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; see Fig. 1) is the major membrane phospholipid in eukaryotic cells[1–3]. 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 lysoPtdCho, phosphatidate, diacylglycerol, lysophosphatidate, 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.

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Complex interactions in phosphatidylcholine synthesis

PtdCho is synthesized by two alternative pathways[1–4]: 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*/*CHO2*-encoded methyltransferase catalyses the first methylation step and the *PEM2*/*OPI3*-encoded methyltransferase catalyses the last two methylation steps. The PtdEtn used in the methylation pathway is derived from phosphatidylserine (PtdSer; see Fig. 3). In yeast, PtdSer is synthesized from CDP-diacylglycerol and serine (Fig. 3), whereas, in mammalian cells, PtdSer is synthesized by an exchange reaction between PtdEtn or PtdCho and serine[4]. Nearly all of the genes that encode the enzymes involved in PtdCho metabolism in these two types of organism have been isolated and characterized, and many of the enzymes have been purified and studied[1,3–8].

In *S. cerevisiae*, PtdCho is synthesized primarily by the PtdEtn-methylation pathway (shown in red in Fig. 3). However, the CDP-choline pathway (shown in blue in Fig. 3) becomes essential for PtdCho synthesis if the enzymes in the PtdEtn-methylation pathway are defective. For example, mutants in which synthesis of PtdSer or PtdEtn, or methylation of PtdEtn, is defective require exogenous choline for growth, because they must synthesize PtdCho through the CDP-choline pathway[3]. Mutants in which PtdSer or PtdEtn synthesis is defective can also synthesize PtdCho if they are supplemented with ethanolamine, which is used for PtdEtn synthesis through the CDP-ethanolamine pathway (Fig. 3)[3]. The PtdEtn subsequently is methylated to form PtdCho in the PtdEtn-methylation pathway.

The prevailing view has been that the CDP-choline pathway is a salvage pathway used by cells when PtdEtn methylation is compromised[9]. However, recent work on mutants that have defective PtdCho 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[10]. The PtdCho synthesized through the PtdEtn-methylation pathway is constantly turned over to yield free choline and phosphatidate (Fig. 3)[10]. This reaction is catalysed by PLD (Fig. 3)[11]. The free choline is then incorporated back into PtdCho through the

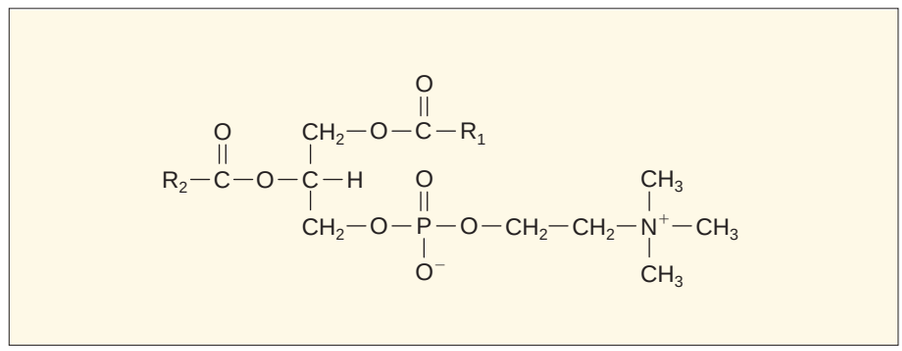

Figure 1

Structure of phosphatidylcholine. R_1 and R_2 are fatty acids; usually, R_1 is saturated and R_2 is unsaturated.

