Molecular Biology of the Cell
Vol. 14, 1801–1807, May 2003

Basolateral Cycling Mediated by a Lumenal Domain Targeting Determinant

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Submitted October 29, 2002; Revised January 7, 2003; Accepted January 23, 2003
Monitoring Editor: Vivek Malhotra

All identified basolateral sorting signals of integral membrane proteins are cytoplasmically disposed, suggesting that basolateral targeting is mediated exclusively by direct interaction with vesicle coat components. Here, we report that GPP130, a *cis*-Golgi protein that undergoes endosome-to-Golgi retrieval using the late endosome-bypass pathway in nonpolarized cells, cycles via the basolateral membrane in polarized MDCK cells. Significantly, the membrane-proximal lumenal domain of GPP130, which mediates GPP130 localization and trafficking in nonpolarized cells, was both necessary and sufficient for basolateral cycling in MDCK cells. The use of lumenal determinants for both basolateral cycling and endosome-to-Golgi retrieval suggests that a novel receptor-mediated mechanism operates at both the trans-Golgi network and distal sites to sort GPP130 along the late-endosome-bypass retrieval pathway in polarized cells.

INTRODUCTION

Simple polarized epithelial cells have two distinct membrane domains: apical and basolateral, separated by tight junctions that prevent lateral diffusion of membrane proteins and lipids. This domain structure is maintained by differential sorting of proteins and lipids destined for the apical and basolateral surfaces. The trans-Golgi network (TGN) serves as the primary sorting station for packaging apical and basolateral cargo molecules into their respective vesicles (Ikonen and Simons, 1998).

Proteins arriving at the TGN are sorted into either one of these pathways depending on the type of targeting information they contain, or they traffic evenly in both pathways if they lack polarized targeting signals. In addition, a hierarchy of signals exists in polarized sorting, because apical sorting occurs only in the absence of a functional basolateral signal. Proteins destined for the apical surface adopt either of two routes to their target membrane: direct transport from the TGN to the apical membrane or transcytosis. The direct route to the apical surface is weak or nonexistent in some polarized cell types, such as hepatocytes (Bastaki *et al.*, 2002). In these cells, apically directed proteins are first taken to the basolateral surface, from which they are internalized and transferred to the apical plasma membrane.

Article published online ahead of print. Mol. Biol. Cell 10.1091/mbc.E02–10–0692. Article and publication date are at www.molbiolcell.org/cgi/doi/10.1091/mbc.E02–10–0692.

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Unlike apical sorting signals, which may be present in the cytoplasmic, transmembrane, or lumenal domains of proteins, all the basolateral sorting signals identified to date lie exclusively in the cytoplasmic domains of the proteins examined (Aroeti *et al.*, 1998; Ikonen and Simons, 1998; Nelson and Yeaman, 2001). Basolateral sorting of many membrane proteins, as well as certain TGN proteins such as TGN38/46 and furin, which cycle to the basolateral membrane, are dependent on interactions of critical tyrosine- or leucine-based motifs in the cytoplasmic tail with specific adaptor proteins. These motifs resemble sequences that specify rapid internalization from the cell surface, suggesting a relationship between basolateral sorting and clathrin-mediated endocytosis (Bonifacino and Dell'Angelica, 1999). Furthermore, an epithelial cell–specific clathrin adaptor subunit called m1B that interacts selectively with a subset of tyrosine-based basolateral targeting signals has been described (Folsch *et al.*, 1999). These observations and the lack of any counter-examples have led to the conclusion that basolateral sorting at the TGN occurs solely by direct interactions of the cytoplasmic sorting signals with coat subunits mediating basolateral delivery.

Nevertheless, nothing in this data set precludes the possibility that basolateral sorting may be mediated by "indirect" interactions, in which a lumenal signal is translated across the membrane by means of a transmembrane receptor, which in turn interacts with coat proteins at the cytoplasmic side. In fact, it is arguable that such a mechanism might operate in basolateral targeting, as similar interactions are used in most other sorting reactions involving vesicle

coat proteins. The most illustrative example of this is the sorting of soluble cargo. Although it is likely that many soluble proteins are targeted for secretion at the basolateral surface, this has not been specifically examined, nor has a receptor for basolateral cargo been identified. In hepatocytes, "soluble" forms of apical transmembrane proteins are secreted basolaterally (Bastaki *et al.*, 2002). However, basolateral targeting might be considered the default in this case, because the primary sorting station for these proteins is the plasma membrane (Nelson and Yeaman, 2001). What is needed, therefore, is identification of a protein that traffics to the basolateral membrane by use of a lumenal signal and the cognate receptor acting to translate the signal to coat proteins.

