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PTPs emerge as PIPs: protein tyrosine phosphatases with lipid-phosphatase activities in human disease

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Abstract

Protein tyrosine phosphatases (PTPs) constitute a family of key homeostatic regulators, with wide implications on physiology and disease. Recent findings have unveiled that the biological activity of PTPs goes beyond the dephosphorylation of phospho-proteins to shut down protein tyrosine kinase-driven signaling cascades. Substrates dephosphorylated by clinically relevant PTPs extend to phospholipids and phosphorylated carbohydrates as well. In addition, non-catalytic functions are also used by PTPs to regulate essential cellular functions. Consequently, PTPs have emerged as novel potential therapeutic targets for human diseases, including cancer predispositions, myopathies, and neuropathies. In this review, we highlight recent advances on the multifaceted role of lipid-phosphatase PTPs in human pathology, with an emphasis on hereditary diseases. The involved PTP regulatory networks and PTP modulatory strategies with potential therapeutic application are discussed.

Introduction

Based on sequence homology some hundred genes have collectively been grouped as 'Class I type' protein tyrosine phosphatase (PTP) genes (1, 2). The proteins they encode all share at least one so-called PTP domain, a ~150-280 amino acid enzymatic core module that carries several highly conserved and characteristic sequence elements among which is the 'CX_5R' signature motif that contains the essential catalytic site cysteine. By means of their canonical phosphotyrosine phosphatase activity, classical (i.e. phosphotyrosine-specific) PTPs are major regulators of protein phosphotyrosine content, which can have both negative and positive signaling outcomes on downstream essential cell functions. As a consequence, classical PTPs may present as tumor suppressor proteins (e.g. *PTPRJ*/DEP1) but also as oncoproteins (e.g. *PTPN1*/PTP1B). Multiple PTP domains, however, appear enzymatically unreactive towards phosphotyrosine-containing proteins or artificial substrates. This especially holds true for the membrane-distal PTP domain in transmembranous, receptor-type PTPs (RPTPs) that usually harbor two tandem cytoplasmic PTP domains. This has led to the suggestion that such inactive PTP domains may serve in fine-tuning the activity of the membrane-proximal PTP domain or help in recruiting appropriate phosphotyrosine-containing target proteins, akin to the phosphotyrosine-binding SH2 and PTB protein domains. Importantly, many PTPs direct their activities to confined subcellular niches by virtue of additional protein-, lipid-, or carbohydrate-interaction domains, and this can be quite relevant in human disease (e.g. *EPM21*/Laforin).

It is now well established that the substrate specificity of many active PTPs goes beyond phosphotyrosine-containing substrates. The PTP subclass of dual-specificity phosphatases (DUSPs), for example, is capable of dephosphorylating phosphoserine- or phosphothreonine-containing proteins as well, but a group of small atypical DUSPs is still orphan in terms of potential physiologic substrates. In addition, several PTPs display dephosphorylation capacity towards non-proteinaceous biomolecules like RNA, phosphorylated glycogen and phospholipids. Here we summarize the latest advances on the involvement in human disease of classical PTPs and DUSPs that bear phosphatase activity towards lipidic substrates, including phosphoinositides (Table 1, Fig. 1, Fig. 2). Phosphoinositides are potent regulators of vesicle transport, chemotaxis, actin dynamics, cell proliferation and survival. Hence the enzymes that generate and degrade phosphoinositides require tight control in a spatio-

temporal manner. For comprehensive reviews on the physiology and pathology associated to PTPs, and on lipid phosphatases that do not belong to the PTP gene family, we refer to recent publications (3-7).

Hear the news about PTPRQ and phogrin

Two classical PTPs dephosphorylate phospohinositides, namely the RPTPs PTPRQ and phogrin (8, 9). Originally, **PTPRQ** was identified in a glomerulonephritis rat model due to its upregulation in mesangial cells (10), but several other cell types, including glomerular epithelial (podocyte) cells, also express PTPRQ. In human tissues, PTPRQ mRNA is abundant in kidney, lung, and testis (11). Alternative promoter use and differential splicing result in multiple protein variants (Fig. 3A), including isoforms that differ in the number of fibronectin type III-like (FNIII) repeats in the long extracellular segment (12), a truncated molecule that misses the catalytic domain (10), and a small cytoplasmic protein that essentially consists of the catalytic domain (11). The single PTPRQ phosphatase domain includes the conserved features of PTPs, but in the WPD-loop the highly conserved aspartic acid is replaced by glutamic acid, the larger carboxylic residue (Fig. 2). As a result, PTPRQ displays very low activity against phosphotyrosine-containing substrates but, like the tumor suppressor PTEN, is quite potent as a phosphoinositide phosphatase (PIP). Substituting the Glu by Asp reverts PTPRQ substrate specificity back into that of a classical PTP and abolishes PIP activity (9). The PTPRQ catalytic domain displays a broad phosphoinositide substrate range *in vitro*, hydrolyzing phosphates from the D3 and D5 positions in the inositol ring. In cells, the PTPRQ PTP domain suppresses PI(3,4,5)P3-dependent signaling, hence diminishes Akt/PKB phosphorylation, growth rates and survival of mammalian glioma cells (9), and likewise prevents adipocyte differentiation of mesenchymal stem cells (13). *PTPRQ* gene mutations are relatively frequent in large intestine-tumor samples (~5%; COSMIC database) but it is as yet unclear whether this is an epiphenomenon due to genomic instability in these tumors or is reflecting a putative PTEN-like tissue-specific tumor suppressor function for PTPRQ.

Ten years ago it was found that PTPRQ in fact encodes the long sought-after 275 kDa hair-cell antigen (HCA), a component of hair-bundle shaft connectors in the inner-ear that is also expressed in kidney glomeruli, and that *Ptprq* knockout mice suffer from progressive loss of

basal-coil cochlear hair cells and ultimately deafness (14). The ordered actin filament protrusions on the hair cell's apical membrane, the stereocilia (Fig. 3B), initially are held together in these mice even though shaft connectors appear absent. Postnatally, however, cochlear hair-bundles gradually deteriorate, transducer currents decline and cells die (14). Detailed immuno-microscopical analyses disclosed that PTPRQ protein is mainly present in a cell surface coat at the stereocilia base, suggesting that it either controls actin filament minus end stability or serves as cargo in transport directed toward the stereocilia base (15). The latter is supported by the finding that PTPRQ immunostaining diffuses out over the entire stereocilia in myosin VI-deficient Snell's waltzer mice and suggests that a PTPRQ-myosin VI complex is critical for the dynamic control of the stereocilia base structure and thus the overall stereocilia bundle (15). Interestingly, in a human ciliopathy disease protein interaction network it is myosin isoform VIIA that, like PTPRQ, associated with deafness phenotypes (16).

