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Membrane localization of the pertussis toxin-sensitive G-protein subunits α_{i-2} and α_{i-3} and expression of a metallothionein–α_{i-2} fusion gene in LLC-PK$_1$ cells

(guanine nucleotide regulatory proteins/renal epithelia/membrane targeting)

LOUIS ERCOLANI*†‡, JENNIFER L. STOW§, JANE F. BOYLE*†, ELIEZER J. HOLTZMAN*†, HERBERT LIN*†, J. RUSSELL GROVE¶, AND DENNIS A. AUSIELLO*†

*Renal and ¶Diabetes Units, Massachusetts General Hospital, and Departments of †Medicine and §Pathology, Harvard Medical School, Boston, MA 02114

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ABSTRACT **The renal epithelial cell line LLC-PK$_1$ has topographically distinct regulatory roles for the α subunits of pertussis toxin-sensitive guanine nucleotide regulatory proteins (α_i subunit); these include the inhibition of adenylyl cyclase at the basolateral membrane and the stimulation of Na^+ channel activity at the apical membrane. We now report that LLC-PK$_1$ cells contain two members of the α_i protein family, α_{i-2} and α_{i-3}, which have distinct cellular locations consistent with their diverse functional roles. By using specific α_i antibodies and immunofluorescence, the α_{i-2} subunit was found to be localized to the basolateral membrane, whereas the α_{i-3} subunit was concentrated in the Golgi and was also detectable at low levels on apical membranes in some cells. Induction of a chimeric mouse metallothionein 1–rat or canine α_{i-2} gene stably transfected into the LLC-PK$_1$ cells produced an increase in the content of the α_{i-2} subunit, which was targeted only to the basolateral membrane. These findings suggest that α_i subunit specificity for effectors may be achieved in polarized renal epithelial cells by their geographic segregation to different cellular membranes. The LLC-PK$_1$ cell stably transfected with the metallothionein–α_{i-2} fusion gene will provide a model for the study of guanine nucleotide regulatory protein function in epithelia.**

The regulation of epithelial function by hormones requires multiple steps for signal transduction. To achieve this, epithelia must maintain a functional polarity by distributing proteins topographically among cellular membranes. Heterotrimeric guanine nucleotide regulatory proteins (G proteins), consisting of α, β, and γ subunits, are sequentially involved at several steps of hormone action and signal transduction in epithelia. These include three pertussis toxin-sensitive G proteins (G_{i-1}, G_{i-2}, and G_{i-3}), which are distinguished by unique α_i subunits (1–3). Our laboratory has utilized the polarized renal epithelial cells, LLC-PK$_1$ and A6, as models to study G_i protein regulated function (4–9). These studies indicated that renal epithelia have G_i proteins that are involved in the regulation of hormone-stimulated adenylyl cyclase and Na^+ transport, on their basolateral and apical membranes, respectively.

We now report that LLC-PK$_1$ cells contain the G_i protein α subunits α_{i-2} and α_{i-3}, localized topographically to either the basolateral (α_{i-2}) or Golgi and apical membranes (α_{i-3}). The overexpression of α_{i-2} subunits in clonal cell lines of LLC-PK$_1$ cells stably transfected with a chimeric mouse metallothionein 1 (MT-1)–α_{i-2} subunit gene confirmed that α_{i-2} subunits are targeted to the basolateral membrane. These stably transfected clonal cell lines maintain a normal phenotype during culture both before and after induction of the α_{i-2} subunit gene with heavy metals. These cells provide a model for the study of G-protein function in renal epithelia.

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MATERIALS AND METHODS

Antibodies. Polyclonal rabbit antibodies raised against peptides of α_i subunits were provided by Allen Spiegel (National Institutes of Health). The antibody AS7 was raised against a synthetic peptide corresponding to the carboxyl-terminal decapeptide of the transducin α subunit and was shown to react on immunoblots with α_{i-1} and α_{i-2} (10). The antibody EC was raised against a synthetic carboxyl-terminal decapeptide from α_{i-3} and recognizes α_{i-3} and α_o (11). In LLC-PK$_1$ cells, the α_{i-1} and α_o subunits are not present, as assessed by Western and RNA blotting (L.E., K. Rossiter, J.L.S., and D.A.A., unpublished observations); we have found in these cells that the AS7 antibody is specific for the native (nondenatured) α_{i-2} subunit and that the EC antibody recognizes only α_{i-3}. Fluorescent and alkaline phosphatase-conjugated anti-rabbit antibodies were obtained from Kirkegaard and Perry Laboratories (Gaithersburg, MD) and ^{125}I-labeled goat anti-rabbit IgG was from New England Nuclear.

