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Structure and Function of Phosphatidylcholine Transfer Protein (PC-TP)/StarD2

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Abstract

Phosphatidylcholine transfer protein (PC-TP) is a highly specific soluble lipid binding protein that transfers phosphatidylcholine between membranes *in vitro*. PC-TP is a member of the steroidogenic acute regulatory protein-related transfer (START) domain superfamily. Although its biochemical properties and structure are well characterized, the functions of PC-TP *in vivo* remain incompletely understood. Studies of mice with homozygous disruption of the *Pctp* gene have largely refuted the hypotheses that this protein participates in the hepatocellular selection and transport of biliary phospholipids, in the production of lung surfactant, in leukotriene biosynthesis and in cellular phosphatidylcholine metabolism. Nevertheless, *Pctp*^{-/-} mice exhibit interesting defects in lipid homeostasis, the understanding of which should elucidate the biological functions of PC-TP.

Keywords

Phospholipid; cholesterol; triglyceride; lipid transfer protein; steroidogenic acute regulatory protein; related transfer (START) domain; peroxisome proliferator activated receptor α

Introduction

Membranes of eukaryotic cells are composed principally of five distinct classes of phospholipids [1–3]. These include four glycerophospholipids: phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines and phosphatidylinositols. Phosphatidylcholines are generally the most abundant phospholipid class in a membrane. They also constitute the major phospholipid class contained in lipoproteins, biliary lipid aggregates and lung surfactant.

As is the case for the other glycerophospholipids, phosphatidylcholines are composed of two fatty acids covalently linked to a glycerol moiety by ester bonds in the sn-1 and sn-2 positions.

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The third carbon of glycerol is esterified to phosphorylcholine. Phosphatidylcholines are synthesized by two distinct enzymatic pathways [4]. One is by addition of CDP-phosphocholine to diglycerides, the enzymatic pathway for which is present in all cells. The other is by sequential methylation of phosphatidylethanolamine by phosphatidylethanolamine *N*-methyltransferase, which is expressed only in the liver. The fatty acyl composition of phosphatidylcholines plays a critical role in regulating the physical properties of membranes [1], with more unsaturated fatty acids functioning to increase fluidity. Phosphatidylcholines are also key sources for biologically potent eicosanoids, which include leukotrienes, prostaglandins and lipoxins. Eicosanoids are enzymatic derivatives of arachidonic acid, which is released from the sn-2 position of cellular membrane phospholipids by cytosolic phospholipase A₂ (cPLA₂) during the inflammatory response.

Although phosphatidylcholine molecules are amphipathic, their monomeric solubility is extremely low (approximately 10⁻¹⁰M) [5]. Consequently, phosphatidylcholines spontaneously form liquid crystalline bilayers, and their spontaneous transfer rate between membranes is negligible. In 1968, Wirtz and Zilversmit [6] observed that rat liver cytosol promotes rapid exchange of phosphatidylcholines between membranes. This suggested the presence of one or more proteins capable of promoting intermembrane phosphatidylcholine transfer and led to the purification of phosphatidylcholine transfer protein (PC-TP) [7]. More than three decades of biochemical, genetic and physiological studies are now beginning to elucidate the biological function of PC-TP.

Biochemical properties of PC-TP

Presumably due to the availability of large quantities of starting material [8,9], earlier studies focused upon PC-TP purified from bovine liver. Bovine PC-TP is a 213 aa protein with a molecular weight of 24.7 kDa and an isoelectric point of 5.8. When initially purified from rat liver, PC-TP was determined to have a molecular weight of 28 kDa and a pI of 8.4, and was not immunoreactive with antibodies to the bovine protein [9]. Whereas these observations raised the possibility that the bovine and rat proteins were substantially different, subsequent cloning and characterization of recombinant rat PC-TP revealed that these proteins are highly conserved, with 76% aa identity [10,11].

