Regulation of CTP:phosphocholine cytidylyltransferase by amphitropism and relocalization

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Phosphatidylcholine (PC) synthesis in animal cells is generally controlled by cytidine 5′-triphosphate (CTP):phosphocholine cytidylyltransferase (CCT). This enzyme is amphitropic, that is, it can interconvert between a soluble inactive form and a membrane-bound active form. The membrane-binding domain of CCT is a long amphipathic α helix that responds to changes in the physical properties of PC-deficient membranes. Binding of this domain to membranes activates CCT by relieving an inhibitory constraint in the catalytic domain. This leads to stimulation of PC synthesis and maintenance of membrane PC content. Surprisingly, the major isoform, CCTα, is localized in the nucleus of many cells. Recently, a new level of its regulation has emerged with the discovery that signals that stimulate PC synthesis recruit CCTα from an inactive nuclear reservoir to a functional site on the endoplasmic reticulum.

AMPHITROPISM refers to a property of proteins whose activities are regulated by interconversion between an inactive soluble form and an active membrane-lipid-bound form. Amphitropism is common among proteins that respond to signals generated on membranes: proteins such as kinases, phosphatases, phospholipases, guanine nucleotide exchange factors and cytoskeletal linker proteins. The weak membrane affinity of these proteins (K_d in the range of 10^{-3}–10^{-6} M) can be modulated by soluble ligands such as Ca^{2+} or nucleotides, or by protein modifications such as phosphorylation or palmitoylation (reviewed in Ref. 1). Alternatively, modulation of membrane lipid composition, rather than modification of the protein, can act as the signal to increase membrane affinity. This is the case for proteins that contain binding pockets for specific lipids such as pleckstrin homology (PH) domains or C1 domains. Other amphitropic proteins contain no binding pockets for lipid monomers, but instead respond to changes in membrane physical properties, such as surface charge density or lipid packing defects, which arise as a consequence of changes in lipid composition[1]. One such protein is cytidine 5′-triphosphate (CTP):phosphocholine cytidylyltransferase (CCT), which catalyzes the formation of cytidine 5′-diphosphate choline (CDPcholine), the head-group donor in the synthesis of phosphatidylcholine (PC; Fig. 1). The membrane affinity of CCT is regulated primarily by lipid compositional changes and also by phosphorylation.

Membrane lipid components are continually turning over through the action of phospholipases, many of which are regulated by extracellular signals. If the rate-limiting phospholipid biosynthetic enzymes, including CCT, were primed to respond quickly to phospholipid catabolism, this would maintain compositional homeostasis[2,3]. There is evidence that CCT responds rapidly to PC catabolism in response to exogenous phospholipase C (PLC)[3] or phorbol ester[2,4] and during progression through the G1 phase of the cell cycle[5]. The mechanism is not known, but given the ability of lipids such as diacylglycerol (DAG), phosphatidic acid (PA) and arachidonate to activate CCT *in vitro*, a reasonable hypothesis is that one or more lipid products of PC catabolism generated in cell membranes directly activate the enzyme by promoting membrane binding (Fig. 1).

CCT is recovered in both soluble and particulate fractions of disrupted cells, in keeping with its weak membrane affinity. The soluble, but not the particulate, enzyme requires exogenous lipids for activity. Its distribution reflects the relative rates of membrane binding and dissociation. While CCT is membrane

