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Article

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Cytosolic phospholipase A_2 regulates Golgi structure and modulates intracellular trafficking of membrane proteins

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The Golgi complex and the *trans*-Golgi network are critical cellular organelles involved in the endocytic and biosynthetic pathways of protein trafficking. Lipids have been implicated in the regulation of membrane-protein trafficking, vesicular fusion, and targeting. We have explored the role of cytosolic group IV phospholipase A_2 ($cPLA_2$) in membrane-protein trafficking in kidney epithelial cells. Adenoviral expression of $cPLA_2$ in LLC-PK_1 kidney epithelial cells prevents constitutive trafficking to the plasma membrane of an aquaporin 2-green fluorescent protein chimera, with retention of the protein in the rough endoplasmic reticulum. Plasma membrane Na^+-K^+-ATPase α-subunit localization is markedly reduced in cells expressing $cPLA_2$, whereas the trafficking of a Cl^-/HCO_3^- anion exchanger to the plasma membrane is not altered in these cells. Expression of $cPLA_2$ results in dispersion of giantin and β-COP from their normal, condensed Golgi localization, and in marked disruption of the Golgi cisternae. $cPLA_2$ is present in Golgi fractions from noninfected LLC-PK_1 cells and rat kidney cortex. The distribution of tubulin and actin was not altered by $cPLA_2$, indicating that the microtubule and actin cytoskeleton remain intact. Total cellular protein synthesis is unaffected by the increase in $cPLA_2$ activity. Thus $cPLA_2$ plays an important role in determining Golgi architecture and selective control of constitutive membrane-protein trafficking in renal epithelial cells.

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Introduction

The polarity of epithelial cells derives from the selective distribution of their plasma-membrane proteins and lipids into distinct plasma-membrane domains (1, 2). Intracellular protein traffic from the endoplasmic reticulum (ER) to the cell surface through the Golgi apparatus is mediated by vesicles that transit between the organelles and different membrane domains. The newly synthesized membrane proteins may be directly addressed to their final destination from the ER (constitutive pathway) or stored in secretory vesicles until an extracellular stimulus induces their release to the membrane (regulated pathway) (1, 3). These pathways have crucial roles in the physiology of polarized cell function in the normal kidney and are perturbed in some pathological states. The asymmetry between basolateral and apical surfaces depends on mechanisms that control the movement of proteins to the correct target membrane in cells (1, 4). Despite intensive research, those processes remain poorly understood.

In addition to their classic roles in the production of second messengers in various aspects of signal transduction (5), lipids of intracellular compartments have been implicated in the regulation of membrane trafficking, vesicular fusion, and targeting (6). Phospholipases have also been implicated in this regulation. Phosphatidic acid, the main metabolite produced by hydrolysis of phospholipids by phospholipase D (PLD), has been proposed to have a direct action on target proteins involved in vesicle movement and to modify the lipid bilayer content and surface charge of membranes (7, 8).

The group IV 85-kDa cytosolic phospholipase A_2 ($cPLA_2$) acts at the 50-2 position of phospholipids to generate free arachidonic acid (AA). AA and its metabolites are important lipid second messengers (5, 9, 10). $cPLA_2$ is regulated by changes in intracellular free-calcium concentration within the physiological range. After release from phospholipids by PLA_2, AA can be converted by cyclooxygenases, lipoxygenases, and cytochrome P-450 monooxygenase to prostaglandins, leukotrienes, and hydroxyeicosanoic acids, products that are involved in the regulation of a number of cellular processes (5, 11). Lysophospholipids generated in vesicle membranes by PLA_2 may regulate the fusion of ER-transport vesicles with the Golgi apparatus (12). Furthermore, Tagaya et al. showed that PLA_2 regulates intra-Golgi protein transport in vitro (13).

We have shown recently that aquaporin 2 (AQP2)/green fluorescent protein (GFP) chimeras are useful constructs for studying intracellular sorting sig-

nals that direct this water-channel protein to specific cellular locations (14). When GFP is fused to the cytoplasmic amino-terminus of AQP2, GFP-AQP2(NT), the water channel enters a vasopressin-regulated (VP-regulated) pathway of plasma membrane insertion. In contrast, the COOH-terminal chimera, AQP2-GFP(CT), localizes constitutively on both apical and basolateral plasma membrane, independently of VP or forskolin stimulation, and traffics in a way that is indistinguishable from wild-type AQP1 (14).

Another protein that is constitutively targeted to the cell membrane in polarized epithelial cells is Na^+-K^+-ATPase, whose correct targeting is critical to normal cell function. Dopamine, which increases $cPLA_2$ activity, induces a decrease in Na^+-K^+-ATPase activity in proximal tubules with internalization of its α- and β-subunits into endosomes via a clathrin-dependent pathway (15).

The goals of this study were to evaluate the role of $cPLA_2$ in membrane-protein trafficking in a polarized renal epithelial cell. In cells expressing $cPLA_2$, cell volume is increased, and plasma membrane Na^+-K^+-ATPase α-subunit is markedly reduced. The amount of AQP2-GFP(CT) is decreased in the plasma membrane and increased in the rough ER. Golgi cisternae are markedly disrupted, and giantin and β-COP are dispersed from their normal, condensed Golgi localization. In contrast, there is normal VP-stimulated membrane localization of GFP-AQP2(NT) and normal constitutive membrane localization of a Cl^-/HCO_3^- anion exchanger. Furthermore, the distribution of tubulin and actin is not altered by $cPLA_2$, indicating that the microtubule and actin cytoskeleton remain intact. These results suggest that $cPLA_2$ plays an important role in the selective regulation of constitutive membrane-protein trafficking by its action on Golgi structure and function.

Methods

Materials. PMA, A23187, vasopressin, AA, indomethacin, SKF 525A, nordihydroguaiacetic acid (NDGA), 4-methylumbelliferone-α-D-glucoside, 4-methylumbelliferone-α-D-mannopyranoside, 4-methylumbelliferone-phosphate, and streptavidin-agarose were purchased from Sigma Biochemical Co. (St. Louis, Missouri, USA). The membrane-impermeant biotin analogue sulfo-NHS-biotin was purchased from Pierce Chemical Co. (Rockford, Illinois, USA). Secondary goat anti-rabbit or anti-mouse IgG Ab's coupled to Cy3 were from Jackson ImmunoResearch Laboratories Inc. (West Grove, Pennsylvania, USA). Peroxidase-conjugated goat anti-rabbit or anti-mouse IgG and reagents for protein measurements based on Bradford's assay were purchased from Bio-Rad Laboratories (Hercules, California, USA). Immobilon-P transfer PVDF membranes were obtained from Millipore Corp. (Bedford, Massachusetts, USA). The Renaissance chemiluminescence system, [^{35}S]-methionine, [^{3}H]-thymidine, [^{3}H]-leucine, and [^{3}H]-AA were from NEN Life Science Products (Boston, Massachusetts, USA). Enhanced chemiluminescence (ECL) Western blotting detection kit, donkey horseradish peroxidase–conjugated anti-rabbit Ab, and sheep horseradish peroxidase–conjugated anti-mouse Ab were purchased from Amersham Life Sciences (Arlington Heights, Illinois, USA); 1,3 phosphatidylcholine 1-steratoyl-2-[1-^{14}C]arachidonyl was purchased from Amersham International (Amersham, United Kingdom). Protease inhibitor cocktail tablets (Complete) were purchased from Boehringer Mannheim GmbH (Mannheim, Germany). Iodixanol solution for formation of density gradients, Optiprep, was obtained from Nycomed Pharma AS (Oslo, Norway).

Cell culture. Cells were maintained at 37°C in DMEM containing 1 g/l glucose and supplemented with 2 mM L-glutamine and 10% FBS (LLC-PK_1) or 10% horse serum (293 cells) and cultured in 95% air and 5% CO_2. Cells were stably transfected with GFP/AQP2 chimeric constructs using the DOTAP transfection reagent (Boehringer Mannheim Gmbh) according to the manufacturer's recommendations. Transfected cells were selected and maintained with 1 mg/ml G418 (Geneticin; GIBCO BRL, Grand Island, New York, USA), and single clones were isolated using cloning rings. Cells were routinely confirmed to be mycoplasma negative.