PC-TP binds phosphatidylcholines exclusively, making it the most specific of the cytosolic lipid transfer proteins [9]. Phosphatidylcholine molecules are bound to the protein in a tight 1:1 stoichiometric complex [12]. When purified from cytosol, PC-TP is always found complexed with phosphatidylcholine, suggesting that apoPC-TP (i.e. PC-TP without a bound phosphatidylcholine molecule) is not present *in vivo* [9]. Even minor chemical modifications of the phosphorylcholine head group greatly reduce or abolish affinity of PC-TP [13].

Binding assays using fluorescent phospholipid analogs demonstrated that PC-TP displays high relative affinities for phosphatidylcholines with sn-1 palmitoyl- and an sn-2 unsaturated acyl chain [9]. In order to identify the molecular species of phosphatidylcholines bound by PC-TP *in vivo*, lipids extracted from purified native protein have been analyzed by mass spectrometry [14]. In contrast to the *in vitro* finding, this analysis revealed that sn-1 stearoyl, sn-2 polyunsaturated phosphatidylcholines were predominantly bound to PC-TP and that sn-1 palmitoyl species are largely absent. This was interpreted as a reflection of the distinct pools of phosphatidylcholine that PC-TP might access within the cell. It is also possible that phosphatidylcholines may have redistributed during protein purification and might not represent molecular species bound within living cells. In this connection, de Brouwer et al. [15] have demonstrated that PC-TP forms less stable complexes with phosphatidylcholines that contain sn-1 palmitoyl compared with sn-1 stearoyl chains.

The phosphatidylcholine exchange activity of PC-TP requires several mechanistic steps. First the protein must bind to a membrane, which is facilitated by interactions with anionic phospholipids contained within the lipid bilayer [16,17]. At the membrane surface, PC-TP releases the phosphatidylcholine within its lipid-binding pocket in exchange for a different phosphatidylcholine molecule. PC-TP then dissociates from the membrane and diffuses to an acceptor membrane, where it binds and exchanges the bound phosphatidylcholine. Important regulators of the membrane association and dissociation of PC-TP are membrane charge and curvature [18–20].

Three dimensional structure of PC-TP

The primary structure of bovine PC-TP was first solved by Edman degradation [21]. Early computer modeling suggested that a lipid binding site was formed by several hydrophobic segments which fold inward to form a hydrophobic pocket shielded from bulk solvent [22]. This prediction was consistent with a prior demonstration that a nitroxide spin-label moiety covalently attached to the phosphatidylcholine sn-2 acyl chain was strongly immobilized when bound to PC-TP and inaccessible for reduction by ascorbate in solution [23]. Photoaffinity labeling further suggested that two distinct hydrophobic regions of the protein participated in binding the sn-2 acyl chain [9,24]. Based on fluorescence depolarization measurements, it was estimated that PC-TP was generally shaped as a prolate ellipsoid with the sn-1 and sn-2 acyl chains immobilized at approximately 60–90° angles to each other [25].

These and other data were clarified when the crystal structure of recombinant human PC-TP in complex with phosphatidylcholine was solved [26]. In this study, recombinant PC-TP was expressed in *E. coli*. Because *E. coli* do not produce or acquire phosphatidylcholines, this allowed synthetic phosphatidylcholines with a precisely defined acyl chain composition to be introduced into the protein during the purification procedure. Figure 1 shows the crystal structure of PC-TP. The overall structure of PC-TP is centered on an antiparallel β sheet of nine strands surrounded by four α -helices. One α -helix rests against the back of the β -sheet, with two α -helices inserted between strands of the β -sheet. Two omega loops are also inserted between strands of the central antiparallel β sheet. A long C-terminal α -helix overlays a tunnel which accommodates a single molecule of phosphatidylcholine. The walls of this tunnel are formed by the β -sheet, three α helices and the omega loops.

Among the key features of the structures is that a single well-ordered phosphatidylcholine molecule occupies a tunnel formed primarily by a central β -sheet and a C-terminal α -helix (Figure 1A). The positively charged choline headgroup of the lipid engages in cation- π interactions within a three-walled aromatic cage formed by the ring faces of Trp 101, Tyr 114 and Tyr 155 (Figure 1B). These features largely explain the exquisite specificity of PC-TP for phosphatidylcholine.

