining was absent in control sections tested without a primary antibody, with

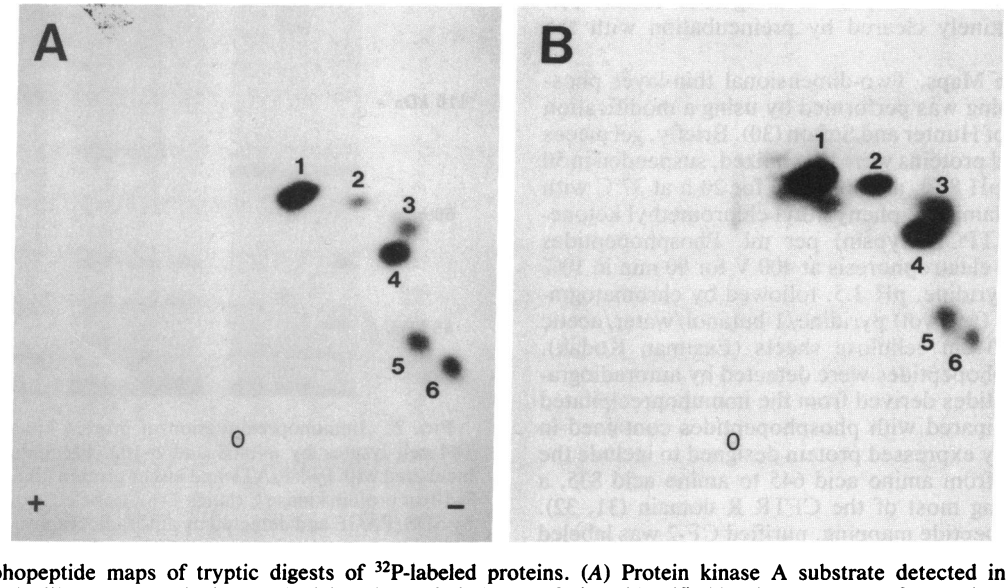

FIG. 4. Phosphopeptide maps of tryptic digests of ^{32}P-labeled proteins. (*A*) Protein kinase A substrate detected in T84 cell lysates immunoprecipitated with α-1468 (equivalent to the labeled protein in lane 2 of Fig. 2 identified by the arrow). (*B*) CF-2, a bacterially expressed protein labeled by using [γ-^{32}P]ATP and protein kinase A. CF-2 contains amino acids 645–835 of the CFTR sequence (31) and includes most of the sites where protein kinase A is predicted to label CFTR (9). Phosphopeptides were loaded at the origin (O) and resolved by electrophoresis (anode and cathode positions as for *A*), followed by chromatography as described.

