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Regulation of Lipid and Glucose Metabolism by Phosphatidylcholine Transfer Protein

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Abstract

Phosphatidylcholine transfer protein (PC-TP, a.k.a. StARD2) binds phosphatidylcholines and catalyzes their intermembrane transfer and exchange *in vitro*. The structure of PC-TP comprises a hydrophobic pocket and a well-defined head-group binding site, and its gene expression is regulated by peroxisome proliferator activated receptor α . Recent studies have revealed key regulatory roles for PC-TP in lipid and glucose metabolism. Notably, *Pctp*^{-/-} mice are sensitized to insulin action and exhibit more efficient brown fat-mediated thermogenesis. PC-TP appears to limit access of fatty acids to mitochondria by stimulating the activity of thioesterase superfamily member 2, a newly characterized long-chain fatty acyl-CoA thioesterase. Because PC-TP discriminates among phosphatidylcholines within lipid bilayers, it may function as a sensor that links metabolic regulation to membrane composition.

Phospholipid transfer proteins

Membrane bilayers of eukaryotic cells are composed principally of five distinct classes of phospholipids [1–3]. These include four types of glycerophospholipids: phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines and phosphatidylinositols. Glycerophospholipids consist of two fatty acids covalently linked by ester bonds to a glycerol molecule in the sn-1 and sn-2 positions, with the third carbon of glycerol esterified to a polar head group. The other common phospholipid is the sphingolipid sphingomyelin, in which a fatty acid and phosphorylcholine are ester-linked to a sphingosine base. Phosphatidylcholines are the most abundant class in membranes, serving as structural constituents and key sources for lipid messengers. Phosphatidylcholines are also the major phospholipid class of lipoproteins and biliary lipid aggregates.

Because phospholipid molecules are highly insoluble molecules with vanishingly small monomeric solubilities [4], they do not spontaneously dissociate from membranes at appreciable rates. The observation that plasma and cytosolic proteins promote rapid transfer and exchange led to the identification and characterization of phospholipid transfer proteins. These include phospholipid transfer protein (PL-TP) from plasma, as well as

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phosphatidylinositol transfer protein (PI-TP), sterol carrier protein 2 (SCP2, a.k.a. non-specific lipid transfer protein) and phosphatidylcholine transfer protein (PC-TP) from cytosol.

In the plasma, PLTP mediates transfer of surface phospholipids from apolipoproteinB-containing lipoproteins to HDL particles [5]. Intracellularly, PLTP participates in late stages of apolipoproteinB lipidation [6]. PLTP *in vitro* catalyzes transfer of all phospholipid classes [7], as well as a variety of other lipids [5].

PI-TP α and PI-TP β bind both phosphatidylinositols and phosphatidylcholines, with PI-TP β also binding sphingomyelins [8]. Although all three phospholipids are transferred, they exhibit marked preference for intermembrane transfer of phosphatidylinositol *in vitro* [8]. PI-TPs play diverse roles in regulating membrane traffic within the Golgi apparatus, neurite outgrowth, cytokinesis and stem cell growth [8] by cell signaling mechanisms that apparently depend upon lipid transfer activity [8,9]. SCP2 catalyzes intermembrane transfer of sterols, as well as a variety of phospholipids and other lipids, including fatty acids and fatty acyl-CoAs. Roles for SCP2 have been demonstrated in the transport and metabolism of sterols and fatty acids [9, 10], as well as in the regulation of lipid rafts and cell signaling [10].

PC-TP *in vitro* catalyzes intermembrane transfer and exchange exclusively of phosphatidylcholines [11]. In this review, we discuss the structure and function of PC-TP, including recent evidence that this highly specific phospholipid lipid transfer protein exerts broad metabolic control in the mouse. PC-TP is expressed early during embryonic development [12], and its tissue distribution is widespread in adult mice and humans [12,13], with highest expression levels in oxidative tissues, including liver, heart, muscle, kidney and brown fat [14,15], and very little expression in white adipose tissue [16].

