Interactions among pathways for phosphatidylcholine metabolism, CTP synthesis and secretion through the Golgi apparatus

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Phosphatidylcholine is the major phospholipid in eukaryotic cells. It serves as a structural component of cell membranes and a reservoir of several lipid messengers. Recent studies in yeast and mammalian systems have revealed interrelationships between the two pathways of phosphatidylcholine metabolism, and between these pathways and those for CTP synthesis and secretion via the Golgi. These processes involve the regulation of the CDP-choline and phosphatidylethanolamine-methylation pathways of phosphatidylcholine synthesis, CTP synthetase, phospholipase D and the phospholipid-transfer protein Sec14p.

**PHOSPHATIDYLCHOLINE (PtdCho)**: (PtdCho, see Fig. 1) is the major membrane phospholipid in eukaryotic cells. In addition to being the major structural component of cellular membranes, PtdCho serves as a reservoir for several lipid messengers: it is the source of the bioactive lipids lysophosphatidylcholine, phosphatidate, diacylglycerol, phosphatidate, platelet activating factor and arachidonic acid. The generation of these lipid messengers from PtdCho depends on metabolism of the latter. Recent studies of Saccharomyces cerevisiae mutants and mammalian systems have uncovered novel interrelationships between the two pathways for PtdCho metabolism, as well as interactions between these pathways and those for CTP synthesis and secretion through the Golgi apparatus. Here, we focus on these interrelationships in yeast and in mammalian systems, and raise questions about the way in which the cell regulates the involvement of PtdCho metabolism with these cellular processes.

**Complex interactions in phosphatidylcholine synthesis**

PtdCho is synthesized by two alternative pathways: the CDP-choline pathway and the phosphatidylethanolamine (PtdEtn)-methylation pathway (Fig. 2). In mammalian cells, all three steps in the PtdEtn-methylation pathway are catalysed by one enzyme, whereas, in yeast, the PEM1/CPE2-encoded methyltransferase catalyses the first methylation step and the PEM2/DEP1-encoded methyltransferase catalyses the last two methylation steps. The PtdEtn used in the methylation pathway is derived from phosphatidylethanolamine, which is used for PtdCho synthesis (shown in blue in Fig. 3). The PtdEtn subsequently is methylated to phosphatidylcholine (PtdCho) through the CDP-choline pathway. The resulting choline can then be reincorporated into PtdCho through the CDP-choline pathway.

The prevailing view has been that the CDP-choline pathway is a salvage pathway used by cells when PtdEtn methylation is compromised. However, recent work on mutants that have defective phosphatidylcholine synthesis and lack phospholipase D (PLD) has shown that the CDP-choline pathway contributes to PtdCho synthesis even when wild-type cells are grown in the absence of exogenous choline. In yeast, PtdSer is synthesized from phosphatidylethanolamine (PtdEtn; see Fig. 3). In mammalian cells, all three steps in the PtdEtn-methylation pathway are catalysed by one enzyme, whereas, in yeast, the PEM1/CPE2-encoded methyltransferase catalyses the first methylation step and the PEM2/DEP1-encoded methyltransferase catalyses the last two methylation steps. The PtdEtn used in the methylation pathway is derived from phosphatidylethanolamine, which is used for PtdCho synthesis (shown in blue in Fig. 3). The PtdEtn subsequently is methylated to phosphatidylcholine (PtdCho) through the CDP-choline pathway. The resulting choline can then be reincorporated back into PtdCho through the

**Figure 1**

Structure of phosphatidylcholine. R₁ and R₂ are fatty acids; usually, R₁ is saturated and R₂ is unsaturated.
CDP-choline pathway and the phosphatidate is recycled back into PtdCho by the PtdEtn-methylation pathway and into other phospholipids – for example, phosphatidylinositol (PtdIns) – via CDP-choline pathway, and the phosphatidylethanolamine (PtdEtn) methylation pathways.

