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A ubiquitous 64-kDa protein is a component of a chloride channel of plasma and intracellular membranes

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ABSTRACT **Chloride channels are present in the plasma and intracellular membranes of most cells. Previously, using the ligand indanyloxyacetic acid (IAA), we purified four major proteins from bovine kidney cortex membrane vesicles. These proteins gave rise to chloride channel activity when reconstituted into phospholipid vesicles. Two of these proteins (97 and 27 kDa) were found to be drug-binding proteins by N-terminal sequence analysis. Antibodies raised to the 64-kDa protein stained only this protein on immunoblots, and only this protein was present after purification on an immunoaffinity column. In addition, these same antibodies were able to deplete IAA-94 inhibitable chloride channel activity from solubilized kidney membranes. Of fractions obtained from the gel filtration of solubilized kidney membranes, only those containing this 64-kDa protein exhibited measurable chloride channel activity. Immunoblots of a variety of species and cell types, both epithelial and nonepithelial, revealed that this protein is ubiquitous and highly conserved. Immunocytochemistry in CFPAC-1 cells revealed staining for this protein on the apical plasma membrane and in the membranes of intracellular organelles. These results demonstrate that the integral membrane protein p64 is a component of chloride channels present in both epithelial plasma membrane and the membranes of intracellular organelles.**

Chloride channels are found in the membranes of almost every cell type, where they play a variety of roles (1). In many cells they act in parallel with cation channels to allow the flux of salt into and out of the cell and thus enable the cell to control its volume. In excitable cells, the ability of chloride channels to conduct charge across membranes is an important component in the control of the membrane potential. In skeletal muscle, chloride channels appear to be responsible for the hyperpolarization that follows contraction, and a reduced chloride conductance leads to the disease myotonia with its high level of depolarization (2). In epithelia, chloride channels are distributed in a polarized fashion, where they mediate the secretion and absorption of salt (3). Hormones such as epinephrine and muscarinic agonists stimulate epithelial secretion by activating apical membrane chloride channels through the action of second messengers and eventually protein kinases (4). In secretory diarrhea or cholera, the opening of chloride channels appears to be responsible for intestinal water secretion (5), whereas in cystic fibrosis, protein kinase A and C are unable to open a chloride channel in the apical plasma membrane (6–10).

Intracellular organelles such as Golgi and endosomes also contain chloride channels, where one of their functions is shunting the electrical potential generated by the ATP-dependent proton pump, enabling a large pH gradient to be generated (11). The pH of individual organelles may be important for the correct processing and recycling of some cellular proteins. Disruption of this function by an abnormally functioning chloride channel might provide an explanation for abnormalities in processing of secreted proteins in cystic fibrosis (12). The chloride channel of intracellular organelles can also be activated by secretagogues, and more recently, it was found that protein kinase A can open the chloride channel of kidney endosomes and brain clathrin-coated vesicles (13, 14).

Chloride channels vary in electrophysiology and sensitivity to biological and pharmacological reagents. Indeed, some epithelial cells have been demonstrated to contain three electrophysiologically distinguishable chloride channels (15, 16). The question arises whether these different channels are derived from a gene family or from alternate splicing of a single mRNA as is the case for the shaker type of potassium channels (17–19). Alternatively, chloride channels might stem from the expression of unrelated genes. To determine the origin of chloride channel diversity and the mechanisms underlying their regulation, immunological, biochemical, and molecular reagents are needed. In an attempt to obtain some of these reagents, we have purified chloride channels from cortex of the bovine kidney and raised antibodies to one component.

Bovine kidney cortex microsomes were used to screen for drugs that might act as useful ligands, and one such drug, an indanyloxyacetic acid (IAA-94), was identified. It had an inhibitory and binding potency in the micromolar range. Using an affinity column based on IAA-94, we were able to purify four proteins from solubilized kidney cortical membranes (20). When reconstituted into phospholipid vesicles, these proteins gave rise to chloride channel activity, indicating that one or more of these proteins constitute a chloride channel. We now present immunological, chromatographic, and sequence data that demonstrate that the 64-kDa protein is a constituent of a chloride channel while eliminating two of the other proteins.

