FEBS Letters 552 (2003) 240–246 FEBS 27619

Differential localization of lipid phosphate phosphatases 1 and 3 to cell surface subdomains in polarized MDCK cells

Yan-Jun Jia, Masahiro Kai, Ikuo Wada, Fumio Sakane, Hideo Kanoh*

Department of Biochemistry, Sapporo Medical University School of Medicine, South-1, West-17, Sapporo 060-8556, Japan

Received 28 May 2003; revised 11 August 2003; accepted 14 August 2003

First published online 28 August 2003

Edited by Felix Wieland

Abstract Lipid phosphate phosphatases (LPPs) are integral membrane proteins with six transmembrane domains that act as ecto-enzymes dephosphorylating a variety of extracellular lipid phosphates. Using polarized MDCK cells stably expressing human LPP1 and LPP3, we found that LPP1 was located exclusively at the apical surface whereas LPP3 was distributed mostly in the basolateral subdomain. We identified a novel apical sorting signal at the N-terminus of LPP1 composed of F(2)DKTRL(7). In the case of LPP3, a dityrosine motif present in the second cytoplasmic portion was identified as basolateral targeting signal. Our work shows that LPP1 and LPP3 are equipped with distinct sorting signals that cause them to differentially localize to the apical vs. the basolateral subdomain, respectively.

Key words: Lipid phosphate phosphatase; Phosphatidic acid phosphatase (type 2); MDCK cell; Apical sorting signal; Basolateral surface

1. Introduction

Mammalian lipid phosphate phosphatases (LPPs) 1 and 3, initially designated as phosphatidic acid phosphatases 2a and 2b [1], have been shown to utilize a broad range of substrates when assayed in vitro. Their substrates thus far include in addition to phosphatidic acid (PA), lysoPA (LPA) [1,2], ceramide-1-phosphate [1,2], sphingosine-1-phosphate [1,2], diacylglycerol pyrophosphate [3], and *N*-oleoyl ethanolamine phosphate [4]. All human LPPs cloned so far, LPP1 [1], LPP2 [4] and LPP3 [1] are integral cell surface membrane proteins with six transmembrane domains and are shown to be *N*-glycosylated at a single conserved site [1,5–7]. These enzymes belong to a phosphatase superfamily containing a novel catalytic site composed of three conserved motifs [5,7–9]. In the case of LPPs, experiments using Dri42 [10], a rat homolog of LPP3, and a computer-predicted transmembrane topology model [8,9] showed that the three phosphatase motifs are distributed in the second and third extracellular loops, thus suggesting the possibility that LPPs act as ecto-enzymes dephosphorylating exogenous lipid phosphates at the cell surface. The action of Drosophila LPP homologs, Wunen [11] and Wunen-2 [12], which generate a repulsive environment for migrating germ cells, may be best accounted for by their ecto-enzyme activities, since these enzymes are considered to catalytically modify the lipid environment surrounding migrating germ cells.

Recently, human LPP3 was found to be involved in the homo- and heterotypic cell–cell and cell–matrix interaction through its Arg-Gly-Asp (RGD) cell adhesion sequence uniquely localized in the second putative extracellular loop of this particular isozyme [13]. This remarkable function of LPP3 as an integrin ligand is apparently independent of its catalytic activity, thus representing a novel non-catalytic function of LPP enzymes. Taken together, LPPs appear to be expressed on the cell surface in a spatially restricted and vectorially regulated manner, in turn enabling them to coordinate cell–cell contacts in the case of LPP3 and to create a chemical signal gradient in the case of the Wunens.

In the present work, we tested to see whether LPPs are sorted to distinct plasma membrane subdomains in polarized cells. For this purpose, we used Madin–Darby canine kidney (MDCK) cells stably expressing LPP isozymes, a widely used and well-characterized model system for studying protein targeting [14]. The plasma membrane of polarized MDCK cells is divided into apical and basolateral subdomains separated by tight junctions. For proper sorting to specific subdomains, LPP isozymes should contain targeting signals that are correctly recognized and interpreted by the cellular sorting machinery. In general, basolateral sorting signals are almost always found in the cytoplasmic domains of transmembrane proteins and frequently contain tyrosine or dileucine motif [15,16]. On the other hand, apical sorting signals are more variable, consisting of glycosylphosphatidylinositol anchor, *N*-glycans, or various other sequences [15–20]. Here we found that LPP1 and LPP3 contain short cytoplasmic sequences responsible for targeting them to distinct subdomains of cell surface membranes.

2. Materials and methods

2.1. Construction of LPP fusion proteins and chimeras

All constructs were C-terminally fused with green fluorescent protein (GFP) by cloning them into the pEGFP-N3 vector (Clontech) [1,21]. For stable transfection experiments, LPP-GFPs were subcloned into the pBI vector (Clontech). LPP1/LPP3 chimeras were generated by sequential PCR (polymerase chain reaction) using LPP1/pEGFP-N3 and LPP3/pEGFP-N3 as templates. Parts of the LPP1 coding

*Corresponding author. Fax: (81)-11-622 1918.
E-mail address: kanoh@sapmed.ac.jp (H. Kanoh).