Cell Culture. LLC-PK$_1$ cells, a polarized epithelial cell line derived from pig kidney, were grown as confluent monolayers and maintained in Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal calf serum in 5% CO_2/95% air as described (4–8). Cells were plated at a density of 1×10^6 per 10 cm^2 and were used for the experiments described below at days 3–6 after plating.

Transfection. Ten micrograms of a eukaryotic expression vector (see below) containing a cDNA for rat α_{i-2} (12) or canine α_{i-2} (13) was cotransfected with 1 μg of pSV2neo (14) into LLC-PK$_1$ cells by the calcium phosphate precipitation method of Gorman *et al.* (15). The rat and canine α_{i-2} cDNAs were the kind gifts of R. Reed (Johns Hopkins University) and C. Homcy (Massachusetts General Hospital), respectively. Cells containing stably integrated plasmids were selected by resistance to geneticin (G418) (GIBCO) at 1 mg/ml.

RNA Gel Blots. Total RNA from LLC-PK$_1$ cells was separated by electrophoresis in 1.0% formaldehyde/agarose gels and then transferred to GeneScreen*Plus* membranes (New England Nuclear). Membranes were prehybridized for 2 hr at 42°C in the presence of 1% SDS/1 M NaCl/10% dextran sulfate/50% (vol/vol) deionized formamide. Hybridization was performed under similar conditions for 24 hr in the presence of denatured full-length rat α_{i-2} cDNA labeled

Abbreviations: G protein, guanine nucleotide regulatory protein; G_i, pertussis toxin-sensitive G protein; α_{i-1}, α_{i-2}, and α_{i-3}, α subunits of G_i; MT-1, mouse metallothionein 1.

‡To whom reprint requests should be addressed at: Renal Unit, 8th Floor, Massachusetts General Hospital, 149 13th Street, Charlestown, MA 02129.

with [α-^{32}P]ATP by priming with random hexamers followed by extension of these primers with the Klenow fragment of DNA polymerase (16). After hybridization, membranes were washed twice for 30 min each in (*i*) 0.3 M NaCl/0.03 M sodium citrate at 23°C, (*ii*) the same buffer with 1% SDS at 65°C, and (*iii*) 15 mM NaCl/1.5 mM sodium citrate at 65°C. The membranes were dried and autoradiographed with Kodak XAR film at −80°C for 12–96 hr. Quantification of hybridization signals was performed by densitometry of the autoradiograms.

Immunofluorescence. LLC-PK$_1$ cells or $\alpha_{i\text{-}2}$-transfected cell clones grown on glass coverslips were rinsed with phosphate-buffered saline (PBS), fixed in 3% paraformaldehyde for 1 hr, permeabilized in 0.1% Triton X-100 for 15 min, and then washed with PBS containing 0.5% bovine serum albumin to reduce nonspecific background staining. The cells were incubated for 2 hr in AS7, EC, or preimmune rabbit serum at 1:100 dilutions, washed three times in PBS, and then incubated for 1 hr with goat anti-rabbit IgG conjugated to Texas red or with sheep anti-rabbit IgG conjugated to fluorescein isothiocyanate. Cells were washed three times in PBS, then mounted in PBS/glycerol, and viewed in an Olympus BHS photomicroscope equipped for epifluorescence. Immunofluorescent staining used to compare $\alpha_{i\text{-}2}$ membrane localization in noninduced and induced cells was repeated in 10 separate experiments.

Immunoblotting of Cell Proteins. A plasma membrane preparation (4–6) and its supernatant fraction were prepared from batches of $\alpha_{i\text{-}2}$-transfected LLC-PK$_1$ cells (clone 2+3s) either noninduced or induced with 5 μM $CdCl_2$ for 16 hr. Proteins in the membrane and supernatant fractions were solubilized by boiling in sample buffer [1% SDS/30 mM Tris, pH 6.8/12% (vol/vol) glycerol], and 150 μg of protein was loaded per lane onto a Laemmli 10% polyacrylamide gel. Following electrophoresis, proteins were transferred onto an Immobilon membrane (Millipore), and the membrane was then stained with Coomassie blue to ensure that all lanes contained equivalent amounts of transferred protein. The destained membrane was then blocked in blotting buffer (5% nonfat dry milk in 20 mM Tris, pH 7.4/0.15 M NaCl/1% Triton X-100), incubated with the AS7 antiserum diluted 1:1000 in blotting buffer, and washed; then bound IgG was detected with goat anti-rabbit IgG conjugated to alkaline phosphatase. For quantification, AS7-reactive bands were labeled with ^{125}I-labeled goat anti-rabbit IgG and visualized by autoradiography, and then the gel bands were cut out and counted in a γ counter.