GFP-AQP2 and GFP-$cPLA_2$ chimeric constructs. The AQP2-GFP(CT), GFP-AQP2(NT), and GFP-$cPLA_2$ fusion chimeras were prepared by ligation of the AQP2 cDNA into the *Eco*RI /*Bam*HI sites of the pEGFP-N1 vector and the *Sal*I/*Bam*HI sites of the pEGFP-C1 vector (CLONTECH Laboratories Inc., Palo Alto, California, USA), as described previously (14).

Construction of a recombinant adenoviral vector carrying the $cPLA_2$ cDNA. The human $cPLA_2$ cDNA was subcloned between the *Not*1 and *Xho*1 sites of the bacterial plasmid vector pAdRSV4 to generate pAdRSV-$cPLA_2$, which contains adenoviral sequences from 0–1.2 and 9.2–16.1 map units, the Rous sarcoma virus long-terminal repeat promoter, and the SV40 early-region polyadenylation signal. The position and the orientation of the $cPLA_2$ cDNA were confirmed by sequencing. Using the calcium phosphate transfection technique, the plasmid vector containing $cPLA_2$ was then cotransfected into 293 cells with pJM17, which encodes the adenoviral cDNA, as described previously (16). The homologous recombinants between the pAdRSV-$cPLA_2$ and pJM17 contain the exogenous cDNA substituted for E1. An individual plaque was then purified and protein expression confirmed with immunoblotting and assay of $cPLA_2$ activity. AdcPLA_2, the recombinant adenovirus, was then prepared in high titer by propagation in 293 cells and purified by CsCl-gradient ultracentrifugation (17). The viral stock was determined to be 2.1×10^{12} particles/ml and 5×10^{10} plaque-forming units/cell (pfu/cell) with a plaque assay (17).

Gene transfer into LLC-PK_1 cells. A recombinant adenovirus carrying the *Escherichia coli LacZ* gene encoding β-galactosidase (AdLacZ) (provided by David A. Dichek, Gladstone Institute for Cardiovascular Diseases, San Francisco, California, USA) was used as a control and to

evaluate the ability of LLC-PK_1 cells to be infected by the adenoviral vector. This adenovirus is similar to $AdcPLA_2$. Cells were infected at different moi and those expressing β-galactosidase were detected 1, 2, 3, 5, 7, 15, and 21 days after infection using 5-bromo-4-chloro-3-indolyl-β-D-galactosidase (X-Gal) substrate as described (18).

In other experiments cells were transfected with pEGFP, pEGFP/$cPLA_2$, or pEGFP/$cPLA_2$(mut) constructs using the Superfect reagent (QIAGEN, Valencia, California, USA). The pEGFP/$cPLA_2$(mut) construct was generated by introducing the NH_2-terminal *Pst*1/*BamH*1 fragment of human $cPLA_2$ (bp 22-375) into pEGFP-N1.

$cPLA_2$ activity. LLC-PK_1 cells were plated on 100-mm tissue-culture plates 2 days before infection with AdLacZ, $AdcPLA_2$, or vehicle. Two days after infection, the cells were washed, harvested, and lysed by sonication in 250 μl of a buffer containing 120 mM NaCl, 50 mM Tris, pH 9.0, and 1 mM EGTA. The lysate was centrifuged at 100,000 *g* for 1 hour at 4°C, and the supernatant was transferred to a new tube. The protein concentration of each sample was determined by Bradford assay. The $cPLA_2$ activity was assayed in duplicate at 37°C for 30 minutes in a 100-μl reaction volume that included 30 μg protein, 0.5 nM 1.3 phosphatidylcholine 1-steratoyl-2-[1-^{14}C]arachidonyl, 2.5 mM $CaCl_2$, 0.2 mM EGTA, 50 mM NaCl, and 75 mM Tris, pH 9.0. The reaction was stopped by adding 800 μl Dole's reagent (32% isopropyl alcohol, 67% *n*-heptane, 1% 1 N H_2SO_4) and mixing using a Vortex-Genie (Fisher Scientific, Pittsburgh, Pennsylvania, USA). After centrifugation, 150 μl of the upper phase was transferred to a new tube containing 50 mg of silica gel and 800 μl of n-heptane. After vortexing and allowing the silica gel to settle, 800 μl of supernatant was counted for radioactivity in a liquid-scintillation counter.

AA release. Forty-eight hours after infection, subconfluent cells in 12-well plates were labeled for 18 hours with 0.30 μCi/ml [^{3}H]AA in DMEM containing 0.1% FBS. The cells were then washed with DMEM containing 0.2% BSA and incubated with the same medium for 30 or 120 minutes or stimulated for 30 minutes with 200 nM PMA and 10 μM A23187. Medium was removed and radioactivity in 400 μl of supernatant was measured in a liquid-scintillation counter. The cells were solubilized with 1% Triton X-100, and the amount of [^{3}H]AA released into the medium was expressed as a percentage of the total (cell-associated plus released).

Immunofluorescence. Cells plated on glass coverslips were infected with either AdLacZ or $AdcPLA_2$, at a moi of 50 pfu/cell, or transfected with pEGF, pEGF/$cPLA_2$ or pEGF/$cPLA_2$(mut). In some experiments, after infection, cells were incubated with either 5 μM of A23187, 10 nM VP, 25 μM indomethacin, SKF 525A, ETYA, or 10 μM NDGA. After fixation for 20 minutes with 4% paraformaldehyde containing 5% sucrose in PBS, cells were permeabilized with 0.1% Triton X-100 for 5 minutes, followed by 10 minutes of blocking in 1% BSA. The inherent fluorescence of the GFP tag was used to detect chimeric proteins. The primary Ab's used for double labeling were an anti–β-COP mAb (clone #M3A5; Sigma Biochemical Co.) to visualize Golgi apparatus–associated vesicles, anti-giantin to visualize Golgi cisternae, anti-tubulin to label microtubules, anti-AE1/2 to label the Cl^-/HCO_3^- anion exchanger, and anti-Na^+K^+-ATPase. BODIPY (Molecular Probes, Eugene, Oregon, USA) 581/591 phalloidin was used to label F-actin. The β-COP and AE1/2 Abs were applied after treatment of cells with 2% SDS in PBS for 4 minutes to unmask antigens (19). All primary Ab's were incubated for 1 hour at room temperature. Secondary Ab's, either Cy3-conjugated goat anti-rabbit IgG or Cy3-conjugated donkey anti-mouse IgG (1:800), were incubated for 1 hour at room temperature. Rhodamine isothiocyanate-conjugated (RITC-conjugated) dextran (Mr 11,000; Sigma Biochemical Co.) was used as a fluid-phase marker of endocytosis. Coverslips were mounted on slides in Vectashield/Tris-Cl (pH 8.9) at a ratio of 1:1. Indirect immunofluorescence was visualized with a Nikon FXA photomicroscope (Nikon Inc., Melville, New York, USA) and a confocal laser-scanning microscope (MRC/600; BioRad Microscopy Division, Hemel Hempstead, United Kingdom). Generated computer images were analyzed using IP Lab Spectrum imaging software (version 3.1; Signal Analytics Corp., Fairfax, Virginia, USA).

Electron microscopy. Forty-eight hours after infection cells were fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 1 hour at room temperature. After rinsing in sodium cacodylate, they were postfixed in 1% OsO_4 in 0.1 M sodium cacodylate for 30 minutes and stained en bloc in 2% aqueous uranyl acetate for 30 minutes at room temperature. Cells were scraped, pelleted, and embedded in 2% agarose, which was minced into 2-mm^2 blocks. Blocks were dehydrated in graded series of ethanol and propylene oxide and polymerized in Epon at 60°C for 24 hours. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips CM 10 electron microscope (Philips Electronics Inc., Mahwah, New Jersey, USA) at 80 kV.