MATERIALS AND METHODS

^{125}I-labeled streptavidin and ^{36}Cl were obtained from NEN. Biotin and horseradish peroxidase-labeled rabbit anti-guinea pig antibodies were obtained from Zymed Laboratories. Staphylococcal protein A-Sepharose 4CL and CNBr-activated Sepharose 4B were from Pharmacia. All other chemicals were obtained from Sigma.

Chloride Channel Purification. Bovine kidney cortex microsomes were prepared as described (21) and stored at −70°C. When needed, the microsomes were rapidly thawed, treated with 1 M KSCN to remove peripheral membrane proteins, solubilized, and purified by binding to and elution from an IAA-23 column as described (20).

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Abbreviations: IAA, indanyloxyacetic acid; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.

Generation of Polyclonal Antisera. Material eluted from a 10-ml IAA-23 affinity column was acetone precipitated, dissolved in Laemmli sample buffer (22), and subjected to SDS/PAGE. Coomassie blue-stained bands were cut from the gels and injected subcutaneously into guinea pigs with complete Freund's adjuvant. Subsequent intraperitoneal injections were performed every 2 weeks for 2 months and then once a month. The antibodies raised to the 64-kDa protein are referred to as anti-64 IgG.

Immunoblots. Kidney membrane vesicles or acetone-precipitated IAA-purified proteins were run on SDS/PAGE gels as above and transferred to nitrocellulose (23). The nitrocellulose was then blocked with 5% nonfat milk and 0.05% Triton X-100 for 1 hr and incubated with antisera preabsorbed against blots of bovine serum albumin. The bound antibody was localized with a biotinylated rabbit secondary antibody, ^{125}I-labeled streptavidin, and autoradiography.

Immunoaffinity Purification. Four milligrams of kidney membrane vesicles was pelleted and resuspended in 1.0 ml of RIPA buffer (150 mM NaCl/50 mM Tris/0.5% cholate/0.1% SDS/1.0% Nonidet P-40) and incubated for 30 min at 4°C. After centrifugation at 100,000 × *g* for 1 hr, the supernatant was incubated first with 50 μl of normal rabbit serum and 50 μl of protein A beads for 16 hr followed by the addition of 50 μl of Pansorbin for 1 hr. The beads and Pansorbin were removed by centrifugation, and the "preabsorbed" proteins were incubated with 50 μl of protein A-agarose beads to which antibodies had been crosslinked. The beads (Affinica antibody orientation kit, Schleicher & Schuell) were first prepared by mixing them with an equal volume of anti-64 IgG or nonimmune IgG and crosslinked and quenched using the manufacturer's instructions. After 2 hr of incubation, the beads were washed twice with 1.0 ml of RIPA buffer and twice with 250 mM sucrose/0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)/10 mM imidazole, pH 7.0. The beads were then suspended in 50 μl of the sucrose/imidazole/CHAPS buffer with 0.1 μl of sulfosuccinimidobiotin (200 mg/ml) in dimethyl sulfoxide. The slurry was incubated at room temperature for 30 min, and the reaction was stopped by the addition of 1.0 ml of RIPA buffer. The beads were washed an additional four times with 1.0 ml of RIPA buffer and finally incubated at 40°C with 80 μl of SDS/PAGE sample buffer. The eluted proteins were then subjected to SDS/PAGE and electroblotted onto nitrocellulose, and the biotinylated proteins were detected by peroxidase-labeled avidin by using enhanced chemiluminescence reagents (Amersham).

Immunodepletion. IgG from the serum was purified by binding to protein A-Sepharose in 0.1 M Tris (pH 8.0) and elution with 0.1 M glycine (pH 3.0). The eluted antibody was immediately titrated to pH 7.0 by adding 1 M Tris (pH 8.0). Solubilized kidney membranes (3 mg/ml) were incubated for 18 hr at 4°C with anti-64 IgG, nonimmune IgG, or no IgG. Antibody–protein complexes were removed with protein A-Sepharose (50 μl/ml) preincubated with 10% guinea pig albumin. The immunodepleted protein was then reconstituted into asolectin (10 mg/ml) by detergent dialysis in the presence of 700 mM sucrose, 10 mM KCl, and 10 mM Hepes (pH 7.0) for 24 hr and then 200 mM KCl and 10 mM Hepes (pH 7.0) for a subsequent 12 hr. Proteoliposomes were frozen to −70°C and, when required, rapidly thawed and sonicated for 25 s in a bath sonicator.