RESULTS

Localization of $\alpha_{i\text{-}2}$ and $\alpha_{i\text{-}3}$ Subunits in LLC-PK$_1$ Cells. The species of α_i protein produced by LLC-PK$_1$ cells were established by RNA gel blotting with specific oligonucleotide probes and immunoblotting of cell proteins with antibodies for $\alpha_{i\text{-}1}$, $\alpha_{i\text{-}2}$, and $\alpha_{i\text{-}3}$. These results demonstrated that only the $\alpha_{i\text{-}2}$ and $\alpha_{i\text{-}3}$ subunits are produced by these cells [as assessed by Western and RNA blotting (L.E., K. Rossiter, J.L.S., and D.A.A., unpublished observations)]. When confluent LLC-PK$_1$ monolayers were stained by immunofluorescence with the α_i antibodies, the $\alpha_{i\text{-}2}$ and $\alpha_{i\text{-}3}$ subunits were localized on distinct membranes. The $\alpha_{i\text{-}2}$ subunit was found mainly on the basolateral cell membrane, with some staining seen diffusely in the cytoplasm (Fig. 1*A*). The $\alpha_{i\text{-}3}$ subunit was concentrated on membranes in the perinuclear region of the cytoplasm in a Golgi-like staining pattern (Fig. 1*B*). The $\alpha_{i\text{-}3}$ subunit was not found associated with basolateral membranes.

Construction of Mammalian Expression Vectors Containing the $\alpha_{i\text{-}2}$ Subunit Gene. In order to provide for the regulated expression of the $\alpha_{i\text{-}2}$ subunit gene products in LLC-PK$_1$ cells, the πLXX vector (17), generously provided by B. Seed (Massachusetts General Hospital) (see Fig. 2*A*), was modified by partial replacement of its avian sarcoma virus long terminal repeat promoter/enhancer segment with a 770-base-pair (bp) segment of the MT-1 5′ flanking region, 64 bp of truncated mouse MT-1 exon A, 14 bp derived from the mp18 polylinker of pSP65, followed by 34 bp of DNA including the Goldberg–Hogness promoter (TATA box; generously provided by R. D. Palmiter, University of Washington; ref. 18) (Fig. 2*B*). To grow the plasmid in JM109 cells, its *supF*, πVX origin of replication, and portions of its simian virus 40 origin of replication were replaced with the plasmid Bluescript II KS + (Stratagene). An *Eco*RI cloning site was introduced (17) along with a rat or canine $\alpha_{i\text{-}2}$ subunit cDNA to form pMXX ($\alpha_{i\text{-}2}$ +) as detailed in Fig. 2*C*.

Stable Transfection of LLC-PK$_1$ Cells with Chimeric MT-1–$\alpha_{i\text{-}2}$ Subunit Genes. LLC-PK$_1$ cells were cotransfected with pMXX ($\alpha_{i\text{-}2}$ +) or the antisense pMXX ($\alpha_{i\text{-}2}$ −) and pSV2neo (14) plasmids, followed by selection of cell clones in medium containing G418. The segment of pMXX containing the SV2 intron and the polyadenylylation signal has several advantages for the expression of eukaryotic mRNA (17). These include