Abbreviations: BSA, bovine serum albumin; GFP, green fluorescent protein; LPA, lysophosphatidic acid; LPP, lipid phosphate phosphatase; MDCK, Madin–Darby canine kidney; nt, nucleotide; PA, phosphatidic acid; PBS, phosphate-buffered saline

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00931-1

sequence were replaced with the homologous regions of LPP3 according to the sequence alignments described previously [1]. The sequences comprising LPP1/LPP3 chimeras were as follows: (the numbers represent the amino acid residues of LPP1 substituted into LPP3) LPP1(88–284)/LPP3, coding nucleotides (nt) 262–852 of LPP1 and nt 1–342 of LPP3; LPP1(127–284)/LPP3, nt 379–852 of LPP1 and nt 1–462 of LPP3; LPP1(44–284)/LPP3, nt 130–852 of LPP1 and nt 1–213 of LPP3; LPP1(14–284)/LPP3, nt 40–852 of LPP1 and nt 1–120 of LPP3; LPP1(1–13)/LPP3, nt 1–39 of LPP1 and nt 121–933 of LPP3; LPP1(1–86)/LPP3, nt 1–258 of LPP1 and nt 346–933 of LPP3. All point mutations were generated using the Quick-Change (Stratagene) site-directed mutagenesis kit, and were confirmed by sequencing. Two clones from separate mutagenesis reactions were selected and transfected separately to confirm the authenticity of the reaction products.

2.2. Generation of MDCK cell line expressing wild-type and mutant LPPs

MDCK cells expressing the tetracycline-regulated transactivator (Tet-Off cells, Clontech), were transfected with LPP-GFPs/pBI and pTK-Hyg vectors according to the manufacturer's instructions. The transfected cells were selected in the presence of hygromycin (200 μg ml^{-1}) and 2 μg ml^{-1} doxycycline for 10 days before surviving clones were picked. Screening of LPP activity and expression was performed on cells grown in the absence of doxycycline, and monitored by fluorescence microscopy and immunoblotting using an anti-GFP antibody (Molecular Probes). The data presented here are from a representative clone, and were reproduced using at least three independently selected clones. For transient transfection of wild-type LPPs, LPP1/LPP3 chimeras and point mutants, all were fused C-terminally with GFP. MDCK cells were plated on coverslips coated with type I collagen (Iwaki-Asahi Techno Glass, Tokyo, Japan). After growing to confluency, the cells were cultured for at least an additional 5 days to allow the development of a tight monolayer. Cells were then transfected with plasmids using LipofectAmine 2000 (Life Technologies) according to the manufacturer's instructions and analyzed by confocal microscopy 24–48 h post-transfection.

2.3. Domain-selective biotinylation

Tight monolayers of stable MDCK transfectants grown on Transwell polycarbonate filters (Corning Costar) were washed twice with ice-cold phosphate-buffered saline (PBS) containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$. The cell monolayers were biotinylated for 30 min at 4°C either apically or basolaterally using 0.5 mg ml^{-1} sulfo-NHS-biotin (Pierce). Cells were washed and lysed in 10 mM Tris–HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, 1% Triton X-100 and protease inhibitor cocktail (Roche Molecular Biochemicals). Cell lysates were centrifuged at 13 000 rpm for 20 min, and the supernatant was incubated with avidin-agarose (Sigma) overnight at 4°C. The biotinylated proteins thus recovered were eluted from the beads by boiling in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer for Western blot analysis using an anti-GFP antibody [21,22] or by boiling in 1% SDS and 10 mM EDTA for peptide-*N*-glycanase F (New England Biolabs) treatment as described previously [1].

2.4. Assay of LPP activity

Radiolabeled LPA was prepared by diacylglycerol kinase (*Escherichia coli*)-catalyzed reaction of monoolein with [γ-^{32}P]ATP as previously described [1,6,21]. The in vitro phosphatase assay using Triton X-100 LPA micelles was done as described previously [1,6,21]. For assaying domain-selective LPP activity in intact cells, MDCK transfectants grown on Transwell 24-well filters were starved for 24 h in Dulbecco's modified Eagle's medium containing 0.1% bovine serum albumin (BSA). The culture medium containing [^{32}P]LPA (5 μM, 5000–20 000 cpm/nmol) was added to the apical or basolateral side of the monolayer and incubated for 10 min at 37°C. The inorganic phosphate liberated was extracted from the medium and determined as described previously [1,21].