Crystal structures of PC-TP were also determined for the protein in complex with dilinoleoyl-phosphatidylcholine and sn-1 palmitoyl, sn-2 linoleoyl phosphatidylcholine [26]. Interestingly, the position and conformation of the two bound phosphatidylcholines are quite similar despite the difference in acyl chain length and unsaturation (Figure 1B). Both the palmitoyl and linoleoyl chains in the sn-1 position adopt a C-shaped configuration, which would not be expected to occur outside the confines of the PC-TP lipid binding pocket. It is noteworthy that these crystal structures do not appear to explain previous observations that PC-TP preferentially binds phosphatidylcholines depending upon acyl chain compositions. Similarly, the conformation of acyl chains of the bound phosphatidylcholine is not easily reconciled with the earlier model based on fluorescence data that was also described above.

The lipid-binding tunnel of PC-TP nicely accommodates a single molecule of phosphatidylcholine (Figure 1C), which occupies approximately 89% of the available volume.

Because this lipid-binding pocket extends to the bulk solvent through only two narrow portals 3–5 Å in diameter, a major conformational change in the protein is clearly required for PC-TP to bind or release a phosphatidylcholine molecule. The C-terminal $\alpha 4$ helix and the $\Omega 1$ loop of PC-TP contact only the acyl chains of the phosphatidylcholine molecule and do not contribute to the headgroup binding site. Therefore, these secondary structural elements could change conformation without disruption of the binding site, such as would be expected to occur when PC-TP binds a phosphatidylcholine molecule at the membrane interface. Further insights would likely emerge via a comparison of the structure of PC-TP in Figure 1 with apoPC-TP, in the event that the latter structure were to be solved in an open conformation.

In agreement with the crystal structure, additional secondary structure analysis indicated that helix $\alpha 4$ in Figure 1A is interrupted by a proline residue to form two shorter helices with distinct physical properties (Figure 2A): a hydrophobic α -helix ($\alpha 4a$) and an amphipathic α -helix ($\alpha 4b$) [27]. Circular dichroic spectra of short synthetic peptides containing one ($\alpha 4b$) or both ($\alpha 4a$ plus $\alpha 4b$) revealed that these C-terminal α -helices in aqueous buffer were most consistent with random coil structures. However, both peptides adopted α -helical configurations in the presence of small unilamellar vesicles. The model in Figure 2B incorporates the membrane binding properties of both α -helices and suggests that the C-terminus of PC-TP facilitates membrane binding and extraction of phosphatidylcholines. Consistent with this hypothesis, truncation of five residues from the C-terminus shortened the predicted amphipathic $\alpha 4b$ helix and decreased PC-TP activity *in vitro* by 50%, whereas removal of 10 residues eliminated the α -helix, both abolished activity and markedly decreased membrane binding [27].

PC-TP is a member of the START domain superfamily

The structure of PC-TP has established it as a member of the steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain superfamily, which was described in 1999 [28]. Based on refinements in database searching algorithms that predict protein structure, it was demonstrated that an apparently diverse group of proteins, including PC-TP, contain a common motif that was capable of binding a lipid molecule [28,29].

START domain proteins are broadly expressed from bacteria to higher organisms, but are amplified in plants [30]. The genomes of humans and mice each contain 15 genes that encode START domains, which may be divided by phylogenetic analysis into six subfamilies [31, 32]. For mammalian START domain proteins, the nomenclature StarD1 - StarD15 has been adopted [31,33]. Whereas PC-TP has been re-designated as StarD2, this new terminology has yet to become widely applied.