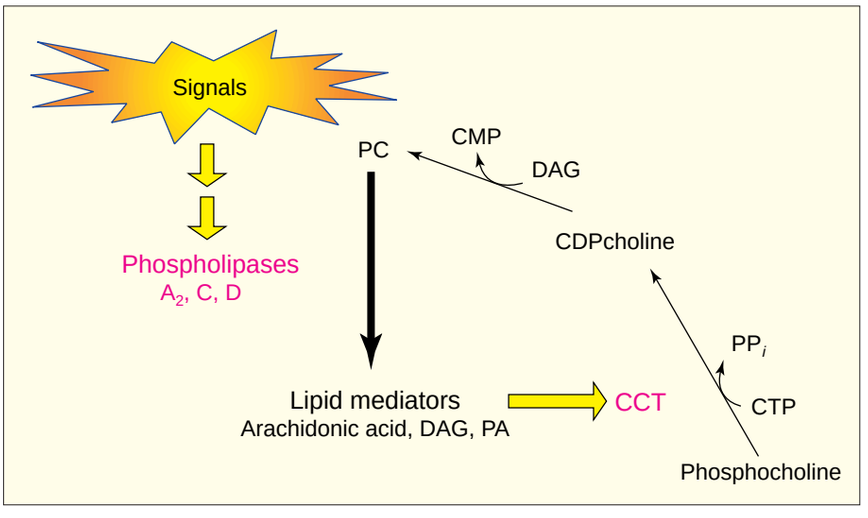

Figure 1
Simplified phosphatidylcholine (PC) metabolic cycle. PC catabolism and anabolism are coordinated. In this model, the lipid products of PC hydrolysis can directly activate CTP:phosphocholine cytidylyltransferase (CCT) by promoting membrane translocation. Abbreviations: CDPcholine, cytidine 5′-diphosphate choline; CMP, cytidine 5′-phosphate; CTP, cytidine 5′-triphosphate; DAG, diacylglycerol; PA, phosphatidic acid.

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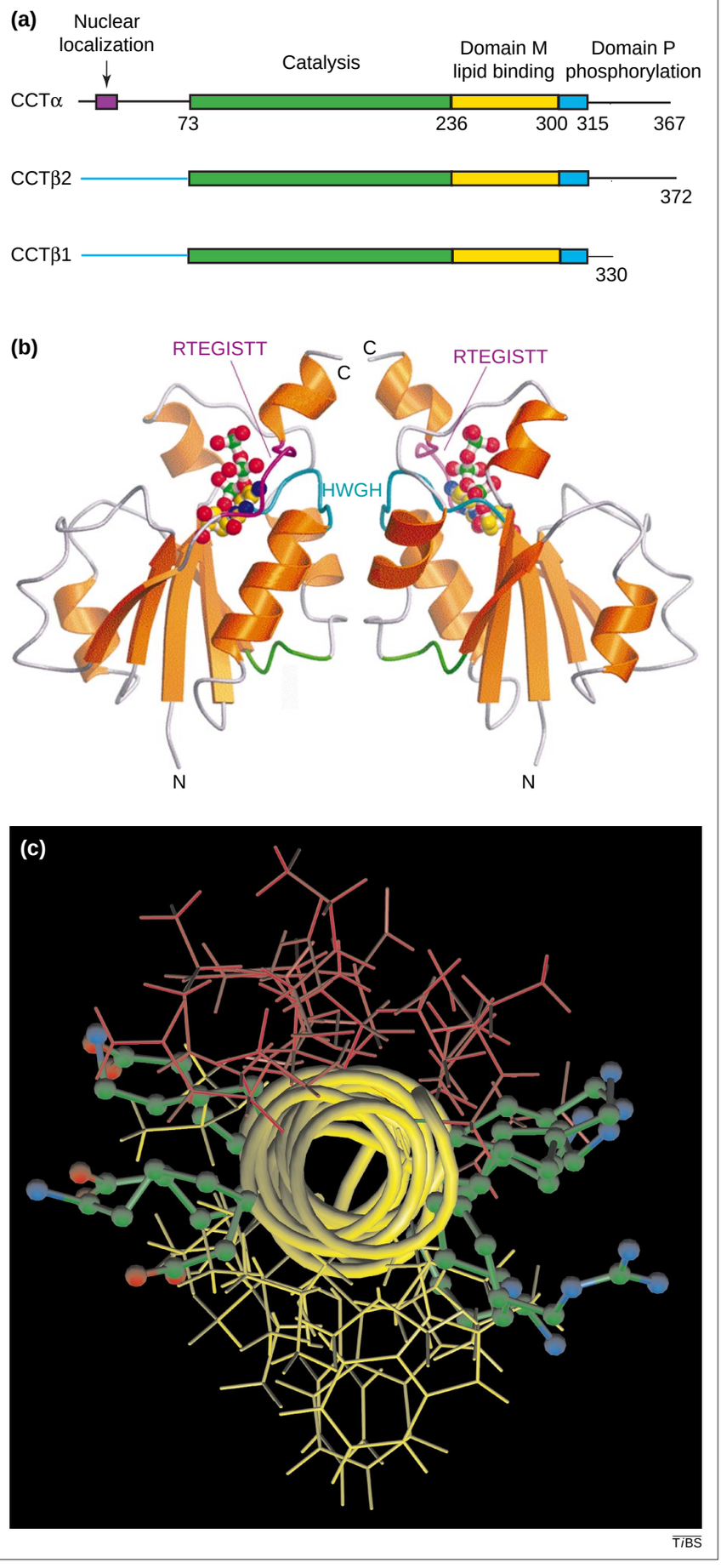

