The protein-tyrosine phosphatase PTPMEG2 is located on the cytoplasmic face of the enclosing membrane of secretory vesicles, where it regulates vesicle size by promoting homotypic vesicle fusion by dephosphorylating N-ethylmaleimide-sensitive factor, a key regulator of vesicle fusion. Here we address the question of how PTPMEG2 is targeted to this subcellular location. Using a series of deletion mutants, we pinpointed the N-terminal Sec14p homology (SEC14) domain of PTPMEG2, residues 1–261, as the region containing the secretory vesicle targeting signal. This domain, alone or appended to a heterologous protein, was localized to intracellular vesicle membranes. Yeast two-hybrid screening identified a number of secretory vesicle proteins that interacted directly with the SEC14 domain of PTPMEG2, providing a mechanism for PTPMEG2 targeting to secretory vesicles. Two such proteins, mannose 6-phosphate receptor-interacting protein TIP47 and Arfaptin2, were found to alter PTPMEG2 localization when overexpressed, and elimination of TIP47 resulted in loss of PTPMEG2 function. We conclude that the N terminus of PTPMEG2 is necessary for the targeting of this phosphatase to the secretory vesicle compartment by association with other proteins involved in intracellular transport.

Proteins destined for secretion are synthesized on membrane-bound ribosomes and are transported through the endoplasmic reticulum and the Golgi apparatus to the trans-Golgi network, where they are sorted into transport vesicles targeted for either the constitutive or the regulated secretory pathways. The latter transport vesicles fuse with each other to form condensing vacuoles referred to as immature secretory vesicles, which subsequently become mature secretory vesicles following an active concentration process that involves influx of more material via transport vesicles from the trans-Golgi network (anterograde transport) as well as removal of excess membrane and proteins by retrograde transport to the trans-Golgi network (1). By electron microscopy, this process can be seen as an increasingly dense core of matrix inside the maturing vesicle. Depending on cell type, mature dense core secretory vesicles have a diameter of 150–600 nm, each cell type containing a relatively narrow range of vesicle sizes. Mature secretory vesicles are stored in the cytoplasm of cells until triggered to undergo exocytosis (2, 3).

Hematopoietic cells utilize the regulated secretory pathway for a variety of functions (1, 3). Platelets contain numerous vesicles with clotting factors, vasoactive agents, and growth factors. Mast cells are specialized to degranulate large, dense core, secretory vesicles that contain histamine, vasoactive molecules, and leukotrienes upon stimulation by antigen-IgE complexes. Cytotoxic T cells contain specialized dense core granules with granzyme, perforin, and other lytic enzymes, which are released in a receptor-triggered and directional manner to kill target cells. In hematopoietic cells, secretory vesicles share many components with lysosomes and have been viewed as specialized lysosomes (4).

A hallmark of the regulated secretory pathway is its response to extracellular stimuli. In both endocrine and hematopoietic cells, the use of this pathway can be up-regulated severalfold, and in some instances, the contents of the vesicles are acutely expelled in response to receptor triggering (4). For example, a key function of helper T cells in the initiation of an immune response is to actively secrete a number of cytokines, which recruit and activate other cell types involved in inflammation and the ensuing innate and adaptive immune responses. Depending on the subtype of helper T cell, these polypeptides include numerous interleukins (e.g. IL-2, -4, -5, and -12), interferons, and growth factors (e.g. for granulocytes and monocytes).

PTPMEG2 (5) is a 68-kDa, class I, cysteine-based, nonreceptor PTP² expressed at highest levels in brain, leukocytes, endocrine, and exocrine cells. In these cells, most of the PTPMEG2 is found on the cytoplasmic face of secretory vesicles (6–9) including those that fuse with the phagosome in neutrophils (8). Structurally, PTPMEG2 is also unique among mammalian PTPs in that it contains a domain in its N terminus with homology to yeast Sec14p, a phosphatidylinositol-binding protein (10). This Sec14p homology (SEC14) domain of PTPMEG2 binds phosphoinositides (8, 11, 12), an event that results in enzymatic activation of the phosphatase domain of PTPMEG2 (8, 11). A physiological substrate for PTPMEG2 is the vesicle