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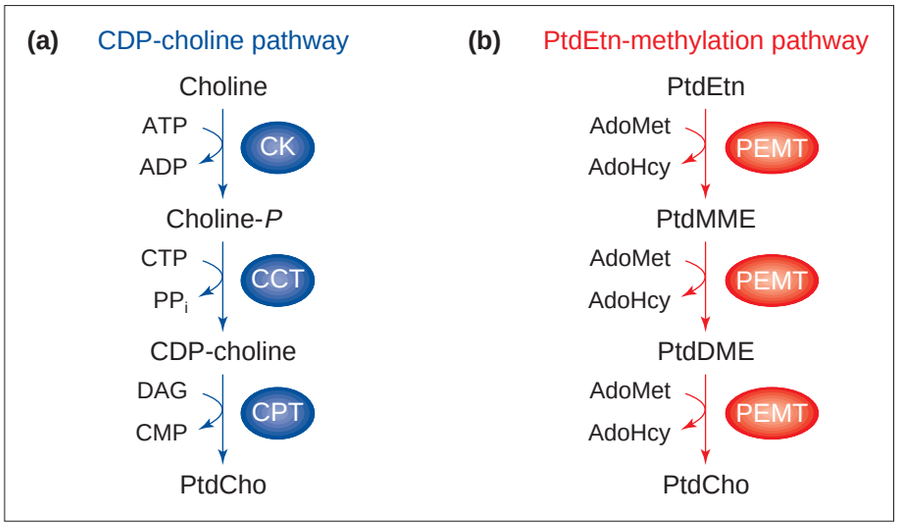

Figure 2

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, adenoasylhomocysteine; AdoMet, *S*-adenosylmethionine; PtdMME, phosphatidylmethylethanolamine; PtdDME, phosphatidyldimethylethanolamine.

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-diacylglycerol (Fig. 3)[10,12].

Whereas, in yeast, PtdCho is derived primarily by methylation of PtdEtn, the major pathway in all mammalian tissues is the CDP-choline pathway. PtdEtn methylation is, however, active in mammalian liver[13]. From 5% to 40% of hepatic PtdCho is estimated to be formed by methylation of PtdEtn[14,15]. Why is this pathway so active in liver but not in other cells? To investigate the role of PtdEtn methylation in liver, Vance and co-workers[16] produced a mouse in which PtdEtn methylation is defective (i.e. a mouse that lacked the gene that encodes PtdEtn *N*-methyltransferase). Under normal laboratory conditions, the homozygous knockouts have no obvious phenotype. However, when they are fed a choline-poor diet, the mice suffer dramatic liver damage within three days[17]. The PtdCho content of the liver decreases by ~50%, and there is an extensive loss of serum lipoproteins – an observation consistent with a defect in the ability of the PtdCho-deficient liver to secrete lipoproteins normally. A possible explanation for this pathology is that the PtdEtn-methylation pathway is a necessary supplement to the CDP-choline pathway in the liver in times of stress, such as starvation[17]. The demand for PtdCho biosynthesis in liver is much greater than in other tissues because liver synthesizes PtdCho for export in serum lipoproteins and bile. The inability of the methyltransferase-deficient mice to synthesize PtdCho for secretion might result in accumulation of deleterious metabolic intermediates or toxic products normally secreted in bile.

In cells other than hepatocytes, little if any PtdCho is made by methylation of PtdEtn. In fact, expression of PtdEtn *N*-methyltransferase cannot be induced in non-hepatic cells, even when the cells are deprived of choline[18,19]. Expression of exogenous PtdEtn methyltransferase can, however, affect the CDP-choline pathway in non-hepatic cells. When cells that normally do not express PtdEtn *N*-methyltransferase are transfected with a cDNA that encodes it, they express the enzyme and synthesize appreciable quantities of PtdCho through methylation of PtdEtn[20,21]. In response to the elevated levels of PtdCho, choline-phosphate cytidylyltransferase (CCT) levels decrease in proportion to the activity of PtdEtn *N*-methyltransferase; this regulation appears to occur at the level of gene expression[21]. We are only beginning to elucidate the factors that control the expression of the gene that encodes CCT; this system will therefore be very useful if we are to decipher how the PtdCho content of a cell can affect CCT synthesis.