Golgi phosphoprotein of 130 kDa (GPP130) is a somewhat unexpected candidate for a protein that uses a lumenal basolateral targeting signal. GPP130 exhibits a steady-state localization in the *cis*-Golgi, mediated by constant traffic to and retrieval from distal compartments, including the plasma membrane and endosomes (Puri *et al.*, 2002). The retrieval mechanism is saturable and requires acidified lumenal compartments, because either overexpression or elevation of lumenal pH induces GPP130 redistribution to the cell surface and endosomes. When acidic pH is restored, GPP130 traffics back to the Golgi (Linstedt *et al.*, 1997; Puri *et al.*, 2002). Importantly, the GPP130 targeting information that mediates its steady-state localization and reversible redistribution resides solely in its lumenal domain in a membrane-proximal stretch of 206 amino acids predicted to form a coiled-coil stem domain (Bachert *et al.*, 2001). Targeting of GPP130 involves at least three key sorting steps: local retrieval at the TGN, internalization at the plasma membrane of molecules that escape this local retrieval, and sorting of internalized molecules at the sorting endosome into a distinct retrieval pathway that bypasses the late endosome on its way back to the TGN (Puri *et al.*, 2002). This late endosome-bypass pathway has only recently been described in nonpolarized cells (Mallet and Maxfield, 1999) and has not yet been identified in polarized cells. However, the best-characterized marker of this pathway, TGN38, is expressed in polarized cells, and its cycling is known to be basolaterally restricted (Rajasekaran *et al.*, 1994). Therefore, GPP130 cycling in polarized cells may be basolaterally restricted and depend on its lumenal targeting sequences.

To test this hypothesis, we investigated polarized trafficking of GPP130 under conditions of overexpression and elevation of lumenal pH. GPP130 cycling was basolaterally restricted in polarized MDCK cells, and the lumenal stem-targeting domain was both required and sufficient for basolateral trafficking. The role of a lumenal signal strongly suggests a novel receptor-mediated process active in basolateral sorting.

MATERIALS AND METHODS

Reagents

Chloroquine (Sigma-Aldrich, St. Louis, MO) and bafilomycin were stored at −20°C as 40 and 500 mM stock solutions in water, respectively. Sodium butyrate (Sigma) was stored at −20°C as 0.5 M stock in growth medium. Anti-GPP130 (A1/118) (Linstedt *et al.*, 1997) and anti-hemagglutinin (12CA5) (Bachert *et al.*, 2001) mouse monoclonal antibodies (mAbs) and anti-GPP130 (Puri *et al.*, 2002), anti-giantin (Puthenveedu and Linstedt, 2001), and anti-transferrin (Apodaca *et al.*, 1994) rabbit polyclonal antibodies were used at the dilutions indicated below. Canine transferrin (Sigma) and transferrin antibodies were kindly provided by Dr. G. Apodaca (University of Pittsburgh, Pittsburgh, PA). Fluorescently conjugated goat secondary antibodies were purchased (Zymed, San Francisco, CA).

Transfection and Cell Culture

Constructs encoding HA-tagged chimeric proteins were generated and cloned into the mammalian expression vector pCB6 as described previously (Bachert *et al.*, 2001). MDCK cells were cultured in minimal essential medium (Sigma) supplemented with 10% FCS and 100 IU/ml penicillin–streptomycin at 37°C with 5% CO_2. Transfection was by $Ca_3(PO_4)_2$ as described previously (Weisz *et al.*, 1992), followed by selection using 0.5 mg/ml G418 (Life Technologies Inc., Grand Island, NY). Transfected cells were isolated by use of cloning rings and expanded. Nonpolarized-cell immunofluorescence experiments were performed on cells seeded on 12-mm coverslips. For polarized-cell experiments, cells were seeded in 12-well transwells (0.4-μm pore; Costar, Cambridge, MA) at $\sim 2 \times 10^5$ cells per well. Experiments were performed after 4 d in culture on filters. Where indicated, the last 14 h of culture before the start of the experiment were in the presence of 5 mM sodium butyrate. Relative expression level was determined from immunoblots on whole-cell detergent lysates as described previously (Linstedt *et al.*, 1997).