² The abbreviations used are: PTP, protein-tyrosine phosphatase; EGFP, green fluorescent protein; NSF, N-ethylmaleimide-sensitive factor; RNAi, RNA interference; ARF, ADP-ribosylation factor; HA, hemagglutinin; TC-PTP, T cell PTP (PTPN2).
Indeed, electron microscopy of thymocytes and splenocytes revealed that these abnormalities may be caused by defective secretion. In particular, they die before birth or within the first neonatal days. Platelet dysfunction is particularly evident as defective skull formation and intracranial bleeding (13). The animals remain small, and the majority of them die before birth or within the first neonatal days. Platelet activation is defective, and T cells stimulated through their antigen receptors secrete very little interleukin-2 (13). All of these abnormalities may be caused by defective secretion. Indeed, electron microscopy of thymocytes and splenocytes from PTPMEG2−/− mice reveals that these cells have very few secretory vesicles of normal size and morphology (13). These animals support the notion that PTPMEG2 is important for secretory vesicle biogenesis. More specifically, we have proposed (7, 9, 11, 13, 14) that PTPMEG2 promotes the homotypic fusion of immature secretory vesicles, which is a key step in the formation of these vesicles from post-Golgi transport vesicles containing cargo destined for secretion. Furthermore, maturing secretory vesicles normally maintain an equilibrium between continuing fusion events with incoming transport vesicles and a retrograde transport of excess membrane and unnecessary material back to the Golgi apparatus. This dynamic balance results in increasing concentration of secretory vesicle contents and control of mature secretory vesicle size. It appears that PTPMEG2 regulates this balance by promoting fusion (anterograde transport) and reducing condensation (retrograde transport), thus increasing the size of secretory vesicles.

An important step toward a more complete mechanistic understanding of PTPMEG2 function would be the elucidation of how this protein is targeted to the proper subcellular location. In this study, we asked which part of PTPMEG2 is responsible for targeting the protein to the secretory vesicle compartment, and then we sought to determine the proteins in this location with which the targeting domain of PTPMEG2 interacts.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Reagents**—The Anti-influenza hemagglutinin (HA) tag epitope mAb16B12, unconjugated or conjugated to Alexa Fluor 488, was from Covance (Princeton, NJ) and Invitrogen, respectively. The anti-V5 tag monoclonal antibody, unconjugated or conjugated to biotin and the rabbit anti-FLAG antibody, was from Sigma. Qdot605- or Qdot655-conjugated goat anti-rabbit antibody, Qdot605-conjugated anti-mouse IgG antibody, and Qdot655-conjugated streptavidin were from Quantum Dot Corp. (Hayward, CA). Guinea pig anti-TIP47 was from Research Diagnostics (Concord, MA). Biotin-conjugated donkey anti-guinea pig antibody was from Jackson ImmunoResearch Laboratories (West Grove, PA).

**Plasmid Construction**—The expression vectors for PTPMEG2 were as described previously (6, 7, 9, 11). To construct an expression vector for the C-terminal deletion mutants of PTPMEG2, cDNA fragments encoding amino acids 1–261, 1–291, and 1–301 of PTPMEG2 were amplified by PCR and ligated into the EcoRI site of pEFHA vector. For expression of enhanced green fluorescent fusion proteins (EGFP), pEF5HA-EGFP vector was constructed by ligating the PCR-amplified EGFP-encoding sequence from pEGFP-N3 into pEF5HA vector using NotI and XbaI sites. The expression vectors for the N-terminal and C-terminal EGFP fusion proteins of PTPMEG2 were constructed by subcloning the cDNA for PTPMEG2 into the pEF5HA-EGFP vector using the XbaI site and EcoRI sites, respectively. The expression vector for the EGFP fusion protein of the N-terminal deletion of PTPMEG2 (ΔN-PTPMEG2-EGFP) was constructed by ligating the PCR-amplified cDNA fragment encoding amino acids 323–593 of PTPMEG2 into the XbaI site of the pEF5HA-EGFP vector.