Figure 2

(a) Domain structure of CTP:phosphocholine cytidylyltransferase (CCT) α, β1 and β2. There is high homology among all three isoforms between amino acids 73 and 323. The beta isoforms differ only at their C termini (after residue 323). The phosphorylation domain of β2 is ~40% identical to that of CCTα. **(b)** Structure of the glycerolphosphate CT (GCT) dimer with bound CTP, a model for the catalytic domain of CCT. Key CTP-binding motifs are highlighted. **(c)** Structure of the membrane-binding amphipathic α helix (residues 243–287), based on the atomic coordinates of two overlapping peptides[28]. Side chains of the polar face are red; side chains of the nonpolar face are yellow. The carbon (green), nitrogen (blue), and oxygen (red) atoms of the interfacial side chains are in ball-and-stick representation. Basic residues dominate the interfacial zone, but three glutamates are present on one side (see Refs 25 and 28, for a discussion of their function). Figure 2b has been reproduced, with permission, from Ref. 22.

bound its K_{cat} is elevated up to 80-fold[6]. CCT partitioning between soluble and membrane-bound forms in cells can be modulated by treatment of cells with PLC, protein phosphatase inhibitors, short-chain diacylglycerol, fatty acids, alkyl-phosphocholines and phorbol esters (reviewed in Refs 2,7,8). These stimuli evoke changes in the phosphorylation state of CCT, changes in membrane lipid composition, or both. However, membrane translocation has not been detected with some conditions that stimulate CCT activity[9–11]. The lack of detectable translocation might reflect sensitivity problems inherent in measuring changes in the membrane affinity of a very weak binder, but it is also apparent that cells can regulate CCT by means that do not involve translocation of existing enzyme. For example, CCT expression can be modulated by effects on the degradation rate of the enzyme[12–15], which appears to be accelerated by dephosphorylation[13–15]. Expression can also be modulated by stabilization of the CCT mRNA (Refs 9,16) and, potentially, by alterations in CCT transcription rate[17]. Unlike regulation of expression, translocation enables very rapid changes in the rate of PC synthesis.

CCT structure

Much progress has been made using cDNA mutagenesis, synthetic peptides and genomics towards the delineation of the structural and functional domains

of CCTs. Three human isoforms have been characterized, CCTα, β1 and β2 (Ref. 18). The beta isoforms are splice variants of the same gene, differing only at their C termini. A separate gene encodes CCTα (Ref. 19). The three isoforms are highly similar in the catalytic and membrane-binding domains (Fig. 2a), and all are regulated by lipids[18]. This review focuses on the regulation of ubiquitously expressed CCTα, which is also the best characterized. Proteolysis of CCTα (Ref. 20), together with secondary structure predictions, give the impression of a protein with a compactly folded (protease-resistant) N-terminal head encompassing amino acids 1–236, linked via a protease-accessible hinge to a long helix followed by an unfolded, protease-sensitive C-terminal tail (Fig. 3b).

The catalytic domain is conserved within the cytidylyltransferase (CT) superfamily, which includes phosphoethanolamine CT and phosphoglycerol CT (ECT and GCT)[21]. Bacterial GCT is a prototypic member of the family, consisting solely of the catalytic domain. The structure of this ~120-amino-acid domain has been solved from crystals of GCT in complex with CTP (Ref. 22). It is an αβ dinucleotide fold consisting of a five-stranded parallel β sheet flanked by five helices (Fig. 2b). The predicted secondary structure elements of the CCTα catalytic domain align well with the GCT fold, and there is ~60% amino acid similarity between this CCT domain and GCT, suggesting that the CCT catalytic domain folds into a structure resembling GCT. CTP binding and transition-state stabilization in GCT are mediated mainly by residues in two highly conserved motifs referred to as the HXGH and RTEGISTS motifs (Refs 21,22; Fig. 2b). A CTP-binding role of the HXGH motif has also been demonstrated by mutagenesis in CCTα (Ref. 23).

Evidence suggests that the catalytic domain of CCT also mediates subunit interactions. Both GCT and CCT are homodimers[22,24]. Crosslinking of a CCTα mutant truncated at residue 236 (Ref. 6) and of CCT proteolytic fragments[20] has localized the subunit interactions to the N-terminal domain. The dimer interface of GCT is mostly hydrophobic and buries ~15% of the surface of each GCT monomer[22] (Fig. 2b). Whether the interface and substrate-binding sites are similar in CCT and whether they are restructured by the activation of CCT are important questions for future research.