PC-TP is a member of the START domain superfamily

The steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain superfamily is a functionally diverse group of proteins that share a unique structural motif for binding lipids (i.e. the START domain) [17,18]. The START domain is conserved in evolution from bacteria to higher organisms [19]. Although START domain proteins are numerous in plants [19], the mammalian genome encodes a total of only 15 (StARD1 - StARD15) [20, 21]. Proteins that contain START domains participate in intracellular lipid transport, lipid metabolism and cellular signaling [21–23]. Whereas these domains most commonly reside at the C-terminus of multidomain proteins, they may also exist as START domain minimal proteins in which the entire amino acid sequence comprises the START domain [19].

PC-TP (a.k.a. StARD2) is an example of a START domain minimal protein. The other phospholipid transfer proteins described above are not members of the START domain superfamily. It is now appreciated that two other START domain minimal proteins exhibit phospholipid transfer activity *in vitro*. StARD7, which was identified as a highly expressed protein in a choriocarcinoma cell line [24], also appears to be specific for phosphatidylcholines and may promote their non-vesicular transfer to mitochondria [25]. StARD10 was discovered as a phosphoprotein that is overexpressed in a significant fraction of primary human breast cancers [26]. It binds and transfers both phosphatidylcholines and phosphatidylethanolamines [27]. In tissue culture, StARD10 cooperates with epidermal growth factor receptor ErbB in eliciting anchorage-independent growth in tissue culture, suggesting it contributes to oncogenesis [26].

Structure and biochemical characteristics of PC-TP

PC-TP complexes phosphatidylcholines in 1:1 stoichiometry [11,28]. Even minor chemical modifications of the phosphorylcholine head group greatly reduce or abolish binding by PC-

TP [29]. Characteristics of START domain structures, the helix-grip fold of PC-TP (Figure 1a) consists of a central β -sheet and a C-terminal α -helix [17], forming a lipid binding pocket that immobilizes a single phosphatidylcholine molecule (Figure 1b) [28]. The specificity for binding the head group of phosphatidylcholines is imparted by Arg 78, which interacts directly with the negatively charged phosphoryl group and forms a salt bridge with Asp 82, and 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 1c) [28].

As evidenced by binding assays using fluorescent phospholipid analogs [11] and by mass spectrometry of bound phosphatidylcholine molecules [30], PC-TP displays high relative affinities for phosphatidylcholines with sn-1 palmitoyl (16:0)- and sn-2 unsaturated- acyl chains, including linoleoyl (18:2), arachidonoyl (20:4) and docosahexaenoyl (22:6). Presumably because these relative affinities exceed those for more saturated molecular species by as much as 10-fold [31], when native or recombinant PC-TP was exposed to microsomes or vesicles composed from microsomal phosphatidylcholines, the protein preferentially bound 16:0–18:2 and 16:0–20:4 phosphatidylcholines, whereas the membranes were mainly composed of 18:0–18:1 and 18:0–18:2 phosphatidylcholines [30]. The observation that enrichment of PC-TP with 16:0–18:2 and 16:0–20:4 phosphatidylcholines was the same for microsomes and vesicles indicates that other membrane proteins do not dictate the phosphatidylcholine molecular species preferences of PC-TP.

The molecular basis for this preferential binding is not readily explained by the crystal structures [28]. Indeed, it was observed that the position and conformation of dilinoleoyl-phosphatidylcholine and sn-1 palmitoyl, sn-2 linoleoyl phosphatidylcholine are quite similar despite the difference in the sn-1 acyl chain length and unsaturation (Figure 1c). It is possible the acyl chain selectivity arises at least in part at the membrane interface, where sn-1 palmitoyl, sn-2 unsaturated phosphatidylcholines would be expected to be more easily removed by PC-TP than more saturated molecular species.