Analysis

Immunofluorescence and image analysis were performed as described (Linstedt *et al.*, 1997), except that 0.3% Triton X-100 was used to permeabilize the cells. Protein localization was performed with anti-GPP130 (diluted 1:200) or anti-hemagglutinin (diluted 1:200) mAbs. Costaining was with anti-giantin (diluted 1:500). For antibody uptake experiments, filters were incubated with antibodies added apically or basolaterally and with transferrin and/or 0.2 mM chloroquine for 3 h or with 5 μM bafilomycin for 1h, where noted, in MEM containing 0.6% BSA at 37°C and 5% CO_2. Uptake was with either anti-GPP130 (used at 1:10) or anti-giantin (used at 1:10) mAbs or polyclonal anti-GPP130 antibody (used at 1:50). Cells were washed with acid wash buffer (0.2 M glycine, 0.5 M NaCl; pH 2.4) for 10 min on ice before fixation. Internalized antibodies were detected by staining with anti-mouse or anti-rabbit secondary antibodies (diluted 1:200). For surface labeling, pairs of filters were incubated for 20 min with apically or basolaterally added anti-GPP130 mAbs (used at 1:50) before washing five times with PBS, fixation, and detection with the conjugated secondary antibodies (1:200). In a modified version of the surface staining, the filters were incubated with anti-GPP130 (1:50) on both surfaces, washed five times in PBS, incubated for 20 min with FITC-conjugated secondary antibody (1:50) added basolaterally and TRITC-conjugated secondary antibody (1:50) added apically, again washed 5 times with PBS, and finally fixed.

RESULTS

GPP130 Is Targeted to the Basolateral Membrane in Polarized MDCK Cells

MDCK cells were transfected with full-length human GPP130 cDNA, and stable clones expressing the protein were isolated. In their nonpolarized state, that is, after growth on coverslips, indirect immunofluorescence staining yielded a GPP130 pattern (Figure 1A) that was strikingly coincident with the Golgi marker giantin in the same cells (Figure 1B). Transfected GPP130 and endogenous giantin patterns were also coincident after growth on filters to allow polarization (not shown). The antibody used to detect trans-

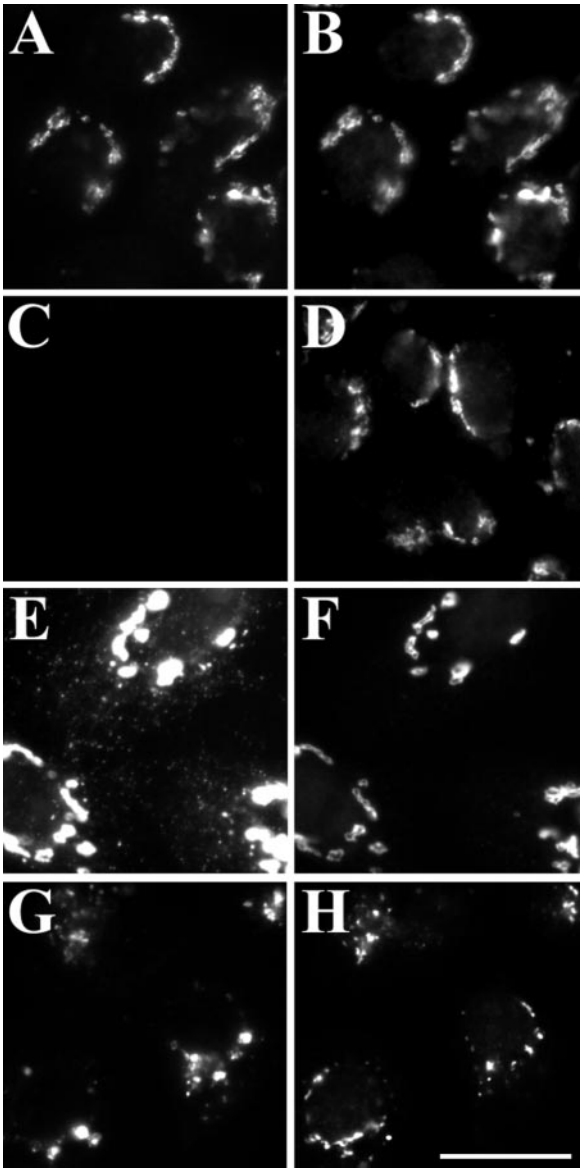

Figure 1. Transfected GPP130 shows saturable and pH-sensitive targeting to the Golgi apparatus similar to endogenous GPP130. MDCK cells stably transfected with GPP130 (A and B) were either induced with sodium butyrate (E and F) or treated with chloroquine (G and H). Nontransfected MDCK cells were used as controls (C and D). All cells were fixed and double-stained with anti-GPP130 (A, C, E, and G) and anti-giantin (B, D, F, and H) antibodies. Bar, 10 μm.

fected GPP130 lacked cross-reactivity with endogenous GPP130, as indicated by staining of nontransfected MDCK (Figure 1C) costained with anti-giantin antibody (Figure 1D). To test whether the transfected GPP130 would traffic to endosomal structures upon overexpression and elevation of lumenal pH, the cells were treated with sodium butyrate (Gorman *et al.*, 1983) and chloroquine (Linstedt *et al.*, 1997), respectively. Sodium butyrate treatment increased GPP130 expression ~2.5- to 3-fold, as determined by immunoblotting (our unpublished results). Under these conditions, ~60% of the cells showed GPP130 staining in peripheral punctate structures (Figure 1E) that lacked staining for giantin (Figure 1F). Cells treated with chloroquine exhibited a striking GPP130 redistribution to punctate structures (Figure 1G) that lacked giantin (Figure 1H). Note that chloroquine treatment altered the giantin pattern but that after treatment, few GPP130 structures remained coincident with giantin.