The membrane-binding domain (domain M) has been identified in CCTα by mutagenesis, proteolysis, as well as studies with synthetic peptides and direct lipid photolabeling and sequencing (reviewed in Ref. 25). Circular dichroism (CD) analyses of synthetic domain M peptides show that anionic lipid vesicles induce a random coil to α-helix transition[26,27]. Two-dimensional nuclear magnetic resonance (NMR) structural analysis of two overlapping, sodium dodecyl sulphate (SDS)-bound peptides spanning residues 236–288 indicate a continuous ~52 residue amphipathic α helix with an N-terminal bend[28]. This bend might constitute part of the hinge to the catalytic domain. The features of domain M are illustrated in Fig. 2c. The polar face is composed of mostly acidic residues, and the interfacial zones are predominantly basic, especially near the N terminus. The nonpolar face contains 18 hydrophobic residues, which should provide ample driving force for membrane binding[28]. In its soluble conformation these residues might be buried, thereby minimizing the hydrophobic driving force and thus the membrane affinity. Other amphitropic proteins use amphipathic helices as membrane-binding domains[1], but of those whose structures have been solved, domain M of CCT is by far the longest. Reversible membrane binding of CCT involves intercalation of domain M as an amphipathic helix into the polar head group and interfacial region of the lipid bilayer (Fig. 3). Quenching of peptide tryptophan fluorescence by bromo adducts at different positions on the acyl chain[26,27] and lipid photolabeling[29] indicate clearly that this domain penetrates the nonpolar core of the bilayer. These findings have led to the hypothesis that lipid compositions that facilitate the folding of domain M into an amphipathic helix and intercalation into the bilayer will be the most effective CCT activators. Although we know the structure of domain M when complexed with SDS as a membrane mimetic, the structure of this domain in the context of the whole enzyme or in the absence of amphiphile is unknown. There is reason to suspect that it interacts with the catalytic domain in an inhibitory capacity, as discussed below.

The phosphorylation domain of CCTα has been mapped to the C-terminal ~50 residues (domain P). In CCTα purified from the baculovirus system, all 16 serines between residues 315 and 367 are >50% phosphorylated, as determined by direct sequencing of high-pressure liquid chromatography-purified phosphopeptides[30]. Phosphopeptide maps of CCTα from insect cells and from other cultured cells are similar[30]. Analysis of CCTα C-terminal deletion mutants expressed in COS cells also indicate multiple phosphorylations confined to domain P (Ref. 31). This proline-rich domain is the most protease-sensitive region of the protein[20,29], suggesting a surface location and flexible structure. CCTα has seven sites for a proline-directed kinase, five potential sites for glycogen synthase kinase 3 and one site for casein kinase II. However, the enzyme has proven to be a poor substrate for phosphorylation *in vitro* by these kinases[31,32]. CCTα can be dephosphorylated *in vitro* by protein phosphatase 1 (Ref. 32). The identities of the kinases and phosphatases that regulate CCT phosphorylation in cells are unknown.

Mechanism of regulation by membrane lipid binding

What properties of PC-deficient membranes promote CCT binding? The lipids that activate purified CCT have been characterized using model membranes and are chemically diverse (see citations in Ref. 25). The most potent are anionic phospholipids and fatty acids, and their effectiveness relates mainly to the negative charge of the headgroup. Other less potent activating lipids include DAG and other neutral lipids with small polar head groups, unsaturated phosphatidylethanolamine (PE), and oxidized PC. The combined action of DAG plus anionic lipid is synergistic at low, physiological membrane compositions[33]. How can CCT respond to such a varied lot of lipids?

The stronger affinity of the enzyme and domain M peptides for anionic membranes compared to pure PC membranes[25–27,33] can be explained by the interfacial positioning of the many basic amino acids, concentrated especially in the N-terminal section of domain M (Fig. 2c). Binding to charged membranes appears to be a two-step process: electrostatic adsorption followed by hydrophobic interactions, which involve intercalation into the nonpolar core of the membrane[25,33]. A two-step binding mechanism is a common feature of amphitropic protein–membrane interactions[1]. Activation of CCT requires the second insertion step. When insertion is blocked by using viscous gel phase rather than fluid phosphatidylglycerol (PG) membranes, the enzyme binds