Metabolic regulation by PC-TP in mice

Influence of PC-TP on the hepatobiliary cholesterol and lipoprotein metabolism

Within the liver, amphipathic bile salt molecules stimulate the biliary secretion of cholesterol together with phosphatidylcholines [32]. Based upon similarities between the molecular species of phosphatidylcholines present in bile and those bound by the protein, it was postulated that PC-TP may play a key role in the biliary secretion of phospholipids and cholesterol [32, 33]. However, this hypothesis was largely disproved when no differences in biliary phospholipids were observed in *Pctp*^{-/-} compared with wild type mice [34]. Although the underlying mechanism remains to be elucidated, regulatory roles for PC-TP in hepatic cholesterol metabolism have been demonstrated [35,36], and the absence of PC-TP expression reduces HDL particle size and hepatic selective uptake of HDL cholesterol [37].

Influence of PC-TP on the metabolism of glucose and fatty acids

The observation in fasting *Pctp*^{-/-} mice of decreased plasma concentrations of glucose and free fatty acids in the absence of increased plasma insulin concentrations suggested that PC-TP might modulate insulin sensitivity. Indeed, hyperinsulinemic-euglycemic clamp studies revealed profound reductions in hepatic glucose production, gluconeogenesis, glycogenolysis and glucose cycling in *Pctp*^{-/-} mice [16]. This increase in hepatic insulin sensitivity was explained in part by the lack of PC-TP expression in liver *per se* and in part by elevated plasma concentrations of the insulin sensitizers leptin and adiponectin that accompanied the increased percentage of body fat in mice lacking PC-TP. Plasma concentrations of TNF α , which promotes

hepatic insulin resistance, were decreased in *Pctp*^{-/-} mice. The increased body fat composition in *Pctp*^{-/-} mice appeared to represent an adaptive response to preferential utilization of fatty acids in oxidative tissues.

Influence of PC-TP on brown fat-mediated thermogenesis

The shift towards utilization of fatty acids for oxidative phosphorylation observed in *Pctp*^{-/-} mice was elucidated in part by a study in brown fat [15], a mitochondria-rich, oxidative tissue that mediates non-shivering thermogenesis. Brown fat consumes more than half of total calories and oxygen in the animal [38]. Suggestive of a regulatory role in thermogenesis, PC-TP expression in brown fat of wild type mice varied inversely as a function of ambient temperature [15]. At thermoneutrality (30°C), PC-TP expression did not influence core body temperature. However, at room temperature, *Pctp*^{-/-} mice exhibited higher body temperatures than wild type controls. They also defended their core body temperature at 4°C more efficiently, as evidenced by reduced upregulation of thermogenic genes in brown adipose tissue compared with wild type mice exposed to the same thermogenic stress. Consistent with these observations, electron microscopy revealed that mitochondria in brown adipocytes lacking PC-TP were enlarged and elongated. Brown adipocytes cultured from *Pctp*^{-/-} mice exhibited higher oxygen consumption rates in response to norepinephrine [15], indicative of increased fatty acid utilization [38]. Taken together, these findings support a key role for PC-TP in regulating adaptive thermogenesis in brown adipose tissue.

PC-TP Polymorphisms

Naturally occurring coding region polymorphisms have been reported in both mice [39] and humans [40]. In each case, the metabolic consequences were consistent with a role for PC-TP in metabolic regulation of glucose and lipid homeostasis.

