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ABSTRACT

Molecular interactions that direct trafficking of secreted proteins are not well-described in salivary glands. Here, we report that the soluble cargo protein Parotid Secretory Protein (PSP) is bound to the membranes of secretory granules isolated from rat parotids. This is apparently due to specific interaction with phosphatidylinositol phosphates (PtdInsP). PSP binds PtdIns(3,4)P₂, 10-fold greater than PtdIns(3,5)P₂ or PtdIns(4)P, and does not bind PtdIns(3)P or PtdIns(5)P. Human PSP synthesized *in vitro* also binds PtdIns(3,4)P₂. Bacterially expressed rat PSP binds PtdIns(3,4)P₂ with a K_d of 2.4 × 10⁻¹¹ M. Other major secretory proteins (amylase, proline-rich protein) are not bound to isolated granule membranes and do not bind phosphatidylinositol phosphates. Immunofluorescence shows PtdIns(3,4)P₂ at the secretory granules, and fluorescent PtdIns(3,4)P₂ can flip from the outer leaflet to the inner leaflet of the membrane. Binding of PSP to PtdInsPs may contribute to sorting during the formation of the secretory granules, or sorting by retention during maturation of the granules.

KEY WORDS: sorting, parotid, secretion, flip-pase, granule, exocrine.

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Parotid Secretory Protein Binds Phosphatidylinositol (3,4) Bisphosphate

INTRODUCTION

Salivary glands contain polarized acinar cells engorged with secretory granules formed at the *trans*-Golgi network (TGN) for targeted release of proteins into ducts leading to the oral cavity. The parotid is the largest salivary gland and secretes proteins having roles in oral host defense and initiation of digestion, including Parotid Secretory Protein (PSP), a family of Proline-Rich Proteins (PRP), and amylase (Geetha *et al.*, 2003; Hu *et al.*, 2005). Parotid and other polarized cells secrete by several pathways, including apical regulated and basolateral pathways (Gorr *et al.*, 2005; Dikeakos and Reudelhuber, 2007; Venkatesh *et al.*, 2007). Specific cargo proteins must be loaded into vesicles destined for the correct pathway. In some tissues, sorting receptors, in concert with pH- and Ca⁺⁺-dependent aggregation, are thought to aid in sorting of proteins to specific pathways (Cool *et al.*, 1997; Dikeakos *et al.*, 2009). However, salivary gland sorting receptors have not been characterized, and aggregation of secretory proteins does not occur in parotid granules (Venkatesh *et al.*, 2004). Sorting for regulated secretion is a central function of salivary gland biology. Defining the molecular mechanisms of sorting would aid in the design of secreted proteins for use as therapeutic transgenes (Vitolo and Baum, 2002; Shan *et al.*, 2005), and would define important markers for studies of parotid differentiation. To determine the mechanisms of sorting into parotid granules, we investigated the binding of secretory proteins with rat parotid gland secretory granule membranes.

MATERIALS & METHODS

Binding of PSP to Secretory Granule Membranes

Parotid secretory granules were isolated from male Sprague-Dawley rats as described in the Appendix. Granules underwent lysis by freezing, and the membranes were washed and used for Western blotting with anti-PSP. Trypsin-treated membranes were incubated with parotid granule lysate (see Appendix).

Protein-Lipid Overlay Assay

Membrane Lipid Strips and Phosphatidylinositol Phosphate (PIP)-arrays (Echelon Biosciences, Salt Lake City, UT, USA) are nitrocellulose membranes with a pattern of spots of specific lipids. These were blocked with 1% (w/v) non-fat milk in PBST [50 mM phosphate buffer, 150 mM sodium chloride (NaCl), and 0.1% (w/v) Tween-20] for 1 hr (Dowler *et al.*, 2000; Kanai *et al.*, 2001). Blocked strips or PIP-arrays were incubated with 2 µg/mL of parotid granule soluble lysate in the same buffer for 1 hr at room temperature. The

membranes were washed three times with PBST, and bound protein was detected with anti-PSP (1:10,000), anti-acidic PRP (1:5000), or anti-amylase (1:5000), followed by horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (1:5000). The signal was detected with a tetramethylbenzidine (TMB) substrate kit (Thermo Scientific, Rockford, IL, USA) on a Kodak ImageStation.

