Minireview

CTP:Phosphocholine Cytidylyltransferase: Paving the Way from Gene to Membrane*

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PC\(^1\) is the most abundant phospholipid in eukaryotic membranes whereas few prokaryotes contain PC. PC is synthesized in eukaryotes by two routes: the CDP-choline pathway (1) and PE methylation (2). PE methylation is the most important contributor to PC formation in *Saccharomyces cerevisiae* (3). In contrast, mammalian cells and tissues predominantly use the CDP-choline pathway (2, 4). PC is the major phospholipid in plant extraplasmatic membranes, and the CDP-choline pathway operates in *Brassica napus* (5), *Arabidopsis thaliana* (6, 7), and *Pisum sativum* (8). PC is present in select prokaryotes, including *Treponema denticola*, whose genome encodes a fusion protein containing both choline kinase and CCT activities (9). On the other hand, *Sinorhizobium meliloti* produces PC by the condensation of choline and CDP-diaclyglycerol catalyzed by the novel PC synthase (10). The CCTs in bacteria do not resemble any of the other known CCTs (11–13).

There are three steps in the CDP-choline pathway. Choline is an essential nutrient in mammals and is transported into the cell and phosphorylated by choline kinase. CCT catalyzes the condensation of CTP and phosphocholine to form CDP-choline, which in turn donates the phosphocholine moiety to diacylglycerol to form PC. CCT mediates the rate-limiting step in this pathway (1) and is unique in that its enzymatic activity is regulated by association with membrane structures (14, 15). A current model posits that bilayer curvature elastic stress is sensed by CCT and governs the degree of membrane association, thus providing a mechanism for both positive and negative regulation of activity (16, 17). The PEMT is the only enzyme in the alternative pathway, and it performs all three of the methylation steps. The significance of PE methylation to PC production is controlled by the expression of PEMT, which is high in liver and testis (4, 18). The goal of this minireview is to highlight recent progress in understanding the regulation of CCT expression and a growing appreciation of its role in cell biology and organ function.

Overview of CCT Biochemistry

The regulatory biochemistry of CCT is discussed in several recent reviews (1, 2, 14, 15, 19–22). The mammalian CCT proteins are divided into four functional domains. The N-terminal domains are distinct and either contain a nuclear localization signal (CCT\(\alpha\)) or not (CCT\(\beta\)). The catalytic domain contains two motifs, HXGH and RTEGISTS, characteristic of the cytidylyltransferase superfamily (23), which are involved in binding CTP (24, 25). A conserved lysine residue between the CTP binding motifs is implicated in phosphocholine binding (25). CCT is a dimer (26, 27). The high resolution structure of the homologous *Bacillus subtilis* G3PCT confirms a role for these features in the catalytic mechanism of the CCT protein family (28–31).

An amphipathic C-terminal \(\alpha\)-helix in the CCTs from mammals, plants, and *Drosophila melanogaster* (22, 32) clearly plays a major role in mediating the lipid interaction and regulation of the enzyme (1, 14, 15, 19, 32). The helical or M domain interacts with both neutral and anionic lipid mixtures and is the primary region of the protein that responds to the bilayer curvature elastic stress (6, 14, 27, 33, 34). Removal of the entire CCT\(\alpha\) C terminus results in an enzyme that retains catalytic activity at high CTP concentrations but cannot respond to lipid regulation (26, 35). The extreme C terminus, or the P domain in mammalian CCT\(\alpha\), can activate the enzyme in response to anionic lipid mixtures when the internal M domain is deleted (35). The CCTs of lower eukaryotes do not have a domain homologous to the M domain but nonetheless are lipid-regulated (22). This group of CCTs includes *S. cerevisiae* (36, 37), *Plasmodium falciparum* (38), and *Caenorhabditis elegans* (39). Clearly more than one sequence motif is capable of forming a structure that can reversibly interact with membrane phospholipids and drive the protein conformational changes associated with catalytic regulation.