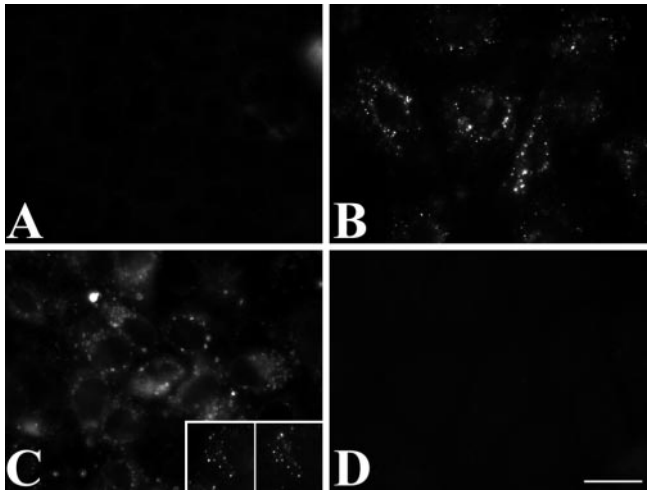

Figure 2. GPP130 cycles to the cell surface and accumulates in endosomes under conditions of overexpression or lumenal pH elevation. MDCK cells stably expressing GPP130 either were exposed to anti-GPP130 mAbs (A) or were induced with sodium butyrate (B) or treated with chloroquine (C) before exposure. (C, inset) Colocalization between internalized FITC–dextran (left) and internalized antibodies (right) in chloroquine-treated cells. Butyrate-induced cells treated with chloroquine were exposed to anti-giantin antibodies as a control (D). Cells were fixed and stained with secondary antibodies to detect internalized antibodies. Bar, 10 μm.

To ensure that transfected GPP130 cycles to the cell surface and accumulates in endosomes under conditions of overexpression or lumenal pH elevation in nonpolarized cells, we allowed uptake of extracellularly applied anti-GPP130 antibody. In the absence of any treatment, anti-GPP130 mAb uptake was not detected (Figure 2A). This was expected for properly targeted GPP130, because no uptake was observed when the same mAb was applied to untreated HeLa cells (Puri *et al.*, 2002). Previous work in HeLa cells suggests that this mAb is not sensitive enough to detect the small amount of GPP130 cycling via the cell surface, whereas this can be detected using a polyclonal antibody. On induction with sodium butyrate, GPP130 cycled via the plasma membrane, as indicated by antibody internalization into punctate peripheral structures (Figure 2B). Similarly, treatment with chloroquine yielded robust antibody uptake, indicating cycling of GPP130 via the surface during its redistribution (Figure 2C). The internalized antibodies colocalized extensively with internalized FITC–dextran after 30 min of uptake (Figure 2C, inset), as previously reported in other cell types (Linstedt *et al.*, 1997). As expected, anti-giantin antibodies were not internalized even when butyrate-induced cells were treated with chloroquine (Figure