^{36}Cl uptake assays were performed according to the method of Garty (24). In brief, external chloride was removed by applying the vesicles to a gluconate-exchange column and eluting them with 250 mM sucrose (21). ^{36}Cl (5×10^6 cpm/ml) was added. Immediately half the sample was removed, and valinomycin was added to a final concentration of 5 μM. At various time points, 0.5-ml samples were taken, and extravesicular ^{36}Cl was removed by passing the vesicles through an 8-cm anion-exchange column. ^{36}Cl was measured by liquid scintillation counting.

Gel Filtration. Kidney microsomes were solubilized with *n*-octyl glucoside as before and applied to a Sephacryl 200 gel-filtration column. Proteins were eluted with 250 mM sucrose and 10 mM imidazole containing 10% glycerol and 0.6% *n*-octyl glucoside. The fractions were concentrated into three samples of 1 ml representing the void volume (>250 kDa), middle fraction (250–80 kDa), and low fraction (<80 kDa). The samples were then tested for chloride channel activity by coreconstitution with bacteriorhodopsin and measuring light-driven ^{36}Cl uptake as described (20). In parallel experiments, fractions were tested for the presence of the 64-kDa protein by immunoblot analysis as described above.

Immunocytochemistry. CFPAC-1 cells were fixed with 3% paraformaldehyde and washed three times with 0.9% NaCl/10 mM sodium phosphate, pH 7.4 (PBS). For intracellular staining, cells were permeabilized with 0.1% Triton X-100. Background staining was blocked by incubation with 0.02% gelatin in PBS. Cells were incubated with anti-64 antisera (1:50), and the bound antibody was detected by goat anti-guinea pig IgG labeled with fluorescein isothiocyanate (Kirkegaard and Perry Laboratories, Gaithersburg, MD; 25 μg/ml). The cells were mounted in PBS/glycerol (50:50, vol/vol) containing *n*-propyl gallate (25).

Preparation of Cell Membranes. Cell membranes were prepared by washing cell monolayers with ice-cold PBS and scraping cells from the culture dish into a solution containing 250 mM sucrose, 5 mM Tris, 1 mM EGTA, and 1 mM $NaHCO_3$ (pH 8.0). The cells were homogenized by 30 strokes in a Dounce homogenizer and subjected to low-speed centrifugation at 4000 × *g* for 15 min. The supernatant was centrifuged at 100,000 × *g* for 1 hr. The resulting membrane pellet was suspended in 250 mM sucrose and 10 mM imidazole (pH 7.0) and frozen at −70°C. When required, membranes were thawed, dissolved in Laemmli sample buffer, and subjected to immunoblot analysis as described above.

RESULTS

We have previously shown that purification of solubilized kidney cortex membranes on an IAA-94 column gave rise to four proteins, which produced chloride channel activity when they were incorporated into lipid membranes (16). Analysis of these eluted proteins by SDS/PAGE and silver staining revealed four proteins (27, 40, 64, and 97 kDa; Fig. 1*A*). However, it was unclear which of these proteins were components of the chloride channel and which were merely drug-binding proteins. To resolve this question, N-terminal sequencing was performed for the 27- and 97-kDa proteins. The sequence obtained from the 27-kDa protein, the most abundant protein, was (in single-letter code) MPPYTIVYFPVRGRCEA, which proved to be a glutathione *S*-transferase. The sequence obtained from the 97-kDa protein was G(R)DKYEPAAVSEHGDKKKAKKXRDM, which identified it as the α subunit of the Na^+,K^+-ATPase. Both the Na^+,K^+-ATPase (26, 27) and glutathione transferase (28) are abundant kidney proteins that are known to bind ethacrynic acid. This compound has great structural similarity to IAA, and it is not surprising that an IAA-23 column should purify these proteins.