The mammalian CCTs are reversibly phosphorylated at multiple serine residues, representing about 25% of the P domain (1). Phosphorylation restricts CCT binding to select lipid vesicles (40) and reduces activity (40, 41). Evaluation of CCT membrane association in lysates or permeabilized cells or in *situ* localization does not reveal a clear relationship between phosphorylation and the cellular location of CCT. Rather, phosphorylation inhibits CCT activity *in vitro* even under optimum lipid activator conditions, consistent with the view that phosphorylation is a mechanism for kinase-regulated signaling (42, 43) to modulate the enzyme activity in addition to membrane lipid composition.

CCT Genes and Isoforms

The CCT protein is derived from two genes on distinct chromosomes in human and mouse. *Pcyt1a* encodes the CCT\(\alpha\) isoform (44, 45), and *Pcyt1b* encodes the CCT\(\beta\)1 (in human only), -\(\beta\)2, and -\(\beta\)3 isoforms (45–47). CCT\(\alpha\) is located on mouse chromosome 16 (3q in human) and CCT\(\beta\) is X-linked in both species. Total cellular CCT activity is the sum of CCT\(\alpha\) and CCT\(\beta\), and any of the four protein isoforms complement a conditional CCT-deficient cell line (45–47). The CCT\(\alpha\) and CCT\(\beta\) proteins are virtually identical in the catalytic and M domains but differ significantly at both termini. CCT\(\alpha\) is found in the cytoplasm associated with membranes, but the majority is in the nucleus as directed by the N-terminal nuclear localization sequence. In contrast, CCT\(\beta\) localizes exclusively outside the nucleus (46). Nuclear CCT\(\alpha\) is associated with chromatin, and a nuclear CDP-choline pathway is active in synthesizing highly saturated, medium chain diacyl- or alkylacyl-PC (48). This observation is consistent with the molecular species distribution of PC produced from the CDP-choline pathway in liver (49) indicating that nuclear CCT participates in PC synthesis. However, nuclear CCT\(\alpha\) is also thought of as a reserve for a prompt response to an extranuclear PC requirement, such as the membrane synthesis associated with mitogenic stimulation (50), or replacement of membrane due to pharmacological disruption of PC synthesis (51). Both hypotheses are possible and are not mutually exclusive. The majority of CCT\(\alpha\) protein is found outside the nucleus associated with glycogen pools (52) in pulmonary cells and tissues (53, 54), which may reflect a high demand for PC synthesis or suggest that the NLS may be cleaved.

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\(^{1}\) The abbreviations used are: PC, phosphatidylcholine; CCT, also known as CT, CTP-phosphocholine cytidylyltransferase; CSF-1, colony-stimulating factor 1; DSPC, disaturated phosphatidylcholine; G3PCT, CTP glycero-3-phosphate cytidylyltransferase; GroPCho, glycerol-3-phosphocholine; HDL, high density lipoprotein; LDL, low density lipoprotein; NLS, nuclear localization sequence; NTE, neuropathy target esterase; PMT, phosphatidylethanolamine N-methyltransferase; PE, phosphatidylethanolamine; PI, phosphatidylinositol; VLDL, very low density lipoprotein.

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Minireview: CCT

CCT in Cell Proliferation and Survival

CCT in Cell Division—The idea that the metabolic pathway regulated by CCT has a critical role in providing PC for cell proliferation arises from the identification of a conditionally lethal cell line lacking CCT activity (55, 56). The alternate route to PC mediated by the PEMT cannot complement this CCT deficiency (57), and PEMT is not expressed at significant levels except in liver and testis (4, 18). The behavior of CCT responds to mitogenic stimulation and contributes to membrane biogenesis in several ways. Progression from G0 to G1 is accompanied by an increased rate of PC synthesis and an immediate, early transient increase in CCT mRNA level due to message stabilization in a macrophase cell line that is dependent on CSF-1 for both growth and survival (58). Following CSF-1 removal, the amount of CCT protein is reduced and located outside the nucleus, and then the mitogenic signal stimulates CCT protein synthesis and the new CCT accumulates and relocates to the cell nucleus (20). The re-addition of serum to lung epithelial cells results in early stabilization of PC synthesis soon after entry into G1 and is accompanied by increased CCT activity where all of the CCT protein is located in the extranuclear compartment and remains there (54). In IEC9 fibroblasts, which are dependent on serum for proliferation but not survival, CCT protein expression is abundant, and a portion of the endogenous nuclear CCT protein migrates to extranuclear membrane sites following serum stimulation (50). On the other hand, serum stimulation of C3H10T1/2 fibroblasts is not accompanied by an early increase in PC synthesis, early changes in CCT mRNA or CCT translocation (54). Thus, the data support the view that if and when prompt extranuclear PC synthesis is required, CCT responds either by gene expression or by translocation. The distribution of CCT remains constant during the cell cycle (54, 59, 60). Membrane PC accumulates during the S phase following increased CCT activity during G1 (54, 60–62), and the CCT activity remains elevated throughout the cell cycle (54, 60, 61) though it is dampened by progressive phosphorylation of the CCTa during the approach to G2 and mitosis (54, 61). CCTa transcription is stimulated 2-fold during the S phase through the phosphorylation (63) and binding (64) of the transcription factor Sp1 to the promoter region (60) in preparation for cell division.