**Figure 2** Phosphatidylcholine (PtdCho) is synthesized by the CDP-choline (a) and phosphatidylethanolamine (PtdEtn)-methylation pathways (b). Choline kinase (CK), choline-phosphate (choline-P) cytidylyltransferase (CCT), and choline phosphotransferase (CPT) catalyse reactions in the CDP-choline pathway (shown in blue). PtdEtn N-methyltransferase (PEMT) catalyses the three-step methylation of PtdEtn in the PtdEtn-methylation pathway (shown in red). In yeast, one methyltransferase enzyme catalyses the first methylation reaction and a second methyltransferase enzyme catalyses the last two methylation reactions. AdoHcy, adenosine homocysteine; AdoMet, S-adenosylmethionine; PtdMME, phosphatidymethylmethionine; PtdDME, phosphatidymethylethanolamine.

Cytidine triphosphate (CTP) and phosphatidic acid (PA) are shown in black. CDP-diacylglycerol (CDP-DAG) synthase (CDS), PtdSer decarboxylase (PSD), and the phosphatidylethanolamine (PtdEtn) methyltransferase (PEMT) catalyse reactions that lead to the formation of PtdCho by the PtdEtn-methylation pathway (shown in red). The reactions catalysed by CTP synthase (CTPS) and phospholipase D (PLD) are shown in black.

**Figure 3** CTP synthetase and phospholipase D play important roles in the synthesis and turnover of phosphatidylcholine (PtdCho). The CDP-choline pathway enzymes choline kinase (CK), choline-phosphate (choline-P) cytidylyltransferase (CCT) and choline phosphotransferase (CPT) are shown in blue. The enzymes CDP-diacylglycerol (CDP-DAG) synthase (CDS), PtdSer decarboxylase (PSD), and the phosphatidylethanolamine (PtdEtn) methyltransferase (PEMT) catalyse reactions that lead to the formation of PtdCho by the PtdEtn-methylation pathway (shown in red). The reactions catalysed by CTP synthase (CTPS) and phospholipase D (PLD) are shown in black.
CTP synthesis and phosphatidycholine metabolism

CTP is essential for phospholipid synthesis in yeast and in mammalian cells. It is the immediate precursor of the activated, energeric phospholipid-pathway intermediates CDP-diacylglycerol, CDP-ethanolamine and CDP-choline (Fig. 3). Proper production of CTP, therefore, should be critical for normal PtdCho biosynthesis. Novel insights into the modulation of PtdCho synthesis by CTP biosynthesis. Proper production of CTP, therefore, should be critical for normal PtdCho biosynthesis. Novel insights into the metabolism of PtdCho synthesis by CTP in yeast have come from studies of a Glu161Lys mutant of the 6-phosphatoate encoded CTP synthetase22. In yeast, CTP synthetase is an essential enzyme that is responsible for the synthesis of CTP from UTP (Fig. 3). The Glu161Lys mutation is not as sensitive to product inhibition by CTP as the wild-type enzyme21. Cells that carry this mutation exhibit elevated levels of CTP and alterations in overall lipid metabolism22. The major affect of the mutation on phospholipid synthesis is an increase in the use of the CDP-choline pathway for PtdCho synthesis. The choline required for this synthesis comes from turnover of PtdCho that is synthesized by the CDP-choline pathway. The Glu161Lys mutation in CTP synthetase causes significant increases in the synthesis of PtdCho and phosphatidate, and a decrease in the rate of PtdSer synthesis. McDougal et al.23 have attributed the increased use of the CDP-choline pathway to an increase in the availability of CTP for the CTP synthetase reaction. The decrease in the rate of PtdSer synthesis is consistent with a decrease in the rate of PtdCho synthesis through the CDP-choline pathway. The mechanism for this regulation might be direct inhibition of PtdSer synthase activity by CTP (Ref. 23). The Glu161Lys mutation also causes an increase in total neutral-lipid content at the expense of total phospholipids22. The increase in total neutral lipids is due to increases in triacylglycerol, free fatty acids, and ergosterol esters22. These changes are reminiscent of the changes in lipid metabolism that occur when wild-type cells enter the stress-like conditions of stationary phase23. Thus, the activation of the CDP-choline pathway as a consequence of the Glu161Lys mutation in CTP synthetase is detrimental to the regulation of lipid metabolism, and proper regulation of PtdCho synthesis through the CDP-choline pathway is important for yeast-cell physiology.