The identification of the 97- and 27-kDa proteins left two candidates as chloride channel proteins: the 64- and 40-kDa proteins. To determine whether one or both of these proteins contribute to chloride channel activity, antibodies were raised to both proteins, where the immunogen was bands cut from SDS/PAGE gels of purified material. Only those raised to the 64-kDa protein reached a useful titer. These antibodies recognized only the 64-kDa protein on immunoblots of crude membranes, and the staining was much greater when purified

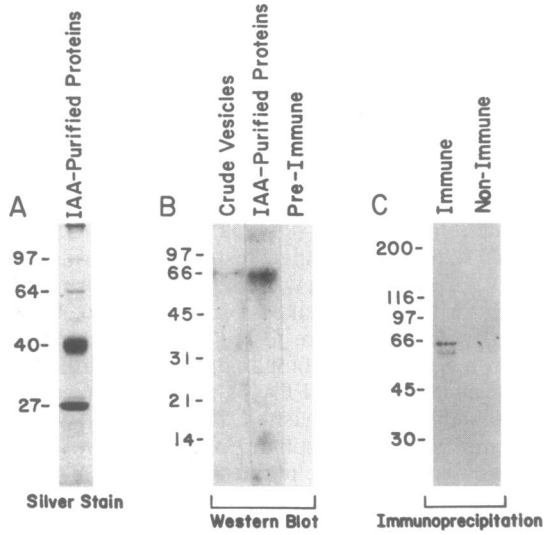

FIG. 1. Analysis of IAA-purified proteins by silver staining of SDS/PAGE gels and immunoblotting with anti-64 IgG. (*A*) Proteins eluted from an IAA-23 affinity column were subjected to SDS/PAGE, revealing four predominant protein bands. (*B*) Antibodies raised against the 64-kDa band recognized this band alone in 30 μg of crude kidney membrane protein or 1 μg of affinity-purified protein following SDS/PAGE and Western blotting. (*C*) Biotinylated eluate from the anti-64 IgG immunoaffinity column electroblotted and probed with enhanced chemiluminescence detection reagent shows only the 64-kDa protein.

material was used (Fig. 1*B*). After determining the specificity of this antibody, an immunoaffinity column was constructed by crosslinking the antibody to protein A beads. Analysis of proteins purified and eluted from this column revealed only the 64-kDa protein (Fig. 1*C*). Attempts to reconstitute chloride channel activity from this eluted protein were unsuccessful. However, reconstitution of crude solubilized membranes showed that the conditions necessary for elution of the protein from the antibody (reduction of pH to 2.5 or treatment with 2% SDS) were too harsh to maintain channel activity. In light of this, immunodepletion was performed (Fig. 2). In brief, solubilized kidney membrane vesicles were incubated with anti-64 IgG, nonimmune IgG, or no IgG. The resulting protein–antibody complexes were removed by binding to protein A-Sepharose and pelleting. The supernatants were assayed for chloride channel activity by reconstitution into asolectin vesicles. The proteoliposomes were formed in the presence of KCl and passed through an anion-exchange column to generate an outwardly directed chloride gradient. Tracer amounts of ^{36}Cl were added to the outside of the vesicles. Vesicles with chloride channels generated an inside positive membrane potential allowing the accumulation of ^{36}Cl in a potential-sensitive manner. Solubilized kidney cortex membranes that were incubated with nonimmune IgG or no IgG and then reconstituted showed ^{36}Cl uptake that was reduced when the electrical potential was dissipated by the potassium ionophore valinomycin (Fig. 2). In experiments not shown, this potential sensitive uptake was reduced by >60% by the addition of 50 μM IAA-94. Supernatants incubated with anti-64 IgG did not exhibit a valinomycin-sensitive ^{36}Cl uptake after reconstitution (Fig. 2). The uptake was similar in magnitude to the valinomycin-insensitive flux of the control experiments. Similar experiments were performed with antibodies directed against glutathione *S*-transferase, but these antibodies were unable to deplete chloride channel activity. Although the contribution of the 40-kDa protein remains to be determined, these results provide compelling evidence that the 64-kDa protein is a component of an IAA-sensitive chloride channel.