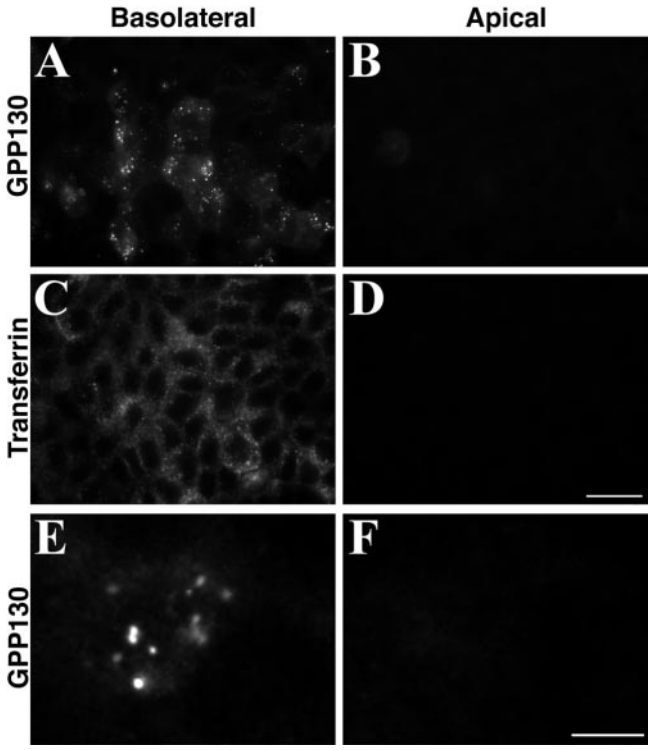

Figure 3. Overexpressed GPP130 cycles via the basolateral plasma membrane in polarized MDCK cells. Butyrate-induced filter-grown cells were concurrently exposed to anti-GPP130 mAbs and canine transferrin on either the basolateral (A and C) or the apical (B and D) surface. Cells were fixed and stained with anti-transferrin and secondary antibodies to detect both internalized anti-GPP130 (A and B) and internalized transferrin (C and D). Bar, 10 μm. Untreated filter-grown cells were also exposed to polyclonal anti-GPP130 antibodies on either the basolateral (E) or apical (F) surface. Total GPP130 in the same cells was detected by costaining using anti-GPP130 mAbs (not shown). The cells shown (E and F) exhibited identical total GPP130 levels. Bar, 2 μm.

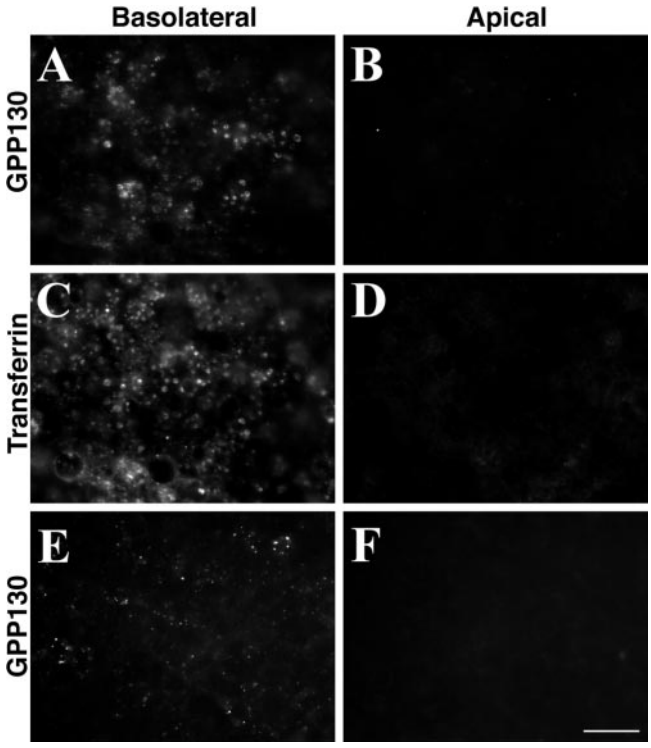

Figure 4. GPP130 traffics through the basolateral plasma membrane after elevation of lumenal pH. Filter-grown MDCK cells were simultaneously exposed to anti-GPP130 mAbs and canine transferrin on either the basolateral (A and C) or the apical (B and D) surface in medium containing chloroquine. Cells were fixed and stained with anti-transferrin and secondary antibodies to detect both internalized anti-GPP130 (A and B) and internalized transferrin (C and D). Anti-GPP130 mAbs were applied on either the basolateral (E) or apical (F) surface of filter-grown MDCK cells treated with bafilomycin. Cells were then fixed and stained for the internalized antibodies. Bar, 10 μm.

2D). In summary, as described previously for endogenous GPP130 in other cell lines (Linstedt *et al.*, 1997), transfected GPP130 in the stable MDCK cell line exhibited pH-sensitive and saturable Golgi targeting such that elevation of lumenal pH or overexpression caused its accumulation in endosomes.

Does overexpressed GPP130 cycle via the apical surface, the basolateral surface, or both? To answer this question, the cells were allowed to polarize by growth on filters, and then anti-GPP130 antibodies were added to either side of the filter. In the absence of any additional treatment, anti-GPP130 mAb uptake was not detected from either surface (our unpublished results). In contrast, after induction of GPP130 expression in polarized MDCK with sodium butyrate, basolaterally applied anti-GPP130 antibodies yielded a strong staining of endosomal structures (Figure 3A). Polarity was intact, and GPP130 trafficking was specific to the basolateral surface, because no uptake of antibody was detected at the apical surface (Figure 3B). Transferrin uptake was used as a further positive control for polarity in the same cells (Fuller and Simons, 1986). As expected, transferrin was internalized preferentially at the basolateral surface (Figure 3C) but not from the apical surface (Figure 3D). Nontransfected MDCK cells did not internalize anti-GPP130 antibodies, and the transfected MDCK cells did not internalize anti-giantin antibodies (our unpublished results). GPP130 trafficking to the basolateral surface depended on GPP130 expression level rather than butyrate treatment per se. Transiently transfected MDCK cells highly overexpressed GPP130 and exhibited antibody uptake predominantly from the basolateral surface (our unpublished results). Further, even in the absence of butyrate treatment, the highest GPP130 expressors after stable transfection exhibited basolateral (Figure 3E) but not apical (Figure 3F) uptake if the more sensitive polyclonal anti-GPP130 antibodies were used.