The amount of PC is not solely controlled by the expression of CCT. PC turnover is another key player in PC homeostasis during the cell cycle (61). Turnover is rapid during G1 and decreases during S phase to coordinate PC synthesis and membrane formation with the doubling of DNA and stable RNA. Although the membrane biogenesis program is coordinated with DNA synthesis, it is independent of DNA replication (61), meaning that inhibitors of DNA synthesis do not stop phospholipid accumulation during S phase and thus cannot be used to synchronize membrane biogenesis. In experiments where CCT overexpression is forced, the flux through the metabolic pathway increases, but the amount of PC mass does not (65, 66). This is due to the stimulation of PC turnover and the formation of GroPCho (65, 66). The enzyme responsible is suggested to be the iPLA2 (66–68) and/or the NTE (69). Overexpression of NTE increases GroPCho production, which is offset by reduced choline uptake and incorporation into CDP-choline, suggesting a homeostatic coupling with CCT. On the other hand, overexpression of iPLA2 does not alter PC synthesis but elevates the GroPCho as well as lys-PC and free fatty acid levels (70), which may feed back and regulate CCT.

CCT in Apoptosis—CCT inhibition triggers apoptosis and it is a protease target in the apoptosis cascade initiated by other stimuli. PC synthesis is essential not only for cell growth and proliferation but also for cell survival (71). Depletion of cellular PC due to either a conditional defect in CCT (72, 73), chemical inhibition of CCT activity (19, 74–78), or bacterial infection (79) leads to cell death by apoptosis or paraptosis, a process that has characteristics of both apoptosis and necrosis (80). Reduction in cellular PC activates caspases (51, 80) and induces the endoplasmic reticulum stress-related protein CHOP without raising a general endoplasmic reticulum stress response (73). During the apoptotic response to reduced PC, CCTa itself translocates from the nucleus and becomes a cytoplasmic target for caspase-mediated cleavage of the N-terminal NLS (51). The retention of CCTa protein outside the nucleus is associated with enhanced CCT activity, suggesting a survival response that operates during cell death pathways initiated by other mechanisms such as tumor necrosis factor (81).

Transcriptional Regulation of CCT Expression

Transcription controls the tissue-specific distribution of CCT isoforms. Although CCTs is expressed in virtually every tissue, the CCTa promoter is particularly active in pulmonary alveolar cells (82) where CCTa is expressed at a high level compared with other tissues (4). The transcription factors Sp1, Sp2, and Sp3 activate CCTa gene expression through their interaction with three cis-acting elements in the proximal promoter region (83). Sp1 activates expression during the cell cycle (63, 64), and Sp3 mediates enhanced expression in Ha-Ras-transformed fibroblasts following phosphorylation by the p42/44 kinase (84). Transcriptional enhancer-4 can regulate CCTa expression either by suppression of transcription via its direct binding to the Ehs promoter element or by activation through interaction with Ets-1 (85, 86). Lipid deprivation increases CCTa mRNA synthesis (87), and direct assessment of promoter activity shows that a sterol response element located in the CCTa promoter governs this response (88, 89). Expression and promoter activity are inhibited when sterols are added to the extracellular medium and induced in lipid-depleted medium. Evaluation of CCTa metabolic activity in cell lines with defective sterol response element-binding protein processing, however, suggests that the regulation of CCTa and the CDP-choline pathway may be indirect (90) due to the lack of correlation between CCT expression (as measured by mRNA or protein) and metabolic activity (as measured by radiolabeling). CCTb2 isoform expression increases during nerve growth factor-induced differentiation of axonal membranes (91), but little is known about the CCTb3 promoter and the factors that contribute to the differential expression of CCTb1, CCTb2, and CCTβ3. CCTb3 is not expressed in mice (4) and may be an aberrant transcript in humans. Two distinct CCT isozymes are found in A. thaliana, and their expression increases differentially in response to cold acclimation (7, 92). A cold-response element and a gibberellin-response element are found in the AtCCTb1 promoter region (93). The research on the transcriptional regulation of CCT is in its infancy, and continued work in this area will reveal how CCT expression responds to differentiation and disease.