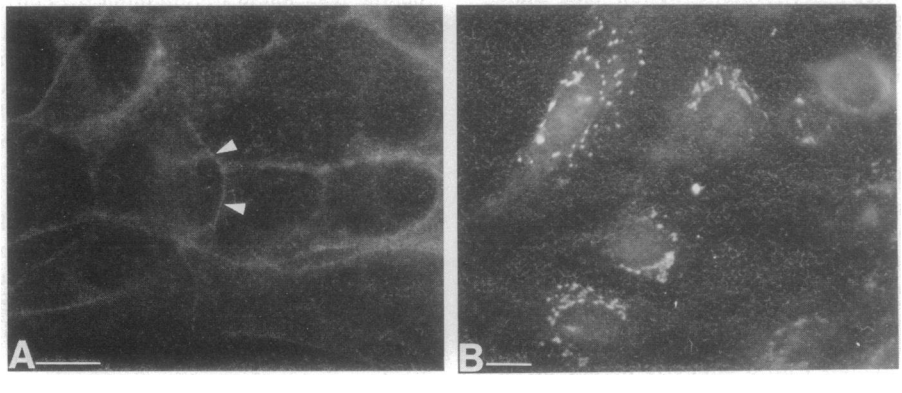

FIG. 1. Confluent monolayers of LLC-PK$_1$ cells showing immunolocalization of $\alpha_{i\text{-}2}$ and $\alpha_{i\text{-}3}$ subunits on cellular membranes. Cell monolayers were fixed in paraformaldehyde, permeabilized with Triton X-100, and incubated with the antibodies as described in the text. (*A*) Cells were incubated with AS7 antibody, which is specific for the $\alpha_{i\text{-}2}$ subunit in these cells. There is some diffuse cytoplasmic staining, but most of the $\alpha_{i\text{-}2}$ is found in a typical cobblestone-like pattern representing staining of the basolateral membranes of these polarized cells (arrowheads). When viewing a monolayer of closely apposed cells, staining on the basolateral membrane is most concentrated at the perimeters of the cells and appears as a single line around each cell border. (*B*) Permeabilized cells were incubated with the EC antibody, which is specific for the $\alpha_{i\text{-}3}$ subunit. No staining of the basolateral membranes in these cells was detected; the staining is concentrated in the perinuclear region of the cytoplasm in a Golgi-like distribution. (Bars = 10 μm.)

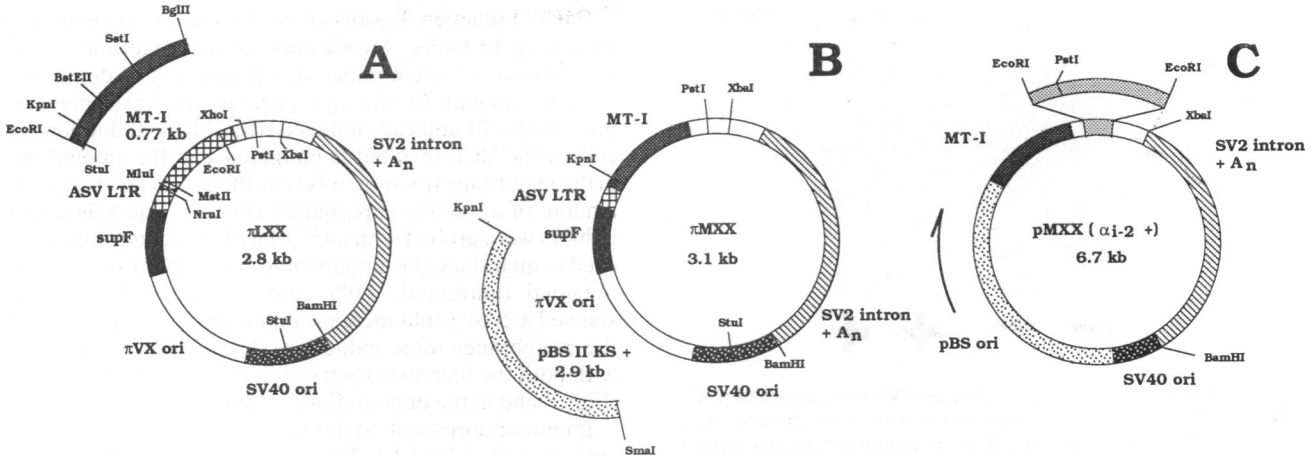

FIG. 2. Construction of eukaryotic expression vectors derived from πLXX, which contains (*i*) SV40 ori, eukaryotic origin of replication from simian virus 40; (*ii*) πVX ori, prokaryotic origin of replication from πVX (originally from pMB1); (*iii*) ASV LTR, long terminal repeat from avian sarcoma virus; and (*iv*) SV2 intron + A_n, simian virus 40 late splice site and polyadenylylation signals from pSV2. (*A*) πLXX was digested with *Nru* I and *Xho* I, followed by purification and blunt ending of the 2.4-kb backbone vector fragment with T4 DNA polymerase. The mouse MT-1 promoter fragment was derived by digesting EV142 with *Eco*RI and *Bgl* II, followed by purification and blunt ending with T4 DNA polymerase. The backbone πLXX vector fragment was ligated to a 0.77-kb MT-1 fragment to obtain the product πMXX. (*B*) πMXX was digested with *Kpn* I and *Stu* I, followed by purification of the 2.0-kb backbone vector fragment. pBS II KS + was digested with *Kpn* I and *Sma* I, followed by purification of the 2.9-kb pBS fragment. The πMXX backbone vector fragment was ligated to the pBS fragment to obtain the product pMXX. (*C*) pMXX was digested with *Pst* I, followed by blunt ending with T4 DNA polymerase. *Eco*RI linkers were then ligated to the linearized vector followed by *Eco*RI digestion and purification. The rat α_{i-2} cDNA was derived by digesting Gi-f2 with *Eco*RI, followed by purification of the 1.8-kb fragment (12). The pMXX backbone vector fragment was then ligated to the α_{i-2} cDNA in the sense or antisense orientation to obtain the products pMXX (α_{i-2} +) and pMXX (α_{i-2} −), respectively. The diagrams are not drawn to scale.