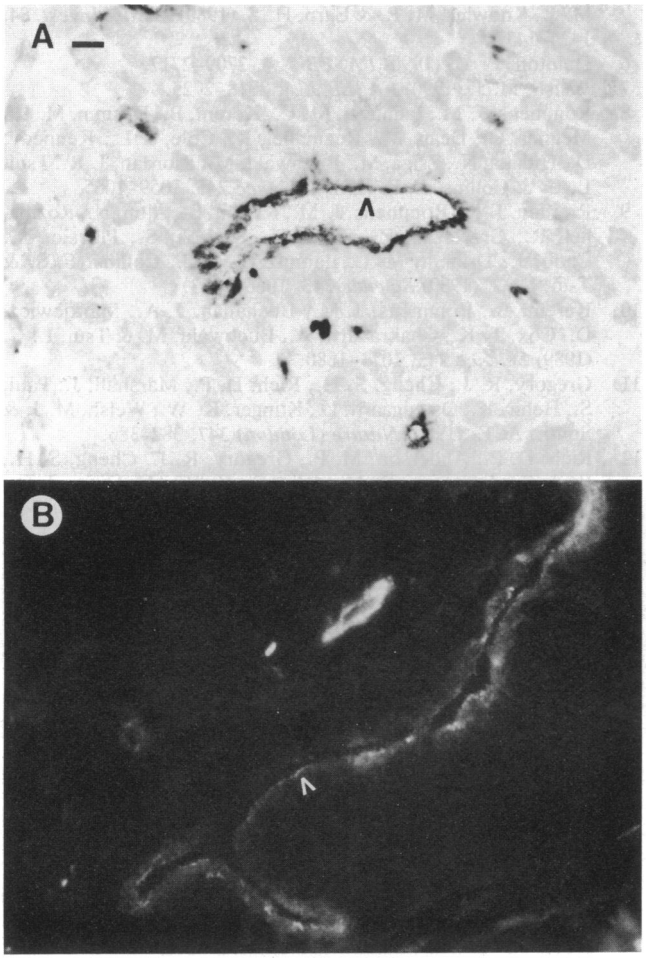

FIG. 5. Localization of CFTR in T84 cells grown as a tumor in an athymic mouse. (*A*) Peroxidase staining with α-1468 (final dilution, 1:100). (*B*) Immunofluorescence staining with α-1468 (1:100 dilution) detected with fluorescein isothiocyanate-conjugated goat anti-rabbit $F(ab')_2$ fragments (1:200 dilution). Hollow arrowheads indicate the apical membrane. (Bar = 9 μm.)

nonimmune rabbit serum, and with α-1468 preincubated with competing peptide (not shown). In additional studies, the apical localization of CFTR in T84 cells was confirmed by using α-102 and α-1468 to stain sections of cells grown as monolayers on collagen-coated filters (Fig. 6 *A* and *C*). With each antibody, staining was specific in that the signal was reduced by preincubation of the antibody with the appropriate peptide (Fig. 6 *B* and *D*).

DISCUSSION

The present study reports several properties of the native form of CFTR detected in T84 cells by an anti-peptide antibody, α-1468. The protein recognized by this antibody is identified as CFTR by three criteria. First, the predominant protein kinase substrate specifically precipitated by α-1468 is a protein of $\approx$170 kDa in agreement with the predicted mass of CFTR, and this protein undergoes phosphorylation by two protein kinases predicted to act on CFTR, protein kinases A and C (9). Second, the same protein kinase substrate is immunoprecipitated from T84 cell lysates both by α-1468 and by a second antibody raised to an unrelated peptide from the predicted CFTR sequence (α-102). Since both antibodies recognize the same protein, it seems likely that α-1468 is recognizing CFTR rather than a cross-reacting protein. Third, two-dimensional phosphopeptide mapping indicates that the immunoprecipitated protein undergoes phosphorylation by protein kinase A at the same sites as does a purified bacterially expressed protein that contains most of the predicted protein kinase A phosphorylation sites in CFTR. This finding rigorously confirms that the precipitated protein is CFTR. Moreover, these peptide maps indicate that most of the phosphorylation events detected when precipitated CFTR is labeled by protein kinase A agree with predictions based both on the occurrence of kinase substrate consensus sequences in the CFTR structure (9) and on the phosphorylation properties of CFTR as expressed in COS-7 cells (19).