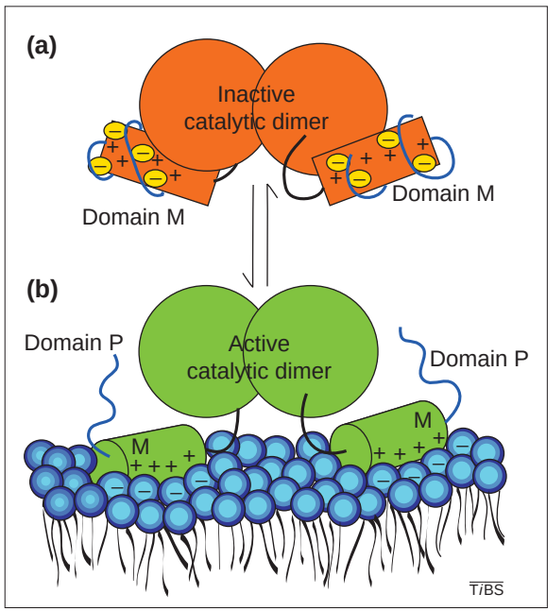

Figure 3

Model of the CTP:phosphocholine cytidylyltransferase (CCT) α dimer in its **(a)** soluble and **(b)** membrane-bound forms. The discrete domains of N-terminal head, hinge, amphipathic helix, and C-terminal tail are best illustrated in (b). In (a), domain M is complexed intramolecularly with the catalytic domain. In (b), partitioning of domain M into the interfacial region of a lipid bilayer disrupts the interaction between the catalytic domain and domain M, leading to activation of the catalytic domain. The conformation of domain M bound to membranes is an α helix, but is not known for the soluble form. Domain P is modeled as a flexible, negatively charged coil, forming electrostatic interactions with the positively charged residues of domain M in the soluble form. The phosphates of domain P could compete with phospholipid negative charges. Dephosphorylation of the membrane-bound form would eliminate this competition and stabilize membrane binding.

electrostatically, but is not active[33]. The first electrostatic step is not involved in binding to membranes containing unsaturated PE, DAG or oxidized PC, and these lipids are generally less potent than the acidic lipids[33,34].

What promotes CCT insertion and activation? Four membrane properties have been suggested: interfacial packing defects, low lateral pressure, acyl chain disorder and curvature strain. That lipid-packing defects (surface cracks) drive membrane binding is supported by an anomalous activation at the gel-to-liquid crystalline phase transition[25]. At this transition, discontinuities in the regular molecular packing occur at the boundaries between gel and fluid phases, which might serve as CCT insertion sites. In addition, lipids with small headgroups, such as DAG, would create surface voids not present in pure phospholipid bilayers[25]. Low lateral surface pressure (i.e. looser packing) is supported by a preference for highly curved versus planar bilayers and the ability of domain M peptides to bind equally well to both anionic and PC monolayers when a low lateral packing pressure is imposed[27]. Disordering of acyl chains by oxidized PC, measured by deuterium NMR, directly correlates with CCT activity, and both the effects on order and CCT activity are reversed by sphingomyelin[34]. Negative curvature strain is a property of lipid activators such as unsaturated PE and DAG. The molecular geometries of these lipids (referred to as type-II lipids) tend to make each monolayer of the bilayer curl towards the water to form a concave surface, but this is prevented by stronger attractive forces between the acyl chains of each monolayer. Upon CCT insertion halfway into one monolayer of such membranes, this curvature strain would be relaxed. That the relaxation of curvature strain can drive CCT binding to some membranes is supported by a correlation between the activation of CCT and the calculated monolayer curvature energy of dioleoyl PE and other type-II lipids[35,36], and the antagonism of lipids that induce positive curvature strain[7,35,37]. These four interrelated properties have not yet been rigorously disconnected to establish their relative importance. It could be that each class of lipid activator promotes a different set of membrane physical features. Solving this problem will be greatly facilitated by the recently developed method for purifying CCT in a lipid-free form[6].

How does phosphorylation affect membrane binding and activation? There is a strong correlation between membrane translocation and CCT dephosphorylation (reviewed in Ref. 8), but the dephosphorylation occurs subsequently to the membrane-binding event[38]. To probe the role of phosphorylation, mutants lacking sets of phosphoserines in domain P have been expressed in the mutant cell line CHO-58, which lacks functional CCTα at the nonpermissive temperature[15]. These studies have revealed that phosphorylation is not required for CCT to produce PC at a rate that will maintain a normal cell growth rate, and that, although the phosphorylated enzyme has lower membrane affinity, activating lipids can overcome the phosphorylation signal[15]. *In vitro* studies have shown that the dephosphorylated wild-type enzyme[32] or a mutant lacking domain P (Ref. 39) requires less anionic lipid for activation. An electrostatic switching mechanism has been suggested in which the phosphates on domain P compete with lipid negative charges for interactions with the positively charged lysines on the adjacent domain M (Ref. 32). In summary, phosphorylation appears to fine-tune the membrane affinity, and in some conditions this could be all that is required as a regulatory switch to modulate CCT activity in cells.