The *Ptprq* knockout data were subsequently corroborated by reports on three different inactivating mutations in human *PTPRQ* that cause autosomal recessive nonsyndromic hearing loss-84 (DFNB84) (12, 17). Intriguingly, although *PTPRQ* is expressed in other cilia-containing tissues only deafness and vestibular dysfunction were encountered in the patients. This may indicate some functional redundancy in these other tissues. Two of the mutations introduce a stop codon in FNIII domains (12) (17) and thus severely truncate PTPRQ (Fig. 3A). The third mutation results in the substitution of a bulky, charged Arg residue by the much smaller, uncharged Gly within FNIII domain 3 (12). This latter mutation suggests that the wild-type protein's extracellular part is essential for PTPRQ's function in hair cells. Its involvement in spatially arranging stereociliar distance and connectivity perhaps parallels the way in which RPTPμ, another transmembrane PTP, participates in the orchestration of intermembrane distance by virtue of its rather rigid stack of FNIII domains (18).

Very recently, the Kremer group detected within a consanguineous deafness family a homozygous nonsense mutation in *PTPRQ* that resides within a catalytic core-encoding exon (Hannie Kremer, personal communication). The resulting mutant would lack some hundred C-terminal residues, including the active site cysteine, and thus will be inactive (Fig. 3A).

Localization studies on this mutant PTPRQ are required to ascertain whether defects in PTPRQ PIP activity are sufficient to pathologically affect the hair cell's acoustic system.

Such a PIP scenario for PTPRQ received unexpected support from a recent case report on a patient that suffers from bilateral Duane retraction syndrome (DRS) and hearing impairment (19). The main feature of DRS is the inability of the eye to move outwards due to aberrant cranial nerve innervation of eye muscles, and often unilateral or bilateral deafness is part of the syndrome. Although the patient's phenotype suggested that homozygous *HOXA1* mutations may be the cause, instead a small *de novo* duplication on chromosome 7 - essentially encompassing gene ***PTPRN2*** – was detected (19). *PTPRN2* encodes **phogrin**, a classical RPTP that is expressed in nervous and endocrine cells. Like its close homolog IA-2, phogrin is a major type 1 diabetes mellitus autoantigen that functions in secretory vesicle release (Fig. 4A) (20). Intriguingly, like PTPRQ also phogrin's catalytic activity is not towards phosphotyrosine-containing proteins but rather towards PI(4,5)P2. Furthermore, proper subcellular localization appeared critical for phogrin's PIP activity (8). Thus, *PTPRN2* expression levels may control developmental steps that are crucial for eye and ear functionality, by impacting on the modulation of phospholipid levels. Undoubtedly, these sensory organs will now be put to the test in the available mouse model and results may add to the portfolio of aberrant PIP activity by PTPs in disease etiology. It is important to note that the gene for microRNA miR-153 resides within *PTPRN2* (21) and thus may contribute to the phenotype resulting from the chromosome 7 microduplication. Mutations in *PTPRN2*, as well as in the phosphatase-inactive paralog *PTPRN* that encodes IA-2, have been detected with a frequency around 5% in several human tumors (COSMIC database). Positive modulation of PTPRQ and PTPRN2 PIP activity may therefore be beneficial in the treatment of human hearing diseases and cancers.

PTENs and MTMs: cancer, myopathies, neuropathies, and more

PTEN (phosphatase and tensin homologue deleted in chromosome ten) and MTMs (myotubularin and myotubularin-related proteins) share substrate specificity towards the D3 position of the inositol ring from distinct phosphoinositides, and both have wide implications in human disease. The canonical function of the tumor suppressor PTEN is the

dephosphorylation of PI(3,4,5)P3 to generate PI(4,5)P2, thereby counteracting the pro-oncogenic function of phosphoinositide-3-kinase (PI3K). MTMs, on the other hand, dephosphorylate PI(3,5)P2 and PI(3)P to generate PI(5)P and PI, respectively. These activities make MTMs major regulators of endosomal vesicular trafficking and dynamics, explaining their link with hereditary neuromuscular diseases (22). Remarkably, both PTEN and MTMs contain additional lipid- and protein-interaction motifs (Fig. 1), underlining the importance of molecular interactions to direct their subcellular functions. PTEN involvement in clinical oncology goes beyond its tumor suppressor activity, and extends to a positive role as sensitizer to different chemotherapies and targeted anti-cancer therapies (23-25). In addition, PTEN deficiency may also be beneficial, in anti-cancer therapies that aim at DNA-repair or that trigger senescence (26, 27). An important non-canonical function of PTEN involves its PTP activity towards a diversity of protein substrates that regulate cancer cell migration or neuronal plasticity (28, 29) (Table 1). Studies were facilitated by the identification of mutants that lack only one of PTEN's activities (30, 31), such as the G129E (destroys PIP but not PTP activity) or Y138L (impairs PTP but not PIP activity) mutations. Also, a growing number of protein interaction-dependent and phosphatase-independent functions have been attributed to PTEN. The protein is thought to play important non-enzymatic roles in apoptosis, gene transcription, cell cycle regulation and DNA repair (27, 32).

A diverse spectrum of hamartoma- and malformation-related hereditary syndromes that manifest highly variable phenotypes (PHTS, PTEN Hamartoma Tumor Syndrome) is attributable to germ-line loss-of-function mutations in *PTEN*. Developmental Delay (DD) and Autism Spectrum Disorders (ASD) are also associated with *PTEN* germ-line mutations, although at lower frequency (33) (Table 1). The causative role of *PTEN* mutations in PHTS, DD, and ASD etiology had two important clinical consequences: (a) the value of PTEN genetic screening and protein function analysis as a robust diagnostic criterion for these syndromes and associated cancer risk (34-36); and (b) the requirement to analyze PTEN protein function and assess the *PTEN* genetic make-up in pre-diagnosed patients (37-40).