Specific cycling via the basolateral domain was also observed when lumenal pH was elevated by use of chloroquine. Anti-GPP130 antibodies were strongly internalized from the basolateral side (Figure 4A) and only minimally from the apical side (Figure 4B). This minimal apical uptake was attributed to a slight disruption of polarity by the chloroquine treatment, as indicated by the internal control.

Transferrin uptake was still robust from the basolateral membrane (Figure 4C), but a small amount of uptake was also observed from the apical side (Figure 4D). Bafilomycin, an inhibitor of the proton pump, was also used as a method to raise the lumenal pH. As expected, in bafilomycin-treated cells, anti-GPP130 antibodies were actively internalized when applied at the basolateral surface (Figure 4E), whereas no internalization of apically applied antibodies was observed (Figure 4F). In summary, GPP130 cycling is basolaterally restricted, suggesting the presence of *cis*-acting sequences that specify polarized targeting.

The Luminal Domain of GPP130 Is Both Required and Sufficient for Basolateral Targeting

To investigate the role of the GPP130 lumenal domain for its basolateral targeting, we transfected MDCK cells with chimeric constructs containing versions of the GPP130 lumenal domain fused in frame to the transmembrane and cytoplasmic domains of DPPIV, an apical plasma membrane protein. The GPP130 lumenal domain is conveniently divided into two segments: the targeting determinant–containing, coiled-coil stem domain and the larger, highly acidic C-terminus (Bachert *et al.*, 2001).

A DPPIV/GPP130 chimera containing the latter segment (GPP130 amino acids 295–696) is plasma membrane localized in nonpolarized cells (Bachert *et al.*, 2001). MDCK cells stably expressing this fusion protein were generated. Under conditions in which the full-length GPP130 was detected only by basolaterally applied antibodies, the chimeric protein was readily detected on the surface with anti-GPP130 antibodies applied either basolaterally (Figure 5A) or apically (Figure 5B). Because not all cells expressed detectable amounts of the protein, a separate experiment was used to confirm that individual cells yielded both basolateral and apical staining. First, filter-grown cells were exposed to anti-GPP130 antibodies concurrently at both surfaces. Second, after washing, the basolateral surface received an FITC-conjugated secondary antibody, and the apical surface received a TRITC-conjugated secondary antibody. Finally, the cells were imaged at three focal planes corresponding to the apical, mid, and basal levels. Single transfected cells showed exclusive TRITC fluorescence at their apical surface (Figure 5, C and D) and comparable levels of FITC fluorescence at their basolateral surfaces in the mid (Figure 5, E and F) and basal regions (Figure 5, G and H). All the cells that expressed the protein, irrespective of the level of expression, showed this nonpolarized distribution.

In contrast, a DPPIV/GPP130 chimera containing the lumenal stem domain (GPP130 amino acids 89–294) behaved similarly to wild-type GPP130. MDCK cells stably expressing this construct yielded a typical Golgi staining pattern (Figure 6A) coincident with costained giantin (Figure 6B). After induction with sodium butyrate, a portion of the transfected chimera was detectable in peripheral punctate structures that lacked giantin staining (our unpublished results). Most importantly, when the cells were allowed to polarize on filter supports and then treated with sodium butyrate to induce expression, externally applied anti-GPP130 antibodies were specifically internalized from the basolateral (Figure 6C), not the apical (Figure 6D), surface. Note that because this chimeric protein did not contain the lumenal epitope recognized by the mAb used above, the uptake

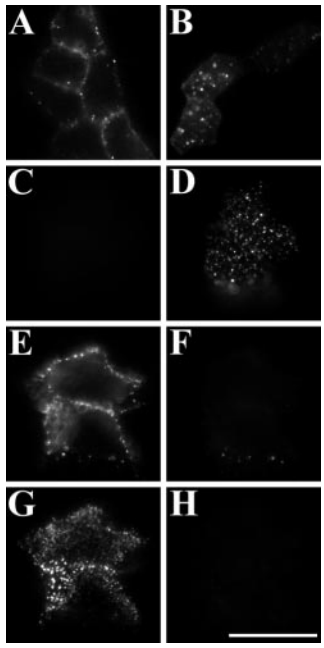

Figure 5. A DPPIV/GPP130 chimera (295–696) lacking the lumenal stem region of GPP130 localizes to both the apical and basolateral domains. Butyrate-induced, filter-grown MDCK cells stably expressing 295–696 were exposed to anti-GPP130 antibodies applied at either the basolateral surface (A) or the apical surface (B). Cells were fixed and stained with secondary antibodies to detect the bound anti-GPP130 antibodies. In a separate experiment, the cells were simultaneously exposed to anti-GPP130 antibodies at both surfaces, followed by washing and exposure to FITC-conjugated secondary antibody at the basolateral surface (C, E, and G) or TRITC-conjugated secondary antibody at the apical surface (D, F, and H). The cells were then imaged at focal planes approximating the apical (C and D), mid (E and F), and basal (G and H) sections. Bar, 10 μm.