How does lipid binding activate the enzyme? This is arguably the most important question. It has been firmly established, based on CD and NMR analyses with peptides, that lipid binding promotes an α-helical conformation for domain M (Refs 26–28). This change in domain M conformation upon membrane insertion is translated to the catalytic domain, in an as yet unknown way, to increase K_{cat} by more than 80-fold[6] and, to a lesser extent, the affinity for CTP (Refs 6,40). The key to understanding the role of domain M came from analysis of CCTα1–236, lacking domains M and P. CCTα1–236 is constitutively active[6,41], whereas the activities of mutants lacking only domain P are lipid dependent[15,31,39]. The K_{cat}/K_m value for CCTα1–236 was 30–50% of the lipid-activated wild-type enzyme value, but the kinetics were the same with or without lipid. Moreover, the truncated enzyme expressed in CCT-deficient CHO58 cells was able to generate PC at a faster rate than the wild-type enzyme[41]. These data suggest that domain M can induce an inhibitory constraint at the active site[6,41]. The function of membrane binding is to relieve this constraint. If domain M is deleted, membrane binding is not required for an active enzyme. This model, invoking an autoinhibitory membrane-binding domain (Fig. 3), is compatible with the general mode of regulation of many enzymes, including others regulated by membrane interactions[1]. The nature of the inhibitory constraint and how it is relieved by changes in the conformation and membrane interactions of domain M is a major quest for future research.

Which cellular signals control CCT translocation?

In contrast to the progress made in defining the functional domains of CCT and in elucidating the mechanism and function of membrane binding, the regulatory mechanisms for CCT translocation to cell membranes in response to physiological signals remain fairly obscure. Many studies have examined the effects on CCT of loading cells with the lipids that regulate the enzyme *in vitro*. Exogenously added fatty acids stimulate massive translocation of CCT to membranes. However, because the free fatty acid content can be elevated to ~15% of the total cellular phospholipid by such manipulations[42], it cannot be concluded from such studies that the membrane distribution of CCT is regulated by fatty acid content. More convincing are the findings that blockade of fatty acid production inhibits glucocorticoid activation of CCT and PC synthesis in aveolar cells[43], and that exogenous fatty acids overcome the inhibition of CCT in a CHO sterol auxotrophic mutant cell line[44]. However, a complication is that fatty acid treatment can also lead to elevated DAG (Refs 4,8). Loading cells with short-chain DAG also promotes CCT binding to membranes independently of protein kinase C (PKC)[45], as does treatment of cells with PLC to generate DAG *in situ* (see Refs 3,7,8 and citations therein). Cells starved for choline have reduced membrane PC:PE ratios, resulting in CCT translocation to membranes. When choline-deprived or PLC-treated cells are supplemented with choline analogs, CCT dissociates from membranes in proportion to the predicted bilayer-stabilizing effect of the modified phospholipid (see Refs 7,8 and citations therein). These studies indicate that the elevation of certain minor lipid components or the depletion of PC in cell membranes can activate the enzyme, and agree with the analyses of pure CCT behavior *in vitro* with lipid vesicles. However, they do not reveal what lipid compositional changes, if any, activate CCT in response to physiological signals.

In an attempt to dissect out the physiological activator of CCT, several investigations have provided evidence for coupling between CCT and the regulated turnover of PC via phospholipase D (PLD; see Fig. 1). In a variety of cells, phorbol ester leads to the PKC-dependent activation of PLD, generating increases in PA and its metabolite, DAG (Ref. 2). Phorbol ester also stimulates CCT translocation and PC synthesis. Neutrophils[4] and HeLa cells[45] treated with phorbol ester showed a very close kinetic correspondence between DAG production via PC turnover and membrane translocation of CCT. Further evidence for coupling between PLD and CCT was obtained with neuroblastoma clonal variants that show no stimulation of the PC metabolic cycle in response to phorbol ester[46]. In these cells overexpression of PKC and MARCKS (a PKC substrate), leading to activation of PLD, restored phorbol ester induction of PC metabolism. Because PA and DAG have synergistic effects on CCT activity and binding to lipid vesicles[32,33], the CCT response to agonists that activate PLD might involve a recognition of membrane properties generated by the combination of PA and DAG (negative surface charge and headgroup spacing–curvature strain).

To which cellular membrane does CCT translocate?