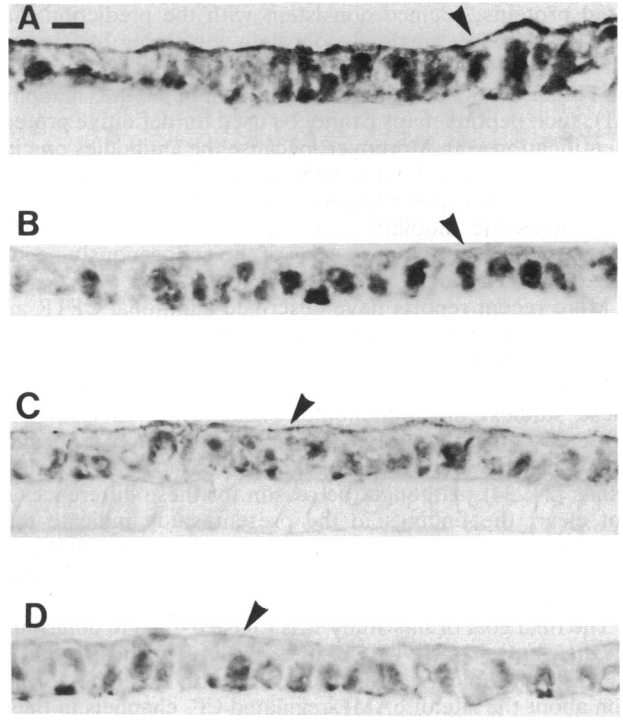

FIG. 6. Localization of CFTR in T84 cells grown as a monolayer on a collagen-coated filter. (*A*) Peroxidase staining with α-102 (final dilution, 1:100). (*B*) Control showing elimination of staining by α-102 after incubating the antibody with competing peptide. (*C*) Peroxidase staining with α-1468 (final dilution, 1:25). (*D*) Control showing elimination of staining by α-1468 after incubating the antibody with competing peptide. Arrowheads indicate the apical membrane. (Bar = 9 μm.)

Three findings support the conclusion that the 155- to 170-kDa immunoblot signal in Fig. 1 represents endogenous CFTR in T84 cells, pancreas, and colon. First, this signal is the only immunoreactive protein detected in several tissues expected to contain CFTR, and this signal is not detected in tissues in which CFTR mRNA occurs at lower levels (9). Second, the electrophoretic mobility of this immunoblot signal resembles that of the precipitated protein identified as CFTR in that each resolves as a broad band or doublet when analyzed by SDS/PAGE. Third, digestion of solubilized cell proteins by N-Glycanase results in an equivalent increase in electrophoretic mobility of both the immunoblot and the immunoprecipitation signals. One implication of this conclusion is that CFTR undergoes similar posttranslational processing in colon, in pancreas, and in T84 cells (Fig. 1*A*). Beyond this, the α-1468 immunoblot signal suggests that CFTR exhibits considerable heterogeneity in its native form.

The observation that CFTR exhibits heterogeneity is consistent with an initial immunochemical study of CFTR in T84 cells in which the predominant detected form of this protein was a diffuse band at a molecular mass of roughly 150 kDa (11). The position and heterogeneity of this band, which was detected by using protein kinase A to label immunoprecipi-

tated proteins, seemed consistent with the prediction that CFTR exists as a single glycoprotein containing 1480 amino acids. Even though this band was tentatively identified as CFTR based on one-dimensional phosphopeptide mapping (11), such peptide maps cannot be used for definitive protein identification (33). Moreover, because the antibodies precipitating this protein did not detect CFTR by immunoblot (11) and because phosphorylation can markedly affect a protein's electrophoretic mobility (33), this initial study left some uncertainty about the properties of the endogenously occurring form of CFTR.

More recent reports have described additional CFTR antibodies that detect immunoblot signals corresponding to proteins with molecular masses of roughly 170 kDa in T84 cells and in other colonocyte cell lines (17, 24, 34). In two of these studies, the immunoblot signals differed from that detected with the precipitation–phosphorylation procedure in that these immunoblot signals occurred as relatively sharp bands (24, 34). Although the reason for these differences is not clear, the findings in the present study indicate that heterogeneity is a characteristic feature of CFTR in T84 cells regardless of whether the protein is detected by immunoblot or by a precipitation–phosphorylation procedure.

The final goal of this study was to use α-1468 to determine the distribution of CFTR within T84 cells. The localization of CFTR to the apical region agrees well with existing information about the site of cAMP-regulated Cl^- channels in these cells (20–23). This staining pattern resembles that recently reported in the epithelial cells lining the intralobular ducts of pancreas (24, 25) and the reabsorptive ducts of sweat gland (26). The polarized expression of CFTR in each of these cell types contrasts with cytochemical observations in transfected COS-7 and Sf9 cells (14, 16), and this discrepancy reinforces the conclusion that CFTR processing may differ in transfected and untransfected cells.