Several findings highlight the importance of steady-state PTEN protein levels for human disease. First, PTEN activity has deleterious pro-apoptotic effects during vascular injury and myocardial infarction, which could be ameliorated by PTEN acute pharmacological inhibition

(41, 42). Second, high levels of PTEN in transgenic mice not only protect against oncogenic transformation, but also result in decreased fat accumulation and increased energy expenditure and life span (43, 44). Conversely, PTEN haploinsufficiency in mice causes enhanced insulin sensitivity and glucose tolerance, which was also observed in PHTS patients and associated with higher obesity risk (45, 46). In this regard, heterozygous loss-of-function mutations in PHTS patients correlate with down-regulated expression of the wild-type allele, and levels of miRNAs targeting PTEN are overexpressed in PHTS patients (35, 47). Moreover, up-regulation of PTEN levels in humans associates with cancer prevention, as well as with protection against allergic inflammation diseases such as asthma (48, 49). In line with this, a complex PTEN expression regulatory network exists that includes different transcription factors and transcriptional regulators, miRNAs, competing endogenous RNAs, and transcribed pseudogenes, as well as stabilizing and destabilizing PTEN-binding proteins (27, 50). PTEN actively shuttles, in a phosphorylation-, ubiquitination-, and sumoylation-dependent manner, between subcellular compartments, including cytosol, internal membranes, and nucleus, which is relevant in disease (27, 51-53). In addition, ubiquitination-related PTEN transfer to recipient cells through exosomes has also been documented (54). Very recently, a long PTEN isoform has been found to be secreted into the extracellular medium, being able to then penetrate into cells by virtue of a HIV-TAT-like cell-penetrating element (55) (Fig. 4A). These findings foster the use of PTEN as a delivering-anti-tumor drug, and open ways to use PTEN levels in serum as a disease marker. Studies are required on the putative relation between PTEN secretion or cell transfer and PTEN-associated pathologies. The notion that PTEN acts as a master regulator of cell physiology and displays a wide plasticity to control pathological processes (56), predicts that not only PTEN mRNA and protein levels need to be determined in human samples, but that inspection of its subcellular location and catalytic properties should be incorporated as well.

TPTE and **TPTE2/TPIP** are two PTEN-related voltage-sensitive phosphatase (VSP)-like proteins, mainly expressed in the testis and presented as different isoforms, including cytosolic and membrane anchored proteins (Fig. 4A) (57-59). TPTE has been described as an inactive phosphatase, although the possibility exists that TPTPE manifests phosphatase activity towards unknown substrates. Changes in *TPTE* gene dosage have been found in patients with Robertsonian Down syndrome, a rare Down syndrome variant displaying the

chromosome 21 Robertsonian translocation (60). Of interest, quite a few tumor-associated mutations have been described for *TPTE* in multiple human epithelial tissues (COSMIC database). Whether this association reflects a causative role in the disease awaits further analysis. TPTE2/TPIP displays phosphatase activity towards the D3 and D5 position of phosphoinositides (59, 61). An alternatively-spliced form of TPTE2/TPIP, lacking most of the catalytic domain but containing an intact C2 domain (TPIP-C2), inhibited cell growth and triggered apoptosis when overexpressed in human cancer cell lines (62, 63). This suggests a putative tumor suppressor role associated with non-catalytic functions of TPTE2/TPIP. It will be relevant to investigate the expression of the TPIP-C2 isoform in tumor samples. Together, these findings warrant for scrutiny of *TPTE* and *TPTE2/TPIP* gene variants and isoforms in human cancer.

The human PTP subfamily of **MTMs** consists of 14 members (MTM1, MTMR1 to MTMR13) that are either catalytically active or inactive PIPs (64). MTMR14, with a substrate specificity that is similar to MTMs, belongs to an evolutionary distinct family (65) (Table 1; Fig. 1). Loss-of-function mutations target several MTM genes in patients with hereditary neuromuscular diseases. Mutations in *MTM1* cause X-linked myotubular myopathy (XLMTM), a severe monogenic disease which manifests with defects in muscle fibre maturation and maintenance. Mutations in *MTMR2* and *MTMR13* are causative of Charcot-Marie-Tooth disease type 4B1 (CMT4B1) and 4B2 (CMT4B2), respectively, severe monogenic demyelinating peripheral neuropathies characterized by muscular weakness and atrophy, and decreased motor nerve conduction velocity in peripheral neurons. Finally, mutations in *MTMR14* are linked to autosomal centronuclear myopathy (ACNM), a disease similar to XLMTM but with milder manifestations and better prognosis (66, 67). Also, a role for MTMR14 in aging sarcopenia has been proposed (68). Although defects in PIP activity in MTMs seem to be important in disease etiology, the pathogenic relevance of non-catalytic MTM functions should also be considered (69). For instance, reconstitution of *Mtm1* knockout mice with catalytically-inactive MTM1 ameliorated the XLMTM-like phenotype without normalizing PI(3)P levels, and some XLMTM-associated MTM1 mutations retain PIP activity (70). Moreover, a duplication in the *MTM1* gene has been found in a XLMTM patient (71). Finally, alterations in the ubiquitin-proteasome and autophagy pathways have been associated with the initiation of XLMTM pathogenesis in *Mtm1* knockout mice (72-74). It is

likely that MTM1 malfunction in its PIP-dependent and -independent roles could determine the onset of XLMTM. As mentioned above, MTMR13 is mutated in CMT4B2. It is, however, enzymatically inactive and rather controls MTMR2 catalytic function and subcellular location through heterotetramerization (75, 76). Both, *Mtmr2* and *Mtmr13* knockout mice display similar CMT4B-like phenotypes, although *Mtmr2* deficiency additionally results in an impairment of spermatogenesis (77-80). Thus, MTMR13-independent physiological roles may exist for MTMR2. In this regard, MTMR2 also binds to the catalytically inactive MTMR5 and MTMR12 family members, and also MTM1 can interact with MTMR12 (81). However, thus far no disease-associated mutations have been found in human *MTMR5* or *MTMR12*, indicating the existence of a complex functional network of catalytic and non-catalytic MTMs governing distinct physiologic activities.