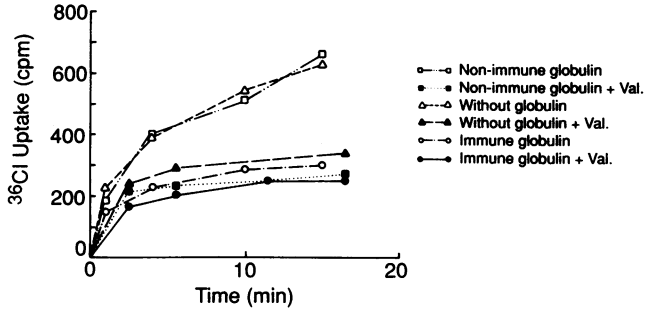

FIG. 2. Depletion of chloride channel activity by anti-64 IgG but not nonimmune IgG. Solubilized kidney membrane proteins demonstrated valinomycin (Val.)-sensitive ^{36}Cl uptake, when reconstituted into asolectin vesicles (triangles). This uptake was completely absent following incubation with anti-64 IgG and removal of the immune complex with protein A (circles). Uptake was unaffected by treatment with nonimmune IgG (squares). Filled symbols show the effect of valinomycin. The data shown are representative of four experiments.

The association of the 64-kDa protein with chloride channel activity was further highlighted by analysis of fractions obtained from gel filtration of solubilized kidney membranes (Fig. 3). Immunoblot analysis revealed that the 64-kDa protein appeared only in the void volume of the column. Concentration and reconstitution of the void fraction (>250 kDa), a middle fraction (150–80 kDa), and a low molecular mass fraction (<80 kDa) revealed chloride channel activity in only the void fraction. This fraction was the only one containing the 64-kDa protein. Elution of chloride channel activity in a high molecular mass fraction suggests that the channel is a large complex consisting of a number of subunits. The existence of the complex is strengthened by the observation that after solubilization of cultured cells in the unrelated detergent CHAPS, the 64-kDa protein was found to elute at 400,000–600,000 kDa from a Superose 6 gel-filtration column. Although these data suggest a high molecular mass complex, its precise size could not be determined because of the unknown contribution of lipid and detergent.

Immunoblot analysis of membranes obtained from T84 cells, Chinese hamster ovary cells, human fibroblasts, and the insect cell line Sf9 demonstrated the presence of immunologically related proteins of a similar molecular mass (Fig. 4). These cells are of epithelial and nonepithelial origin and are from diverse species, suggesting that this channel protein is highly conserved and might play a role outside of epithelia.

We determined the cellular location of this chloride channel protein by immunocytochemistry in CFPAC-1 cells, a

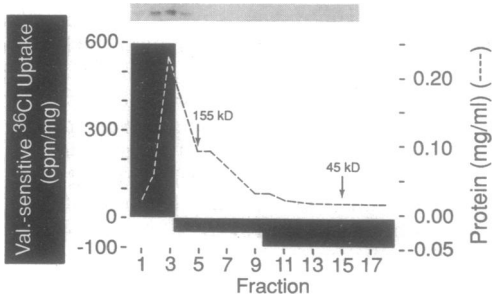

FIG. 3. Comigration of chloride channel activity and the 64-kDa protein through a Sephacryl 200 gel-filtration column. Immunoblotting of fractions eluted from a Sephacryl 200 gel-filtration column revealed the presence of the 64-kDa protein in the void volume. After concentration and reconstitution, only proteins in the void volume (>250 kDa) demonstrated electrogenic ^{36}Cl uptake. Uptake is given as cpm/mg of protein reconstituted. The data shown are the average of three experiments.

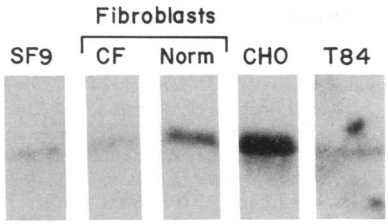

FIG. 4. Immunoblots of Sf9, CHO, human fibroblasts, and T84. All five cell lines show the presence of an immunologically related protein of similar molecular mass. Norm, normal; CF, cystic fibrosis.

pancreatic carcinoma epithelial cell line derived from a patient with cystic fibrosis (29). Immunocytochemical staining of confluent CFPAC-1 cells revealed the presence of the 64-kDa protein in these cells (Fig. 5). In lightly fixed cells (Fig. 5*A*) staining appeared on the apical surface of the monolayer, but the intensity of staining was quite variable: only 30% of the cells were brightly stained. When the cells were permeabilized (Fig. 5*B*), staining was present in intracellular membranes, but was not visible on the basolateral membrane. The staining was apparent in vesicles around the nucleus and is consistent with the location of the Golgi, but it is also present in other compartments toward the periphery of the cell. The exact identity of these compartments awaits double-staining procedures at the light and electron microscopic level. That the antibody did not stain all permeabilized cells of the monolayer is likely to be a technical artifact since double labeling of the same field with a proton ATPase antibody showed staining only in cells stained with the anti-64-kDa antisera. Staining of the other cells mentioned above with the antibody was below the detection limit of fluorescent antibody methods, confirming our suspicion that the protein is of very low abundance.