Three-dimensional structures have been solved for three START domains. In addition to PC-TP [26], these are MLN64 [34] and StARD4 [35]. These structures share the same helix-grip fold configuration [29], which creates a unique type of hydrophobic pocket that is formed by the central β -sheet and the C-terminal α -helix [29] and is distinct from other classes of lipid binding proteins (e.g. fatty acid binding proteins (FABPs) [36], sterol carrier protein 2 (SCP-2) [37], and phosphatidylinositol transfer protein (PI-TP) [38]). Apart from PC-TP, ligands have been identified for a very limited number of other family members [32]. These observations, taken together with the common feature of a hydrophobic pocket, indicate that other START domains also bind lipids, and that ligand specificity is most likely conferred by the configuration of amino acids that form the lining of the lipid-binding cavity.

START domains are found in multidomain proteins, but also as START-domain minimal proteins (i.e. proteins in which the amino acid sequence of the protein comprises the entire START domain). PC-TP is an example of a START domain minimal protein. With multidomain proteins, START domains are associated with domains of established function, including signal transduction and transcriptional control [30]. In plants, START domains are

principally associated with homeodomain transcription factors [28,30]. Homeodomains are DNA binding motifs, the functions of which include regulation of development in eukaryotes. Their incorporation together with START domains in multidomain proteins is unique to plants [30]. In humans, about half of START domains are found within multidomain proteins. Considering their putative functions, it has been postulated that START domain proteins constitute a diverse group of lipid transfer and lipid sensing molecules [28–30,32].

Gene structure and conservation

The *Pctp* gene consists of six exons [11,39,40] and resides on chromosome 11 in the mouse and in the syntenic region of human chromosome 17q21-22 [39]. There appears to be a single transcription initiation site [11]. cDNAs encoding PC-TP have been cloned from rat, bovine, mouse, and human, and their nucleotide, as well as deduced amino acid sequences are highly conserved [11,39–42].

Tissue distribution and regulation of *Pctp* expression

The tissue distribution of PC-TP varies among species and among mouse strains. In humans, PC-TP mRNA is widely expressed in tissues (except thymus) with highest levels present in liver, placenta, testis, and kidney [39]. By comparison, a radioimmunoassay to quantify PC-TP in rat revealed highest concentration in liver followed by intestine, kidney, spleen, lung and adrenals [43]. In mice [40], the tissue distribution of PC-TP generally correlates with the rat. Wu et al. [44] demonstrated relatively high levels of PC-TP in livers of adult C57BL/6J mice compared with low levels in FVB/NJ mice. By contrast, PC-TP expression in livers of a mixed FVB/NJ-129/Ola genetic background falls to undetectable levels within 12 weeks following birth [40]. An immunohistochemical analysis [40] has demonstrated that PC-TP expression in the liver is attributable to hepatocytes. In the testis, PC-TP is expressed in all cells that participate in spermatogenesis, and in the epididymis, it is expressed in epithelial cells that overlie the sperm ducts.

The putative gene promoter region of rat *Pctp* has been cloned and partially characterized [11]. Database searching revealed a number of consensus nucleotide sequences for binding of transcription factors, which might contribute to *Pctp* expression in liver. Using 5'-deleted promoter-firefly luciferase constructs transfected into a rat hepatoma cell line compared with rat fibroblasts, a 637 bp promoter region was identified, which might contribute to liver expression. This region contained a consensus response element for the liver-enriched transcription factor C/EBP β . Also present in this region are two overlapping DR-1 motifs for binding of PPAR/RXR heterodimers. Interestingly, a consensus sequence for HNF-4 binding overlaps one of the two PPAR binding sites.

The nuclear hormone receptor PPAR α , is a ligand-induced transcription factor that is enriched in liver and plays a key role in regulating hepatic lipid metabolism [45]. Target genes of PPAR α promote binding, transport and oxidation of fatty acids, as well as the biliary elimination of plasma cholesterol [45]. Indeed, mice treated with fibrate drugs, which are PPAR α ligands, revealed 2- to 25-fold induction of hepatic PC-TP protein [46–48]. Upregulation of PC-TP in response to fibrates occurs at the transcriptional level and depends upon PPAR α expression, as evidenced by an absence of upregulation in *Ppara*^{-/-} mice [47]. Induction of PC-TP mRNA in liver by 10.6-fold has also been observed in response to adenovirus-mediated overexpression of PPAR γ [49].