2.5. Indirect immunofluorescence and immunoblotting

Cells grown on coverslips or polycarbonate filters were fixed with methanol, blocked with 1% BSA in PBS and treated with an anti-human E-cadherin monoclonal antibody (Transduction Laboratories), followed by Alexa-594-conjugated anti-mouse IgG (Molecular Probes)

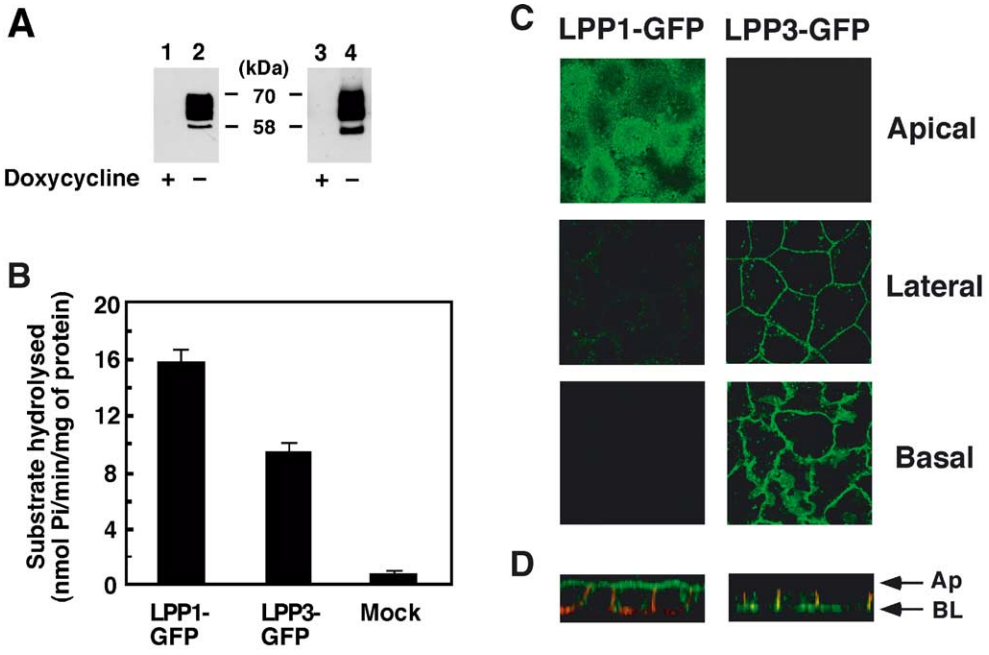

Fig. 1. Steady-state localization of LPP-GFPs in polarized MDCK cells. A: MDCK cells stably expressing LPP1-GFP (lanes 1 and 2) or LPP3-GFP (lanes 3 and 4) were cultured in the presence or absence of doxycycline. The cell lysates were probed with an anti-GFP antibody using Western blotting. B: Cells expressing LPP-GFPs or EGFP alone (MOCK) were lysed and LPP activity was assayed in the clude membrane fractions. The results are means ± S.E.M. of triplicate measurements. C: MDCK cells were grown on polycarbonate filters, fixed, and processed for immunostaining of endogenous E-cadherin (red). The cells were then analyzed by confocal laser scanning microscopy. The optical *x–y* sections corresponding to the apical and the basal surfaces, or to an equidistant intermediate plane of the cells are shown. The scale bar is 10 μm. D: the *x–z* section reconstitution of MDCK cells expressing LPP-GFPs (green) and endogenous E-cadherin (red) is shown. Three independent clones were established for each construct and the results shown are obtained from representative clones.

using procedures described in a previous publication [23]. Images were taken with an inverted confocal laser scanning microscopy (Zeiss LSM510) and processed using the Adobe Photoshop version 5.0 software. Quantitative analysis of the apical versus basolateral distribution of GFP fusion proteins was done using the NIH Image program. Immunoblotting analysis of GFP fusion proteins was done as described previously [1,22].

3. Results

3.1. Polarized localization of LPP1 and LPP3

We first characterized the MDCK cell lines stably expressing LPP1 and LPP3 under the control of the tetracycline-repressor. In vitro LPP activity in representative clones cultured in the absence of doxycycline was markedly increased with a concomitant expression of anti-GFP-reactive protein bands in the 60–70 kDa range on Western blots (Fig. 1). *N*-glycosylation of wild-type or epitope-tagged LPPs has been noted to yield multiple bands on Western blots [1,6,13,21,24]. Confocal microscopy of monolayers showed that LPP1-GFP expressed was almost exclusively distributed to the apical surface, whereas LPP3 was localized mostly in the basolateral subdomain (Fig. 1C). In a latter case, immunostained endogenous E-cadherin, a basolateral domain marker [25], colocalized with LPP3 and also indicated that the cells were correctly polarized under the experimental conditions used (Fig. 1D).

To further characterize the steady-state polarization of the two LPPs, cells cultured on the Transwell polycarbonate filters were subjected to apical or basolateral cell surface biotinylation. Quantification of the biotinylated LPP-GFP proteins confirmed the results of the microscopic observation and showed that approximately 90% of LPP1-GFP was located on the apical surface, whereas most of LPP 3 (70%) was expressed on the basolateral surface (Fig. 2). *N*-glycanase treatment of the biotinylated LPPs showed that the two enzymes

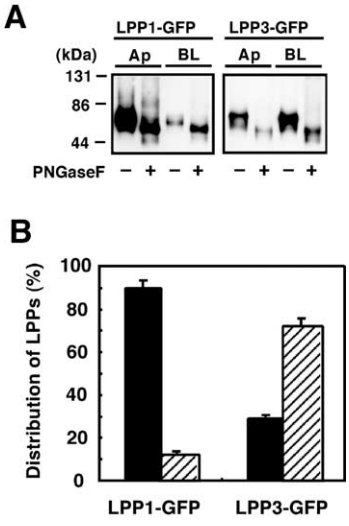

Fig. 2. Domain-selective biotinylation of LPP-GFPs in MDCK cells. A: Stably transfected cells were grown on polycarbonate filters and biotinylated on either the apical (Ap) or the basolateral (BL) domain. The cell lysates were then treated with peptide *N*-glycanase F (PNGase F). The biotinylated proteins were detected using immunoblotting with an anti-GFP antibody. B: The blots were quantified using NIH Image and the percentages of apically localized (solid black bars) and basolateral (hatched bars) proteins are shown. The results represent the means ± S.E.M. obtained from four experiments.