Liposome Pull-down and Translocase Assay

Parotid granule soluble lysate was incubated with artificial liposomes spiked with 3% of either phosphatidylinositol (PtdIns) or phosphatidylinositol biphosphate (PtdInsP₂), prepared by standard methods (see Appendix). PtdIns-translocation assays followed well-characterized methods, described in the Appendix. Whole parotid granules were isolated by differential centrifugation as described previously (Venkatesh and Gorr, 2002). Fluorescent C16-4-nitrobenzo-2-oxa-1,3-diazole (NBD)-tagged PtdIns or PtdInsPs were incorporated for the measurement of translocation across the membrane (Natarajan and Graham, 2006). To test for the integrity of the granules during the assay, we probed granule supernatants for leaked PSP by Western blot.

Immunoblotting and Immunohistochemistry

Sucrose-gradient purified parotid secretory granule membranes were probed with antibodies to PtdInsP as detailed (Appendix). The specificity and relative affinities of the anti-lipid antibodies were quantified on PIP-arrays, and the dilution series was used as a standard curve for calculation of the amount of each lipid in the granule membrane. Immunohistochemistry on rat parotid tissue sections was performed by standard methods (Appendix).

Cloning, Expression, and Affinity Measurement of PSP

rPSP and hPSP (human PSP) lacking the signal sequence were cloned into pcDNA6.2-V5 vector for reticulocyte lysate expression, or into pGEX-6P vector for bacterial expression and purification (Appendix). Expressed rat and human PSP each included a V5 tag for immunodetection. Control proteins included glutathione-S-transferase (GST)-V5 and chloramphenicol acetyl transferase-V5 (CAT-V5).

Data Analysis

The data obtained from densitometry of immunoblots by the Kodak ImageStation were analyzed by one-way analysis of variance (ANOVA) with Student-Neuman-Keuls multiple-comparisons post-test or an unpaired *t* test. A *p* value < 0.05 was considered statistically significant. Densitometric analysis of the protein-lipid overlay assays was used to calculate the binding affinity, with the use of GraphPad Prism software version 5.01.

RESULTS

PSP Is the Only Major Cargo Protein on Secretory Granule Membranes

Western blot analysis of extensively washed purified granule membranes showed 35% of PSP bound to the membrane. Other abundant secretory proteins, such as amylase and acidic PRP,

were not detected (Fig. 1A), suggesting that PSP is selectively bound. Binding of PSP to the granule membrane was consistent with the presence of a sorting receptor protein; however, numerous experiments with purified membranes failed to detect cross-linking of PSP to any membrane protein (not shown). To confirm the absence of a protein-binding site, we subjected granule membranes to extensive proteolytic digestion with trypsin prior to incubation with PSP. Subsequent incubation of washed trypsinized membrane with parotid granule soluble lysate resulted in exogenous PSP binding to the membrane (Fig. 1B, lane TM+L), suggesting that PSP does not require membrane proteins for binding. In contrast, amylase was always in the unbound fraction (Fig. 1B). Similar results were obtained with Pronase-treated parotid granule membranes (data not shown).

PSP Binds to Phosphatidylinositol (3,4)biphosphate [PtdIns(3,4)P₂]

We used soluble granule lysate in lipid-overlay assays to determine whether salivary proteins bind specific lipids (Dowler *et al.*, 2000). None of the granule lysate proteins bound the most abundant membrane lipids [phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), cholesterol, or sphingomyelin] (Fig. 1C). Similarly, acidic PRP never bound any lipid spots, and amylase generally showed no binding. Conversely, PSP bound selectively only to PtdInsPs (Fig. 1C). Binding occurred across the range of pHs present in parotid granules (from pH 6.0 at the TGN to 6.8 after maturation) (Arvan *et al.*, 1984).

Binding of native PSP depends on the location of the phosphate groups. We observed a 3- to 5-fold higher binding of PSP to PtdIns(3,4)P₂ compared with PtdIns(4,5)P₂ or PtdIns(3,4,5)P₃, and 10-fold greater than PtdIns(3,5)P₂ or PtdIns(4)P (Fig. 2A). PSP did not bind PtdIns(3)P, PtdIns(5)P, or PtdIns. This specificity indicates that PSP binds the phosphorylated head group of PtdInsPs (Fig. 2B), similar to known PtdInsP-binding proteins (Dowler *et al.*, 2000). Key contacts with phosphates at specific locations are required for binding of PSP. Alternatively, neither amylase nor PRP was detected in parallel blots.