Cellular distribution of PC-TP

Early studies of the subcellular distribution using a radioimmunoassay indicated that approximately 60% of rat liver PC-TP is present in the cytosolic fraction, with the remainder

being evenly distributed over the particulate fractions [43]. More recent experiments using confocal laser scanning microscopy have demonstrated that fluorescence-labeled PC-TP is found throughout the cytosol, and photobleaching experiments have revealed that PC-TP is highly mobile in the cytoplasm [50]. PC-TP was also present in the nucleus [50], suggesting that it could regulate gene transcription, possibly by interacting with a transcription factor.

Interestingly, de Brouwer et al. [50] demonstrated that, in response to exposure of endothelial cells to clofibrate, PC-TP associates with mitochondria. Because this occurred over a period of minutes, the effect was too rapid to be explained by transcriptional regulation. Rather, it appeared that clofibrate-stimulated phosphorylation of PC-TP at serine 110 which may have led to its rapid relocation. Consistent with this hypothesis, relocation did not occur when serine 110 was mutated or in HepG2 cells, in which fibrate exposure does not induce phosphorylation. Notwithstanding this suggestive finding, there is no direct evidence to indicate that PC-TP is phosphorylated.

Biological functions of PC-TP

Based on its substrate specificity and tissue distribution, a variety of functions of PC-TP have been proposed since its purification more than 30 year ago [9]. The more recent cloning of cDNAs encoding PC-TP [11,39,41,42] and the creation of *Pctp*^{-/-} mice [40] has permitted testing of these and other possibilities.

Biliary lipid secretion

Based upon its accentuated expression in the liver and its high relative affinities for phosphatidylcholine molecular species that are secreted by the liver into bile [51,52], it was proposed that PC-TP might transport phosphatidylcholines from their site of synthesis in the smooth endoplasmic reticulum to the canalicular membrane of hepatocyte for secretion into bile [53,54]. In support of this possibility, *in vitro* studies revealed that bile salts at submicellar concentrations that might be present in hepatocytes markedly increased that activity of PC-TP [53]. Moreover, PC-TP was found to be downregulated in patients with hepatolithiasis, in whom biliary phospholipid secretion was decreased [55]. Nevertheless, an essential function of PC-TP in the hepatocellular selection and transport of biliary phosphatidylcholines was refuted by the observation that biles of *Pctp*^{-/-} mice contained normal compositions and concentrations of phosphatidylcholines. The observation that PC-TP expression falls to undetectable levels in 12 week old FVB/NJ-129/Ola mice also suggests that PC-TP is not essential and that vesicular phosphatidylcholine transfer combined with a small component of diffusional transfer might be sufficient for basal activity. Alternatively, some other protein that binds phosphatidylcholine could mediate transfer under basal conditions. Hepatocytes do express other proteins with phosphatidylcholine transfer activity (e.g. SCP-2, PI-TP and StarD10) [56]. However, there was no evidence for upregulation of SCP-2 or PI-TP in livers of mice lacking PC-TP [40]. The possibility that a compensatory mechanism was operative in *Pctp*^{-/-} mice was largely excluded by the observation that knockout mice infused intravenously with tauroursodeoxycholate, a hydrophilic bile salt that promotes maximal biliary phospholipid secretion, secreted phosphatidylcholines into bile as robustly as did wild type mice [40].

Notwithstanding these observations, a regulatory role of PC-TP in biliary secretion was demonstrated when *Pctp*^{-/-} mice were challenged with a high fat, high cholesterol diet [44]: Upregulation in secretion rates of biliary phosphatidylcholines, cholesterol and bile salts were all impaired in *Pctp*^{-/-} mice. Alterations in biliary lipid secretion could not be attributed to transcriptional regulation of the expression of canalicular membrane lipid transporters (i.e. Abcb11, Abcb4 and Abcg5/g8), but may have been the result of a defect in the cellular trafficking of these transporters to the canalicular membrane.