In conclusion, this study has shown that a single high-affinity, monospecific antibody, α-1468, can be used to study CFTR in T84 cells by immunocytochemical, immunoblot, and immunoprecipitation procedures. The endogenous form of CFTR in T84 cells has been identified, and several properties of this protein have been characterized. Its apical localization, heterogeneous electrophoretic mobility, and identification as a protein kinase substrate are each consistent with predictions based on knowledge of the CFTR cDNA sequence (9), of the cell biology of CF (6, 7), and of the secretory physiology of T84 cells (20–23, 28). These findings validate the use of α-1468 as an immunochemical reagent for cell staining and indicate that this antibody should be useful for studying other aspects of the regulation and function of CFTR.

We thank Dr. Richard Metzgar (Duke University) for assistance in growing the T84 cell tumors and Dr. Richard Boucher (University of North Carolina) for suggestions concerning the experiment shown in Fig. 3. Technical assistance was provided by Andrew Kaz, Mark Rigby, Lesley Page, Wei Wang, Kathleen Dittrich, and Denise Clark. This work was supported by grants from the Cystic Fibrosis Foundation (to J.A.C. and A.C.N.), the National Institutes of Health (to J.A.C. and C.R.M.), and the Veterans Administration (to J.A.C.).

1. Schoumacher, R. A., Shoemaker, R. L., Halm, D. R., Tallant, E. A., Wallace, R. W. & Frizzell, R. A. (1987) *Nature (London)* **330**, 752–754.
2. Li, M., McCann, J. D., Liedtke, C. M., Nairn, A. C., Greengard, P. & Welsh, M. J. (1988) *Nature (London)* **331**, 358–360.
3. Hwang, T., Lu, L., Zeitlin, P. L., Gruenert, D., Huganir, R. & Guggino, W. B. (1989) *Science* **244**, 1351–1353.
4. Li, M., McCann, J. D., Anderson, M. P., Clancy, J. P., Liedtke, C. M., Nairn, A. C., Greengard, P. & Welsh, M. J. (1989) *Science* **244**, 1353–1356.
5. Boucher, R. C., Cheng, E. H. C., Paradisio, A. M., Stutts, M. J., Knowles, M. R. & Earp, H. S. (1989) *J. Clin. Invest.* **84**, 1424–1431.
6. Quinton, P. M. (1990) *FASEB J.* **4**, 2709–2717.
7. Welsh, M. J. (1990) *FASEB J.* **4**, 2718–2725.
8. Rommens, J. M., Iannuzzi, M. C., Kerem, B., Drumm, M. L., Melmer, G., Dean, M., Rozmahel, R., Cole, J. L., Kennedy, D., Hidaka, N., Zsiga, M., Buchwald, M., Riordan, J. R., Tsui, L.-C. & Collins, F. S. (1989) *Science* **245**, 1059–1065.
9. Riordan, J. R., Rommens, J. M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J., Drumm, M. L., Iannuzzi, M. C., Collins, F. S. & Tsui, L.-C. (1989) *Science* **245**, 1066–1073.
10. Kerem, B., Rommens, J. M., Buchanan, J. A., Markiewicz, D., Cox, T. K., Chakravarti, A., Buchwald, M. & Tsui, L.-C. (1989) *Science* **245**, 1073–1080.
11. Gregory, R. J., Cheng, S. H., Rich, D. P., Marshall, J., Paul, S., Hehir, K., Ostedgaard, L., Klinger, K. W., Welsh, M. J. & Smith, A. E. (1990) *Nature (London)* **347**, 382–386.