We have known for a long time that CTP levels are an important factor in determining the rate of PtdCho synthesis in mammalian cells. Choy and Vance24 provided the first demonstration that CTT is rate-limiting in the CDP-choline pathway: they showed that the elevated rate of PtdCho synthesis in poliovirus-infected HeLa cells is due to the elevated level of CTP (Ref. 24). Subsequent studies have suggested that exogenous cytidine stimulates PtdCho synthesis by increasing the levels of CTP25-27, a CTT substrate. Of course, the activity of any enzyme will be modified by changes in the concentration of its substrate – but CTT concentration is not saturating. Enzymological studies of purified CTT, however, have suggested a mechanism for the relationship between cellular CTP levels and CTT activity that is more elaborate than this. CTT is a lipid-activated phosphoenzyme that becomes dephosphorylated upon activation in the cell28. The kinetic effect of the binding of lipids to CTT is a significant decrease in the K_m for CTT (Ref. 27). Moreover, the phosphorylation state of CTT affects lipid binding: the dephosphoenzyme has a higher affinity for lipids than does the phosphorylated enzyme28. The phosphorylation state of the enzyme, therefore, can affect the K_m for CTT via modulation of lipid binding. Thus, this enzyme can be sensitive to and can balance the importance of CTP levels, the membrane composition and the state of its own phosphorylation in the cell.

Phosphatidycholine metabolism and the secretory pathway

Yeast. Trafficking of proteins from the Golgi complex involves considerable membrane flux and, given that PtdCho is the major membrane phospholipid, there is probably a regulatory link between secretion and PtdCho metabolism. Recent studies, in fact, have suggested that there is an interaction between PtdCho metabolism and the secretory pathway that involves enzymes in the CDP-choline pathway, a phosphatidyltransfer protein (Sec14p) and PLD (PLDp). In vitro, Sec14p catalyses the transfer of PtdIns and PtdCho between membrane surfaces29. In vivo, Sec14p function is essential for cell viability and budding of vesicles from the Golgi complex (Fig. 4)29. The sec14 mutation is suppressed (i.e. bypassed) by mutations in the genes that encode the CDP-choline pathway enzymes choline kinase (chi1), CTT (ctt1) and choline phosphotransferase (cct1) – the chi1 sec14 double mutant, for example, is viable29. In other words, the synthesis of

PtdCho by the CDP-choline pathway is lethal in the absence of a functional Sec14p. Synthesis of too much PtdCho by the CDP-choline pathway appears to be detrimental to the secretory process. Sec14p could regulate the PtdIns/PtdCho ratio in Golgi membranes and downregulate PtdCho synthesis by directly inhibiting CTT activity (Fig. 4)29 and/or by preventing consumption of the diacylglycerol used for PtdCho synthesis through the CDP-choline pathway29. The enzymes in the PtdIns-metabolism if that are not to be involved with Sec14p function: mutations in this pathway do not bypass its essential function29.

Recent work by Henry and co-workers30,31 has indicated that PLD activity plays a role in connecting the CDP-choline pathway and secretion. Cells that carry mutations in the CDP-choline pathway enzymes exhibit a choline-excretion phenotype30. This phenotype depends on PLD1/SPO14-encoded-PLD-mediated turnover of PtdCho synthesized by PtdCho and the inability of cells to reincorporate free choline into PtdCho through the CDP-choline pathway31. The choline-excretion phenotype is exacerbated if mutations in genes for CDP-choline-pathway enzymes are combined with a temperature-sensitive sec14 (sec14ts) mutation (when grown at the restrictive temperature)31. The increase in choline excretion in these mutants requires activation of PLD (Ref. 11). Furthermore, such mutations fail to bypass the sec14ts phenotype if the gene that encodes PLD is also deleted, and secretion is not restored in these triple mutants32. These results indicate that the correct levels of the CDP-choline-pathway enzymes and PLD are important for secretion and viability in yeast. Henry and co-workers30,32 have argued that phosphatidate derived from the PLD-mediated turnover of PtdCho in the chi1 sec14 double mutant is the basis for the suppression of the sec14 mutation. This hypothesis is supported by the work of Bankaitis and co-workers32, who have shown that phosphatidate levels increase in response to PLD activity. These workers have previously proposed that diacylglycerol plays an essential role in the Golgi secretory function33. They now propose that PLD activity yields sufficient phosphatidate for phosphatidate-phosphatase enzymes to provide a diacylglycerol pool that mediates protein secretion33. However, a specific requirement for
phosphatidate rather than diacylglycerol is further supported by studies in mammalian systems.  