Subcellular fractionation of LLC-PK_1 cells. To localize AQP2-GFP(CT) and GFP-AQP2(NT) to plasma or intracellular membranes, total cellular membranes were separated according to the technique described by van't Hof et al. (20). For each gradient, cells from three subconfluent 100-mm dishes were used. Two days after infection, cells were harvested in an isotonic buffer containing protease inhibitors and homogenized in a 1.5-ml Dounce homogenizer. Nuclei and unbroken cells were removed by centrifugation for 10 minutes at 500 *g*, and the supernatants were centrifuged for 60 minutes at 100,000 *g* at 4°C. The resulting pellet, containing total cellular membranes, was resuspended in 1 ml of the previous buffer and layered on top of continuous iodixanol density gradients (2.5–25% wt./vol. Optiprep in 0.25 M sucrose/TE). Gradients were centrifuged for 3 hours at 100,000 *g* and fractionated into 700-μl samples. For analysis of marker-enzyme activities, 50-μl samples were taken from each

fraction and mixed with 200 μl of enzyme assay buffer in 48-well plates. The α-glucosidase II activity (ER membranes) was assayed in 150 mM citrate/phosphate, pH 6.5, 1 mM 4-methylumbelliferone-α-D-glucoside, α-mannosidase II (Golgi membranes) in 150 mM phosphate buffer, pH 6.0, 1 mM 4-methylumbelliferone-α-D-mannopyranoside, and alkaline phosphatase activity (plasma membranes) was measured in 100 mM Tris, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$, and 1 mM 4-methylumbelliferone-phosphate. Reactions were allowed to proceed for 4 hours at 37°C and stopped by addition of 100 μl ice-cold 1 M glycine, pH 10, 1 M Na_2CO_3. Fluorescence was measured at λ_{ex} 360 nm, λ_{em} 460 nm using a DNA fluorometer TKO 100 (Hoefer Scientific Instruments, San Francisco, California, USA).

To evaluate whether $cPLA_2$ localizes to the Golgi apparatus of native nontransfected LLC-PK_1 cells, the cells were grown to 80% confluence. Approximately 30×10^6 cells were resuspended in 1 ml of homogenization buffer (0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4, with Complete cocktail of protease inhibitors) and homogenized by passing ten times through a ball-bearing homogenizer (EMBL Precision Engineering, Heidelberg, Germany). The postnuclear supernatant was generated by centrifugation at 1,000 *g* for 5 minutes and placed on a 5–20% linear Optiprep gradient formed in separation buffer (78 mM KCl, 4 mM MgCl, 8 mM $CaCl_2$, 10 mM EGTA, 50 mM HEPES/KOH, pH 7.0) using a gradient former (Model 385; Bio-Rad Laboratories). Gradients were spun at 100,000 *g* for 17 hours at 4°C using Beckman L8-M ultracentrifuge (SW41Ti rotor, 27,000 rpm). Gradients were recollected using an ISCO Density Gradient Fractionator (MODEL 640; ISCO, Lincoln, Nebraska, USA), and proteins were precipitated as described previously (21) and solubilized in SDS-PAGE sample buffer.

Purification of Golgi stacks from kidney tissue. Isolation of Golgi stacks and cytosol from kidney cortex was performed using a discontinuous sucrose gradient as described previously (22).

Western blot analysis. Cells and subcellular lysates were solubilized in buffer containing 20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM β-glycerophosphate, 1 mM DTT, 1 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, and protease inhibitors. An aliquot of protein lysates was separated using SDS-Tris-glycine-polyacrylamide gels (23). A graphite electroblotter system (MilliBlot; Millipore Corp.) was used to transfer proteins from the gels to Immobilon PVDF membranes. Before immunoanalysis, nonspecific binding sites were blocked by incubating the membrane with 5% nonfat dry milk. The membranes were incubated with Ab's against $cPLA_2$ (1:5,000), β-COP (1:200), GP-58 (1:5,000), Arf1 (1:1,000), Rab11 (1:1,000), or carbonic anhydrase-2 (CA-2; 1:1,000) diluted in TBS-Tween-albumin buffer (15 mM NaCl, 0.1% Tween-20, 3% BSA, 5 mM Tris-HCl, pH 7.0). Polyclonal anti-$cPLA_2$ Ab was provided by Andrey Cybulsky (McGill University, Montreal, Canada). Monoclonal anti-Golgi 58K protein (anti–GP-58, clone 58K-9) Ab was purchased from Sigma Biochemical Co. Rabbit polyclonal Ab against Rab11 (marker of the recycling endosomes) was from Zymed Laboratories Inc. (South San Francisco, California, USA). Rabbit polyclonal Ab against CA-2 was a gift from Bill Sly (St. Louis University School of Medicine, St. Louis, Missouri, USA). Production and characterization of the rabbit polyclonal anti-Arf1 was described previously (24). After washing in TBS-Tween buffer, the membranes were exposed to horseradish peroxidase–conjugated donkey anti-rabbit or sheep anti-mouse Ab's, and the specific bands were revealed using the ECL technique. Quantitative densitometry was performed with NIH Image Analysis software.

Metabolic labeling and biotinylation. Two days after infection, cells (in 100-mm dishes) were incubated with methionine-free DMEM for 1 hour. Cellular proteins were metabolically labeled for 6 hours with 0.5 mCi/ml of [^{35}S]methionine and [^{35}S]cysteine (Translabel; ICN Radiochemicals, Irvine, California, USA) in methionine-deficient medium, followed by a 1-hour chase in methionine-rich DMEM medium. At the end of the chase period, dishes were rinsed three times with ice-cold PBS, and cell surface proteins were labeled by biotinylation, using the membrane-impermeant biotin analogue sulfo-NHS-biotin (0.5 mg/ml; Pierce Chemical Co.) in PBS-C/M (containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$), for 30 minutes at 4°C. Cells were then washed four times with ice-cold PBS-C/M, harvested by scraping with a rubber policeman, pelleted by centrifugation, and stored at –80°C until analysis. Total (plasma membrane and intracellular) Na^+-K^+-ATPase and AE 1 protein was immunoprecipitated using monoclonal anti–Na^+-K^+-ATPase (to α-subunit, clone VIF12; gift from D. Fambrough, Johns Hopkins University, Baltimore, Maryland, USA) and polyclonal anti-AE1/2 Ab's (gift from S. Alper, Beth Israel Hospital, Boston, Massachusetts, USA), respectively. Biotinylated (plasma-membrane) proteins were separated from nonbiotinylated (intracellular) proteins by precipitation with streptavidin-agarose and analyzed with 4–20% SDS-PAGE and fluorography.

Protein synthesis. Protein synthesis analysis in LLC-PK_1 cells infected at a moi of 50 pfu/cell with AdLacZ and $AdcPLA_2$ or noninfected (control) was assessed by [^{3}H]-leucine incorporation and expressed as cpm/10^3 cells, as described previously (16). After stimulation, cell were pulse labeled with 1μCi/ml [^{3}H]-leucine for 6 hours, washed twice with ice-cold PBS, and fixed on ice for 30 minutes in 10% trichloroacetic acid (TCA). The medium was then removed, the cells were washed twice with 5% TCA, and once with water. The radioactivity incorporated into the TCA-precipitated material was determined by liquid-scintillation counting after solubilization in 0.25 M NaOH.

Statistical analysis. Data are expressed as mean plus or minus SD. A Student's *t* test was used to compare the means of normally distributed continuous variables. A value of $P < 0.05$ was chosen to determine statistical significance. Each experiment was performed independently at least three times.

Table 1
AA release in LLC-PK$_1$ cells expressing cPLA$_2$ or β-galactosidase

	30 min (%)	120 min (%)	PMA + A23187 (%)
Control	0.93 ± 0.10	2.09 ± 0.95	1.26 ± 0.35
AdLacZ	0.92 ± 0.10	2.13 ± 0.39	2.48 ± 0.97
AdcPLA$_2$	2.14 ± 0.22^A	4.78 ± 1.98^A	15.10 ± 1.56^A

Cells were infected at a moi of 50 pfu/cell, and AA release was determined as described in Methods. The cells were stimulated by vehicle (for 30 and 120 minutes), or PMA (200 nM) and A23187 (10 μM) for 30 minutes. All values are means ± SD of a triplicate experiment. $^AP < 0.05$, AdcPLA$_2$ vs. control and AdLacZ.