We made synthetic liposomes to determine whether PSP binds to PtdInsPs in intact membranes. Granule lysate PSP did not bind to liposomes of PC, PE, and PtdIns (77:20:3), or to liposomes with PtdIns(3,5)P₂ (Fig. 2C). PSP bound consistently to PtdIns(3,4)P₂ and PtdIns(4,5)P₂-spiked liposomes, showing that native PSP binds PtdIns(3,4)P₂ and PtdIns(4,5)P₂ in a lipid membrane in the absence of a transmembrane sorting receptor (Fig. 2C). Amylase did not bind to any of these liposomes.

We used *in vitro*-expressed PSP to investigate whether binding to lipids was mediated by other granule proteins. Rat PSP-V5 expressed in rabbit reticulocyte lysates bound lipids with a pattern similar to that of native rPSP, binding only PtdIns(3,4)P₂ and PtdIns(4,5)P₂ (Fig. 2D), indicating that the interaction was independent of other parotid granule proteins. The amino acid sequences of rat and human PSP (Splunc2, C20ORF70) have only 52% similarity. Nevertheless, hPSP-V5 translated *in vitro*, bound selectively to PtdIns(3,4)P₂ and PtdIns(4,5)P₂, demonstrating that this activity is conserved between species (Fig. 2D). The negative control protein CAT-V5, having a size and isoelectric point similar to that of rPSP, did not bind lipids.

Figure 2. PSP binds phosphatidylinositol (3,4)bisphosphate. **(A)** PIP-arrays were incubated with parotid granule lysate (2 $\mu\text{g}/\text{mL}$). Bound protein was detected with anti-PSP. This result was obtained in 3 separate experiments. **(B)** Structure of PtdIns(3,4) P_2 showing the critical phosphate groups. **(C)** To ascertain if PSP binds to PtdInsPs in lipid bilayers, we incubated soluble granule lysate with liposomes of PC and PE (77:20) spiked with 3% PtdIns, PtdIns(3,4) P_2 , PtdIns(3,5) P_2 , or PtdIns(4,5) P_2 . The washed liposome pellets (with bound PSP) were analyzed by SDS-PAGE and Western blotting with anti-PSP. Each lane is a separate incubation, done in triplicate or duplicate. The arrow indicates that PSP bound only to PtdIns(3,4) P_2 or PtdIns(4,5) P_2 . **(D)** Rat PSP-V5, human PSP-V5, and CAT-V5 were translated in reticulocyte lysates *in vitro* and incubated (50 $\mu\text{L}/10\text{ mL}$) with lipid strips having spots of 5 different lipids. Bound protein was detected with anti-V5 antibody. U: unprogrammed reticulocyte lysate. Similar results were obtained in 4 experiments. **(E)** The binding of bacterially expressed rPSP-V5 to PtdIns(3,4) P_2 was measured by densitometry of protein-lipid overlay experiments (Appendix). Different concentrations of protein were incubated with 50 pmol of PtdIns(3,4) P_2 and PtdIns. To construct a standard curve, we spotted known amounts of rPSP-V5 (0.25 ng to 0.1 μg) on a nitrocellulose membrane and probed it with anti-V5-HRP antibody (see Appendix). Specific bound protein and free protein were calculated according to the standard curve. The Fig. is representative of 3 experiments, which gave an average $K_d = 2.4 \times 10^{-11}\text{ M}$.

Essentially all of the integrated lipid was in the outer leaflet, since dithionite and BSA destroyed the fluorescence (Fig. 3C, column 1). Incubation for 1 hr at 37°C allowed 15% of integrated NBD-PtdIns(3,4) P_2 to translocate to the protected inner leaflet (Fig. 3C, column 2). Subsequent addition of Triton X-100 caused a loss of fluorescence, showing that the NBD-PtdIns(3,4) P_2 had been protected by the membrane. Parallel Western blot analysis with anti-PSP, to determine granule integrity, showed less than 2% leakage, thus demonstrating that granules remained “sealed” during the assay.