Tissue-specific Roles for CCT

CCT in the Brain—In brain, CCTb1 and CCTb2 expression are about equal in contrast to other tissues where CCTb3 isoforms are found at roughly 10-fold lower levels. The amounts of CCTa and CCTb2 transcripts become equalized upon differentiation of cultured neuronal cells (4, 91), similar to the levels found in brain. Also, CCTβ3 expression is stimulated in hippocampal neurons treated with the neuropeptide arginine-vasopressin4–8 (94). Lipodystrophies associated with some neuropathological disorders are linked to aberrant CCT activity. For example, Gaucher disease cells accumulate glucosylceramide, which directly activates CCT and stimulates PC synthesis (95). In Sandhoff disease, an accumulation of ganglioside GM2 is associated with reduced CCT activity and PC mass in the brain (96). In both diseases, altered PC metabolism may contribute to the pathology (97). Changes in CCT activity affect the utilization of the diacylglycerol intermediate, changing not only the rate of membrane PC synthesis but also the rate of triglyceride synthesis in an inverse manner (57, 98). Altered PC synthesis can also affect gene expression, as illustrated by the up-regulation of the genes involved in triglyceride formation when PC formation is deficient (99). The heightened expression of CCTβ in the brain suggests that it has a function in this tissue. However, the CCTβ−/− mice do not have a brain phenotype (4).

CCT in the Lung—CCT has a regulatory role in the production of lung DSPC, a major surfactant component secreted into the alveolus. CCTa mRNA stabilization during late gestational maturation of type II pneumocytes increases protein expression (100), glucocorticoid treatment of pulmonary alveolar cells elevates membrane-associated CCT activity (100, 101), and both processes are accompanied by stimulated DSPC synthesis. Signaling regulates CCT activity through a number of mechanisms. Calpain-mediated proteolysis in response to tumor necrosis factor α (102) or oxidized LDL treatment (103) removes both the extreme N terminus and the
regulatory domains of the protein and reduces activity. Altered sphingomyelin (101, 104, 105) or fatty acid metabolism (106, 107) changes the membrane composition and can influence the membrane association of CCT. Sphingomyelin hydrolysis generates sphingosine, an inhibitor of CCT (101, 108), and also reduces CCT expression in alveolar type II cells by a mechanism that is dependent on a negative regulatory element in the promoter region (109). Long chain ceramide is thought to inhibit CCT (104, 105, 110), but the expression of CCT is up-regulated in response to a range of metabolic changes and signaling stimuli. There is no solid evidence that CCT functions with macromolecular partners, but small molecule lipid regulators of CCT are plentiful and transiently produced at diverse intracellular locations. Second, comparative genomics will continue to uncover new CCTs, some of which will be nearly identical to the known proteins, but the investigation of the dissimilar CCTs will provide new insights into protein-lipid interactions at a molecular level. CCT redundancy within a system clearly provides a backup mechanism for cell survival. However, there are also tissue-specific functions of the CCT isoforms that are just beginning to be appreciated. This issue is difficult to address in vitro because the enzyme activity in cell lysates may not reflect its activity in intact cells because of the regulation imposed by the membrane lipid composition, the availability of metabolic intermediates, and the action of degradative pathways. It appears that the genetic approach will be required to sort out the importance of CCT in health and disease. Innovative genetic techniques will be required to pave the way to understanding the intricacies of these systems.

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