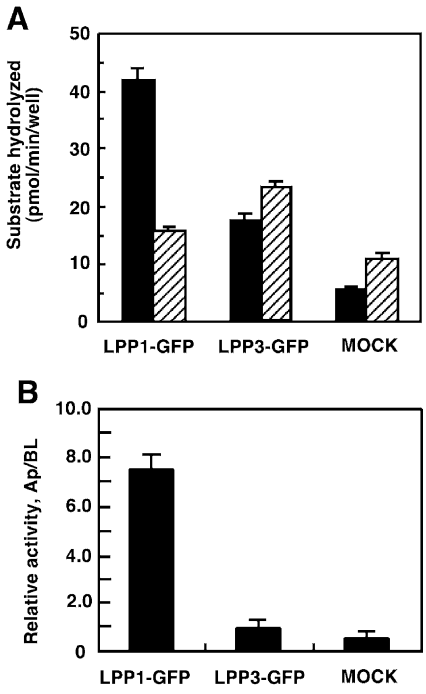

Fig. 3. Domain-selective LPP activity in intact cells. A: MDCK cells stably expressing LPP-GFPs and EGFP (MOCK) were grown on 24-well polycarbonate filters. The tight monolayers were first serum starved for 24 h, and then incubated for 10 min at 37°C with [^{32}P]LPA bound to BSA added apically (solid black bars) or basolaterally (hatched bars). B: LPP activity is presented as the ratio of the apical (Ap) to the basolateral (BL) enzyme activities. The endogenous activity obtained in MOCK cells was subtracted from the total LPP enzyme activities in the transfectants. The results show the mean of three experiments ± S.E.M.

targeted to different subdomains were glycosylated similarly, indicating that *N*-glycosylation was not the reason for their differential targeting. Moreover, mutation of the N-linked glycosylation site of LPP1 did not affect its apical distribution (data not shown).

Next, we determined LPP ecto-enzymatic activity present on the apical and basolateral domain using established procedures [21]. LPP1 [26–28] and LPP3 [21] expressed in non-polarized cells have been shown to act as ecto-enzymes hydrolyzing exogenous LPA and other lipid phosphates. As shown

Table 1
Determination of steady-state distribution of wild-type and chimeric LPP-GFPs in MDCK cells

	Ap/BL ratio
LPP1	10.1
LPP3	0.12
LPP3/LPP1(127–284)	0.18
LPP3/LPP1(88–284)	0.30
LPP3/LPP1(44–284)	0.85
LPP3/LPP1(14–284)	0.92
LPP1(1–86)/LPP3	9.1
LPP1(1–13)/LPP3	4.2

Quantitative analysis of the fluorescence signals in the plasma membrane of MDCK cells was performed using NIH Image as described in Section 2. The Ap/BL ratio is the ratio of the mean fluorescence intensities in the apical versus the basolateral plasma membrane domains. The data are the mean of three to four images for each construct and the S.E.M. was less than 5% of the mean values.