DISCUSSION

Reconstitution of solubilized kidney cortex vesicles showed the appearance of voltage-sensitive ^{36}Cl uptake. Although we have interpreted this process to indicate the presence of chloride channels, this kind of uptake could be produced by electrogenic carriers. However, previous studies in these vesicles have made this conclusion highly unlikely (21). There are several known anion transport carriers including Na/K/Cl cotransport and the Cl–HCO_3 exchanger. Both of these processes are neutral and would not be expected to respond to changes in membrane potential. In principle, several carriers can demonstrate potential-sensitive behavior under some conditions. The potential-sensitive uptake was not affected by the presence of Na and was independent of pH at pH values of 5–8 and of extravesicular calcium concentrations of 0–1 mM; these experiments suggest that the known carriers could not mediate the flux. This uptake could also be demonstrated in the absence of Cl on the opposite side of the membrane, excluding the presence of a voltage-sensitive Cl–Cl exchange. Finally, an antibody against a single protein was able to abolish all the voltage-sensitive uptake. These results provide compelling evidence that the flux measured is produced by a chloride channel.

Kidney cortex membranes that we used were enriched for Golgi markers as well as Na^+,K^+-ATPase; hence, they contained intracellular membranes as well as basolateral membranes. The proximal tubule, the major component of the kidney cortex, does not contain any basolateral chloride channel activity (30). Hence, it is likely that the channel we purified resides in intracellular membranes. The evidence presented here shows that the 64-kDa IAA-binding protein is necessary for the kidney cortex chloride channel. There is suggestive evidence that it is also sufficient for this activity. The anti-64 antibody depletes all the chloride channel activity from the solubilized membranes, but only one protein is immunoprecipitated. Recent studies have shown that kidney cortex endosomes contain a chloride channel that can be activated by protein kinase A (13). Biochemical evidence shows that the only protein specifically phosphorylated in these membranes is a 66-kDa protein (31). In addition, attempts at purification of the chloride channel of brain clathrin-coated vesicles also yielded a protein with a molecular mass of 65–70 kDa. These results suggest that the 64-kDa protein in epithelial and nonepithelial cells might serve as the Golgi–endosome chloride channel. However, more direct