The expression vectors for TIP47, ARL6IP, Rab28, BNIP3, FKBP8, and amino acids 283–353 of TC-PTP were constructed by amplifying those cDNAs by PCR from the Jurkat cDNA library and ligating them into the BamHI and EcoRI sites of the pEF5/V5 and pEF5F expression plasmids, which we constructed from pEF5HA by replacing the 5′ HA epitope tag encoding sequence with a sequence encoding the V5 tag (GKIPNPPLLGLDST) or the triple FLAG tag (DYKDDDDK)3. All constructs were verified by nucleotide sequencing.

**Cells and Transfections**—RBL-2H3 mast cells, Jurkat T leukemia cells, and HeLa cells were kept at logarithmic growth in RPMI supplemented with 10% heat-inactivated fetal calf serum, l-glutamine, and antibiotics. Transient transfections of Jurkat cells were carried out by electroporation and RBL, HeLa, and 293T cells by liposome transfection using Lipofectamine 2000 (Invitrogen). Electroproporation conditions typically involved 2 × 107 cells and a total of 1–10 μg of plasmid DNA; in each transfection the amount of DNA was kept constant by adding empty vector. Cells were used for experiments 48 h after transfection.

HeLa cells stably transfected with an RNA interference (RNAi) plasmid for TIP47 used the sequence CCC GGG GCT CAT TTC AAA C, starting at position 1435 in the 3′-untranslated region of the human TIP47 mRNA as a target. In brief, two complementary oligonucleotides were synthesized containing the above target sequence followed by a spacer of 9 nucleotides, the target sequence in reverse orientation, and a sequence of 5 thymidines as a terminator of transcription. Both primers were annealed and cloned into the pSHH plasmid (Biocarta, Carlsbad, CA) using the XhoI and XbaI restriction sites. The plasmid was transfected using Effectene (Qiagen) as a transfection reagent. Co-transfection of a plasmid containing a neomycin phosphotransferase gene was used to allow the selection of cells stably expressing the RNAi vector. The derived HeLa clones were assayed by immunofluorescence, immunoprecipitation, and Western blotting for the suppression of TIP47. A single clone in which TIP47 expression was reduced by more than 90% was used for all further experiments.

**Immunoblotting and Immunoprecipitation**—Immunoprecipitation was performed as before (9, 11). Immunoblotting was developed by the enhanced chemiluminescence technique (ECL kit, Amersham Biosciences) according to the manufacturer's instructions.

**Confocal Microscopy**—Immunofluorescence staining was done as described previously (6, 7, 9, 11). Briefly, cells were washed in phosphate-buffered saline and fixed in freshly made 3.7% formaldehyde. Fixed cells were permeabilized with 0.1%...
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saponin and 2.5% normal goat serum in phosphate-buffered saline, and the cells were mounted onto glass slides and viewed under a confocal laser scanning microscopy FluoViewTM FV1000 (Olympus American, Melville, NY). Differential interference contrast images were taken of most cells.

For three-dimensional reconstructions, 20 serial z-sections were taken at 0.5-μm increments. The serial sections were then processed by using the software program Volocity (Volocity2 Pro Image3, LLC).

Yeast Two-hybrid Screening—The cDNA encoding the N-terminal SEC14 domain of PTPMEG2 (amino acids 1–326) was subcloned into the pGilda vector to generate in-frame fusion proteins with the LexA DNA-binding domain and used as bait. Library screening by the yeast two-hybrid method was performed according to the manual of Origen Technologies (Rockville, MD). Briefly, the yeast EGY48 strain of Saccharomyces cerevisiae (MAT, trp1, ura3, his, leu2::plexApo6-leu2) was first transformed with the bait plasmid and the lacZ reporter plasmid pSH18-34 and then with a human Jurkat T cell pG4-5 cDNA library. A total of 6 million transformants were assayed for growth on leucine-deficient SD plates. Positive colonies were then retested for growth and for β-galactosidase activity on the leucine-deficient SD plates. The cDNA inserts of isolated library plasmids from these LEU+/lacZ+ clones were amplified by PCR and sequenced. The obtained sequence tags were subjected to BLAST searches to identify the genes.