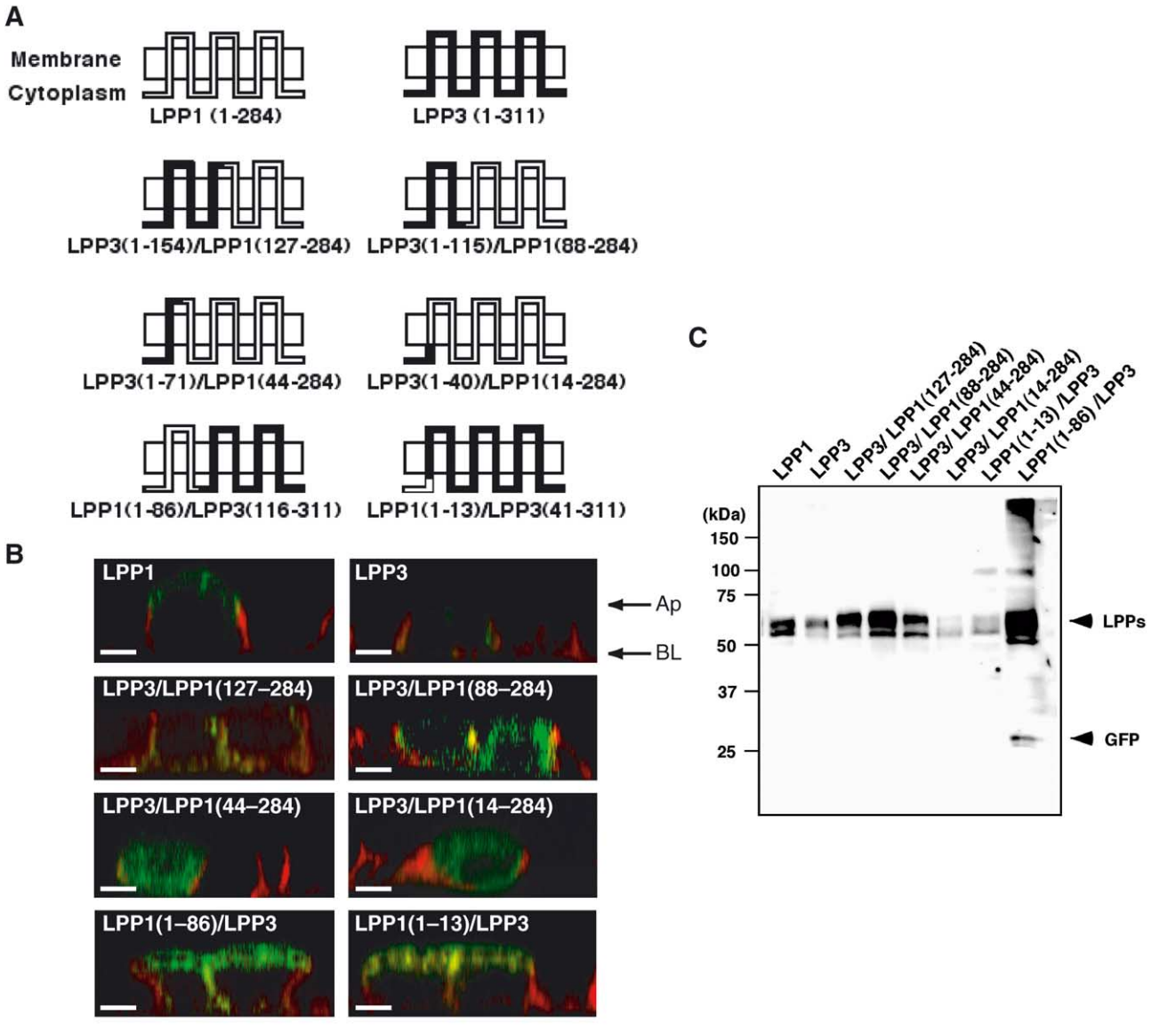

Fig. 4. Schematic representation of LPP1/LPP3 chimeras and their expression patterns. A: LPP1 (white, GFP portion at the C-terminus omitted) and LPP3 (black) were used as the parent constructs from which LPP1/LPP3 chimeric enzymes were derived as described in the text. The numbers in parentheses represent the amino acids of LPP1 and LPP3 that were substituted into the homologous regions of the enzymes. B: The *x–z* section reconstitution of MDCK cells transfected with chimeric enzymes. Chimeras fused with GFP and endogenous E-cadherin are shown in green and red, respectively. Scale bar: 10 μm. C: The cell lysates of transfectants (20 μg of protein) were analyzed by Western blotting using an anti-GFP antibody. Throughout these experiments, three to four images were analyzed for each construct and representative results are shown. Quantification of LPP-GFP distribution obtained from these images is shown in Table 1.

in Fig. 3, LPP1-transfectant possessed apical surface activity nearly eight-fold higher than that of the basolateral surface. On the other hand, LPP3 ecto-activity of intact cells did not increase substantially despite its high activity in lysates (Fig. 1A), and its basolateral activity was similar to that present on the apical surface (Figs. 1 and 2). The reasons for this discrepancy remain to be explored, however, it appears that LPP3 localized at the basolateral surface is less accessible to exogenous substrates as compared with LPP1 on the apical surface.

3.2. Apical and basolateral targeting signals present in LPPs

We next identified the targeting signals responsible for differential sorting of the two LPP isozymes. For this purpose we constructed LPP1/LPP3 chimeras as shown in Fig. 4 and examined their steady-state distribution in the membrane. First, we confirmed that all fusion proteins were expressed, although the level of expression showed an experimet-to-experiment variability (Fig. 4C). The LPP3/LPP1(127–284) and LPP3/LPP1(88–284) constructs displayed basolateral localization (Fig. 4B and Table 1), suggesting that the N-terminal half of LPP3 contains a basolateral sorting signal and that the corresponding N-terminal region of LPP1 is required for its apical sorting. Since LPP3/LPP1(44–284) exhibited non-polarized distribution (Fig. 4B and Table 1), we hypothesized that the signal for basolateral localization of LPP3 may reside in the sequence between Ser(72)-Arg(115) residues. The LPP1(1–13)/LPP3 chimera, where the short N-terminal cytoplasmic

sequence of LPP1, Met(1)-Asp(13), replaced that of LPP3, was preferentially (~80%) localized to the apical surface, dramatically altering its original basolateral localization. Similar data were obtained with the LPP1(1–86)/LPP3 chimera (Fig. 4B and Table 1).