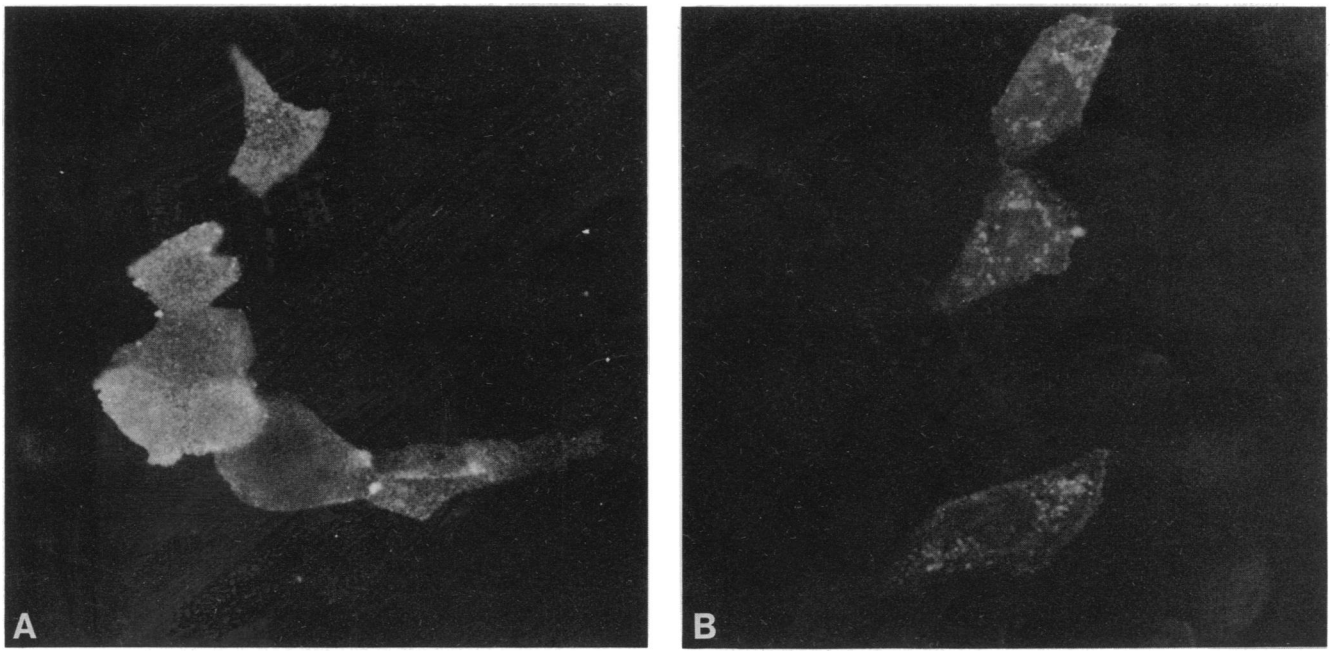

FIG. 5. Immunolocalization of the chloride channel protein in CFPAC-1 cells. Immunofluorescence in intact cells (*A*) and permeabilized cells (*B*) revealed the presence of the chloride channel protein in the apical plasma membrane and intracellular membranes, but not in the basolateral membrane.

immunocytochemical and biochemical studies need to be done before this conclusion can be drawn with certainty. That immunocytochemistry shows it to be located in the apical membrane of an epithelial cell demonstrates that one of the epithelial chloride channels is either the same protein or one structurally related to it.

Recently several proteins have been implicated in mediating chloride channel activity. A cDNA from *Torpedo* electric organ that encodes a 90-kDa protein yielded voltage-sensitive Cl currents when expressed in *Xenopus* oocytes (32). A 38-kDa protein from bovine trachea has been shown to have chloride channel activity (33). Injection of CFTR, the gene product of the cystic fibrosis gene, caused the appearance of cAMP-activated Cl channels in cells that did not possess them. Finally, a monoclonal antibody to a 210-kDa protein from *Necturus* gallbladder was found to inhibit cAMP-activated Cl channels in that epithelium (34). A number of groups have succeeded in expressing the CFTR message in a variety of nonepithelial cells (35, 36). This expression led to the appearance of cAMP-regulated chloride channels in the plasma membrane of these cells that was not present before transfection, leading the authors to propose that the CFTR protein is itself a cAMP-regulated chloride channel. Further studies using mutated CFTR also showed that the anion selectivity and regulation by cAMP were affected, providing stronger evidence for this proposal (37, 38). The relation of these proteins to the 64-kDa one discussed here is unknown at present. In particular, it is not known whether each of those proteins can by itself form anion channels or requires additional subunits, since heterologous cells might constitutively express these subunits. Because we were unable to reconstitute chloride channel activity from the immunoprecipitated 64-kDa protein, similar questions regarding this protein will need to be resolved.