The similar substrate specificities of the catalytically active MTM members and their differential disease involvement form an intriguing aspect on MTM biology. This is important when considering the modulation of MTM activities for therapeutic purposes. Although at the protein level a comparative tissue-expression analysis is lacking, mRNA expression data reveal that some MTMs are expressed ubiquitously whereas others display tissue-restricted expression patterns (82). Importantly, differential tissue-specific expression as well as differences in subcellular localisation of MTMs may impact on physiologic functions. For instance, depletion of *Mtmr2* in Schwann cells, but not in motor neurons, conferred a CMT4B1-like phenotype in mice (83). MTMs are found in the cytosol and are associated with endosomal vesicles and plasma membrane domains (Fig. 4B). Differential regulation of MTM subcellular location may thus confer functional specificity by targeting the enzymes to distinct phosphoinositide pools (81). In the case of MTM1 this would regulate the remodelling and maintenance of the sarcoplasmic reticulum in the skeletal muscle (66). In addition, MTM1, MTMR1, and MTMR2 display different PDZ-binding motifs at their C-termini, which could mediate differential subcellular targeting by binding to specific PDZ domain-containing proteins (84, 85). Interestingly, PTEN also displays a functional type I PDZ-binding motif at its C-terminus (32). Whether these different phosphatases could form part of interconnected PDZ-domain functional networks with relevance in human disease remains to be seen. The possibility exists that targeting non-mutated MTM paralogs to

specific subcellular compartments through interference with their binding to lipid or protein partners, we could help to alleviate pathologies caused by mutations on other MTMs.

Non-canonical functions of MTMs may be related with putative protein phosphatase activities. MTMR4 binds SMAD proteins in early endosomes and the cytosol (Fig. 4B), and expression of catalytically active MTMR4 correlates with SMAD dephosphorylation, which attenuates TGFβ– and BMP-induced signals (86, 87). Although demonstration of direct SMAD dephosphorylation by MTMR4 is lacking, it is tempting to speculate that SMADs represent direct targets for MTMRs' PTP activity, which could impact on cell growth and invasion. In this regard, a physical association between PTEN and SMAD3, which impaired TGFβ-induced gene expression and invasiveness during cell transformation, has been reported (88). Since both TGFβ and BMP down-regulate PTEN expression (89, 90), it would be interesting to analyse the expression of MTMR4 upon TGFβ and BMP cell stimulation. Additional studies have associated MTMs with the positive regulation of Akt/mTOR-pathway-mediated signalling and cell growth/survival responses (67), which suggests that MTMs could be targets for inhibition in apoptotic-resistant tumors. However, negative regulation of Akt by some MTMs has also been reported (91). Finally, the pseudophosphatase SBF1/MTMR5 has been linked to cell-growth regulation in NIH-3T3 fibroblasts, and displayed transforming activity in association with nuclear localization (92). This indicates that alternative, PIP-independent subcellular location-specific MTM functions may exist. Further work is required to unveil the expression and function of MTMs in human cancer.

PRL-3 and PTPMT1: so small, so important

PRL-3 and PTPMT1 are two small DUSPs that display PIP activity, lack regulatory domains but do possess specific subcellular targeting motifs. PRL-3 is targeted to cell membranes through C-terminal prenylation, whereas PTPMT1 is targeted to the mitochondrial matrix by an N-terminal mitochondrial translocase motif (93, 94) (Fig. 4B). **PRL-3** (encoded by gene *PTP4A3*) belongs to the PRL subfamily of DUSPs, which also includes the highly conserved PRL-1 and PRL-2 paralogs (Fig. 2). PRLs are overexpressed in different human tumors and their catalytic activity is associated with oncogenicity and metastasis, making them potential targets for

human cancer therapies (95). Physiologic substrates of PRLs, however, remain unknown. Thus far some proteinaceous substrates that relate to the cytoskeleton and cell adhesion have been proposed for PRL-3 (Table 1). In addition, PRL-3 displayed activity *in vitro* towards PI(4,5)P2, likely targeting the D5 position, which correlates with enhanced cell migration (96). PI(4,5)P2 is important in mammalian cells for regulation of cytoskeletal-, focal adhesion-, and cell migration-related processes (97). Nevertheless, *in vivo* evidence of PRL-3 PIP activity is currently lacking as changes in phosphoinositide levels in PRL-3 knockout mice have not been documented (98). Germ-line loss-of-function mutations in the inositol/PIP 5-phosphatase *OCRL/INPP5F* and *INPP5E* genes are causative of the hereditary cerebrorenal human OCRL and Joubert syndromes, respectively, two pathologies linked to cilia defects (99) and thus reminiscent of the PTPRQ -DFNB84 connection discussed above. However, no disease-associated *PTP4A3* germ-line mutations have been reported. INPP5E is not related with PTPs, but similar to PRL-3 it also contains a C-terminal prenylation motif, and its expression is also altered in several human cancers (100). Further studies are thus required to link PRL-3 PIP activity with cancer and other human diseases.

PTPMT1 belongs to the heterogeneous group of small atypical DUSPs. Initially identified as a PTEN-like PTP, PTPMT1 is a unique PTP both in terms of subcellular localization and substrate specificity (Table 1, Fig. 4B). PTPMT1 is anchored in the inner mitochondrial membrane with its catalytic domain facing the mitochondrial matrix, where it acts as a phosphatidylglycerophosphate phosphatase in the cardiolipin biosynthetic pathway (93, 101). However, a linkage of PTPMT1 with cardiolipin metabolism-related diseases has not been documented. PTPMT1 is required for mitochondrial integrity and oxidative respiration, and its ablation increases insulin secretion in pancreatic β cells, as well as apoptosis in cancer cell lines (93, 102). This suggests an active role for PTPMT1 in type 2 diabetes and cancer. No significant number of mutations in tumors is reported for *PTPMT1* (COSMIC database). *Ptpmt1* knockout mice show early embryonic lethality, and ablation of *Ptpmt1* in embryonic stem cells decreased differentiation and proliferation, but not survival, of these cells (101, 103). Thus, the role of PTPMT1 in the control of cell growth and survival seems to be cell-type specific. PTPMT1 also displays *in vitro* activity towards phosphoinositides (103, 104). Although the physiologic relevance of PTPMT1 PIP activity is still uncertain, it may account for PTPMT1 cardiolipin synthesis-independent functions.

Conclusions

In recent years, the substrate specificity of PTP family enzymes has expanded to include a diversity of non-proteinaceous substrates. From this, both non-canonical and even non-catalytic functions with importance in physiology and disease have emerged. In fact, the PIP activity displayed by the PTPs discussed here currently represents the most relevant link of this protein superfamily to human disease states, including a variety of hereditary syndromes. Remarkably, even within the 'classical PTP subfamily' (105) a direct link to a congenital disease is provided by PIPs: PTPRQ and phogrin. Perhaps more of the classical PTPs will turn out to display non-canonical substrate specificities. Although DUSP enzymes such as PRLs are widely involved in cancer etiology, their physiologic substrates remain poorly defined. It is tempting, though, to suspect phosphoinositides as being high up in their substrate list. The ascription of phosphatidylglycerophosphate phosphatase activity to the mitochondrial PTPMT1 also gives a new perspective on how PTPs can be involved in human disease, including metabolic diseases and their linkages with cancer. Finally, elucidation of the physiologic substrates of PTPs of unclear biological activity, such as substrate-orphan atypical DUSPs, may yet grant to these enzymes a place as actors in the course of human pathologies of unknown molecular origin.