Results

PLA$_2$ expression in cells infected with AdcPLA$_2$. There is a dose-dependent increase in cPLA$_2$ activity in AQP2-GFP(CT) LLC-PK$_1$ cells infected at a moi of 0 (uninfected controls), 10, 50, 100, 200, and 400 pfu/cell for 48 hours with AdcPLA$_2$. No increase is seen in uninfected cells or cells infected with AdLacZ. Normal MDCK and rat kidney mesangial cells have cPLA$_2$ activity within the range seen in LLC-PK$_1$ cells infected with AdcPLA$_2$ at a moi of 50 to 100 pfu/cell (Figure 1a). Because we wanted to establish conditions in which the expression of cPLA$_2$ did not exceed that normally measured in other renal epithelial and mesangial cells, we chose a concentration of 50 pfu/cell for our studies. PMA and A23187 treatment results in more AA release into the medium in AdcPLA$_2$-infected cells than in control cells or cells infected with AdLacZ (Table 1). In cells infected with AdcPLA$_2$, PLA$_2$ activity increases with time, with a peak of activity between 3 to 6 days after infection (Figure 1b). Immunoblot analysis confirmed that the progressive increase in cPLA$_2$ activity of cells exposed to increasing moi of AdcPLA$_2$ was a direct consequence of progressive increases in the expression of cPLA$_2$ (Figure 1c). When infected with 50 pfu/cell, the total amount of cPLA$_2$ protein was less in the infected LLC-PK$_1$ cells than it was in the MDCK or mesangial cells.

Greater than 90% of the cells infected with AdLacZ or AdcPLA$_2$ expressed either β-galactosidase or cPLA$_2$, respectively, as assessed by immunocytochemistry (data not shown). There was no measurable toxicity over 48 hours after infection as assessed by the absence of an increase of lactate dehydrogenase (LDH) release (data not shown).

AQP2-GFP(CT), but not GFP-AQP2(NT), trafficking is disrupted by cPLA$_2$. Constitutive and hormone-regulated pathways of protein trafficking were examined using GFP/AQP2 chimeras as model systems. The AQP2-GFP(CT) chimera has been shown previously to traffic constitutively to the membrane. Constitutive AQP2-GFP(CT)–membrane localization is normal in AdLacZ-infected cells (Figure 2, a and b). By contrast, in cells expressing cPLA$_2$, there is a considerable reduction of AQP2-GFP(CT) on the plasma membrane and an increase in perinuclear, intracellular staining both in the absence (Figure 2c), or presence, of VP (Figure 2d).

GFP-AQP2(NT) localization in intracellular vesicles is unchanged under unstimulated conditions in cells infected with AdcPLA$_2$ (Figure 3a) (14). A shift from intracellular vesicles to the basolateral membrane is observed in these cPLA$_2$-expressing cells upon stimulation with 10 nM VP for 30 minutes (Figure 3b) in a pattern identical to that seen in noninfected cells (14).

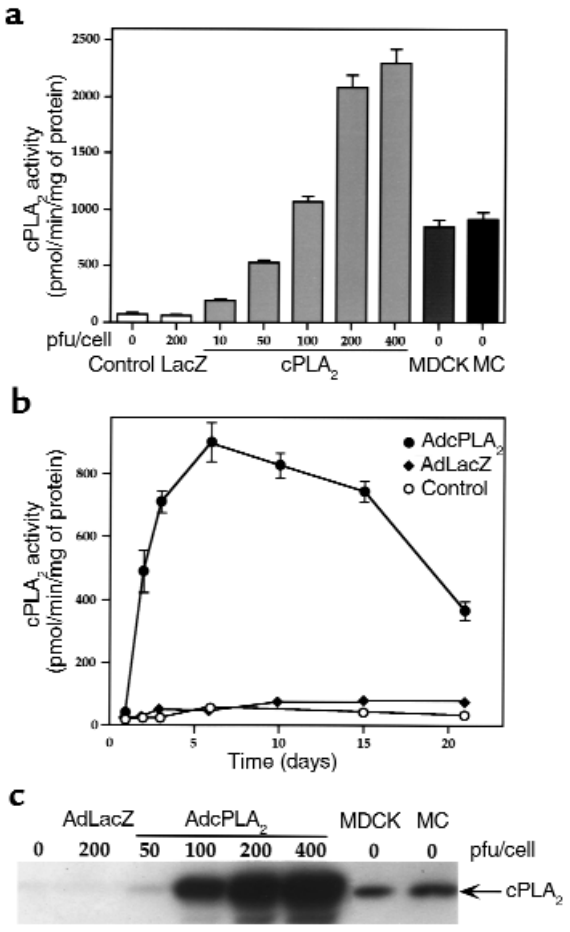

Figure 1
(**a** and **b**) Assay of cPLA$_2$ activity in LLC-PK$_1$ cells expressing LacZ and cPLA$_2$ and in uninfected cells. (**a**) AQP2-GFP(CT) LLC-PK$_1$ cells were incubated with either AdLacZ or AdcPLA$_2$ at different moi (10, 50, 100, 200, and 400 pfu/cell). Forty-eight hours later, the cells were lysed and centrifuged at 100,000 *g* for 1 hour at 4°C. Each assay was performed three times in duplicate. Cell lysates of MDCK and rat mesangial cells (MC) were processed at the same time and used to compare the level of activity in the different cell lines. cPLA$_2$ activity was significantly greater ($P < 0.01$) in cells infected with AdcPLA$_2$ at 10, 50, 100, 200, and 400 pfu/cell when compared with control noninfected cells and AdLacZ infected cells. (**b**) cPLA$_2$ activity was determined 1, 2, 3, 6, 10, 15, and 21 days after infection with AdLacZ or AdcPLA$_2$ at 50 pfu/cell or in control cells ($n = 3$). AdcPLA$_2$-treated cells had significantly greater ($P < 0.01$) cPLA$_2$ activity at each time point when compared with AdLacZ and control cells. (**c**) Immunoblot of cPLA$_2$ from control cells and cells infected with AdLacZ and AdcPLA$_2$. Cells were infected with AdLacZ or AdcPLA$_2$ at different moi. Forty-eight hours later, the cells were solubilized, and 25 μg of each lysate were separated by SDS-PAGE and a Western blot generated with 1:5000 diluted polyclonal anti-cPLA$_2$ Ab.

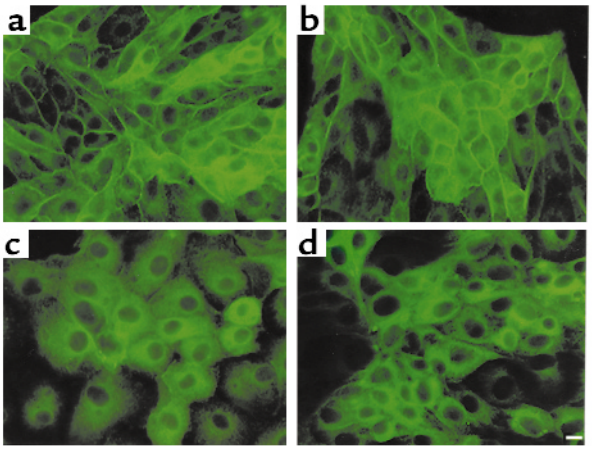

Figure 2
Immunofluorescence localization of AQP2-GFP(CT) in transfected LLC-PK$_1$ cells. Two days after infection with either AdLacZ or AdcPLA$_2$, cells were fixed and examined for GFP expression using indirect immunofluorescence. In control cells (**a**) and cells expressing β-galactosidase (**b**), AQP2-GFP(CT) is localized on the basolateral and apical membrane. By contrast, in cells expressing cPLA$_2$ in the absence (**c**) or presence (**d**) of VP (10 nM, 30 minutes), there is a complete loss of AQP2-GFP(CT) basolateral-membrane staining. Bar, 5μm.