Because these results clearly indicated that the N-terminal short sequence of LPP1 contains an apical targeting signal, which is dominant over the basolateral signal of LPP3, we performed alanine scanning mutagenesis in this region to pinpoint the critical residues (Fig. 5). Substitution with Ala of

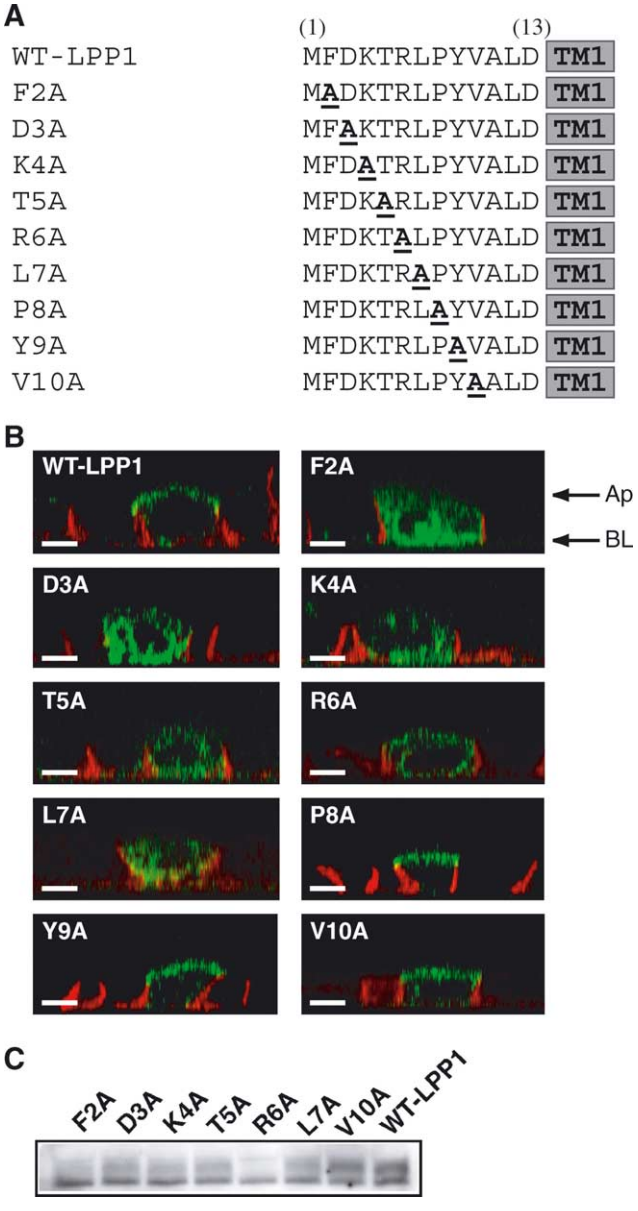

Fig. 5. The N-terminal six-residue sequence, Phe(2)-Leu(7), serves as an apical targeting signal for LPP1. All residues in the sequence, F(2)-V(10) were individually substituted with Ala as shown in A, and the confocal fluorescence micrographs reconstituted in the *x–z* section are shown for the LPP1 mutants (B). C: Cell lysates (20 μg of protein) were analyzed by Western blotting for the expression level of LPP1-GFP fusion proteins. WT-LPP1, wild-type LPP1; TM1, the first transmembrane region. E-cadherin is shown in red. Scale bar: 10 μm. Representative images obtained for four to six cells expressing each construct are shown.

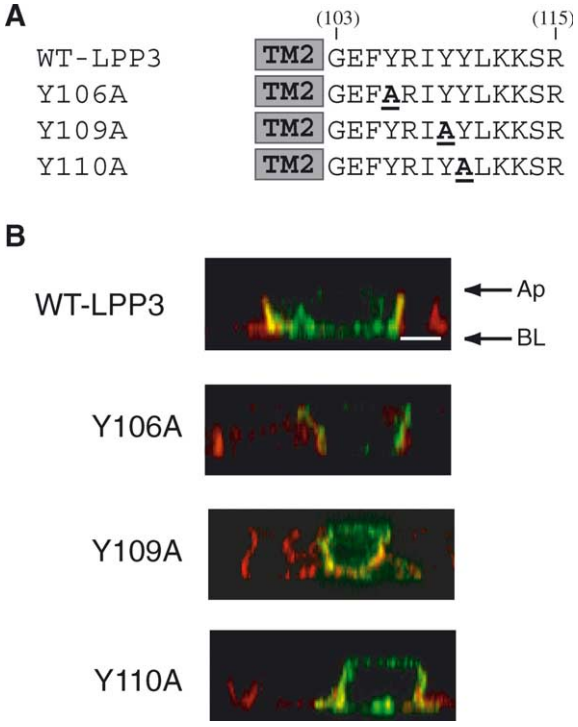

Fig. 6. A dityrosine motif present in the second cytoplasmic loop of LPP3 serves as a basolateral targeting signal. The three Tyr residues found in the G(103)-R(115) sequence were individually substituted with Ala (A), and confocal micrographs (*x–z* section) of MDCK cells expressing the LPP3 mutants are shown (B). TM2, second transmembrane region; LPP3-GFP, green; E-cadherin, red. Scale bar: 10 μm. Representative images obtained for four to six cells expressing each construct are shown.

any amino acids from Phe(2) to Leu(7) invariably resulted in their non-polarized distribution, whereas mutation of other residues did not affect the apical localization of LPP1. Thus, the signal targeting LPP1 to the apical membrane consists of six amino acid residues at the N-terminus, F(2)DKTRL(7). We also note that the TRL sequence represents a class I PDZ-binding motif [29] similar to that present at the C-terminus of cystic fibrosis transmembrane conductance regulator, which is responsible for its apical targeting [30,31].