Surfactant production

Phosphatidylcholines are the major lipid of lung surfactant. Therefore, another proposed function for PC-TP has been secretion of lung surfactant from type II alveolar cells [42,43, 57]. Because the lungs of *Pctp*^{-/-} mice develop normally, and the molecular species and contents of phosphatidylcholines in lung surfactant were unchanged in the absence of PC-TP expression [40], this argues against a key role for PC-TP in surfactant formation.

Leukotriene biosynthesis and cellular phosphatidylcholine metabolism

Geijtenbeek et al. [14] observed that the sn-2 acyl chain of PC-TP-bound phosphatidylcholines was typical of the precursor of leukotrienes. This finding suggested that PC-TP might function in the biosynthesis of these biologically active lipids. Because a tissue distribution survey in mouse revealed that bone marrow-derived mast cells express high levels of PC-TP, this raised the possibility that PC-TP may play a role in leukotriene synthesis and secretion, perhaps by supplying arachidonic acid-containing phosphatidylcholines to calcium-sensitive phospholipase A₂ [40]. However, following stimulation with a calcium ionophore, there were no differences in formation of lyso-phosphatidylcholine or the synthesis and secretion of leukotrienes in cultured bone marrow-derived mast cells from *Pctp*^{-/-} and wild type mice.

PC-TP-deficient bone marrow-derived mast cells also displayed normal phosphatidylcholine labeling kinetics and phospholipid compositions, arguing against a role for PC-TP in phosphatidylcholine metabolism [40]. This is supported by the observation that overexpression of PC-TP in Chinese hamster ovary (CHO) cells did not alter cellular phospholipid compositions, phosphatidylcholine contents or phosphatidylcholine synthetic rates [58].

HDL metabolism

Formation of HDL begins when apoA-I molecules interact with the plasma membrane to form particles with pre β mobility on agarose gels [59–63]. Assembly of pre β -HDL requires the presence on the plasma membrane of ATP-binding cassette A1 (ABCA1) [64,65]. Pre β -HDL particles formed by exposing cultured cell lines [66–70] or primary hepatocytes [71] to apoA-I are highly enriched (80–90%) in phosphatidylcholines. Moreover, the molecular species of phosphatidylcholines in HDL particles contain predominantly sn-1 16:0 (palmitoyl) fatty acyl chains, with an unsaturated sn-2 acyl chain, typically 18:2 (linoleoyl) [72]. To explore a role for PC-TP in the formation of nascent pre β -HDL, CHO cells were stably transfected with a PC-TP cDNA [58]. PC-TP accelerated apoA-I-mediated phospholipid and cholesterol efflux as pre β -HDL particles in proportion to its level of cellular expression, suggesting a function of PC-TP in promoting pre β -HDL particle formation.

Because PC-TP was found to be expressed abundantly in macrophages [73], additional insights into a potential role in HDL metabolism were gleaned from studies of peritoneal macrophages cultured from *Pctp*^{-/-} and wild type mice. Macrophages scavenge oxidatively modified lipoproteins, while defending themselves against unesterified cholesterol-induced cytotoxicity by adaptive mechanisms that depend in part upon Abca1-dependent efflux of phosphatidylcholines [74]. In cholesteryl ester-loaded macrophages from *Pctp*^{-/-} mice, apoA-I-mediated efflux of phospholipids and cholesterol was reduced. In contrast to CHO cells in which overexpression of PC-TP did not influence the expression of Abca1, reduced apoA-I-mediated phosphatidylcholine efflux in cholesterol loaded mouse peritoneal macrophages was attributable to proportional decreases in cellular expression levels of Abca1.