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Figure legends

Figure 1 – Structural representation of lipid phosphatase activity-displaying protein tyrosine phosphatases and their close paralogs, and of their phospholipid substrates.

A - Domain structures for the various PIP-type PTPs and some subfamily members that are discussed in the text. Structures of the major isoforms and protein names are used, and drawings are to scale (size indication is included in the legend box). In the case of PTEN and TPIP an additional isoform, that is of relevance for the discussion accompanying figure 4, is shown between brackets. Domain acronyms: PTP/PIP, lipid phosphatase activity-displaying protein tyrosine phosphatase; FNIII, fibronectin type III; TM, transmembrane; inactive, PTP/PIP for which no activity has been demonstrated (yet); C2, lipid-binding C2 region; PBM, PDZ-domain binding motif; PH-GRAM, Pleckstrin Homology - Glucosyltransferases Rab-like GTPase activators and Myotubularins; CC, coiled-coil; FYVE, phosphatidylinositol-binding Zn finger as found in Fab 1, YOTB, Vac 1 and EEA1; DENN, differentially expressed in neoplastic versus normal cells; PH, pleckstrin homology; CBM, carbohydrate-binding module; Prenylation, C-terminal consensus sequence for prenylation; Mito TS, N-terminal mitochondrial targeting signal; N-PP, HIV-TAT-like N-terminal Penetrating Peptide.

B – Rough chemical structure of phosphoinositides (left) and phosphatidylglycerophosphate (right) that serve as substrates for PIP-type PTPs and PTPMT1, respectively.

Figure 2 - Amino acid alignment of WPD- and P-catalytic-loops from PTPs with lipid phosphatase activity or related proteins. Sequences are grouped by PTP subfamily and similarity, with conserved residues in gray. Human sequences are shown, and amino acid numbering is in brackets. The catalytic Cys and Arg in the P-loop, and the Asp conserved in the WPD-loop, are bolded and highlighted in yellow. Note that the Asp conserved in the WPD-loop is absent in PTPRQ and PTPRN/IA-2, as well as in MTMs. The Asp conserved in the P-loop of MTMs is highlighted in cyan, and might serve as the proton donor/acceptor catalytic residue. MTMR14 has the conserved C(X)5R catalytic motif and displays similar substrate specificity to active MTMs, but it belongs to a different gene family. The relative alignment of the WPD-loops is based on amino acid sequence- or three-dimensional-

alignments. MTMs, with the exception of MTMR14, were aligned with MTMR2 using BLAST, and the three-dimensional alignment of MTMR2 with PTEN (Asn355 from MTMR2 aligns with Asp92 from PTEN, double headed arrow; (106)) was taken as a reference to align MTMs with the rest of proteins. The alignment of the MTMR14 WPD-loop is arbitrary, using as a reference the amino acid distance between the P- and WPD-loops from MTMR2. The left columns indicate the amino acid length of the protein (longer isoforms) and the PDB IDs for three-dimensional structures (RPTP catalytic domains, or DUSP or MTM proteins, from human proteins, with the exception of PTPMT1, which is from mouse protein). * indicates inactive enzymes. Note that inactive MTMs lack the catalytic residues at the P-loop; on the other hand, PTPRN and TPTE possess these residues, making possible the existence of enzyme activity towards unknown substrates.

Figure 3 – Loss of PTPRQ function leads to stereocilia defects and hair cell loss in autosomal recessive nonsyndromic hearing loss-84.

A - Schematic depiction of seven PTPRQ isoforms that have been reported. FNIII, TM and PTP domains are indicated. The canonical isoform with protein data base accession number Q9UMZ3 is depicted on top. Below, four different mutants that are causatively involved in inherited deafness in man are shown.

B - Drawing of the organization of stereocilia and hair bundle cross-links in the cochlear hair cells. Examples of deafness-associated proteins that make up these structures are usherin and VLGR1 (ankle), PTPRQ (shaft), stereocilin (top) and cadherin 23 (tip link). For a recent review on the vertebrate sensory hair cell we refer to Hackney & Furness (107).

Figure 4 – Subcellular localization and substrate accessibility of lipid-phosphatase PTPs.

A - PTENs and PTPRN2. PTEN distributes actively between cytosol, plasma membrane and nucleus, which is controlled by protein and lipid interactions and dictated by conformational changes driven by PTEN postranslational modifications, including phosphorylation, sumoylation, and ubiquitination. In the nucleus, PTEN exerts gene-transcription and DNA-repair functions, mostly independent of its PIP activity. PTEN is also exported to recipient

cells by the exosome pathway. In addition, a PTEN isoform with an extra N-terminal region (PTEN-long) is secreted to the medium through the secretory pathway, and is transferred into recipient cells using an HIV-TAT-like internalizing sequence. Thus, PTEN is a versatile master regulator of cell physiology acting at multiple cell locations and displaying a variety of biological activities. TPTE and TPIP exist as several transmembranal and non-transmembranal isoforms, some of which are depicted. A detail of the topology transmembrane regions from TPTE and TPIP long isoforms is included. The localization of the longer TPTE isoform (TPTE γ) to the plasma membrane is uncertain. Note that the TPIP-C2 isoforms lacks the PTP domain. PTPRN2 and PTPRN (not depicted) possess a single TM region and are found at dense core vesicles.

B – MTMs, PRL-3, and PTPMT1. Most of MTMs are associated to intracellular membranes by means of lipid-binding domains, and distribute amongst components of the endocytic vesicular pathway. Subcellular location of specific MTMs is based on Hnia et al. (67). PRL-3, as well as PRL-1 and PRL-2 (not depicted), associate with internal membranes through a C-terminal prenylation motif, which is essential for their biological activities. PRLs may enter the nucleus upon interference with the prenylation-dependent cell membrane-targeting, which could be relevant for subcellular location-specific PRL functions. PRL-3, but not PRL-1, dephospohorylates PI(4,5)P2. In addition, PRL-3 associates functionally with different adhesion/motility-related proteins. PTPMT1 is anchored in the inner mitochondrial membrane, with its PTP domain facing the mitochondrial matrix, where it converts phosphatidylglicerophosphate in phosphatidylglicerol as part of the cardiolipin synthesis metabolic pathway.