The bulk of CCTα is nuclear. For many years it had been held that the two CCT forms were cytosolic and endoplasmic reticulum (ER)-bound, based on sedimentation patterns. Eventually, immunofluorescence analysis with CCTα-specific antibody confirmed a predominantly diffuse nuclear localization in a variety of cell cultures and in liver slices[47]. In primary hepatocytes expressing both α and β isoforms, immunofluorescence and electron microscopy with an antibody recognizing both isoforms revealed CCT in both the cytoplasm and the nucleus[48]. Recently, a careful evaluation of CCT localization in several cell types using isoform-specific antibodies has demonstrated that CCTβ isoforms are confined to the ER, and that CCTα is present on the ER in addition to the nucleus[18].

Nuclear localization of CCTα requires the N-terminal nuclear localization sequence (NLS). Deletion of residues 8–28 in CCTα, containing the ^{12}RKRRK^{16} motif, transformed the nuclear expression into predominantly cytoplasmic expression[49]. Deletion of domains M and P, or substitution of the 16 phosphoserines with alanine in domain P, did not affect the nuclear localization when expressed in the CCT-defective CHO-58 cells[15,41], suggesting that dephosphorylation does not drive nuclear export. As long as the NLS signal is present the enzyme appears nuclear.

The function of nuclear CCT is a puzzle, as the enzyme preceding it in the CDP-choline pathway is cytosolic and the enzyme succeeding it appears to be ER bound. Given that other enzymes involved in the production of lipid second messengers from PtdIns(4,5)P_2 and PC have a nuclear localization[50], perhaps the nuclear presence of CCT is connected with lipid signaling. Alternatively, the nucleus might serve as a holding bin for an inactive CCT reserve. Nuclear sequestration as a mechanism to control protein function is emerging[51] alongside the well-established mechanism of cytoplasmic sequestration. The molecules that CCT interacts with in the nucleus are unknown.

Cell-cycle regulation of CCT localization. The localization studies described above did not take the cell-cycle status into account. Northwood *et al.* examined the cellular localization of CCT in IIC9 fibroblasts during the release from quiescence (G0) into the cell cycle[52]. CCT expression was unchanged during this transition, but cellular distribution was modulated, as assessed by immunofluorescence. In quiescent cells, CCT was confined to the nucleus. Between 10 min and 4 h after addition of serum to stimulate entry into the cell cycle, it translocated to the cytoplasm where it co-localized with an ER marker protein. Between 4 and 8 h post serum, CCT returned to the nucleus. The expression and cytoplasmic localization of CCTβ isoforms were unaffected by serum stimulation; the movements were restricted to CCTα. The shuttling of CCTα between the nucleus and the ER closely paralleled an increase in its membrane affinity, enzymatic activity and stimulation of PC synthesis. These data suggest that the nuclear enzyme serves as an inactive reserve, which is recruited to the ER, where it is activated by membrane binding. Studies with CHO-58 cells containing a temperature-sensitive CCTα allele provide evidence for a large reserve of inactive CCTα (Ref. 53), which might explain the predominantly nuclear location usually observed (see Box 1).

It is clear that ER binding is not essential for activation of CCT. Enrichment of cells with oleic acid or treatment with PLC results in strong translocation from the diffuse nuclear pool, not to the ER, but to the nuclear envelope, and this movement is accompanied by an activation of CCT (Refs 54,55). Perhaps in these model systems, the enzyme binds to the membrane of nearest proximity that is enriched in the lipid activators. To verify that the nuclear to cytoplasmic transport of CCT is required for its activation during re-entry into the cell cycle, an inhibitor of this process must

be identified. The nuclear export mechanism for CCTα has not yet been characterized. There is no obvious leucine-rich export sequence in CCTα, and its export is not inhibited by leptomycin[52], which targets proteins containing this motif.

Because the phosphorylation state of CCT does change in concert with activity changes during the cell cycle[5], Northwood *et al.* examined whether phosphorylation changes provide the signal for the nuclear→ER translocation during re-entry into the cell cycle[52]. In quiescent cells, nuclear CCT was highly phosphorylated; it was progressively dephosphorylated upon re-entry into the cell cycle, but the kinetics of dephosphorylation lagged behind the activation of CCT and its translocation to the ER. CCT also returned to the nucleus in dephosphorylated form[52]. Clearly, net phosphorylation changes are not the driving force for CCT shuttling between nucleus and ER.

An alternative hypothesis, that CT translocation to the ER during the G0→G1 transition is controlled by lipid second messengers, remains to be tested. If the signals for translocation were purely lipid mediated, why would CCT be targeted to the ER and not other cell membranes? Do the regulatory lipids accumulate predominantly in the ER at the appropriate time to coordinate with other signals? Other signals downstream of external ligands could assist the recruitment of CCT to the ER, such as modification of a CCT-binding protein to direct targeting to the ER or to eliminate retention in the nucleus. The question of specificity in membrane targeting goes beyond the case of CCT and applies to the translocation of all amphitropic proteins[1].