RESULTS

Subcellular Location of PTPMEG2 Deletion Mutants—Expression of PTPMEG2 in Jurkat T cells, RBL mast cells, or COS cells results in the accumulation of nearly all the epitope-tagged protein on the surface of large vesicles, which represent fused and enlarged secretory vesicles (6, 7, 9, 11). Such vesicles also formed when a fusion protein of PTPMEG2 and EGFP (Fig. 1a) was expressed in RBL mast cells (Fig. 1b), Jurkat T cells, or HeLa cells (not shown). They were readily visualized in live cells as well and remained stable for many hours in untreated cells. Similar results were obtained with both N- and C-terminal fusions of EGFP, except that the former gave rise to somewhat smaller vesicles. In contrast, a fusion protein of PTPMEG2 lacking the first 322 amino acid residues with EGFP (ΔN-PTPMEG2-EGFP) was diffusely cytoplasmic (Fig. 1c). Thus it appears that amino acid residues 1–322, which comprise the SEC14 domain and the linker region (residues 251–322), contain the necessary motif(s) for vesicle targeting.

In a reciprocal approach, HA-tagged constructs of the N terminus of PTPMEG2 with increasing portions of the linker region were constructed, expressed in RBL cells (Fig. 1d), fixed, and stained with Alexa488-conjugated anti-HA antibody. Right panel, Nomarski phase contrast image. e, similar staining of RBL cells expressing the C-terminally truncated HA-PTPMEG2-(1–301). f, similar staining of RBL cells with HA-PTPMEG2-(1–291). g, similar staining of RBL cells with HA-PTPMEG2-(1–261).

FIGURE 1. Targeting of PTPMEG2 to secretory vesicles by its N-terminal SEC14 domain. a, domain structure of PTPMEG2 and the constructs used in this study. b, left panels, confocal microscopy of RBL mast cells expressing a fusion protein of PTPMEG2 with EGFP attached to its C terminus (PTPMEG2-GFP). Middle panels, Nomarski phase contrast images. Right panels, a merge of the left and middle panels. c, similar experiments with ΔN-PTPMEG2-GFP, which lacks the first 250 amino acids of PTPMEG2. d, confocal images of RBL cells transfected with N-terminally HA-tagged PTPMEG2 (HA-PTPMEG2), fixed, and stained with Alexa488-conjugated anti-HA antibody. Right panel, Nomarski phase contrast image. e, similar staining of RBL cells expressing the C-terminally truncated HA-PTPMEG2-(1–301). f, similar staining of RBL cells with HA-PTPMEG2-(1–291). g, similar staining of RBL cells with HA-PTPMEG2-(1–261).
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Identification of Proteins that interact with the N terminus of PTPMEG2.

**a.** Growth and β-galactosidase (β-gal) activity of positive clones from a yeast two-hybrid screen expressing the indicated proteins. b, top panel, anti-HA epitope immunoblot of anti-FLAG immunoprecipitates from 293T cells transfected with HA-PTPMEG2-(1–326) plus empty vector (lane 1) or FLAG-tagged putative interacting proteins as indicated. Middle panel, anti-HA immunoblot of a lysate sample from the same transfecants. Bottom panel, anti-FLAG immunoblot of a lysate sample from the same transfecants.

FIGURE 2. Identification of proteins that interact with the N terminus of PTPMEG2. a, growth and β-galactosidase (β-gal) activity of positive clones from a yeast two-hybrid screen expressing the indicated proteins. b, top panel, anti-HA epitope immunoblot of anti-FLAG immunoprecipitates from 293T cells transfected with HA-PTPMEG2-(1–326) plus empty vector (lane 1) or FLAG-tagged putative interacting proteins as indicated. Middle panel, anti-HA immunoblot of a lysate sample from the same transfecants. Bottom panel, anti-FLAG immunoblot of a lysate sample from the same transfecants.

dues 1–261, 1–291, 1–301, and 1–326 were vesicular (Fig. 1, e–g). Their intracellular pattern was very similar to that of the catalytically inactive full-length PTPMEG2-C515S (6, 7). Very similar results were obtained using EGFP fusion proteins of the same PTPMEG2 constructs (not shown). We concluded that amino acids 1–261 contain sufficient vesicle targeting information. This region coincides with the SEC14 domain.