Table 1. PTPs with non-proteinaceous substrates and link to susceptibility or to inheritance of human disease

PTP subfamily[1] / PTPs[2]	Non-proteinaceous substrates[3]	Proteinaceous substrates[4]	Link to *Hereditary disorder*[5] / disease[5]
Classical PTPs			
RPTPs:			
PTPRQ	PIPs *(D3, D5 position)*	.....	*DFNB84*
PTPRN2/phogrin/IA-2β	PIPs	.....	*DRS*, Major autoantigen in T1DM, Mood- and drug-dependence-Disorders[6]
PTPRN/IA-2*	.....	.....	Major autoantigen in T1DM
DUSPs			
PTENs:			
PTEN	PI(3,4,5)P3 *(D3 position)*	FAK, Shc1, PDGFR, Src, β-catenin *(pTyr);* Creb, 5-HT2cR, SMAD3 *(pSer)*[7] PTEN *(pSer, pThr, pTyr)*	*PHTS*[8], *ASD*, *DD*, Cancer, T2DM, Asthma, Drug Dependence[9]
TPTE2/TPIP	PI(3,4,5)P3, PI(3,4)P3 *(D3 position)*... PI(4,5)P2 *(D5 position)*	.....	Cancer
TPTE*	.....	.....	*Robertsonian Down syndrome*. Cancer
MTMs:			
MTM1	PI(3,5)P2, PI(3)P *(D3 position)*	.....	*XLMTM*
MTMR1	PI(3,5)P2, PI(3)P *(D3 position)*	.....	*cDTM1*
MTMR2	PI(3,5)P2, PI(3)P *(D3 position)*	.....	*CMT4B1*
MTMR3	PI(3,5)P2, PI(3)P *(D3 position)*	.....	Cancer
MTMR4	PI(3,5)P2, PI(3)P *(D3 position)*	SMADs *(pSer)*[7]	Hypercholesterolemia[6]
MTMR6	PI(3,5)P2, PI(3)P *(D3 position)*	.....	Unknown
MTMR7	PI(3,5)P2, PI(3)P *(D3 position)*	.....	Creutzfeldt-Jakob variant[6]
MTMR8	PI(3,5)P2, PI(3)P *(D3 position)*	.....	Unknown
MTMR14	PI(3,5)P2, PI(3)P *(D3 position)*	.....	*ACNM*, Aging sarcopenia
SBF1/MTMR5*	.....	.....	Cancer
SBF2/MTMR13*	.....	.....	*CMT4B2*
MTMR9*	.....	.....	Metabolic syndrome, Obesity[6]
MTMR10*	.....	.....	Unknown
MTMR11*	.....	.....	Unknown
MTMR12*	.....	.....	Unknown
PRLs:			
PTP4A1/PRL-1	.....	ATF-5 *(pTyr)*	Cancer
PTP4A2/PRL-2	.....	.....	Cancer
PTP4A3/PRL-3	PI(4,5)P2 *(D5 position)*	Ezrin *(pThr)*; Integrin β1 *(pTyr)*; Keratin 8 *(pSer)*[7]; EF-2 (*pThr*)[7]; Stathmin (*pSer*)[7]; Nucleolin (*pThr*)[7]	Cancer
Atypical DUSPs:			
EPM2A/Laforin[10]	Phosphoglycogen	GSK3β, Tau *(pSer)*	*LD*, Cancer
PTPMT1	Phosphatidylglycerophosphate, PIPs	.....	T2D, Cancer

[1] Class I Cys-based PTPs. Subfamily classification according to Alonso et al (ref). PTP, protein tyrosine phosphatase; RPTP, transmembrane receptor-like PTP; DUSP, dual-specificity phosphatase; PRL, phosphatase of regenerating liver; PTEN, phosphatase and tensin homologue deleted in chromosome 10; MTM, myotubularin

[2] PTPs with non-proteinaceous substrate specificity and links to human disease are given. In some cases, links to disease are indirect and based on correlation-studies or *in vitro* cellular experiments. Paralog proteins with unknown role in human disease are listed. Official gene names and protein names are provided. PRLs and MTMs are comprehensibly listed.

[3] The major non-proteinaceous substrates are indicated. Note that, in some cases, conclusive proof on the identity of the physiological substrate(s) is lacking

[4] Proposed protein substrates are given

[5] Hereditary diseases transmitted by germ-line mutations are in italics. T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; *DFNB84*, Autosomal Recessive Nonsyndromic Hearing Loss 84; *LD*, Lafora disease; *PHTS*, PTEN Hamartoma Tumor Syndrome; *ASD*, Autism Spectrum Disorder; *DD*, Developmental Delay; *XLMTM*, X-linked Myotubular Myopathy; *cDMT1*, Congenital Myotonic Distrophy; *CMT4B1*, Charcot-Marie-Tooth Disease type 4B; ACM, Autosomal Centronuclear Myopathy

[6] Based on SNP associations.