12. Rich, D. P., Anderson, M. P., Gregory, R. J., Cheng, S. H., Paul, S., Jefferson, D. M., Klinger, K. W., McCann, J. D., Smith, A. E. & Welsh, M. J. (1990) *Nature (London)* **347**, 358–363.
13. Drumm, M. L., Pope, H. A., Cliff, W. H., Rommens, J. M., Marvin, S. A., Tsui, L.-C., Collins, F. S., Frizzell, R. A. & Wilson, J. M. (1990) *Cell* **62**, 1227–1233.
14. Cheng, S. H., Gregory, R. J., Marshall, J., Paul, S., Souza, D. W., White, G. A., O'Riordan, C. R. & Smith, A. E. (1990) *Cell* **63**, 827–834.
15. Gregory, R. J., Rich, D. P., Cheng, S. H., Souza, D. W., Paul, S., Manavalan, P., Anderson, M. P., Welsh, M. J. & Smith, A. E. (1991) *Mol. Cell. Biol.* **11**, 3886–3893.
16. Kartner, N., Hanrahan, J. W., Jensen, T. J., Naismith, A. L., Sun, S., Ackerley, C. A., Reyes, E. F., Tsui, L.-C., Rommens, J. M., Bear, C. E. & Riordan, J. R. (1991) *Cell* **64**, 681–691.
17. Tabcharani, J. A., Chang, X.-B., Riordan, J. R. & Hanrahan, J. W. (1991) *Nature (London)* **352**, 628–631.
18. Anderson, M. P., Gregory, R. J., Thompson, S., Souza, D. W., Paul, S., Mulligan, R. C., Smith, A. E. & Welsh, M. J. (1991) *Science* **253**, 202–205.
19. Cheng, S. H., Rich, D. P., Marshall, J., Gregory, R. J., Welsh, M. J. & Smith, A. E. (1991) *Cell* **66**, 1027–1036.
20. Mandel, K. G., Dharmsathaphorn, K. & McRoberts, J. A. (1986) *J. Biol. Chem.* **261**, 704–712.
21. Halm, D. R., Rechkemmer, G., Schoumacher, R. A. & Frizzell, R. A. (1988) *Am. J. Physiol.* **254**, C505–C511.
22. Cliff, W. H. & Frizzell, R. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4956–4960.
23. Anderson, M. P. & Welsh, M. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6003–6007.
24. Crawford, I., Maloney, P. C., Zeitlin, P. L., Guggino, W. B., Hyde, S. C., Turley, H., Gatter, K. C., Harris, A. & Higgins, C. F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9262–9266.
25. Marino, C. R., Matovcik, L. M., Gorelick, F. S. & Cohn, J. A. (1991) *J. Clin. Invest.* **88**, 712–716.
26. Cohn, J. A., Melhus, O., Page, L. J., Dittrich, K. L. & Vigna, S. R. (1991) *Biochem. Biophys. Res. Commun.* **181**, 36–43.
27. Murakami, H. & Masui, H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3464–3468.
28. Dharmsathaphorn, K., McRoberts, J. A., Mandel, K. G., Tisdale, L. D. & Masui, H. (1984) *Am. J. Physiol.* **246**, G204–G208.
29. Bacallao, R. & Stelzer, E. H. K. (1989) *Methods Cell Biol.* **31**, 437–452.
30. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
31. Marshall, J., Martin, K. A., Picciotto, M., Hockfield, S., Nairn, A. C. & Kaczmarek, L. K. (1991) *J. Biol. Chem.* **266**, 22749–22754.
32. Picciotto, M. R., Bertuzzi, G., Greengard, P. & Nairn, A. C. (1991) *Ped. Pulmon. Suppl.* **6**, 224 (abstr.).
33. Dunbar, B. S. (1987) *Two Dimensional Electrophoresis and Immunological Techniques* (Plenum, New York).
34. Zeitlin, P. L., Crawford, I., Luo, L., Woel, S., Cohen, M. E., Donowitz, M., Montrose, M. H., Hamosh, A., Cutting, G. R., Guenert, D., Huganir, R., Maloney, P. & Guggino, W. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 344–347.