Immunoblot analysis of membrane fractions. To determine the intracellular localization of AQP2-GFP(CT) and GFP-AQP2(NT) in cells expressing LacZ or cPLA$_2$, total cellular membranes were separated by ultracentrifugation on a continuous iodixanol density gradient according to the technique described by van't Hof et al. (20). Fractions were analyzed for enzyme activities identifying plasma membrane, Golgi apparatus, and ER by the markers alkaline phosphatase, α-mannosidase, and α-glucosidase II, respectively. An equal sample of each fraction was separated by SDS-PAGE and blotted with a monoclonal anti-GFP Ab. In cells expressing either LacZ or cPLA$_2$, GFP-AQP2(NT) was localized principally within the intracellular fractions as expected, although some plasma-membrane localization was apparent (Figure 4a). In contrast, AQP2-GFP(CT) was expressed mainly in the plasma-membrane fractions in cells expressing LacZ, whereas in cells expressing cPLA$_2$, AQP2-GFP(CT) was shifted markedly to the ER fractions (Figure 4a). Marker-enzyme activities of each of the fractions are presented in Figure 4b.

Effect of cPLA$_2$ on membrane localization of Na$^+$-K$^+$-ATPase and AE 1. To test whether cPLA$_2$ had an effect on the trafficking of other constitutive membrane proteins, LLC-PK$_1$ cells infected with either AdLacZ or AdcPLA$_2$ were stained with Ab's against the α-subunit of Na$^+$-K$^+$-ATPase or AE1/2. In cells expressing cPLA$_2$, the normal Na$^+$-K$^+$-ATPase basolateral membrane localization (Figure 5a) was almost completely abolished, and it was difficult to tell where the protein was relocated (Figure 5b). However, AE1/2 basolateral localization was unaffected (Figure 5, c and d).

To confirm that the effect of cPLA$_2$ on trafficking of the Na$^+$-K$^+$-ATPase and the anion exchanger was not related to a difference in the stability of the proteins at the plasma membrane, cells expressing either LacZ or cPLA$_2$ were labeled with [^{35}S]-methionine. Two days after infection with either AdLacZ or AdcPLA$_2$, specific activities of postnuclear supernatants of LLC-PK$_1$ cells (Figure 6a), SDS-PAGE profiles of the metabolically labeled proteins (Figure 6b), and total amount of cellular Na$^+$-K$^+$-ATPase (Figure 6c) are not affected by expression of cPLA$_2$. Expression of cPLA$_2$ results in a marked inhibition of trafficking of Na$^+$-K$^+$-ATPase to the plasma membrane of LLC-PK$_1$ cells (Figure 6d). The trafficking of AE 1 protein to the plasma membrane is not affected in cPLA$_2$-expressing cells.

Trafficking in cPLA$_2$-expressing cells treated with inhibitors of AA metabolism. To determine if the effect of cPLA$_2$ on protein trafficking was due to an increase of synthesis of one of the AA metabolites, 24 hours after infection with AdLacZ or AdcPLA$_2$ the cells were incubated with either indomethacin (2.5×10^{-5} M), NDGA (10^{-5} M), or SKF 525A (2.5×10^{-5} M), cyclooxygenases, lipoxygenases, or cytochrome P-450 monooxygenase inhibitors, respectively. After 24 hours, the localization of AQP2-GFP(CT) and Na$^+$-K$^+$-ATPase was examined in controls and in cells expressing cPLA$_2$. Indomethacin, NDGA, and SKF 525A did not change the abnormal protein distribution of either AQP2-GFP(CT) or Na$^+$-K$^+$-ATPase α-subunit in the cells expressing cPLA$_2$ (data not shown). In addition, incubation of the cells with 5 μM AA for 2 or 4 days had no effect on the localization of either AQP2-GFP(CT) or AE1. To stimulate AA release, cells expressing cPLA$_2$ or β-galactosidase were incubated for 2 hours with 10 μM of A23187, a calcium ionophore. A23187 did not modify the localization of GFP-AQP2(NT), AQP2-GFP(CT), Na$^+$-K$^+$-ATPase, or AE1 in cells expressing β-galactosidase or cPLA$_2$ (data not shown).

Protein synthesis in cells expressing cPLA$_2$. To determine if the effect of cPLA$_2$ on protein trafficking was related to an inhibition of total protein synthesis, we studied [^{3}H]-leucine incorporation 48 hours after infection of LLC-PK$_1$ cells with either AdLacZ or AdcPLA$_2$. There was no significant difference between control cells and cells expressing β-galactosidase or cPLA$_2$ (368 ± 40, 502 ± 49, and 471 ± 31 cpm/10^3 cells, respectively). In addi-

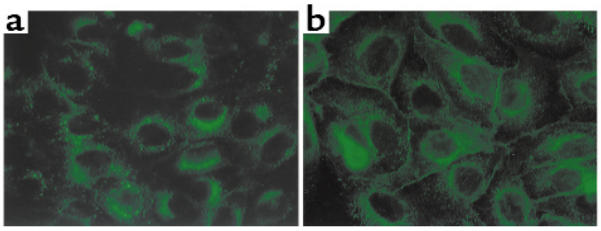

Figure 3
Normal vesicular trafficking of GFP-AQP2(NT) in cPLA$_2$-expressing LLC-PK$_1$ cells. Two days after infection with AdcPLA$_2$, cells were fixed and examined for GFP expression by immunofluorescence. Under nonstimulated conditions (**a**), GFP-AQP2(NT) was localized to intracellular vesicles. After stimulation with 10 nM VP for 30 minutes (**b**), staining shifted to the plasma membrane.

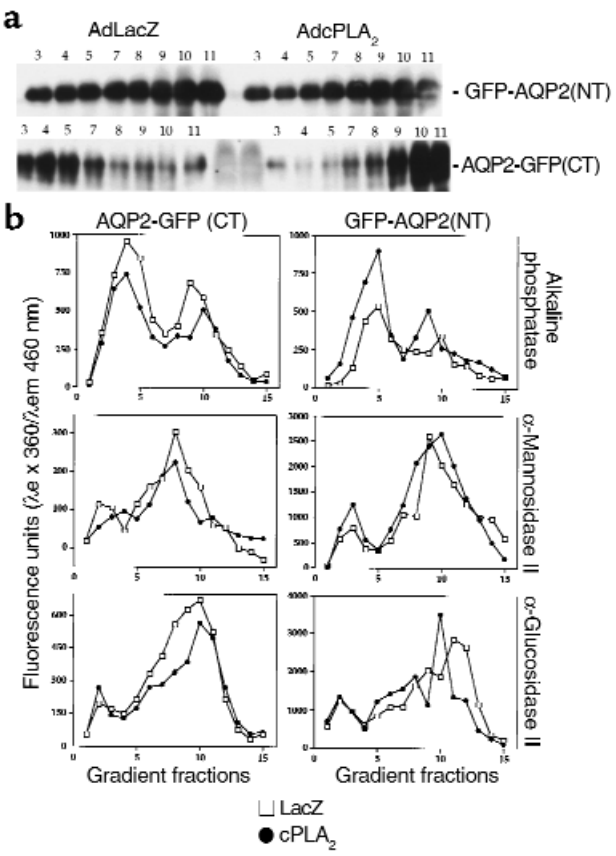

Figure 4
Alteration in AQP2-GFP(CT) localization with $cPLA_2$. (**a**) Immunoblot of GFP-AQP2(NT) and AQP2-GFP(CT) in cellular-membrane fractions. Two days after infection with either AdLacZ or $AdcPLA_2$, cellular membranes were separated by ultracentrifugation on a continuous iodixanol density gradient. Each fraction was assayed for marker-enzyme activities of plasma membrane (alkaline phosphatase), Golgi apparatus (α-mannosidase II), and ER (α-glucosidase II). Proteins were separated by SDS-PAGE and detected with 1:1000 anti-GFP Ab. GFP-AQP2(NT) is expressed principally in the ER fractions in cells infected with AdLacZ or $AdcPLA_2$. AQP2-GFP(CT) is expressed principally in the plasma-membrane fractions in cells infected with AdLacZ, whereas this protein is principally detected in the ER fractions of cells expressing $cPLA_2$. (**b**) Analysis of marker-enzyme activities of each fraction of the density gradient. Cellular-membrane fractions from LLC-PK_1 cells expressing GFP-AQP2(NT) or AQP2-GFP(CT) and infected with either AdLacZ or $AdcPLA_2$ were separated as described in Methods. Gradients were fractionated from the top to the bottom (left to right in the figure) and 50 µl of each fraction was assayed for marker-enzyme analysis. Enzymatic activities were determined as described in Methods.

tion, cycloheximide treatment (25 µg/ml) for 1–3 hours had no detectable effect on the distribution of GFP-AQP2(NT) and AQP2-GFP(CT) (data not shown).