Identification of Proteins Interacting with the MEG2 N Terminus—To gain some insight into the mechanism by which the SEC14 domain of PTPMEG2 targets to the secretory vesicle membrane, we performed a yeast two-hybrid screen using the SEC14 domain of PTPMEG2 to target the secretory vesicle membrane. The SEC14 domain plus linker as bait. Amino acid residues 1–326 of PTPMEG2 were cloned into the “bait” vector pGilda, which was transfected into yeast together with a Jurkat cDNA library in the “prey” vector pJG4-5. 43 positive clones, retained after two rounds of specificity testing, were subsequently sequenced and found to encode eight different proteins, all of which are known to be at least partly localized to post-Golgi vesicle membranes, including transport vesicles, secretory vesicles, endoplasmic reticulum, and mitochondria. These proteins were the mannose 6-phosphate receptor-associated protein TIP47 (gi 20127486), Arfaptin2 (gi 6912602), vesicle-associated transporter 1 (VAT1; gi 18379349), the GTPase Rab28 (gi 4758994), adenovirus E1B-19K/Bcl2-interacting protein 3 (BNIP3; gi 7669481), and FK506-binding protein 8 (FKBP8; gi 20127486) (Fig. 2a), as well as Arf-like protein 6-interacting protein (ARL6IP; Gi 24308007) and the C terminus of the 48-kDa isoform of TC-PTP (not shown). These eight proteins were taken as candidates for PTPMEG2 interactors potentially involved in targeting of PTPMEG2 to secretory vesicles or possibly to other intracellular membranes.

Co-immunoprecipitation of Vesicle Traffic Proteins with PTPMEG2—To determine whether the potential interactors physically associate with PTPMEG2 strongly enough to withstand detergent lysis and immunoprecipitation, we cloned seven of the potential interactors into the pEF5F expression plasmid, which we constructed from pEF5HA by replacing the 5′ HA epitope tag-encoding sequence with a sequence encoding a triple FLAG tag. All seven proteins expressed well in transfected Jurkat cells (Fig. 2a, bottom row) or 293T cells (not shown), and they all co-immunoprecipitated PTPMEG2-(1–326), albeit to various extents (Fig. 2b, top row). Arfaptin2 and FKBP8 co-immunoprecipitated the highest amounts of PTPMEG2, whereas TIP47 immunoprecipitated much less and VAT-1 not at all. The reciprocal experiment, namely co-immuno-
decrease the size of the PTPMEG2-containing fused vesicle and to increase the amount of PTPMEG2 on small granules. BNIP3 showed a pattern similar to VAT1 (not shown), whereas Rab28 staining resembled FKBP8 staining (not shown).

In Jurkat T cells, most of the interacting proteins showed very similar staining patterns as in RBL cells, with two exceptions: TIP47 was expressed at lower levels and accumulated in a set of small vesicles in the close vicinity of the large PTPMEG2-con-

FIGURE 3. Co-localization of PTPMEG2 with putative interacting proteins in RBL mast cells. a, confocal microscopy of RBL mast cells transfected with empty pEF5F and HA-PTPMEG2 and stained with rabbit anti-FLAG antibody plus Qdot605-conjugated anti-rabbit Ig (red; left panel) and Alexa488-conjugated anti-HA (green; middle panel). The right panel is a Nomarski phase contrast image of the same cell. b, same staining of cells transfected with FLAG-tagged TIP47 and HA-PTPMEG2. The right panel is a merge of the first two panels. c, same staining of cells transfected with FLAG-tagged Arfaptin2 and HA-PTPMEG2. d, same staining of cells transfected with FLAG-tagged VAT1 and HA-PTPMEG2. e, same staining of cells transfected with FLAG-tagged ARL6IP and HA-PTPMEG2. f, same staining of cells transfected with FLAG-tagged FKBP8 and HA-PTPMEG2.

taining vesicle, which also was somewhat larger in these cells when TIP47 was co-expressed (Fig. 4, b–d). Interestingly, some of the TIP47-positive vesicles touched the PTPMEG2-positive vesicle, and there appeared to be vesicles with both proteins (Fig. 4e). This may indicate a role for TIP47 in the transport of PTPMEG2 from the Golgi/trans-Golgi network to the large fused secretory vesicle. Also VAT1 had a somewhat different subcellular distribution in Jurkat cells (Fig. 4f) compared with RBL cells; more VAT1 was close to the plasma membrane, and a small portion of VAT1 co-localized with PTPMEG2 on the fused vesicle.