Unphosphorylated PtdIns is translocated by flippase at a rate similar to that for phosphatidylcholine (Vishwakarma *et al.*,

2005). However, PtdInsPs have not previously been tested. We compared the translocation of NBD-PtdIns with several NBD-PtdInsPs. Approximately 10% of PtdIns was flipped to the inner leaflet of parotid granules, whereas from 15 to 18% of PtdIns(4)P, PtdIns(3,4) P_2 , or PtdIns(3,5) P_2 was translocated (Fig. 3D). Hence, the flippase activity was relatively non-selective, and the phosphate groups did not block translocation.

DISCUSSION

The present study demonstrates a new activity for the major salivary protein PSP. We found that PSP binds to the membrane

Figure 3. PtdIns(3,4)P₂ is present in parotid secretory granule membranes. **(Ai)** To determine the relative amounts of PtdInsPs in granule membranes, we spotted lipids from sucrose-gradient-purified membranes on PVDF and incubated them with antibodies to PtdIns(4)P, PtdIns(3,4)P₂, PtdIns(4,5)P₂, or PtdIns(3,4,5)P₃. **(Aii)** The specificity and relative affinity of the antibodies were determined by binding to PIP-arrays. The order of lipid spots on the arrays is as in Fig. 2A. **(Aiii)** The amount of PtdInsP in the granule membrane was quantified based on densitometry of the spots (i) and the standard curves (ii). This approach detected 50% more PtdIns(3,4)P₂ than PtdIns(4)P. Bars are the means of 2 experiments. **(B)** Immunohistochemistry shows that PtdIns(3,4)P₂ colocalized to secretory granules of the parotid acinar cells. Rat parotid tissue sections were stained with monoclonal anti-PtdIns(3,4)P₂ (i, green) and polyclonal anti-rPSP (ii, red) as described in MATERIALS & METHODS. Overlays of the fluorescence images (in both iii and vi) show co-localization (yellow). Negative controls for anti-PtdIns(3,4)P₂ (iv) and anti-rPSP (v) were incubated with highly specific secondary antibodies alone. The boundary of the acinus is encircled (dotted line). The boxed area of the Nomarsky image (panel vi) is enlarged in the inset. The bar is 5 μm. Panel (vii) shows hematoxylin and eosin staining of a parotid acinus. Arrow indicates the acinar duct area. **(C)** Parotid membranes can translocate NBD-PtdIns(3,4)P₂. Fluorescent NBD-PtdIns(3,4)P₂ was incubated with intact parotid secretory granules for 5 min on ice, and then at 37°C for 0, 1, or 4 hrs. The percent of NBD-PtdIns(3,4)P₂ in the inner leaflet was determined at each time-point by BSA extraction and dithionite-quenching of NBD-lipid in the membrane outer leaflet. Subsequently, Triton X-100 was added to disrupt the membranes, to demonstrate that lysis of the granule led to complete quenching. Data are the mean ± SE of 3 experiments in triplicate (*p < 0.01 compared with 0 hr). **(D)** Translocation of NBD-labeled PtdIns, PtdIns(3,4)P₂, PtdIns(3,5)P₂, PtdIns(5)P, or PtdIns(4)P across parotid granule membranes. PtdIns is a positive control previously shown to be flipped (Vishwakarma *et al.*, 2005). Results are the percent of NBD-lipid flipped after 0 or 1 hr of incubation. *p < 0.05, ***p < 0.01 compared with PtdIns. Data are mean ± SE of 3-6 experiments in triplicate.

of the secretory granule, apparently by binding PtdIns(3,4)P₂. Many proteins are anchored to membranes by specific PtdInsP-binding domains (Vicinanza *et al.*, 2008); however, the involvement of PtdInsPs in the localization of any luminal protein is a novel observation. Consistent with this binding, both PI3-kinase and PI4-kinase are present on granules and TGN of several cell types (Weixel *et al.*, 2005; Graham and Burd, 2011), and we found PtdIns(3,4)P₂ present in the parotid secretory granule membrane (Fig. 3B).