sequences that facilitate mRNA nuclear to cytoplasmic export and stabilize the expressed mRNA. The addition of these sequences to the 3′ untranslated region of the expressed mRNA theoretically adds 1.2 kilobases (kb) or more to its length. Normal LLC-PK_1 cells express an α_{i-2} mRNA of 2.6 kb by RNA gel blot analysis (data not shown). Thus the identification of cell clones expressing chimeric α_{i-2} was facilitated, as functional chimeric mRNAs should be 3.7 kb or greater. RNA gel blot analysis of several stable transfectants is seen in Fig. 3. Following a 24-hr exposure to 2 μM $CdCl_2$, neomycin-resistant LLC-PK_1 clones, such as those designated as 2+1s, 2+3s, 2+5s, 2−8s, and 2−12s [the prefixes 2+ and 2− indicate transfection with pMXX (α_{i-2} +) and pMXX (α_{i-2} −), respectively], produced chimeric α_{i-2} mRNAs of various sizes as shown by hybridization with the ^{32}P-labeled rat α_{i-2} cDNA probe. Some clones, such as 2+3s, expressed two recombinant mRNAs of 2.6 and 3.8 kb. Some LLC-PK_1 clones (e.g., 2+2s) only integrated the neomycin-resistance gene and gave a single weaker hybridization signal at 2.6 kb. These cells did not increase their α_{i-2} mRNA content following $CdCl_2$ exposure (data not shown). We selected cell lines (e.g., 2+3s) that had stably integrated chimeric MT-1−α_{i-2} genes for further studies. There were no growth or phenotypic changes observed in LLC-PK_1 cells transfected with the α_{i-2} gene. The morphology of the cells was routinely examined by phase-contrast and confocal microscopy.

Kinetic Analysis of $CdCl_2$ Induction of Chimeric MT-1–α_{i-2} mRNA in Stable LLC-PK_1 Cell Lines. To determine the rate and magnitude of chimeric MT-1–α_{i-2} mRNA induction in our selected cell lines, individual clones were treated with 0–10 μM $CdCl_2$ over a 24-hr period of culture. RNA from these cells was then analyzed by gel blotting. A representative experiment, using LLC-PK_1 clone 2+3s, is shown in Fig. 4. Over a 24-hr period these cells constitutively expressed low levels of mRNAs of 2.6 and 3.8 kb, which hybridized with a ^{32}P-labeled rat α_{i-2} cDNA fragment. After induction of these cells with 1, 2, or 10 μM $CdCl_2$, the content of chimeric α_{i-2} mRNAs increased over 270 min by 1.5-, 3-, and 10-fold, respectively, and then declined by 24 hr. No increase in α_{i-3} hybridizable mRNA was detected (data not shown).

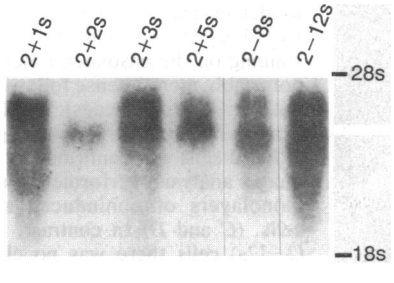

FIG. 3. RNA gel blot analysis of neomycin-resistant LLC-PK_1 cells obtained by a limiting-dilution technique. Cells were treated with 2 μM $CdCl_2$ for 24 hr. The α_{i-2} cDNA was of canine origin. Ten micrograms of total RNA was examined for the expression of chimeric mRNA, which hybridized to a ^{32}P-labeled full-length rat α_{i-2} cDNA fragment. Neomycin-resistant cells initially transfected with pMXX (α_{i-2} +) are designated with 2+ signs for sense chimeric genes (e.g., 2+1s), and cells transfected with pMXX (α_{i-2} −) are designated with 2− signs for antisense chimeric genes (e.g., 2−8s).