The influence of PC-TP expression on HDL metabolism has also been examined *in vivo* [75]. In chow fed mice, neither plasma cholesterol concentrations nor concentrations and compositions of plasma phospholipids were influenced by PC-TP expression. However, in *Pctp*^{-/-} mice there was an accumulation of small α -migrating HDL particles. A high fat, high

cholesterol diet, normalized HDL particle sizes and increased plasma cholesterol and phospholipid concentrations compared with wild type mice. This finding was attributable to reduced HDL uptake from plasma into livers of *Pctp*^{-/-} mice. Whereas PC-TP is not essential for the enrichment of HDL with phosphatidylcholines *in vivo*, it does modulate particle size and rates of hepatic clearance.

PC-TP expression regulates hepatic lipid metabolism

Because PC-TP expression regulates both HDL metabolism and biliary lipid secretion, its influence on hepatic lipid homeostasis was studied [76]. The activity of acyl CoA:cholesterol acyltransferase (Acat), which esterifies cholesterol in liver, was markedly increased in chow fed *Pctp*^{-/-} compared with wild type mice. The activity of 3-hydroxy-3-methylglutaryl-(HMG)-CoA reductase, the rate limiting enzyme of cholesterol biosynthesis, was unchanged in liver, whereas activity of cholesterol 7 α -hydroxylase (Cyp7A1), which initiates cholesterol catabolism to bile salts, was reduced. Consistent with increased Acat activity, esterified cholesterol concentrations in livers of *Pctp*^{-/-} mice were increased and unesterified cholesterol concentrations were decreased. Because hepatic phospholipid concentrations were also decreased in the absence of PC-TP, unesterified cholesterol/phospholipid ratios in liver remained unchanged.

A high fat, high cholesterol diet downregulated HMG-CoA reductase in *Pctp*^{-/-} and wild type mice, whereas Acat was increased only in wild type mice. A greater reduction in Cyp7A1 activity in *Pctp*^{-/-} mice could be attributed to increased size and hydrophobicity of the bile salt pool. Despite higher hepatic phospholipid concentrations, the unesterified cholesterol/phospholipid ratio increased. The lack of Acat upregulation in *Pctp*^{-/-} mice suggested that, in the setting of the dietary challenge, the capacity for esterification to defend against hepatic accumulation of unesterified cholesterol was exceeded in the absence of PC-TP expression. Taken together, these findings indicate that regulation of cholesterol homeostasis is a physiological function of PC-TP in liver, which can be overwhelmed in mice by a cholesterol-rich diet.

Influence of PC-TP expression on the development of atherosclerosis in apoE-deficient mice

In response to loading with unesterified cholesterol, the absence of PC-TP in macrophages was associated with marked increases in apoptotic cell death [73], such as occurs in atherosclerotic plaques [77]. This, taken together with the apparent roles in HDL metabolism and in the response of biliary lipid secretion to excess dietary cholesterol, suggested that PC-TP with homozygous disruption of both *Pctp* and *ApoE* genes (*Pctp*^{-/-}/*ApoE*^{-/-}). *ApoE*^{-/-} mice are hypercholesterolemic and develop atherosclerosis spontaneously on a chow diet, and this can be accelerated by feeding a Western-type diet [79]. Unexpectedly, the absence of PC-TP expression was associated with a modest reduction in atherosclerosis during a six month period. In male mice, this could be attributed to changes in plasma lipids. In female mice, adjustment for plasma lipids did not eliminate the influence of PC-TP expression on atherosclerotic lesion area. These findings suggested that PC-TP expression within the arterial wall predisposes to atherosclerosis in the setting of hyperlipidemia.

Regulation of triglyceride metabolism by PC-TP

Emerging evidence suggests that PC-TP expression also influences triglyceride metabolism. This is consistent with its upregulation by PPAR α , which regulates numerous genes of fatty acid and triglyceride homeostasis [45]. In addition to demonstrating the rapid association of PC-TP with mitochondria in cells exposed to clofibrate [50], de Brouwer et al. [48] reported that plasma triglyceride concentrations in *Pctp*^{-/-} mice failed to decrease in response to fibrate feeding. Despite increased hepatic triglyceride secretion rates, livers of *Pctp*^{-/-} mice fed chow accumulate triglycerides [80,81]. This was not attributable to increased synthesis because the