Three types of lipid translocases have been described at the TGN or in post-Golgi vesicles. P-type ATPase translocases, present in the TGN and secretory granules (Suzuki *et al.*, 1997), are important for secretion (Liu *et al.*, 2008; Muthusamy *et al.*, 2009). Additionally, the ATP-binding cassette (ABC) superfamily of transporters includes lipid translocases, which

are found in cytoplasmic organelles including the TGN, lysosomes, and secretory vesicles of lung type II cells (Stahlman *et al.*, 2007; Eckford and Sharom, 2010). Similarly, the phospholipid scramblase family mediates bidirectional flipping of phospholipid across the membrane, and PLSCR1 is present in neutrophil secretory vesicle membranes (Frasch *et al.*, 2004). Unphosphorylated PtdIns is translocated by an ER flippase (Vishwakarma *et al.*, 2005). However, none of the translocase families has been tested for the ability to flip PtdInsPs. We found that parotid membranes support flipping of phosphorylated forms to a greater extent than PtdIns. This is reasonable, since other lipids with larger headgroups are known to be flipped (Daleke, 2007). The presence of PtdInsPs in the luminal leaflet of membranes raises the possibility that other intra-organelle proteins may be localized by PtdInsP anchors.

The locations of phosphate groups on the inositol headgroup are important for strong binding of PSP to PtdInsPs. A high affinity for binding to PtdIns(3,4)P₂ was calculated. However, additional studies are necessary to characterize the interaction, including mutagenesis of PSP to localize the binding site(s), and surface plasmon resonance studies to quantify the interaction with PtdInsP₂ in a lipid membrane. PtdInsP-binding domains typically contain a critical group of lysines and/or arginines to interact with the negatively charged phosphates (Dowler *et al.*, 2000). PSP does contain groups of basic amino acids; however, sequence analysis shows only weak similarities to known PtdInsP-binding domains.

The current models for trafficking of soluble cargo proteins into the correct secretory vesicles often involve sorting receptors which bind the luminal cargo (Park and Loh, 2008; Dikeakos *et al.*, 2009). We suggest an alternative wherein PtdIns(3,4)P₂ produced by the coat protein complex PI-kinases on the forming granule is flipped to the luminal leaflet and bound by soluble PSP within the TGN. These interactions may help collect PSP into the forming granule, and support 'sorting by retention' during maturation of the granule. Given the known roles of PI-kinases in forming coat protein complexes, similar mechanisms for sorting may be active in other tissues.

In summary, we determined that PSP is a novel PtdIns(3,4)P₂-binding protein. This contributes to the observed ability of PSP to anchor to the granule membrane. This represents a previously unrecognized luminal role for PtdIns(3,4)P₂.