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1. Franciolini, F. & Petris, A. (1990) *Biochim. Biophys. Acta* **1031**, 247–259.
2. Rudel, R. & Lehmann-Horn, F. (1985) *Physiol. Rev.* **65**, 310–356.
3. Greger, R., Kunzelmann, K. & Gerlach, L. (1989) *Ann. N.Y. Acad. Sci.* **574**, 403–415.
4. McCann, J. D. & Welsh, M. J. (1990) *Annu. Rev. Physiol.* **52**, 115–135.
5. Field, M., From, D., Al-Awqati, Q. & Greenough, W. B., III (1972) *J. Clin. Invest.* **51**, 796–804.
6. Welsh, M. J. (1990) *FASEB J.* **4**, 2718–2725.
7. Schoumacher, R. A., Shoemaker, R. L., Halm, D. R., Tallant, E. A., Wallace, R. W. & Frizzell, R. A. (1987) *Nature (London)* **330**, 752–754.
8. Li, M., McCann, J. D., Liedtke, C. M., Nairn, A. C., Greengard, P. & Welsh, M. J. (1988) *Nature (London)* **331**, 358–360.
9. 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.
10. Hwang, T. C., Lu, L., Zeitlin, P. L., Gruenert, D. C., Huganir, R. & Guggino, W. B. (1989) *Science* **244**, 1351–1353.
11. Barasch, J., Gershon, M. D., Nunez, E. A., Tamir, H. & Al-Awqati, Q. (1988) *J. Cell Biol.* **107**, 2133–2147.
12. Barasch, J., Kiss, B., Prince, A., Saiman, L., Gruenert, D. & Al-Awqati, Q. (1991) *Nature (London)* **352**, 70–73.
13. Bae, H.-R. & Verkman, A. S. (1991) *Nature (London)* **348**, 637–639.
14. Mulberg, A. E., Tulk, B. M. & Forgac, M. (1991) *J. Biol. Chem.* **266**, 20590–20593.
15. Cliff, W. H. & Frizzell, R. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4956–4960.
16. Worrell, R. T., Butt, A. G., Cliff, W. H. & Frizzell, R. A. (1989) *Am. J. Physiol.* **256**, C1111–C1119.
17. Schwarz, T. L., Papazian, D. M., Carretto, R. C., Jan, Y. N. & Jan, L.-Y. (1990) *Neuron* **4**, 119–127.
18. Timpe, L. C., Jan, Y. N. & Jan, L. Y. (1988) *Neuron* **1**, 659–667.
19. Pongs, O., Kecskemethy, N., Muller, R., Krah-Jentgens, I., Baumann, A., Kiltz, H. H., Canal, I., Llamazares, S. & Ferrus, A. (1988) *EMBO J.* **7**, 1087–1096.
20. Landry, D. W., Akabas, M. A., Redhead, C. R., Edelman, A. E., Cragoe, E. J. & Al-Awqati, Q. (1989) *Science* **244**, 1423–1425.
21. Landry, D. W., Reitman, M., Cragoe, E. J., Jr., & Al-Awqati, Q. (1987) *J. Gen. Physiol.* **90**, 779–798.
22. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
23. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
24. Garty, H., Rudy, B. & Karlish, S. J. D. (1983) *J. Biol. Chem.* **258**, 13094–13099.
25. Giloh, H. & Sedat, S. W. (1982) *Science* **217**, 1252–1255.
26. Charnock, J. S., Potter, H. A. & McKee, D. (1970) *Biochem. Pharmacol.* **19**, 1637–1641.
27. Davis, P. W. (1970) *Biochem. Pharmacol.* **19**, 1983.
28. Habig, W. H. & Jakoby, W. B. (1981) *Methods Enzymol.* **77**, 398–405.
29. Schoumacher, R. A., Ram, J., Iannuzzi, M. C., Bradbury, N. A., Wallace, R. W., Tom Hon, C., Kelly, D. R., Schmid, S. M., Gelder, F. B., Rado, T. A. & Frizzell, R. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4012–4016.
30. Ishibashi, K., Rector, F. C. & Berry, C. A. (1990) *Am. J. Physiol.* **258**, F1569–F1578.
31. Reenstra, W. W., Sabolic, I., Bae, H.-R. & Verkman, A. S. (1992) *Biochemistry* **31**, 175–181.
32. Jentsch, T. J., Steinmeyer, K. & Scwarz, G. (1990) *Nature (London)* **348**, 510–514.
33. Ran, S. & Benos, D. J. (1991) *J. Biol. Chem.* **266**, 4782–4788.
34. Finn, A. L., Tsai, L.-H. & Falk, R. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7649–7652.
35. Anderson, M. P., Rich, D. P., Gregory, R. J., Smith, A. E. & Welsh, M. J. (1991) *Science* **251**, 679–682.
36. 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.
37. Anderson, M. P., Gregory, R. J., Thompson, S., Souza, D. W., Sucharita, P., Mulligan, R. C., Smith, A. E. & Welsh, M. J. (1991) *Science* **253**, 202–204.
38. Rich, D. P., Gregory, R. J., Anderson, M. P., Manavalan, P., Smith, A. E. & Welsh, M. J. (1991) *Science* **253**, 205–207.