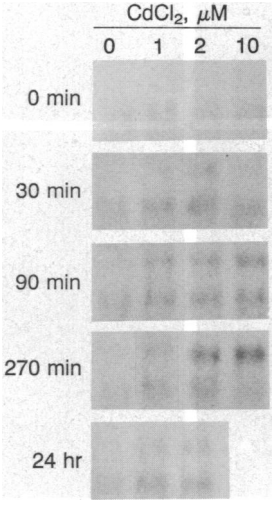

FIG. 4. RNA gel blot analysis of a clonal LLC-PK_1 cell line (2+3s) stably transfected with pMXX (α_{i-2} +) and showing induction with $CdCl_2$ over 24 hr. Twenty-five micrograms of total RNA from these cells was hybridized with a ^{32}P-labeled full-length rat α_{i-2} cDNA fragment. All lanes were matched for RNA content as determined by ethidium bromide staining of 18S and 28S ribosomal bands in each lane and by hybridization with a ^{32}P-labeled human actin cDNA fragment (data not shown). Upper and lower bands represent mRNA of 3.8 and 2.6 kb, respectively.

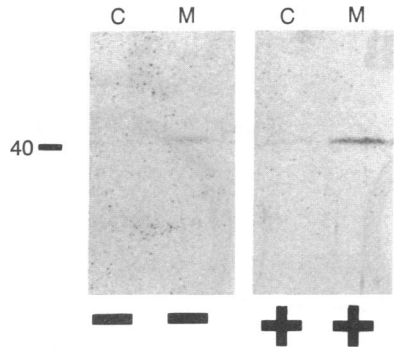

FIG. 5. Immunoblotting of membranes (M) and supernatant (C) fractions from LLC-PK_1 (2+3s) cells that were noninduced (−) or induced (+) with 2 μM $CdCl_2$ for 16 hr. Immunoblotting was carried out with the AS7 serum followed by an alkaline phosphatase-conjugated second antibody. The AS7 serum reacts specifically with a band at 40 kDa corresponding to the α_{i-2} subunit. There was an increased amount of the 40-kDa protein in the membrane fraction of the induced cells, but there was no change in the amount of this protein in the supernatant.

$CdCl_2$ Induction Results in an Increased Amount of α_{i-2} Protein in LLC-PK_1 Membranes. Immunoblotting of LLC-PK_1 (2+3s) cell membranes was performed to compare the relative amount of the α_{i-2} protein (40 kDa) present in untreated cells and cells induced with $CdCl_2$. Induction of the cells with $CdCl_2$ resulted in an increase in the amount of α_{i-2} in the membrane fraction, whereas there was no change in the amount of α_{i-2} in the supernatant (Fig. 5). Equivalent immunoblots were probed with an ^{125}I-labeled second antibody and used to quantitate the amount of α_{i-2} protein present. The data obtained (untreated, 1505 cpm vs. induced, 3879 cpm) showed a 2- to 3-fold increase in the amount of α_{i-2} protein in the membranes after induction. These data are consistent with both the increase observed in the amount of α_{i-2} mRNA (Fig. 3) and in the immunofluorescent staining of α_{i-2} (Fig. 6).

Immunofluorescent staining of both sense (2+3s) and antisense (2−12s) LLC-PK_1-transfected cell lines with α_{i-2} and α_{i-3} antibodies showed that the localization of the α_{i-2} subunit predominantly on the basolateral membranes and the α_{i-3} subunit in the Golgi region was consistent with their localization in normal LLC-PK_1 cells (Fig. 6*A*). The immunofluorescent localization of the α_{i-2} subunit was compared in the induced and uninduced transfected cells. Following overnight induction of the LLC-PK_1 (2+3s) cells with 5 μM