A polymorphism in mouse PC-TP related to glucose tolerance

The New Zealand Obese (NZO) and Nonobese Nondiabetic (NON) mouse strains have been utilized to identify *Nidd* quantitative trait loci (QTL) that govern non-insulin dependent diabetes (NIDDM) [41]. NZO mice are characterized by juvenile-onset obesity and maturity-onset non-insulin dependent diabetes. Approximately 50% of NZO males eventually develop obesity-associated diabetes. NON is an unrelated mouse strain with defective insulin secretion by pancreatic β -cells. These mice develop impaired glucose tolerance, but do not progress from impaired glucose tolerance to hyperglycemia [41]. When NON and NZO are crossed, their male (NZOxNON)F1 progeny become obese, but unlike the NZO strain, their frequency of diabetes is increased to 90–100% [41,42]. The gene encoding PC-TP was observed to reside within the QTL for *Nidd3*, a recessive NZO-derived locus on Chromosome 11 [39], which was identified by prior segregation analysis between NZO and NON [41]. Sequence analysis revealed that the NZO-derived PC-TP contained a non-synonymous point mutation that resulted in an Arg120His substitution [39]. Consistent with the structure-based predictions, functional studies demonstrated that Arg120His PC-TP was inactive *in vitro*, most likely due to defective binding of phosphatidylcholines. Although the (NZOxNON)F1 mice have multiple metabolic defects that may contribute to the increased development of diabetes, NZO mice could be relatively protected from diabetes by the presence of two alleles for Arg120His PC-TP.

A polymorphism in human PC-TP that influences LDL size

The accumulation in plasma of small dense LDL particles is a consequence of insulin resistance and confers increased risk for cardiovascular disease [43]. In a genome-wide scan, a QTL affecting LDL particle size and density was localized in a region of chromosome 17 (17q21) [40] that was previously shown to contain the PC-TP gene [13]. A coding polymorphism

(Glu10Ala) was identified in individuals who harbored larger LDL particles than those with the wild type protein. For subjects carrying Glu10Ala PC-TP, there was also a three-fold lower risk of having the atherogenic small dense LDL phenotype. Although this study did not test associations of PC-TP polymorphism with direct measures of insulin resistance, the relationship between small dense LDL and insulin resistance suggests a potential role for PC-TP in modulating insulin sensitivity. However, the functional consequences of the Glu10Ala substitution on PC-TP activity have yet to be explored.

Cellular biology of PC-TP

Studies of subcellular distribution using cell fractionation approaches have indicated that PC-TP is largely present in the cytosolic fraction, with the remainder being evenly distributed over the particulate fractions [44,45]. Confocal laser scanning microscopy using immunofluorescence and fluorescence-labeled PC-TP detect PC-TP in both the cytoplasm and nucleus [46,47]. Consistent with a potential function in fatty acid metabolism, de Brouwer *et al.* [46] demonstrated that, in response to the exposure of endothelial cells to clofibrate, PC-TP associates with mitochondria. This rapid effect appeared to be explained by a posttranslational mechanism involving clofibrate stimulated, PKC-mediated phosphorylation of PC-TP at serine 110. This was evidenced by mutational studies in which relocation did not occur when serine 110 was mutated, but not by a direct demonstration that the protein was phosphorylated.

PC-TP–Interacting proteins

START domains largely reside within multidomain proteins, suggesting that binding of a hydrophobic ligand might regulate the activity of another domain within the same protein [19]. Because PC-TP is a START domain minimal protein (i.e. consists solely of the lipid-binding motif), we hypothesized that the biological activities of PC-TP might necessitate protein-protein interactions [47]. PC-TP is expressed in a variety of tissues of the adult animal [12,13] and as early as embryonic stem cells [12], suggesting potentially distinct roles in metabolism and development. To explore this possibility, yeast two-hybrid screens were performed utilizing cDNA libraries prepared both from adult mouse liver and from embryos [47]. PC-TP-interacting proteins included thioesterase superfamily member 2 (Them2) from liver and the homeodomain transcription factor Paired box gene 3 (Pax3) from the embryo [47]. As illustrated in Figure 2, these were noteworthy because the START superfamily contains multidomain proteins that consist of START plus thioesterase domains in mammals, as well as START plus homeodomains in plants [19,21,22]. Them2 is a mitochondria-associated long chain acyl-CoA thioesterase of uncertain biological function [45,48]. The acyl-CoA thioesterase activity of purified recombinant Them2 was enhanced by recombinant PC-TP [45]. In tissue culture, PC-TP co-activated the transcriptional activity of Pax3, which plays a key role in neural and cardiac development [49]. These findings suggest that PC-TP may engage in distinct regulatory activities that depend upon the tissue expression of different interacting proteins.