**Mammals.** One might expect that mechanisms that interconnect secretion and PtdCho biosynthesis would be found in mammals as well as in yeast. As in yeast, mammalian phospholipid-transfer proteins have been implicated in secretion, but direct interactions between these proteins and PtdCho biosynthesis have not been demonstrated. An indirect relationship might exist, however. Vesicle budding requires hydrolysis of PtdCho by PLD; PLD would then neesaptate PtdCho biosynthesis to achieve homeostasis. CCT is activated in response to PtdCho degradation. Work by Weinhold and co-workers suggested that there is another connection between PtdCho metabolism and secretion. They discovered that transcytosis-associated protein (TAP), also known as p115) interacts with CTP synthetase, PLD and Sec14p. A unifying theme is evident: the rate-limiting step in the CDP-choline pathway plays a role in the secretory pathway. Data indicate that the PtdCho biosynthesis is regulated coordinately through the action of CTP synthetase, PLD and Sec14p. How are these proteins regulated, and how does this regulation impact on the secretory process and cell physiology? Multiple forms of CTP synthetase, PLD and Sec14p exist; multiple forms of several other lipid-biosynthesis enzymes are common in eukaryotic systems. Are the multiple forms of CTP synthetase, PLD and Sec14p localized to different cellular compartments, and how would such compartmentation impact on regulation? What mechanisms control the communication between pathways if they are localized in different places? We still have much to learn about the relationships between phospholipid metabolism and other metabolic processes. This is an exciting time in science because the molecular tools for addressing such relationships are becoming more available.

**References**

HLA-DM – an endosomal and lysosomal chaperone for the immune system

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Molecular chaperones are involved in a variety of cellular processes, including stabilization of newly synthesized polypeptide chains, assembly of oligomers, transport of proteins and organelle biogenesis. They are known to exert their activity in the cytosol, endoplasmic reticulum, mitochondria and chloroplasts. HLA-DM is the first example of a molecular chaperone that operates in lysosomes: it plays a crucial role in endosomal and lysosomal proteolytic systems.

As part of the immune response to pathogenic invaders, several types of vertebrate cell present proteolytic fragments of endocytosed foreign proteins in the context of specialized peptide receptors, the major histocompatibility complexes (MHC) class II molecules, specialized peptide receptors that are expressed by antigen-presenting cells of the immune system.

For the generation of antigenic peptides from endocytosed proteins, MHC class-II-positive antigen-presenting cells (APCs) generally exploit the conventional proteolytic system of endosomal and lysosomal organelles. The lysosomal environment is characterized by low pH, reducing conditions and high proteolytic activity, and thus provides optimal conditions for protein unfolding and degradation, thereby giving rise to peptides. However, in order to be loaded onto MHC class II molecules, peptides must fulfill some structural requirements. They must possess certain amino acid side chains at so-called ‘anchor’ positions; these side chains allow binding to the groove in the MHC class II molecule, owing to the high degree of variation in the antigenic peptide-binding groove. The specificity of any such protective molecules would have to be rather broad. The degree of variation in the antigenic peptide-binding groove is at least 13–15 residues in length. The peptide-binding groove in the MHC class II molecule, owing to the high degree of variation in the antigenic peptide-binding groove, must be at least 13–15 residues in length. Consequently, peptide antibodies to specific types of such protective molecules would have to be rather broad. The specificity of any such protective molecules would have to be rather broad. Consequently, peptide antibodies to specific types of such protective molecules would have to be rather broad.

To capture antigenic fragments before quantitative proteolysis is for MHC class II molecules to become present in proteolytic compartments; this is in fact the case in APCs. Consequently, binding

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