experiments presented in Figure 6 were performed with a polyclonal antibody developed against the GPP130 stem domain. This antibody yielded a slight background visible at all surfaces, including those of noninduced cells (Figure 6, E and F). In transfected HeLa cells, the lumenal stem domain of GPP130 does not interact in a stable manner with endogenous GPP130 (Bachert *et al.*, 2001). Furthermore, the same construct is Golgi-localized in HeLa cells lacking endogenous GPP130 after siRNA-mediated knockdown (manuscript in preparation). Thus, Golgi localization of the stem domain does not require its interaction with endogenous GPP130 and it is likely that the same holds true for its basolateral targeting. In sum, specific antibody uptake from the basolateral surface of polarized MDCK cells overexpressing a DPPIV/GPP130 chimera containing the lumenal stem domain of GPP130 strongly suggests the presence of a basolateral sorting signal positioned in the GPP130 lumenal domain.

To test whether the stem domain containing this sorting signal is not only sufficient but also required for basolateral targeting of GPP130, a construct lacking this region, Δ40–247 (Bachert *et al.*, 2001), was expressed by transient transfection. In contrast to wild-type GPP130, which yielded ba-

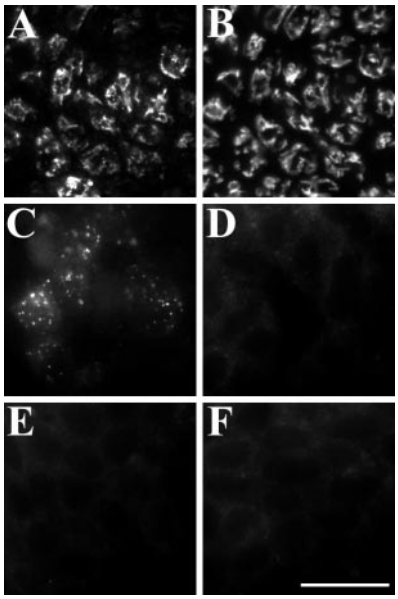

Figure 6. A DPPIV/GPP130 chimera (89–294) containing the lumenal stem region of GPP130 cycles via the basolateral membrane. Filter-grown MDCK cells stably expressing 89–294 were fixed and stained for the transfected protein (A) and giantin (B). Filter-grown cells were induced with butyrate and exposed to anti-GPP130 antibodies applied at either the basolateral surface (C) or the apical surface (D). As a control, noninduced cells were also subjected to antibodies applied basolaterally (E) or apically (F). Cells were fixed and stained with secondary antibodies to detect the internalized anti-GPP130 antibodies. Bar, 10 μm.

solateral (Figure 7A) but not apical (Figure 7B) antibody uptake, the stem-deleted version of GPP130 was detected on the surface of all expressing cells by antibodies applied either basolaterally (Figure 7C) or apically (Figure 7D).

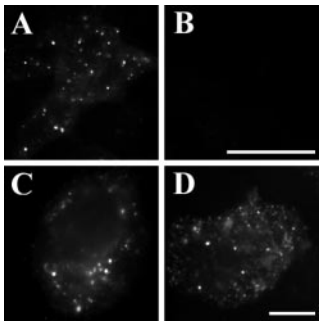

Figure 7. The lumenal stem domain of GPP130 is required for its basolateral targeting. Butyrate-induced, filter-grown MDCK cells expressing either full-length (A and B) or Δ40–247 (C and D) versions of GPP130 were exposed to anti-GPP130 mAbs applied either at the basolateral surface (A and C) or the apical surface (B and D). Cells were fixed and stained with secondary antibodies to detect the bound anti-GPP130 antibodies. Bars: (A and B) 10 μm and (C and D) 2 μm.