Regulatory role for PC-TP in response to peroxisome proliferator activated receptor- α (PPAR α)

Consistent with the influence of PC-TP on lipid and glucose metabolism is its regulation by PPAR α , a ligand-activated nuclear hormone receptor transcription factor that is enriched in oxidative tissues [50]. When activated by endogenous fatty acids and synthetic fibrates, PPAR α regulates genes that promote binding, transport and oxidation of fatty acids, as well as the biliary elimination of plasma cholesterol and glucose metabolism [50]. The promoter region of the PC-TP gene contains peroxisome proliferator response elements (PPREs) [51], and

dietary administration to mice of fibrate drugs upregulate hepatic PC-TP mRNA [52,53] and protein [53–55]. Evidence is now emerging for regulatory roles of PC-TP in the metabolic responses that occur in response to PPAR α activation [53]. Microarray profiling of livers from fenofibrate fed wild type and *Pctp*^{-/-} mice revealed differential expression of numerous metabolic genes, as well as their regulatory transcription factors. Moreover, in tissue culture, PC-TP expression controlled activity of PPAR α , as well as hepatocyte nuclear factor 4 α (HNF4 α) [53], a liver-enriched transcription factor that promotes gluconeogenesis; it also increased lipoprotein-mediated lipid secretion from the liver [56,57]. When taken together, these findings suggest that the mechanism by which PC-TP modulates hepatic metabolism is at least in part via the activation of transcription factors that govern nutrient homeostasis.

Models for metabolic control by PC-TP

Notwithstanding significant gaps in our understanding of the mechanisms by which PC-TP regulates metabolism and energy utilization, it is feasible to construct speculative models (Figure 3). Endogenous fatty acids liberated from adipose tissue during fasting are taken up by the liver and activate PPAR α [50,58]. This upregulates expression of both PC-TP [14,53] and Them2 [45]. Binding of PC-TP to Them2 at the mitochondrial membrane stimulates its fatty acyl-CoA thioesterase activity [47], and this association can be enhanced by PC-TP phosphorylation [46]. Although it is appreciated that StarD1 facilitates cholesterol entry into mitochondria after it binds to the outer mitochondrial membrane and undergoes a phospholipid-dependent conformational change (i.e. the molten globule transition) [59], it is not known whether a similar mechanism contributes to the activity of PC-TP. Because fatty acids gain access to mitochondria when converted to fatty acyl-CoAs by a long chain acyl-CoA synthetase [60], PC-TP-stimulated Them2 activity may limit entry. This would be expected to redirect fatty acids, so that they could become ligands for activation of HNF4 α [61]. However, it is anticipated that some fatty acids would be converted to fatty acyl-CoAs by one of several long chain fatty acyl-CoA synthetases [60]. Upon conversion to diacylglycerols, fatty acyl-CoAs could then limit insulin sensitivity by reducing hepatic insulin signaling [62]. The combination of HNF α activation and reduced insulin signaling would be expected to increase hepatic glucose production. This model is consistent with the observations that the absence of PC-TP in mice is associated with profound increases in hepatic insulin sensitivity and decreases in gluconeogenesis [16].