Conclusion and outlook

The amphitropism of CCT involves a long amphipathic α helix (domain M) that partitions into membrane bilayers deficient in PC. The enzyme responds to one or more physical properties of the PC-deficient bilayer, such as surface charge, packing density and curvature strain, but the relative importance of each is unclear. The membrane activation of CCT is apparent from analyses of the binding of pure enzyme to lipid vesicles, as well as studies of the enzyme in cultured cells. In cells, however, the amphitropism of CCT involves more than simple membrane partitioning and might be regulated by shuttling between nuclear and ER compartments, although additional examples are needed to establish control of CCTα by relocalization. The factors regulating the movement in and out of the nucleus need to be resolved, as does the function of CCTα in the nucleus and the roles of the individual isoforms. Finally, resolution of the mechanism of activation of the catalytic domain upon binding of domain M to membranes will require intensive structural analysis.

Acknowledgements

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Box 1. Note added in proof

While this report was in press, a paper was published presenting an alternative view of the localization of CCT during cell cycle progression[56].

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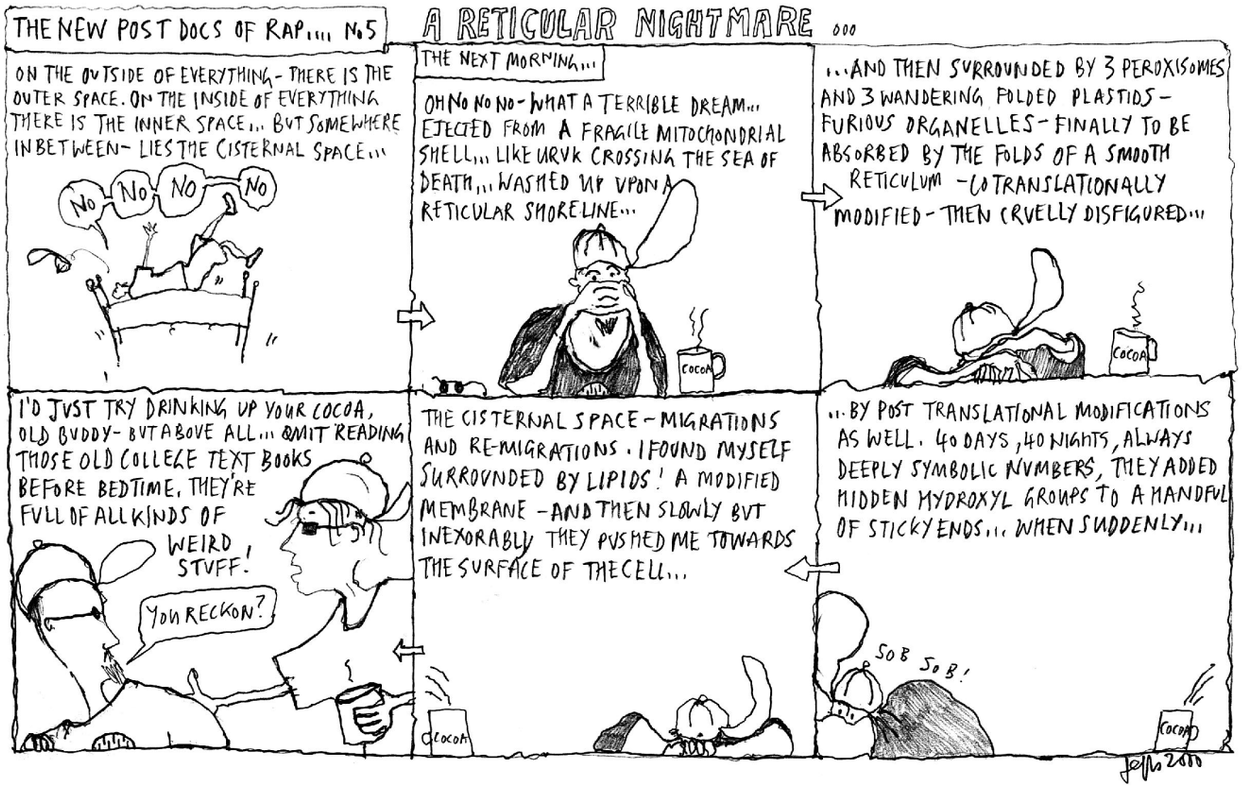

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