DISCUSSION

The absence of identified lumenal determinants specifying basolateral targeting has led to the idea that all basolateral sorting is mediated by cytoplasmic signals that interact directly with vesicle coat proteins. GPP130 provides an important counter-example. In nonpolarized cells, GPP130 is retrieved to the Golgi together with TGN38 via the late endosome-bypass pathway (Puri *et al.*, 2002). Although this pathway has not yet been identified in polarized cells, TGN38 is expressed in polarized cells and is known to be basolaterally restricted. This suggested that GPP130 also cycles specifically via the basolateral domain. If so, it seemed likely that such targeting would depend on lumenal sequences, because GPP130 targeting in nonpolarized cells is mediated exclusively by lumenal determinants (Bachert *et al.*, 2001). Indeed, this was the case. Cycling of GPP130 to the cell surface, induced by either overexpression or elevation of lumenal pH, was accompanied by anti-GPP130 uptake only from the basolateral surface. Importantly, the GPP130 lumenal stem domain was both necessary and sufficient for the basolaterally restricted surface cycling of GPP130.

On the basis of the lumenal location of the GPP130 basolateral determinant, GPP130 sorting into the basolateral pathway is most likely mediated by indirect interactions with cytoplasmic vesicle coat proteins at the TGN. Thus, it is likely that a basolateral-specific transmembrane "receptor" mediates packaging of GPP130 into carrier vesicles. This is distinct from, and possibly competes with, any interactions that mediate retrieval of the protein from the TGN back to the *cis*-Golgi. Consistent with such a basolateral-specific receptor interaction, sorting of GPP130 to the basolateral surface appeared to be saturable. High-level overexpression of GPP130, achieved by transient transfection, yielded comparatively weak but detectable antibody uptake from the apical surface of polarized MDCK cells (not shown). Further indirect evidence of receptor-mediated targeting comes from analysis of an early Golgi protein, GP73, that shares many characteristics with GPP130. GP73 also depends on lumenal stem determinants for retrieval from the cell surface and endosomes via the late endosome-bypass pathway (Puri *et al.*, 2002). Importantly, GPP130 overexpression causes mistargeting of endogenous GP73, yet they do not seem to interact. Therefore, it is likely that GP73 trafficking is also basolaterally restricted and that both GP73 and GPP130 depend on lumenal interactions with the same receptor for their targeting.

In contrast to the situation for basolateral sorting, apical sorting frequently involves lumenal determinants, which are primarily sequence elements that serve as acceptor sites for glycosylation (Matter, 2000). The mechanism by which glycans act in apical sorting is not clear, although interactions with transmembrane lectins might assist enrichment of glycoproteins in apically targeted vesicles (Rodriguez-Boulan and Gonzalez, 1999; Matter, 2000). Although GPP130 is glycosylated at either of two adjacent sites (Linstedt *et al.*, 1997), these sites are outside the basolateral targeting domain. Indeed, the DPPIV/GPP130 chimera containing the GPP130 lumenal stem domain lacked glycosylation sites yet was basolaterally restricted. Also, the GPP130 constructs lacking the stem domain contained the glycosylation sites and yielded a nonpolarized distribution. Therefore, glycosyla-

tion of the GPP130 lumenal domain does not mediate polarized targeting of GPP130.

The extent to which GPP130 cycles to distal basolateral compartments in the absence of overexpression or elevation of lumenal pH is unknown. However, the late Golgi lumen is only mildly acidic. This, together with other considerations (Bachert *et al.*, 2001), suggests that the pH sensitivity of GPP130 Golgi targeting is best explained by a model in which GPP130 normally traffics into the late endosome-bypass pathway, where it encounters a pH-dependent retrieval step. Inhibition at this step would, over time, cause redistribution of GPP130 and TGN38 to the same endosomes. Therefore, a significant amount of GPP130 may move from the TGN to endosomes in untreated cells. The possibility that this involves, at least in part, cycling via the cell surface is suggested by surface labeling of GPP130 and anti-GPP130 polyclonal antibody uptake in untreated HeLa cells (Puri *et al.*, 2002). It is not known why GPP130 and TGN38 bypass late endosomes as they move back to the TGN. Perhaps this pathway presents less chance for spillage into the degradative lysosomal pathway.

Our results suggest the presence of the late endosome-bypass pathway from the basolateral membrane in polarized MDCK cells. Furthermore, because the GPP130 lumenal stem domain mediates both basolateral targeting and endosome-to-Golgi retrieval, a relationship is suggested between the sorting mechanisms active in this pathway and those at the TGN. Therefore, the identification of a lumenal determinant mediating basolateral targeting not only suggests the presence of a basolateral targeting receptor but also provides reagents that could reveal its identity and allow its characterization. This, in turn, may reveal unexpected shared mechanisms in basolateral targeting and endosome-to-Golgi retrieval.

ACKNOWLEDGMENTS

We thank C. Bachert for expert help with the expression plasmids and T. Lee and G. Apodaca for a critical reading of the manuscript. This work was supported by National Institutes of Health grant GM-56779–02 to A.D.L.

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