Cell morphology and Golgi apparatus organization. The actin cytoskeleton and microtubule network appeared unaffected by $cPLA_2$, as determined by anti-tubulin and phalloidin labeling (data not shown). To examine the structure of the Golgi apparatus, anti–β-COP Ab was used to label coat proteins on transporting vesicles within the *cis*-medial Golgi apparatus, and anti-giantin Ab was used to label the Golgi cisternae. In LacZ control cells, β-COP and giantin are present in condensed, perinuclear patterns, as expected (Figure 7, a and c, respectively, for β-COP and giantin). In contrast, in cells expressing $cPLA_2$, β-COP and giantin localization is dramatically dispersed in a manner that suggests disruption of the Golgi organization (Figure 7, b and d). After transfection with a construct encoding a GFP-$cPLA_2$ fusion protein, there is marked disruption of the Golgi apparatus of cells that express the protein, whereas a mutant of $cPLA_2$, which is catalytically inactive, has no effect on β-COP distribution (Figure 8).

Using electron microscopy, the morphology of the Golgi apparatus was analyzed in LacZ- and $cPLA_2$-expressing cells. The Golgi stacks were well-formed cisternae in the β-galactosidase–expressing cells (Figure 9a, arrows), whereas they were scattered and vesiculated in cells expressing $cPLA_2$ (Figure 9b, arrows). Nuclear membranes and ER are intact.

$cPLA_2$ is localized to Golgi apparatus of kidney cortex and noninfected LLC-PK_1 cells. Given the dramatic effects of $cPLA_2$ on Golgi structure, it is of importance to evaluate whether $cPLA_2$ is associated with the Golgi apparatus in normal renal epithelium. $cPLA_2$ colocalizes with postnuclear fractions that stain positively with anti–GP-58, a marker of the Golgi complex (Figure 10, a and b) in LLC-PK_1 cells. CA-2 was used as cytosolic marker and Rab-11 as recycling endosome marker. In addition,

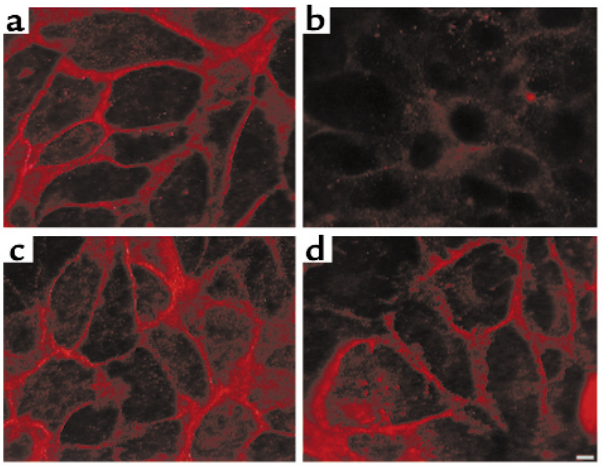

Figure 5
Immunofluorescence localization of Na^+-K^+-ATPase α-subunit and AE1/2 in AQP2-GFP(CT) LLC-PK_1 cells. Cells grown on coverslips were incubated with either AdLacZ or $AdcPLA_2$ at a moi of 50 pfu/cell. Two days later, cells were fixed and permeabilized with 0.1% Triton, followed by indirect-immunofluorescence staining with an Ab against either the α-subunit of Na^+-K^+-ATPase or AE1/2. The secondary goat anti-rabbit IgG Ab was coupled to Cy3. In cells infected with AdLacZ, as in control cells, Na^+-K^+-ATPase (**a**) and AE1/2 (**c**) are localized to the basolateral membrane. However, in cells expressing $cPLA_2$, the basolateral-membrane staining of Na^+-K^+-ATPase (**b**) was almost completely lost, whereas there was no change in the basolateral localization of AE1/2 (**d**). Bar, 3µm.

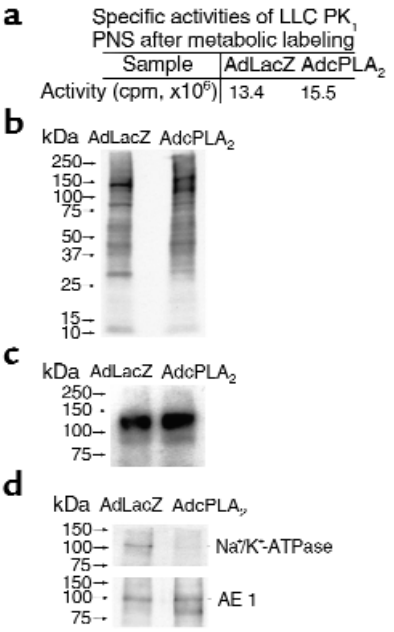

Figure 6
Inhibition of Na^+-K^+-ATPase trafficking in $cPLA_2$-expressing cells. Two days after infection with either AdLacZ or $AdcPLA_2$, LLC-PK_1 cells were metabolically labeled with 0.5 mCi [^{35}S]-methionine (6 hours of pulse followed by 1 hour of chase). (**a**) Specific activities of postnuclear supernatants of LLC-PK_1 cells, as well as (**b**) SDS-PAGE profiles of the metabolically labeled proteins, are not affected by expression of $cPLA_2$. (**c**) Western blot analysis indicates that total amount of cellular Na^+-K^+-ATPase is also unaffected after expression of $cPLA_2$. (**d**) Using plasma-membrane biotinylation and immunoprecipitation (see Methods), we see that expression of $cPLA_2$ results in marked inhibition of trafficking of [^{35}S]Na^+-K^+-ATPase to the plasma membrane of LLC-PK_1 cells (upper panel). The trafficking of [^{35}S]AE 1 protein to the plasma membrane is not affected in $cPLA_2$-expressing cells (lower panel).

and other cellular functions such as volume regulation. Abnormal cell trafficking, including abnormal localization of the Na^+-K^+-ATPase, is characteristic of several renal diseases, including polycystic kidney disease (25) and pathophysiological states, such as ischemia/reperfusion injury (26).

Replication-deficient recombinant adenoviral vectors facilitate the titrated expression of a transgene in a high percentage of cells (> 90% in these studies) so that "physiological" levels of a protein are produced (27, 28). Using stable transfectants of GFP-AQP2 fusion proteins (14), we have found that $cPLA_2$ alters constitutive [AQP2-GFP(CT)] but not VP-regulated [GFP-AQP2(NT)] intracellular trafficking. To confirm that this effect was also relevant to endogenous proteins, it was demonstrated that basolateral membrane localization of Na^+-K^+-ATPase was almost completely abolished, whereas localization of the Cl^-/HCO_3^- exchanger (AE1/2) was unaffected. [^{35}S]-methionine labeling experiments confirm that trafficking of the α-subunit of Na^+-K^+-ATPase to the cell membrane is altered, whereas AE1 trafficking is intact, confirming that there is a level of specificity to the effects of $cPLA_2$ on constitutive trafficking pathways. The absence of an effect of $cPLA_2$ on VP-induced membrane expression of GFP-AQP2(NT) may be explained by the localization of this protein in preformed post-Golgi vesicles that traffic to the membrane upon addition to VP. This process is unaffected by $cPLA_2$ activity.

While the redistribution of basolateral Na^+-K^+-ATPase in the proximal tubule with ischemia has been attributed to disruption of epithelial-cell cytoskeletal structural organization (29, 30), our data suggest that $cPLA_2$-induced changes in intracellular vesicle–trafficking processes may also account for changes in distribution of Na^+-K^+-ATPase.