Presumably under similar control by PPAR α , both PC-TP and Them2 are expressed in extrahepatic oxidative tissues. In these tissues, it is proposed that PC-TP-Them2 interactions also limit access of fatty acids to mitochondria, as illustrated for brown fat (Figure 4). In response to cold stimulus, norepinephrine is released by the sympathetic nervous system and stimulates G protein-coupled β_3 -adrenergic receptors in brown fat [38]. This activates protein kinase (PK) A by increasing cAMP levels. Activated PKA phosphorylates hormone sensitive lipase [63], which hydrolyzes triglycerides from lipid droplets into fatty acid and glycerol. Activated PKA also leads to phosphorylation of PKC through phosphoinositide-3-kinase. This could lead to phosphorylation of PC-TP and translocation to mitochondria [46]. As described for liver (Figure 3), the interaction of PC-TP and Them2 might limit fatty acyl-CoA translocation into mitochondria. Within mitochondria of brown fat, β -oxidation of fatty acids leads to the production of NADH, which is then utilized for the production of heat via uncoupling protein 1. In order to maintain body temperature, this occurs at a higher rate than does ATP production via the electron transport chain. Feedback inhibition occurs when heat provided by brown fat reduces systemic adrenergic stimulation. Feedback inhibition also occurs within brown adipocytes when ATP production becomes sufficient to increase reactive oxygen species (ROS), which results in inhibition of adenylyl cyclase [64] (Figure 4). In the absence of PC-TP expression, increased utilization of fatty acids for heat production might not

be associated with increased ROS production, leading to the observed hypersensitivity to norepinephrine [64].

Future directions

Among the important unresolved questions in the biology of PC-TP is the relationship of the metabolic phenotypes observed for *Pctp*^{-/-} mice and the capacity of the protein to promote intermembrane exchange of phosphatidylcholines *in vitro*. Whereas it is possible that the distribution of phosphatidylcholines among membranes is central to its biological activity, it is attractive to speculate that PC-TP may instead function as a sensor of membrane composition.

Phosphatidylcholine molecular species play critical roles in regulating the physical properties of membranes, including lipid ordering and microdomain formation [65,66]. Fasting is an example of a physiological stress that can alter membrane composition. Activation of PPAR α promotes the synthesis of polyunsaturated fatty acids [67,68] and modulates the molecular species phosphatidylcholines [69]. Although the specific membrane(s) that provide the phosphatidylcholine ligands for PC-TP are not known, presumably the endoplasmic reticulum makes the greatest contribution based on its extent within the cell. Considering the marked preference of PC-TP for binding phosphatidylcholines containing sn-2 polyunsaturated fatty acids [11], changes in these molecular species within membranes would be expected to disproportionately influence the distribution of phosphatidylcholine molecules bound to PC-TP. Because a phosphatidylcholine molecule almost completely occupies the lipid binding pocket (Figure 1b), changes in the spatial configuration of the bound molecule could result in distinct conformations of PC-TP when the lipid-binding site becomes occupied with sn-2 polyunsaturated phosphatidylcholine molecular species. If interactions between PC-TP-protein interactions were to be regulated by these conformational changes, this could provide a cellular mechanism to ascertain changes in membrane composition. For example, phosphatidylcholine-dependent interactions between PC-TP and Them2 could provide a novel link between membrane composition and metabolic control of energy substrate utilization. By modulating transcriptional activity during development, PC-TP could also allow cells to integrate information on membrane composition into decisions concerning Pax3-determined cell fate [70].

Small molecule inhibitors of the phosphatidylcholine transfer activity of PC-TP have recently been identified [71], and these could prove useful in establishing the relationship between the binding and transfer of phosphatidylcholines by PC-TP *in vitro* and its function *in vivo*. Whether different phosphatidylcholine molecular species influence the three dimensional structure of PC-TP or the interactions between PC-TP and other proteins is also an important area for future investigation. Gene targeting of Them2 in mice will also be required to test whether loss of expression yields similar phenotypes as observed in *Pctp*^{-/-} mice. Finally, considering the phenotypes of *Pctp*^{-/-} mice, it is possible that small molecule inhibitors of PC-TP could be of therapeutic value in the management of type 2 diabetes.