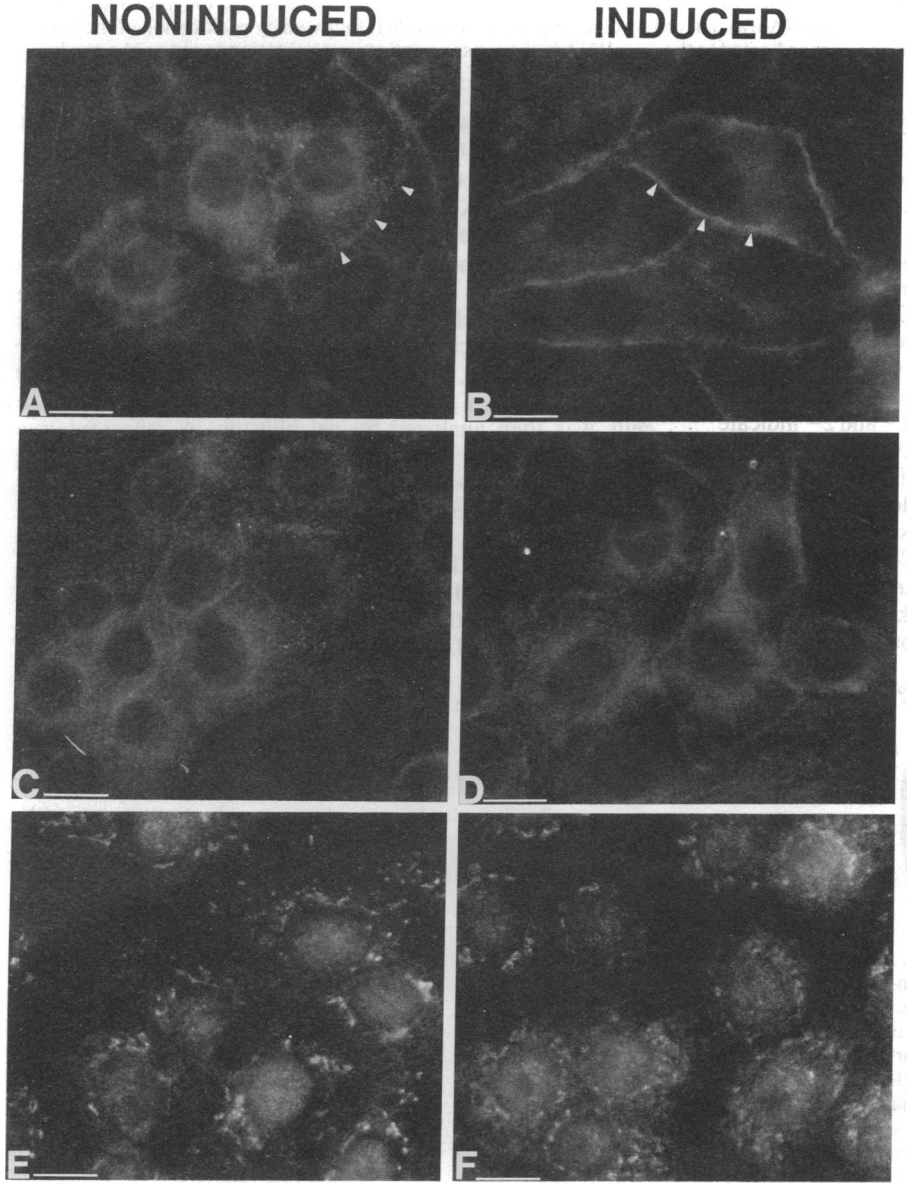

FIG. 6. Immunofluorescent staining of transfected cell clones showing the distribution of α_{i-2} and α_{i-3} subunits in noninduced cells or cells induced with 2–5 μM $CdCl_2$ for 16 hr. Cell monolayers were fixed and stained. LLC-PK_1 (2+3s) cells (*A* and *B*) and LLC-PK_1 (2−12s) cells (*C* and *D*) were incubated with the AS7 antiserum to localize the α_{i-2} subunit. (*E* and *F*) LLC-PK_1 (2+3s) cells were incubated with the EC antiserum to detect the α_{i-3} subunit. (*A* and *B*) In LLC-PK_1 (2+3s) cells the amount of α_{i-2} staining on the basolateral membranes was noticeably more intense following induction with $CdCl_2$ (arrowheads). A 3-fold increase in basolateral membrane staining intensity in induced cells was confirmed by quantitative image analysis performed on equivalent monolayers of noninduced and induced cells. (*C* and *D*) In contrast, in LLC-PK_1 (2−12s) cells there was no change in the amount of staining for α_{i-2} or in its distribution following induction. (*E* and *F*) To show that the overexpression of α_{i-2} in the LLC-PK_1 (2+3s) cells was specific for this subunit, the cells were also stained with the EC antibody to α_{i-3}. There was no change in the Golgi-like distribution of this α_{i-3} subunit following the same induction that caused the increase in basolateral α_{i-2} staining seen in *B*. (Bars = 10 μm.)

$CdCl_2$, there was a 2- to 3-fold increase in the intensity of immunofluorescent staining of α_{i-2} on the basolateral membranes of these cells compared to the noninduced cells (Fig. 6*B*). In contrast, there was no change in the amount of basolateral membrane staining in the antisense transfected LLC-PK_1 (2−12s) cells after induction (Fig. 6 *C* and *D*). Similarly there was no change in the amount of staining or in the distribution of the α_{i-3} subunit in LLC-PK_1 (2+3s) cells (Fig. 6 *E* and *F*) or in LLC-PK_1 (2−12s) cells (not shown) after induction. Thus induction of the LLC-PK_1 (2+3s) cells resulted in a specific overexpression of the α_{i-2} subunit and this overexpressed α_{i-2} subunit was correctly targeted to the basolateral membrane. Since α_{i-2} localized to the basolateral membrane, it is likely that α_{i-2} is responsible for the pertussis toxin-sensitive regulation of vasopressin-stimulated adenylyl cyclase in this membrane (6). This was confirmed by the demonstration that inhibition of vasopressin-sensitive adenylyl cyclase was unaffected in transfected noninduced cells but increased by ≈30% in induced cells (L.E. and D.A.A., unpublished observations).