absence of PC-TP expression was associated with reduced mRNA levels of sterol regulatory element binding protein-1c (SREBP-1c), a transcription factor that promotes hepatic lipogenesis, as well as its downstream targets. Similarly, there was no evidence for decreased fatty acid oxidation: plasma β -hydroxybutyrate concentrations were unchanged, and the absence of PC-TP did not influence hepatic mRNA levels for carnitine palmytoyl transferase I or II. Rather, it appeared that hepatic triglyceride accumulation in *Pctp*^{-/-} mice might be primarily attributable to increased hepatic uptake of plasma fatty acids, as evidenced by increased mRNA levels of fatty acid transport protein 2 and decreased plasma free fatty acid concentrations. Finally, although its pathophysiological significance is not yet known, a naturally occurring mutation that inactivates PC-TP has been described in New Zealand Obese mice, which spontaneously develop obesity and diabetes [82].

Summary and future directions

PC-TP is a highly specific lipid binding protein that is a member of the START domain superfamily. Since its discovery, its biochemical properties and structure have been well characterized. A key issue that remains is to define the major conformational change of PC-TP that occurs during binding to phosphatidylcholines. If apoPC-TP could be crystallized in an open conformation, this would provide important new information.

The loss of PC-TP expression is associated with a variety of metabolic abnormalities in the mouse, particularly in the setting of a dietary cholesterol challenge. The mechanistic basis of these observations remains unclear and awaits further study. One important question is whether the biological functions of PC-TP are related to the intermembrane phosphatidylcholine transfer activity of PC-TP, which has been so extensively characterized *in vitro*. In this connection, the PC-TP polymorphism in New Zealand Obese mice that inactivates PC-TP *in vitro* may provide additional insights [82]. An additional area for study is the potential function (s) of PC-TP that is present within the nucleus. If PC-TP were demonstrated to modulate transcription events, this could establish an important new connection between membrane phospholipid composition and gene regulation.

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Figure 1.

A) Overall structure of human PC-TP. The α -helix identifiers and residue ranges for human PC-TP are $\alpha 1$ (9–22), $\alpha 2$ (64–74), $\alpha 3$ (75–82) and $\alpha 4$ (184–209). The β -strand identifiers and residue ranges are $\beta 1$ (31–36), $\beta 2$ (39–46), $\beta 3$ (51–61), $\beta 4$ (84–93), $\beta 5$ (96–104), $\beta 6$ (111–123), $\beta 7$ (130–138), $\beta 8$ (150–162) and $\beta 9$ (168–178). The Ω -loop identifiers and residue ranges are $\Omega 1$ (105–110) and $\Omega 2$ (139–149). B) Interactions of PC-TP (blue) with the glycerol-3-phosphorylcholine moiety of 1-palmitoyl,2-linoleoyl-sn-glycerol-3-phosphorylcholine (palmitoyl-linoleoyl phosphatidylcholine) (yellow). The structure of 1,2-dilinoleoyl-sn-glycerol-3-phosphorylcholine (dilinoleoyl phosphatidylcholine) from the PC-TP-phosphatidylcholine complex is superimposed (gray). C) Solvent accessible volume of the binding pocket containing phosphatidylcholine. The phosphatidylcholine molecule occupies approximately 89% of the lipid binding pocket, which extends through two narrow portals 3–5 Å in diameter to bulk solvent. (Reprinted with permission from reference [26]).

Figure 2.

The C-terminus of PC-TP is the membrane binding domain. A) The $\alpha 4$ helix of the C-terminus of PC-TP can be subdivided into two functionally distinct types, a membrane spanning helix ($\alpha 4a$) and an amphipathic helix ($\alpha 4b$). B) A model of PC-TP-membrane binding that occurs when $\alpha 4b$ aligns at the surface of a bilayer. Extraction of a phosphatidylcholine may be accomplished when $\alpha 4a$ penetrates into the membrane.