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REFERENCES

- Arvan P, Rudnick G, Castle JD. (1984). Osmotic properties and internal pH of isolated rat parotid secretory granules. *J Biol Chem* 259: 13567-13572.
- Cool DR, Normant E, Shen F, Chen HC, Pannell L, Zhang Y, *et al.* (1997). Carboxypeptidase E is a regulated secretory pathway sorting receptor: genetic obliteration leads to endocrine disorders in Cpe(fat) mice. *Cell* 88:73-83.
- Daleke DL. (2007). Phospholipid flippases. *J Biol Chem* 282:821-825.
- De Matteis MA, Godi A. (2004). PI-loting membrane traffic. *Nat Cell Biol* 6:487-492.
- Dikeakos JD, Reudelhuber TL. (2007). Sending proteins to dense core secretory granules: still a lot to sort out. *J Cell Biol* 177:191-196.
- Dikeakos JD, Di Lello P, Lacombe MJ, Ghirlando R, Legault P, Reudelhuber TL, *et al.* (2009). Functional and structural characterization of a dense core secretory granule sorting domain from the PC1/3 protease. *Proc Natl Acad Sci USA* 106:7408-7413.
- Dowler S, Currie RA, Campbell DG, Deak M, Kular G, Downes CP, *et al.* (2000). Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities. *Biochem J* 351(Pt 1):19-31.
- Dumaresq-Doiron K, Savard MF, Akam S, Costantino S, Lefrancois S. (2010). The phosphatidylinositol 4-kinase PI4KIIIalpha is required for the recruitment of GBF1 to Golgi membranes. *J Cell Sci* 123(Pt 13): 2273-2280.
- Eckford PD, Sharom FJ. (2010). The reconstituted *Escherichia coli* MsbA protein displays lipid flippase activity. *Biochem J* 429:195-203.
- Falkenburger BH, Jensen JB, Dickson EJ, Suh BC, Hille B. (2010). Phosphoinositides: lipid regulators of membrane proteins. *J Physiol* 588(Pt 17):3179-3185.
- Frasch SC, Henson PM, Nagaosa K, Fessler MB, Borregaard N, Bratton DL. (2004). Phospholipid flip-flop and phospholipid scramblase 1 (PLSCR1) co-localize to uropod rafts in formylated Met-Leu-Phe-stimulated neutrophils. *J Biol Chem* 279:17625-17633.
- Geetha C, Venkatesh SG, Dunn BH, Gorr SU. (2003). Expression and anti-bacterial activity of human parotid secretory protein (PSP). *Biochem Soc Trans* 31(Pt 4):815-818.
- Gorr SU, Venkatesh SG, Darling DS. (2005). Parotid secretory granules: crossroads of secretory pathways and protein storage. *J Dent Res* 84:500-509.
- Graham TR, Burd CG. (2011). Coordination of Golgi functions by phosphatidylinositol 4-kinases. *Trends Cell Biol* 21:113-121.
- Hu S, Xie Y, Ramachandran P, Ogorzalek Loo RR, Li Y, Loo JA, *et al.* (2005). Large-scale identification of proteins in human salivary proteome by liquid chromatography/mass spectrometry and two-dimensional gel electrophoresis-mass spectrometry. *Proteomics* 5:1714-1728.
- Kanai F, Liu H, Field SJ, Akbary H, Matsuo T, Brown GE, *et al.* (2001). The PX domains of p47phox and p40phox bind to lipid products of PI(3)K. *Nat Cell Biol* 3:675-678.
- Liu K, Surendhran K, Nothwehr SF, Graham TR. (2008). P4-ATPase requirement for AP-1/clathrin function in protein transport from the trans-Golgi network and early endosomes. *Mol Biol Cell* 19:3526-3535.
- Muthusamy BP, Natarajan P, Zhou X, Graham TR. (2009). Linking phospholipid flippases to vesicle-mediated protein transport. *Biochim Biophys Acta* 1791:612-619.
- Natarajan P, Graham TR. (2006). Measuring translocation of fluorescent lipid derivatives across yeast Golgi membranes. *Methods* 39:163-168.
- Park JJ, Loh YP. (2008). How peptide hormone vesicles are transported to the secretion site for exocytosis. *Mol Endocrinol* 22:2583-2595.
- Shan Z, Li J, Zheng C, Liu X, Fan Z, Zhang C, *et al.* (2005). Increased fluid secretion after adenoviral-mediated transfer of the human aquaporin-1 cDNA to irradiated miniature pig parotid glands. *Mol Ther* 11:444-451.
- Stahlman MT, Besnard V, Wert SE, Weaver TE, Dingle S, Xu Y, *et al.* (2007). Expression of ABCA3 in developing lung and other tissues. *J Histochem Cytochem* 5:71-83.
- Suzuki H, Kamakura M, Morii M, Takeguchi N. (1997). The phospholipid flippase activity of gastric vesicles. *J Biol Chem* 272:10429-10434.
- Venkatesh SG, Gorr SU. (2002). A sulfated proteoglycan is necessary for storage of exocrine secretory proteins in the rat parotid gland. *Am J Physiol Cell Physiol* 283:C438-C445.
- Venkatesh SG, Cowley DJ, Gorr SU. (2004). Differential aggregation properties of secretory proteins that are stored in exocrine secretory granules of the pancreas and parotid glands. *Am J Physiol Cell Physiol* 286: C365-C371.
- Venkatesh SG, Tan J, Gorr SU, Darling DS. (2007). Isoproterenol increases sorting of parotid gland cargo proteins to the basolateral pathway. *Am J Physiol Cell Physiol* 293:C558-C565.
- Vicinanza M, D'Angelo G, Di Campli A, De Matteis MA. (2008). Function and dysfunction of the PI system in membrane trafficking. *EMBO J* 27:2457-2470.
- Vishwakarma RA, Vehring S, Mehta A, Sinha A, Pomorski T, Herrmann A, *et al.* (2005). New fluorescent probes reveal that flippase-mediated flip-flop of phosphatidylinositol across the endoplasmic reticulum membrane does not depend on the stereochemistry of the lipid. *Org Biomol Chem* 3:1275-1283.
- Vitolo JM, Baum BJ. (2002). The use of gene transfer for the protection and repair of salivary glands. *Oral Dis* 8:183-191.
- Weixel KM, Blumental-Perry A, Watkins SC, Aridor M, Weisz OA. (2005). Distinct Golgi populations of phosphatidylinositol 4-phosphate regulated by phosphatidylinositol 4-kinases. *J Biol Chem* 280:10501-10508.