Basolateral sorting signals usually reside in the cytoplasmic portion of membrane proteins ([15,16], reviewed in [32]) and frequently contain a Tyr residue [15,32]. For this reason we searched for a potential basolateral signal in LPP3 that would reside in the Ser(72)-Arg(115) sequence suggested by the expression experiments of chimeras (Fig. 4). We noted that the Gly(103)-Arg(115) sequence resides in the second cytoplasmic loop of LPP3 [1,5,7–9]. This region contains three Tyr residues, all of which were individually mutated to Ala (Fig. 6A). We found that mutation of either Y(109) or Y(110) resulted in non-polarization of LPP3 in contrast to Y(106) mutation that did not affect its basolateral distribution (Fig. 6). Thus, LPP3 contains a dityrosine basolateral targeting motif, similar to that present in the human low density lipoprotein receptor [15].

4. Discussion

Using two independent techniques, confocal microscopy

and cell surface biotinylation, here we demonstrate that LPP1 and LPP3 are sorted to opposite subdomains of cell surface by possessing apical and basolateral targeting sequences, respectively. The apical targeting signal detected at the N-terminus of LPP1 is unique in that the six-residue sequence contains TRL, a PDZ-binding motif, which thus far has only been detected at the C-terminus of various proteins [29]. However, it remains unknown whether this sequence of LPP1 indeed interacts with PDZ domain-containing apical proteins. This apical targeting signal was found to be dominant over the basolateral targeting signal in LPP3. This is unusual because basolateral signals tend to be dominant over apical signals when present in the same molecule [15,32]. In LPP3 the basolateral targeting signal was identified as a dityrosine motif in the second cytoplasmic loop, although it remains to be seen whether this isozyme contains other additional basolateral targeting motifs. We do not understand the reasons why only 70% of expressed LPP3 was distributed in the basolateral region in contrast to LPP1, 90% of which was localized to the apical surface. Although more work is needed to define the implication of distinct targeting of the two highly homologous LPPs, it now becomes clear that spatially segregated modes of action should be taken into consideration when addressing the functions of LPPs.

LPP3 activity present in the basolateral compartment was surprisingly low and we could not detect a differential increase in activity that corresponded to the difference in localization of this enzyme. This contrasts our findings with LPP1, which showed an increased apical localization and ecto-activity. LPP3 was recently shown to act as a ligand for specific subsets of integrins, resulting in a tight cell–cell interaction that is critically involved in the formation of vascular capillaries [13]. In this respect, MDCK cells express several integrins that can bind to the RGD core sequence of LPP3 [33]. Since the cell adhesion sequence is present in the second extracellular loop of LPP3 adjacent to the first phosphatase catalytic sequence, and because a considerable portion of LPP3 is targeted to the lateral surface, the resulting tight cell–cell contacts might limit the bioavailability of the exogenous substrates. However, we cannot exclude the possibility that LPP3 is under the control of yet unidentified regulatory mechanisms.

Since all lipid phosphate substrates, in particular LPA and sphingosine-1-phosphate, are known to possess signaling functions mediated by their specific cell surface receptors [34–36], LPPs are generally thought to participate in the regulation of lysophospholipid-mediated cellular signaling. Indeed, the cell surface LPP activities to exogenously added LPA have been detected [21,26–28]. LPP1 was further shown to be functionally linked to Edg2 receptor, and overexpression of this isozyme in rat fibroblasts resulted in the attenuation of LPA signaling through this particular receptor, rather than in decreasing bulk concentration of extracellular LPA [28]. In this context, although no data have been available concerning the polarized distribution of lysophospholipid receptors, our work raises the interesting possibility that certain lysophospholipid receptors may be also distinctly polarized on the cell surface, facilitating their functional coupling with specific LPP isozymes residing in the same subdomains. Although more work is needed to clarify the implication of polarized distribution of LPPs, our work has revealed a novel aspect of spatially distinct targeting of these isozymes.

Acknowledgements: This work has been supported in part by Grants-in Aid for scientific research from the Ministry of Education, Culture, Sports and Technology, Japan. We thank Dr. G. Tigyi (University of Tennessee Health Science Center) for critical reading of the manuscript.