DISCUSSION

The critical role of heterotrimeric G proteins as intermediaries in signal transduction has been established for a variety of effector systems, including adenylyl cyclase and ion channel regulation (1, 2). It is now evident from the molecular cloning of the α subunits of G proteins that they are each distinct members of a large supergene family (3). What is not clear, however, is whether there is functional specificity to each α subunit. A variety of α subunits may be expressed in the same cell type and may each be involved in one or more signaling pathways. For example, *in vitro* reconstitution experiments utilizing excised membrane heart patches have demonstrated that three different α_i subunits are equally capable in their ability to activate an ion channel for potassium (19). In other studies, the same α subunit (α_s; α subunit of stimulatory G protein) may regulate both adenylyl cyclase and calcium channels in skeletal muscle (20).

We have previously demonstrated two topographically distinct roles for G_i proteins in polarized renal epithelium LLC-PK_1 cells: modulation of adenylyl cyclase at the basolateral membrane (4–6) and regulation of the amiloride-sensitive Na^+ channel at the apical membrane (7, 8). This cell line has provided the opportunity to define the specific cellular location of α_i subunits and to develop a clonal transfected cell model for the regulation of α_i gene products. Both have been achieved in the present study.

The present data suggest that the multiple pertussis toxin-sensitive pathways in LLC-PK_1 cells are mediated by either α_{i-2} or α_{i-3} protein subunits, which were the only such subunits found. We have found distinct locations for the α_{i-2} and α_{i-3} subunits at the basolateral and Golgi membranes, respectively. The localization of the α_{i-2} protein at the basolateral cell membrane is consistent with its role as a modulator of the vasopressin-sensitive adenylyl cyclase system previously defined (4–6). The predominant localization of α_{i-3} subunits in Golgi suggests a possible role for them in signal transduction in intracellular vesicle trafficking. Recent evidence implicates monomeric G proteins in this process (21), but to our knowledge this is the first localization of heterotrimeric α_i subunits on Golgi membranes. Alternatively, α_{i-3} could be shuttled from the Golgi to other cellular membranes such as the apical membrane. While α_{i-3} appears at only low levels in the apical membranes of LLC-PK_1 cells, we have shown it is more abundant at the apical membrane of the A6 epithelial cell, where it regulates the sodium channel (9).

On the basis of the above data, we decided to express α_i genes in a temporal and stoichiometric manner that would be appropriate for membrane targeting. To achieve this, we developed a "Trojan horse" strategy of introducing MT-1-driven chimeric genes into cells. These chimeric genes remain relatively quiescent until their expression is stimulated by exposure of cells to a suitable heavy metal inducer. This allows for the stable integration of these genes into cells without altering their phenotypic behavior. A similar strategy has previously been successful for the introduction of other MT-1 chimeric genes, such as neuropeptide hormones into nonepithelial cells (22). In our present studies in epithelial cells, the incremental increases in mRNA and resultant α_{i-2} protein following stimulation of a MT-1 chimeric gene allowed for controlled expression of the new protein that could be appropriately targeted to the site of its function at the basolateral membrane. Thus, we have demonstrated the overexpression and correct targeting of an endogenous membrane-associated protein in an epithelial cell. The second form of α_i subunit found in these cells, α_{i-3}, was neither targeted to the basolateral membrane nor altered in its distribution following the induction of the α_{i-2} gene. These data would suggest that the targeting mechanism for these subunits may be coupled to small amino acid sequence differences that exist between α_{i-2} and α_{i-3} proteins (3). Thus, it appears that one mechanism to allow for the specificity of α_i subunit function in renal epithelial cells is by their distribution on topographically distinct membranes.

Our strategy for introduction of MT-1 chimeric genes that encode α_i subunits has provided a tool for the study of heterotrimeric G protein-coupled reactions in renal epithelia. This cell model should provide information on the function of α_i proteins and, more generally, on the cell biology of membrane-associated proteins in epithelia.

The generosity of Randall Reed for providing the rat α_{i-2} cDNA, Charles Homcy for providing the canine α_{i-2} cDNA, and Allen Spiegel for providing the AS and EC antibodies is greatly appreciated. We thank Inwha Kang for constructing the πMXX vector. This work was supported by Grant AM19406 from the National Institutes of Health.

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