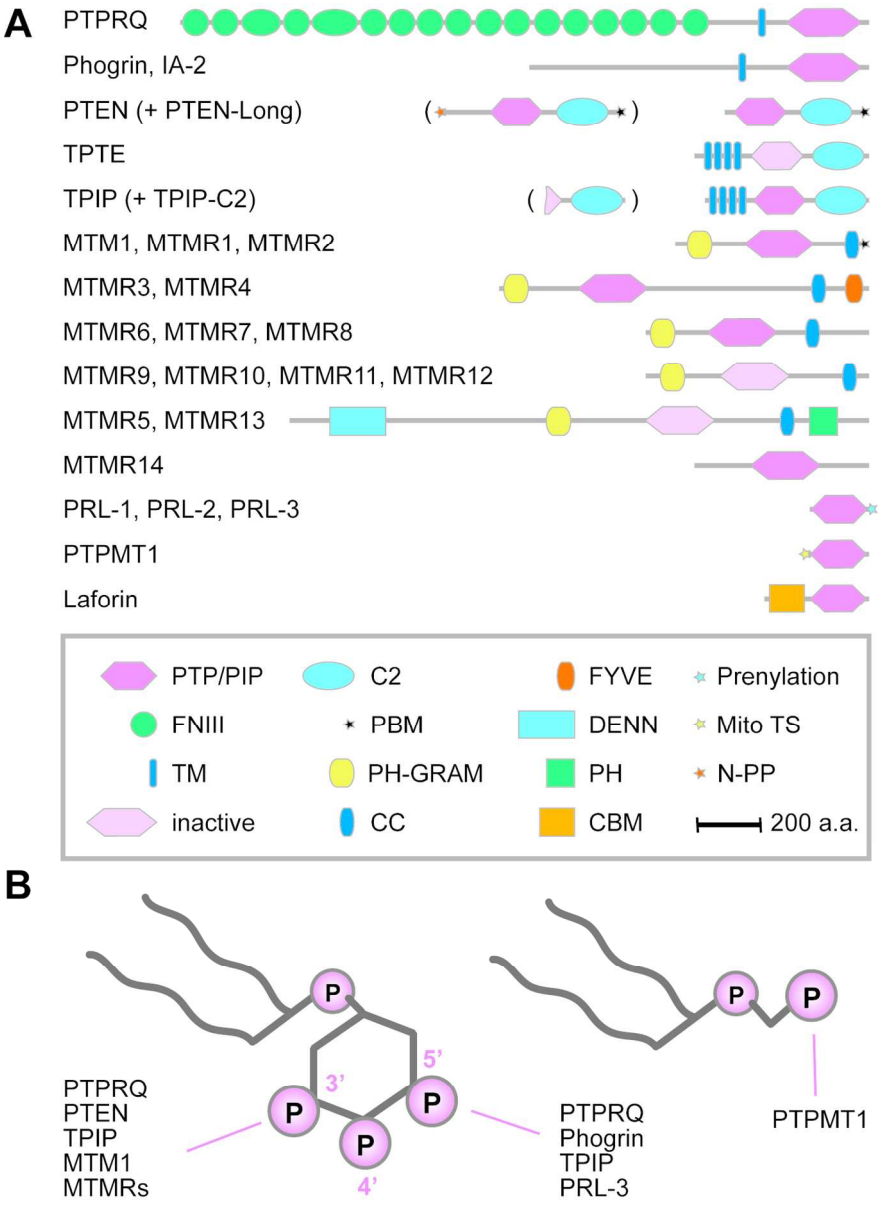
[7] Based on physical association. No evidence for direct dephosphorylation

[8] Major manifestations of PHTS include Cowden syndrome, Lhermitte-Duclos disease, Bannayan-Riley-Ruvalcaba syndrome, and Proteous and Proteous-like syndromes

[9] Associated with PTEN protein phosphatase activity towards 5-HT2cR

[10] Laforin does not display lipid phosphatase activity and it has not been addressed in this review.

* Inactive enzymes, based on their amino acid sequences at the active site (see Fig. 2) or on lack of activity towards putative substrates.



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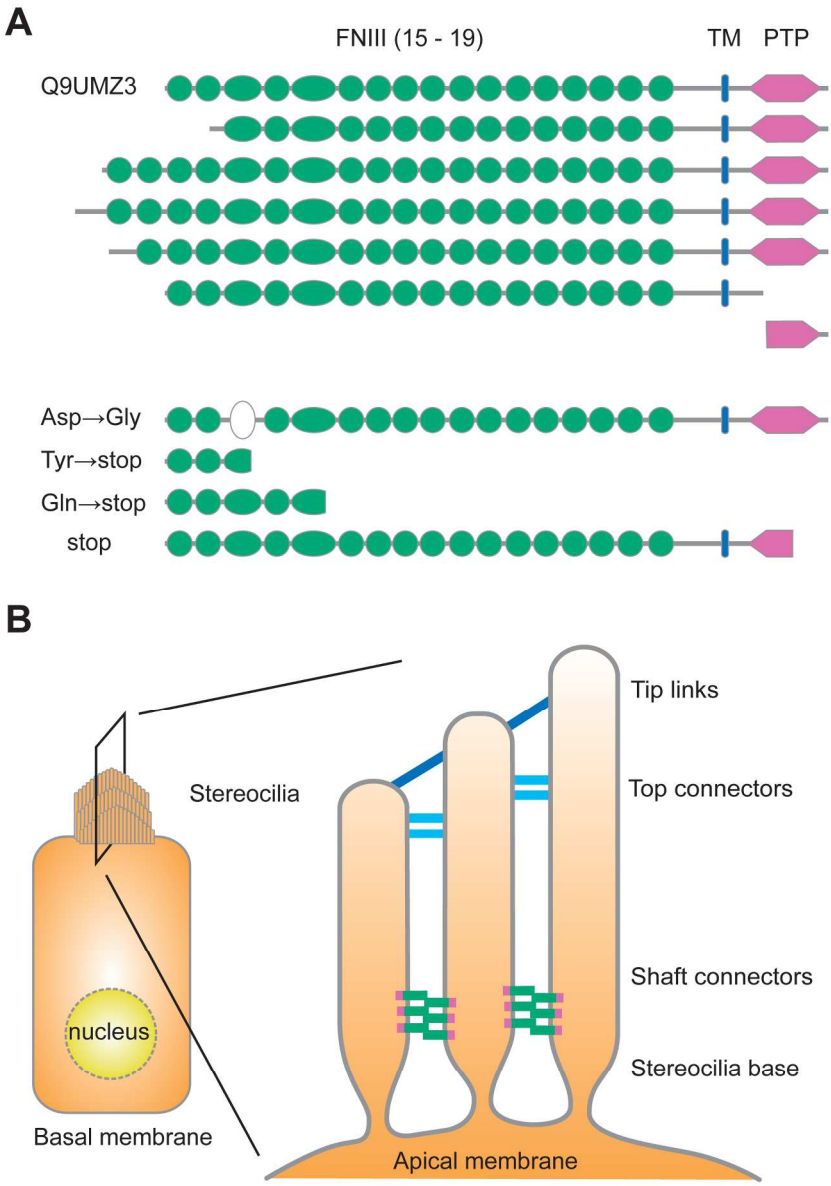
	WPD-loop		P-loop	*lenght*	PDB ID	
PTPRQ	(2164) TAWPEHGVPENSAPL (2178)		(2199) HCSAGVGRT (2207)	*(2299)*	-	**Classical RPTPs**
PTPRN2/IA-2β	(909) LSWYDRGVPSSSRSL (923)		(944) HCSDGAGRS (952)	*(1015)*	2QEP	
PTPRN/IA-2*	(873) LSWPAEGTPASTRPL (887)		(908) HCSDGAGRT (916)	*(979)*	2I1Y	
PTEN	(88) YPFEDHNPPQLELIK (102)		(123) HCKAGKGRT (131)	*(403)*	1D5R	**PTENs**
TPTE2/TPIP	(284) IMIDDNHNVPTLHEM (298)		(319) HCKGGKGRT (327)	*(522)*	-	
TPTE*	(302) IMIDDNHNVPTLHQM (316)		(337) HCKGGTDRT (345)	*(551)*	-	
MTM1	(307) FLDIHNIHV	(315)	(374) HCSDGWDRT (382)	*(603)*	-	**MTMs**
MTMR1	(371) FLEIHNIHV	(379)	(437) HCSDGWDRT (445)	*(665)*	-	
MTMR2	(350) FLDIHNIHV	(358)	(416) HCSDGWDRT (424)	*(643)*	1LW3	
MTMR3	(346) FMGMANIHS	(354)	(412) HCSDGWDRT (420)	*(1198)*	-	
MTMR4	(340) FMGMANIHA	(348)	(406) HCSDGWDRT (414)	*(1195)*	-	
MTMR6	(268) FVGIENIHV	(276)	(335) HCSDGWDRT (343)	*(621)*	2YF0	
MTMR7	(270) FIGIENIHV	(278)	(337) HCSDGWDRT (345)	*(660)*	-	
MTMR8	(270) FMGIENIHV	(278)	(337) HCSDGWDRT (345)	*(704)*	-	
MTMR9*	(265) HKSIERYHI	(273)	(332) HGTEGTDST (340)	*(549)*	-	
SBF1/MTMR5*	(1376) PIEVFEARQ	(1384)	(1446) GLEDGWDIT (1454)	*(1893)*	-	
SBF2/MTMR13*	(1340) PVEFHEIRQ	(1348)	(1409) CLEEGWDIT (1417)	*(1849)*	-	
MTMR10*	(336) LPNIQEVQA	(344)	(402) QEEEGRDLS (410)	*(777)*	-	
MTMR11*	(309) LPSLADVQL	(317)	(374) QERGDRDLN (382)	*(709)*	-	
MTMR12*	(321) FLSLQEIQT	(329)	(390) LEENASDLC (398)	*(747)*	-	
MTMR14	(263) YMAEGLIFN	(271)	(329) HCISGWDRT (337)	*(650)*	-	
PTP4A1/PRL-1	(68) WPFDDGAPPSN	(78)	(103) HCVAGLGRA (111)	*(173)*	1XM2	**PRLs**
PTP4A2/PRL-2	(65) WPFDDGAPPPN	(75)	(100) HCVAGLGRA (108)	*(167)*	-	
PTP4A3/PRL-3	(68) WPFDDGAPPPG	(78)	(103) HCVAGLGRA (111)	*(173)*	1V3A	
EPM2A/Laforin	(231) MPTPDMSTEGRV	(242)	(265) HCNAGVGRS (273)	*(331)*	-	**Atypical DUSPs**
PTPMT1	(97) LSTVDMTGIPTL	(108)	(131) HCKAGRSRS (139)	*(201)*	3RGQ	