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Figure 1. Structure of PC-TP in complex with phosphatidylcholines

(a) Structure of human PC-TP (blue) in complex with phosphatidylcholine (carbon, yellow; nitrogen, dark blue; oxygen, red; phosphorous, orange). (b) Solvent accessible volume of the binding pocket containing phosphatidylcholine. The phosphatidylcholine molecule (color coding as in panel a) occupies approximately 89% of the lipid binding pocket. (c) Interactions of PC-TP amino acid residues (carbon, light blue; nitrogen, dark blue; oxygen, red) with the glycerol-3-phosphorylcholine moiety of 1-palmitoyl,2-linoleoyl-sn-glycerol-3-phosphorylcholine (palmitoyl-linoleoyl phosphatidylcholine) (color coding as in panel a). The structure of 1,2-dilinoleoyl-sn-glycerol-3-phosphorylcholine (dilinoleoyl phosphatidylcholine) is superimposed (gray). (Modified with permission from reference [28].)

Figure 2. PC-TP interacts with protein domains that are found in multidomain START proteins
 (a) Protein-protein interactions. Double-headed dashed arrows depict PC-TP (green) interactions with Them2 and the homeodomain (HD) transcription factor Pax3. These interactions were identified by yeast two-hybrid screening of cDNA libraries from adult liver (left) and mouse embryo (right), respectively. (b) Multidomain protein. Corresponding multidomain START proteins from mammals (left) and plants (right), which contain START domains together with two thioesterase (Thio) domains and a HD plus a leucine zipper (LZ) domain, respectively. BFIT, brown fat inducible thioesterase; CACH, cytosolic acetyl CoA thioesterase; THEA, thioesterase adipose associated. (Reprinted with permission from reference [47].)

Figure 3. Postulated function of PC-TP in the fasting liver

Hepatic uptake of fatty acids during fasting activates (i) PPAR α and increases expression of PC-TP and Them2. (ii) The interaction of PC-TP and Them2 stimulates thioesterase activity and prevents fatty acyl-CoA uptake into mitochondria via carnitine palmitoyl transferase I (CPTI). Within the cytoplasm, these fatty acids may activate HNF4 α , which promotes hepatic export of lipids and glucose. (iii) Fatty acids may also be reconverted by long chain acyl-CoA synthetases (ACSL) into fatty acyl-CoA, which downregulate insulin signaling. Solid arrows indicate metabolic pathways. Relative thicknesses of lines are drawn in proportion to flux of substrate. Hashed lines indicate stimulatory (arrowheads) and inhibitory (line-stops) regulation.

Figure 4. Postulated function of PC-TP in brown fat

During cold exposure in brown fat, (i) norepinephrine binds to β_3 -adrenergic (β_3 -AR) receptors coupled with a G protein. This leads to increased cAMP level and phosphorylation of protein kinase A (PKA). (ii) Activated PKA phosphorylates hormone sensitive lipase (HSL), which hydrolyzes triglycerides (TG) into fatty acids and glycerol. (iii) Fatty acids are converted to fatty acyl-CoA by a long-chain acyl-CoA synthetase (ACSL). (iv) Norepinephrine-induced PKA also leads to activation of PKC through phosphoinositide-3-kinase (PI3K). This could cause (v) phosphorylation of phosphatidylcholine transfer protein (PC-TP), which relocates to mitochondria and interacts with thioesterase superfamily member 2 (Them2). This stimulates thioesterase activity and prevents fatty acyl-CoA uptake into mitochondria. (vi) Within mitochondria, fatty acyl-CoAs are oxidized, yielding NADH that is used for production of heat via uncoupling protein 1 (UCP1), which dissipates the proton gradient established by the electron transport chain (ETC). (vii) Under conditions where ATP production by the ETC exceeds heat production, reactive oxygen species (ROS) produced through the ETC provide feedback inhibition of this signaling pathway. Solid arrows indicate metabolic pathways. Hashed lines indicate stimulatory (arrowheads) and inhibitory (line-stops) regulation.