Endogenous TIP47 and FKBP8 Co-localize with Endogenous PTPMEG2—To determine whether some of the interacting proteins that partially co-localize with overexpressed PTPMEG2 also interact with endogenous PTPMEG2 under normal conditions, we stained RBL cells with a monoclonal antibody against PTPMEG2 plus a quantum dot 605-conjugated anti-mouse Ig and rabbit antisera against FKBP8 plus Qdot655-conjugated anti-rabbit Ig or with guinea pig anti-TIP47 plus biotin-labeled anti-guinea pig Ig plus streptavidin-conjugated Qdot655. These experiments (Fig. 5) showed that the endogenous proteins indeed co-localized in a granular manner, presumably on a set of cytoplasmic vesicles. The co-localization with TIP47 was of a higher degree, whereas the co-localization with FKBP8 occurred only in a smaller number of pixels. Nevertheless, these experiments demonstrated that endogenous TIP47 and FKBP8 co-localize with endogenous PTPMEG2, suggesting that they can indeed interact under normal circumstances.

Elimination of TIP47 by RNAi Prevents Proper PTPMEG2 Localization—The positive effect of TIP47 on the ability of PTPMEG2 to enlarge the vesicle on which it is located prompted us to ask whether endogenous TIP47 is required for PTPMEG2 targeting to secretory vesicle membranes. When a stable TIP47 RNAi-transfected line of HeLa cells3 was

3 A. Bulankina and S. Höning, unpublished observations.
transfected with PTPMEG2, we found that the phosphatase was unable to cause secretory vesicle fusion, whereas this response was as usual in control HeLa cells (Fig. 6). In the TIP47-deficient cells, PTPMEG2 remained distributed throughout the cytosol in a finely granular manner, with some enrichment only in the Golgi region (Fig. 6). This pattern was seen in 71% of all PTPMEG2-positive cells. In 27% of the cells, there was some accumulation of PTPMEG2 in smaller vesicle, and only 2% of cells had the majority of PTPMEG2 in one or a few large fused vesicles. In comparison, 98% of PTPMEG2-transfected control HeLa cells had all of their PTPMEG2 in such fused vesicles, and only 2% had some PTPMEG2 in more numerous smaller granules. Furthermore, reintroduction of low levels of TIP47 by transfection restored the ability of PTPMEG2 to induce secretory vesicle fusion. This effect was dose-dependent. We conclude that TIP47 is required for the function and/or targeting of PTPMEG2.

DISCUSSION

To gain more mechanistic insights into the physiological function of PTPMEG2 in secretory vesicle biology, we have determined here that the SEC14 domain of PTPMEG2 is responsible for targeting the protein to the secretory vesicle membrane and have identified several proteins that interact with this domain, and possibly with the linker region as well, of PTPMEG2. These proteins may have many possible different types of functions; they may include binding partners that escort newly synthesized PTPMEG2 from the Golgi and trans-Golgi network to the transport vesicles that fuse with immature secretory vesicles. Our data support this kind of function for TIP47. The interactors may also include proteins that retain PTPMEG2 on secretory vesicles or that retrieve PTPMEG2 from the plasma membrane upon exocytosis to recycle it to the secretory pathway or traffic it to lysosomes. Finally, interacting proteins may regulate PTPMEG2 activity positively or negatively, facilitate substrate access, or be downstream effectors or substrates themselves. The different interactors we have found probably represent several of these alternative functions.
Association of PTPMEG2 with Vesicle-trafficking Proteins

TIP47 was first cloned as a protein that binds the cytosolic tail of mannose 6-phosphate receptors and is reportedly required for transport of these receptors from late endosomes to the Golgi (15). TIP47 binding was found to depend on a phenylalanine-containing diaromatic motif and a loop with the sequence PATAPRPD (residues 3–10). The role of TIP47 in endocytosis clearly is not the only function of this molecule. TIP47 also binds to lipid droplets through its PATAPRPD (residues 3–10) and with guinea pig anti-TIP47, biotin-anti-guinea pig Ig plus Qdot655-conjugated streptavidin (red), and mouse monoclonal anti-PTPMEG2 and the same secondary antibody as in a and b.