$cPLA_2$ is associated with Golgi complex isolated from the rat kidney cortex, which consists primarily of proximal epithelial cells (data not shown).

Cell volume and endocytosis in cells expressing $cPLA_2$. The size of the cells increased after $cPLA_2$ expression. To quantitate this effect, 50 β-galactosidase–expressing cells and 50 $cPLA_2$-expressing cells were measured. By determining the two-dimensional size of cells expressing $cPLA_2$, the estimated volume was eightfold larger than that of cells infected with AdLacZ. Rhodamine conjugated to the cell-impermeant agent dextran was used to evaluate endocytosis. Normal endocytosis is found in cells expressing $cPLA_2$ (Figure 11). The glycoprotein characteristics of the apical surface of β-galactosidase– and $cPLA_2$-expressing cells are similar as evaluated by staining with wheat germ agglutinin conjugated to rhodamine (data not shown).

Discussion

The polarized renal epithelial cell is an excellent model for studying differential targeting of proteins to cell membranes. Normal cell-membrane trafficking is necessary for the maintenance of transepithelial transport

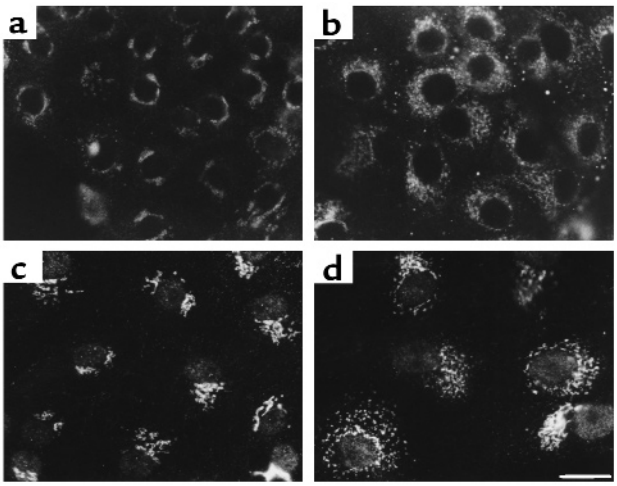

Figure 7
Immunofluorescence localization of β-COP and giantin distribution in AQP2-GFP(CT) LLC-PK_1 cells. (**a**) In cells infected with AdLacZ, β-COP localizes to a condensed Golgi region, whereas $cPLA_2$-expressing cells (**b**) exhibit a dramatic dispersion of β-COP. (**c**) In cells infected with AdLacZ, giantin localizes to the Golgi cisternae. (**d**) By contrast, giantin localization is completely dispersed within the cytoplasm in $cPLA_2$-expressing cells. Bar, 5 μm.

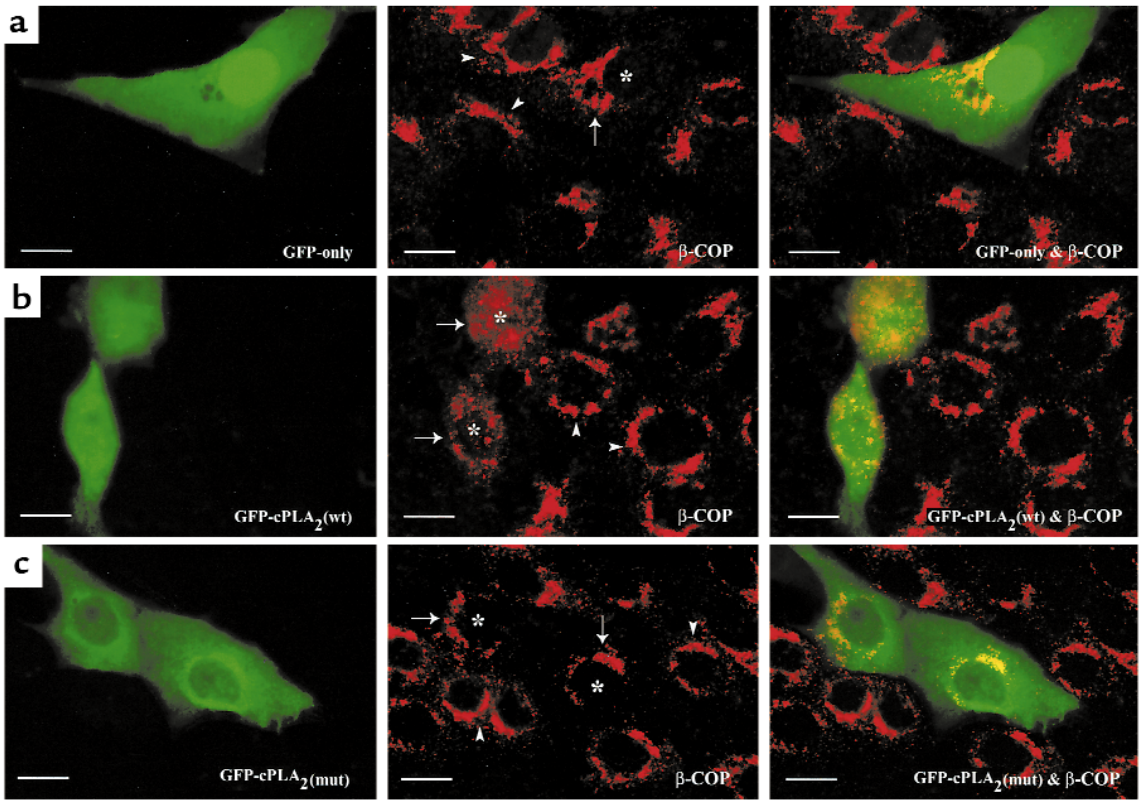

Figure 8
Catalytically deficient mutant of $cPLA_2$ has no effect on β-COP localization. Kidney LLC-PK_1 epithelial cells were transfected with pEGFP only (**a**), pEGFP/$cPLA_2$ (wt) (**b**), or with pEGFP/$cPLA_2$ (mut), encoding a fusion protein of GFP with the calcium lipid-binding region of $cPLA_2$ (**c**) and stained with Ab's against β-COP. Left column represents GFP-expressing, GFP-$cPLA_2$ (wt)–expressing, and GFP-$cPLA_2$ (mut)–expressing cells, middle column shows endogenous β-COP distribution, and right column shows the merging of the two images in each row. Asterisks indicate transfected cells. Arrowheads indicate Golgi complex of nontransfected cells, and arrows indicate the Golgi structure in transfected cells in the same cell culture. Bar, 5 μm. wt, wild-type.

The $cPLA_2$ activity is significantly increased in rat proximal tubule-enriched kidney homogenates after ischemia and reperfusion (31). The decrease in plasma membrane Na^+-K^+-ATPase cannot be due to proteolysis, since equivalent amounts of the protein are found in control and $cPLA_2$–expressing cells. The α-subunit is localized to intracellular membranes in $cPLA_2$-expresssing cells (data not shown). The decrease in Na^+-K^+-ATPase activity likely accounts for the increase in cell volume measured in the absence of morphological changes in actin cytoskeleton and microtubule network (32).