Fig.2



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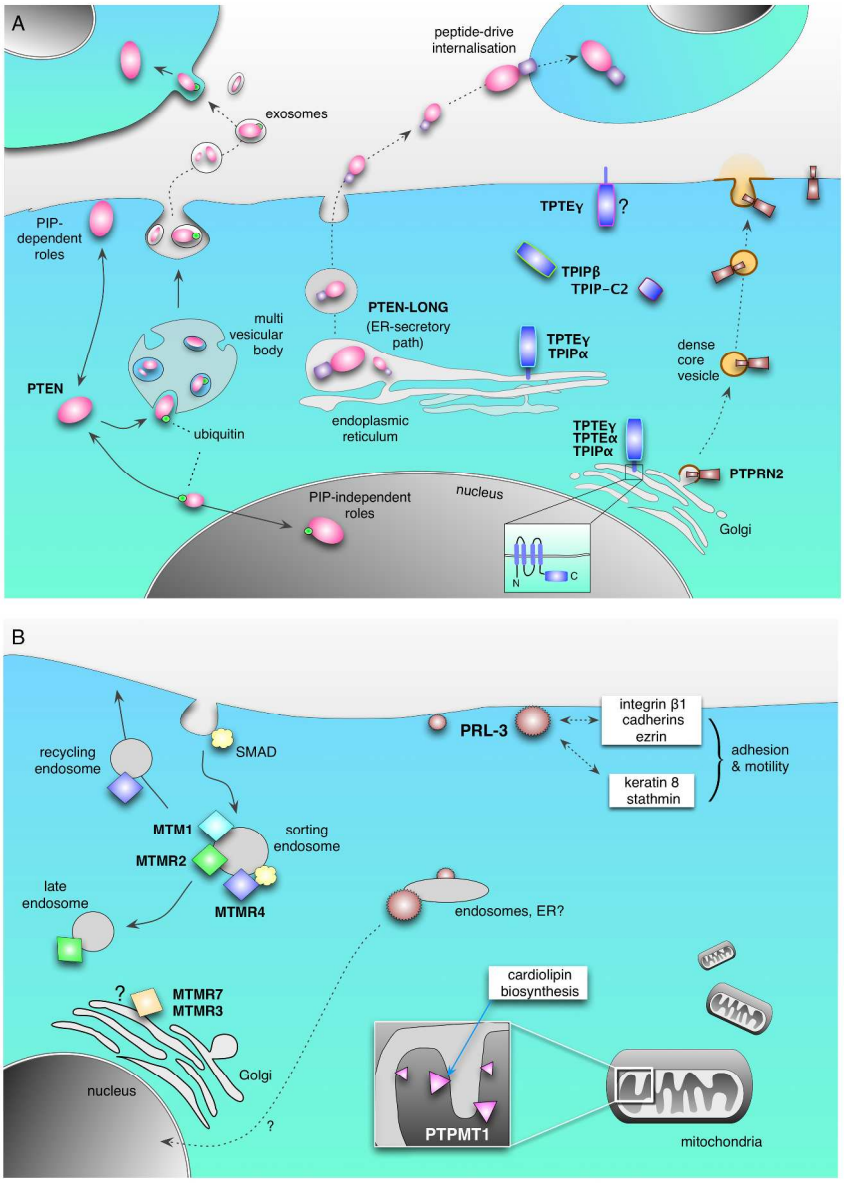

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