Our finding that Rab28 (rather than Rab9) interacts with PTPMEG2 may also be related to TIP47 recruitment for anterograde transport of PTPMEG2 to the immature secretory vesicle compartment. Fig. 4e illustrates a snapshot of this process. This model would explain the positive effect of TIP47 on PTPMEG2 function; increased levels of TIP47 would facilitate transport of PTPMEG2 and secretory vesicle material to the secretory vesicle compartment, resulting in its further enlarge-
shaped domain that binds to lipid bilayers rich in acidic phospholipids and drives their curvature into tubules/vesicles (25, 26). These interactions offer little explanation for our observations but provide many potential leads to follow. ARL6IP, which interacts with the ARF-like protein ARL6 (27) and the Rab28 GTPase, may also have roles in anterograde Golgi to post-Golgi vesicle traffic or retrograde traffic, which is important for immature secretory vesicle concentration. The physiological function of the FK506-binding protein, FKBP8, is poorly understood, but it is known to play an important role in embryonic development (28), particularly in the brain where it counteracts the sonic hedgehog signaling pathway (28). Thus, its relatively high degree of co-immunoprecipitation and co-localization with PTPMEG2 and its effects on PTPMEG2 function may indicate that the functions of the two proteins are related. This again may be relevant for the many developmental defects seen in the PTPMEG2−/− mouse (13). It has been proposed that FKBP8 is located mostly on mitochondria (28), but our experiments do not support this notion (data not shown). Instead, a portion of FKBP8 was present on the PTPMEG2-positive enlarged vesicle, and endogenous FKBP8 co-localized with endogenous PTPMEG2.

BNIP3, which contains a BH3 domain and a C-terminal transmembrane sequence, can promote apoptosis by heterodimerization with Bcl-2-related BH3 proteins (29). The connection to PTPMEG2, if any, is not apparent, particularly because BNIP3 has been shown to reside on mitochondria. Thus, the interaction of the SEC14 domain of PTPMEG2 with both BNIP3 and FKBP8 in the yeast may have been an artifact of the yeast system, or, alternatively, these two proteins may reside to some extent on other internal membranes. Although we have not detected any PTPMEG2 on mitochondria, we cannot categorically exclude the possibility that some PTPMEG2 associates transiently or permanently with this organelle.

The VAT1 protein is an integral membrane protein found on synaptic vesicles in neuronal cells, but it is broadly expressed (30). It belongs to the quinone oxidoreductase subfamily of zinc-containing alcohol dehydrogenase proteins and has ATPase activity. Other than a probable role in vesicle traffic, its relationship with PTPMEG2, if any, remains entirely speculative. Finally, our yeast two-hybrid screen detected the C terminus of the 48-kDa isoform of TC-PTP as a possible PTPMEG2 interactor. Because this isoform of TC-PTP is associated largely with the endoplasmic reticulum due to a targeting motif present in the C terminus of this isoform, we considered the interaction in yeast to be, most likely, an artifact.

It is intriguing to note that most of the putative PTPMEG2-interacting proteins identified in this study are proteins associated with vesicle traffic, small GTPases, and lipid interaction. Although lipid binding by the SEC14 domain is not required for membrane association (11), it seems probable that binding of highly acidic phospholipids, like phosphoinositides (8, 11, 12) or phosphatidylycerine (31), promotes the interaction of the SEC14 domain with other proteins such as the BAR domain of Arfaptin2 or the PAT domain of TIP47. This would provide a plausible explanation for the requirement of phosphoinositides for the function of PTPMEG2 in cells (8, 11). Future work will be aimed at solving this question. The role of small GTPases of the Ras superfamily, such as Rab28 and possibly ARL6, in PTPMEG2 trafficking, targeting, and function also warrants further investigation.

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