The $cPLA_2$–induced changes in trafficking are possibly due to a direct effect of the enzyme on lipid composition of the Golgi membrane or may be an effect of AA itself. The asymmetry of the lipid bilayer, with choline-containing phospholipids located preferentially in the external leaflet and the aminophospholipids in the cytoplasmic leaflet, is necessary to maintain the fundamental properties of the different intracellular compartments within the cell (33). Hydrolysis of membrane phospholipid into lysophospholipid and free fatty acid by $cPLA_2$ may modify the composition of the two leaflets of the

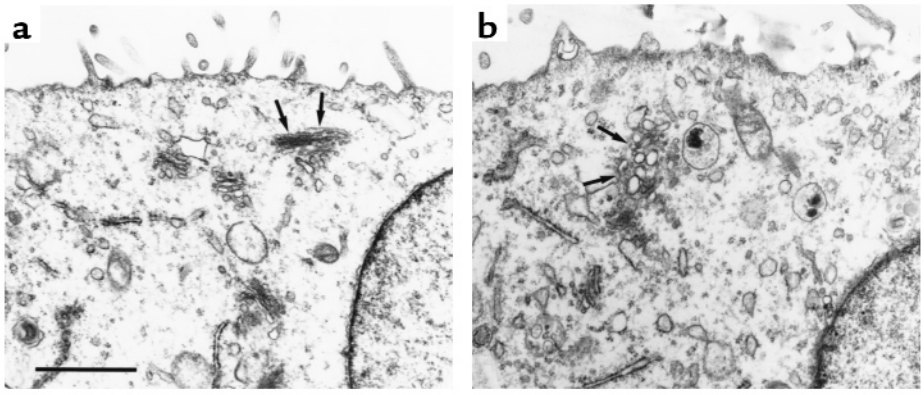

Figure 9
Electron microscopy examination of cellular morphology AQP2-GFP(CT) LLC-PK_1 cells. (**a**) The Golgi complex (arrow) appears normal in cells infected with AdLacZ. (**b**) In contrast, the Golgi complex in $cPLA_2$-expressing cells was markedly disrupted and vesiculated. ER and nuclear membranes appear normal. Bar, 1 μM.

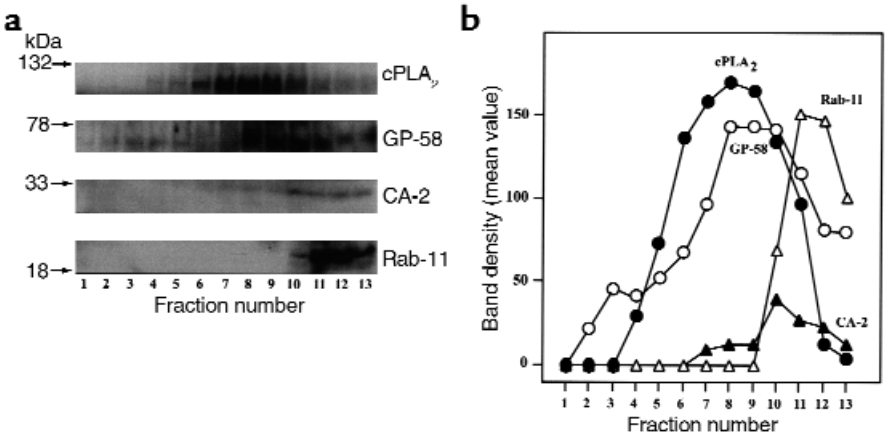

Figure 10
Localization of $cPLA_2$ in subcellular fractions of noninfected LLC-PK_1 cells. $cPLA_2$ colocalizes with GP-58, a marker of the Golgi complex. (**a**) Western blot analyses of LLC-PK_1 cell postnuclear iodixanol density-gradient fractions with $cPLA_2$, GP-58 (Golgi marker), CA-2 (cytosolic marker), and Rab-11 (recycling endosomes marker) Ab's. (**b**) Densitometry analysis of distribution and colocalization of $cPLA_2$ with LLC-PK_1 cell Golgi complex.

phospholipid bilayer. In addition, the modulation of the lipid content induced by $cPLA_2$ could alter the substrate of phospholipase D, thus affecting phosphatidic acid production locally, with secondary effects on trafficking due to the change in this important lipid (33, 34).

The complex organization and dynamic nature of the Golgi subcompartments make it very difficult to study their lipid composition (35). Phospholipase D is present in Golgi membranes (36), is activated by ADP-ribosylation factor (37), and is responsible for catalyzing the transphosphatidylation reaction of phosphatidic acid and diacylglycerol to form bisphosphatidic acid (38). The removal of an acyl chain from bisphosphatidic acid leads to the production of semilysobisphosphatidic acid, which has been localized to the most dynamic pleiomorphic regions of the Golgi complex, the transvesicular networks on both sides of the Golgi stacks (35). Thus, $cPLA_2$ activity might alter the lipid balance of the Golgi apparatus in such a way that stack formation or steady-state integrity is not favored. Since inhibitors of cyclooxgenases, lipoxygenases, and P-450 monooxgenase, or incubation of the cells with AA for 2–4 days, did not modify or mimic the effects of $cPLA_2$, it is unlikely that an AA product, or AA itself, is responsible for the effects observed.

Membrane-associated or secreted proteins contain structural information that is recognized by the intracellular sorting machinery (39). Usually, this information is conveyed by short hydrophobic amino acid sequences in the cytoplasmic tail or phosphorylation of sugar residues or specific patterns of glycosylation, mainly in the rough ER and the Golgi apparatus (40). It is possible that $cPLA_2$ could act on enzymes involved in posttranslational glycosylation within the Golgi apparatus. In addition, by modifying Golgi lipid composition, $cPLA_2$ could modify the conformational environment of Golgi membranes, which may alter critical membrane-fission events (41) or composition of lipid rafts (42).

The $cPLA_2$-induced modification of the Golgi apparatus is similar to the Golgi complex fragmentation that occurs in mitotic cells (43). Since $cPLA_2$ is constitutively phosphorylated in mitotic cells (44), and $cPLA_2$ activity increases in proliferating cells (data not shown), it is possible that $cPLA_2$ is responsible, at least in part, for the Golgi complex fragmentation observed in mitosis.

As reported by de Figueiredo et al., the ability of brefeldin A to stimulate Golgi and *trans*-Golgi network tubulation was inhibited by a number of antagonists of PLA_2 (45). However, because some of the antagonists used in this work are not specific and could have exerted their effects through changes in phosphatidic acid metabolism and on more than one form of PLA_2 (46), it is not possible to attribute the changes observed to a particular enzymatic activity.

In conclusion, our studies demonstrate the potential importance of intracellular group IV $cPLA_2$ activity for

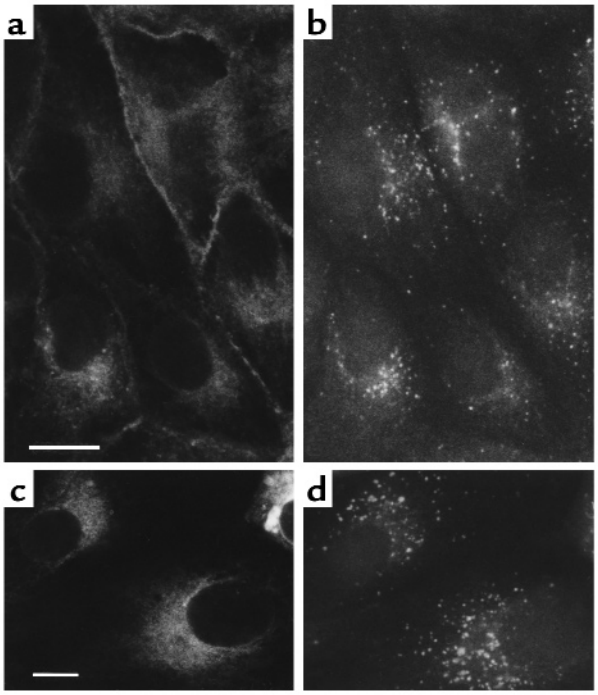

Figure 11
Double-labeling immunofluorescence demonstrating endocytosis of RITC-dextran in AQP2-GFP(CT) LLC-PK_1 cells. (**a**) AQP2-GFP(CT) localizes on the basolateral membrane and in a perinuclear vesicular pattern in AdLacZ-infected cells. (**b**) RITC-dextran is endocytosed into a perinuclear location in the same cells. (**c**) In $cPLA_2$-expressing cells, the basolateral membrane is devoid of AQP2-GFP(CT). (**d**) In the same cells as shown in (**c**), RITC-dextran is endocytosed into perinuclear vesicles. No consistent difference between AdLacZ and AdcPLA_2-infected cells was observed with regard to RITC-dextran endocytosis. Bar, 5 μM.

Golgi apparatus structure and function. Our results also demonstrate the selectivity of these effects of $cPLA_2$ for the constitutive pathway of membrane-protein trafficking and for a subset of polypeptides. These effects of $cPLA_2$ are likely mediated by changes in Golgi membrane lipid composition and may also explain changes observed in the Golgi structure during the cell cycle.

Acknowledgments

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