References

[1] Kai, M., Wada, I., Imai, S., Sakane, F. and Kanoh, H. (1997) J. Biol. Chem. 272, 24572–24578.
[2] Waggoner, D.W., Gomez-Munoz, A., Dewald, J. and Brindley, D.N. (1996) J. Biol. Chem. 271, 16506–16509.
[3] Dillon, D.A., Chen, X., Zeimetz, G.M., Wu, W.-I., Waggoner, D.W., Dewald, J., Brindley, D.N. and Carman, G.M. (1997) J. Biol. Chem. 272, 10361–10366.
[4] Hooks, S.B., Ragan, S.P. and Lynch, K.R. (1998) FEBS Lett. 427, 188–192.
[5] Brindley, D.N. and Waggoner, D.W. (1998) J. Biol. Chem. 273, 24281–24284.
[6] Kai, M., Wada, I., Imai, S., Sakane, F. and Kanoh, H. (1996) J. Biol. Chem. 271, 18931–18938.
[7] Kanoh, H., Kai, M. and Wada, I. (1999) Chem. Phys. Lipids 98, 119–126.
[8] Stukey, J. and Carman, G.M. (1997) Protein Sci. 6, 469–472.
[9] Neuwald, A.F. (1997) Protein Sci. 6, 1764–1767.
[10] Barila, D., Plateroti, M., Nobili, F., Muda, A.O., Xie, Y., Morimoto, T. and Perozzi, G. (1996) J. Biol. Chem. 271, 29928–29936.
[11] Zhang, N., Zhang, J., Purcell, K.J., Cheng, Y. and Howard, K. (1997) Nature 385, 64–67.
[12] Starz-Gaiano, M., Cho, N.K., Forbes, A. and Lehman, R. (2001) Development 128, 983–991.
[13] Humtsoe, J.O., Feng, S., Thakker, G.D., Yang, J., Hong, J. and Wary, K.K. (2003) EMBO J. 22, 1539–1554.
[14] Matter, K. and Mellman, I. (1994) Curr. Opin. Cell Biol. 6, 545–554.
[15] Matter, K., Hunziker, W. and Mellman, I. (1992) Cell 71, 741–753.
[16] Hunziker, W. and Fumey, C. (1994) EMBO J. 13, 2963–2967.
[17] Schiffele, P., Peranen, J. and Simons, K. (1995) Nature 378, 96–98.
[18] Chuang, J.-Z. and Sung, C.-H. (1998) J. Cell Biol. 142, 1245–1256.
[19] Muth, T.R., Ahn, J. and Caplan, M.J. (1998) J. Biol. Chem. 273, 25616–25627.
[20] Jacob, R., Preuss, U., Panzer, P., Alfalah, M., Quack, S., Roth, M.G., Naim, H. and Naim, H.Y. (1999) J. Biol. Chem. 274, 8061–8067.
[21] Ishikawa, T., Kai, M., Wada, I. and Kanoh, H. (2000) J. Biochem. (Tokyo) 127, 645–651.
[22] Sakane, F., Imai, S., Yamada, K., Murakami, T., Tsushima, S. and Kanoh, H. (2002) J. Biol. Chem. 277, 43519–43526.
[23] Nagaya, H., Wada, I., Jia, Y.-J. and Kanoh, H. (2002) Mol. Biol. Cell 13, 302–316.
[24] Sciorra, V.A. and Morris, A.J. (1999) Mol. Biol. Cell 10, 3863–3876.
[25] Miranda, K.C., Khromykh, T., Christy, P., Le, T.L., Gottardi, C.J., Yap, A.S., Stow, J.L. and Teasdale, R.D. (2001) J. Biol. Chem. 276, 22565–22572.
[26] Roberts, R., Sciorra, V.A. and Morris, A.J. (1998) J. Biol. Chem. 273, 22059–22067.
[27] Jasinska, R., Zhang, Q.-X., Pilquil, C., Singh, I., Xu, X., Dewald, J., Dillon, D.A., Berthiaume, L.G., Carman, G.M., Waggoner, D.W. and Brindley, D.N. (1999) Biochem. J. 348, 677–686.
[28] Xu, J., Love, L.M., Singh, I., Zhang, Q.-X., Dewald, J., Wang, D.-A., Fischer, D.J., Tigyi, G., Berthiaume, L.G., Waggoner, D.W. and Brindley, D.N. (2000) J. Biol. Chem. 275, 27520–27530.
[29] Hung, A.Y. and Sheng, M. (2002) J. Biol. Chem. 277, 5699–5702.
[30] Moyer, B.D., Denton, J., Karlson, K.H., Reynolds, D., Wang, S., Mickle, J.E., Milewski, M., Cutting, G.R., Guggino, W.B., Li, M. and Stanton, B.A. (1999) J. Clin. Invest. 104, 1353–1361.

[31] Moyer, B.D., Duhaime, M., Shaw, C., Denton, J., Reynolds, D., Karlson, K.H., Pfeiffer, J., Wang, S., Mickle, J.E., Milewski, M., Cutting, G.R., Guggino, W.B., Li, M. and Stanton, B.A. (2000) J. Biol. Chem. 275, 27069–27074.
[32] Yeaman, C., Grindstaff, K.K. and Nelson, W.J. (1999) Physiol. Rev. 79, 73–98.
[33] Schoenenberger, C.-A., Zuk, A., Zinkl, G.M., Kendall, D. and Matlin, K.S. (1994) J. Cell Sci. 107, 527–541.
[34] Moolenaar, W.H. (1995) J. Biol. Chem. 270, 12949–12952.
[35] Spiegel, S. and Milstien, S. (2000) Biochim. Biophys. Acta 1484, 107–116.
[36] Hla, T. (2001) Prostaglandins 64, 135–142.