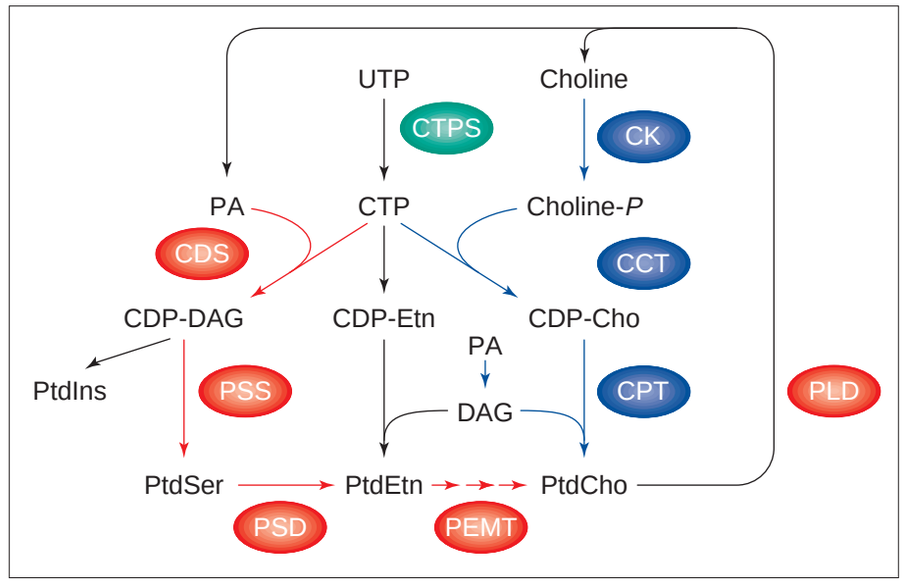

Figure 3

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 synthase (PSS), PtdSer decarboxylase (PSD), and the phosphatidylethanolamine (PtdEtn) methyltransferases (PEMT) catalyse reactions that lead to the formation of PtdCho by the PtdEtn-methylation pathway (shown in red). The reactions catalysed by CTP synthetase (CTPS) and phospholipase D (PLD) are shown in black. CDP-Etn, CDP-ethanolamine; PA, phosphatidate; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine.

CTP synthesis and phosphatidylcholine metabolism

CTP is essential for phospholipid synthesis in yeast and in mammalian cells. It is the immediate precursor of the activated, energy-rich 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 in yeast have come from studies of a Glu161→Lys mutant of the *URA7*-encoded CTP synthetase[22]. In yeast, CTP synthetase is an essential enzyme that is responsible for the synthesis of CTP from UTP (Fig. 3). The Glu161→Lys mutant is not as sensitive to product inhibition by CTP as the wild-type enzyme[22]. Cells that carry this mutation exhibit elevated levels of CTP and alterations in overall lipid metabolism[22]. 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 PtdEtn-methylation pathway. The Glu161→Lys mutation in CTP synthetase causes significant increases in the rate of synthesis of PtdCho and phosphatidate, and a decrease in the rate of PtdSer synthesis. McDonough *et al.*[23] have attributed the increased use of the CDP-choline pathway to an increase in the availability of CTP for the CCT reaction. The decrease in the rate of PtdSer synthesis is consistent with a decrease in the rate of PtdCho synthesis through the PtdEtn methylation pathway. The mechanism for this regulation might be direct inhibition of PtdSer synthase activity by CTP (Ref. 23).

The Glu161→Lys mutation also causes an increase in total neutral-lipid content at the expense of total phospholipid[22]. The increase in total neutral lipids is due to increases in triacylglycerol, free fatty acids, and ergosterol esters[22]. These changes are reminiscent of the changes in lipid metabolism that occur when wild-type cells enter the stress-like conditions of stationary phase[9]. Thus, the activation of the CDP-choline pathway as a consequence of the Glu161→Lys 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 Vance[24] provided the first demonstration that CCT 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 CTP[25,26], a CCT substrate. Of course, the activity of any enzyme will be modified by changes in the concentration of its substrate – if that concentration is not saturating. Enzymological studies of purified CCT, however, have suggested a mechanism for the relationship between cellular CTP levels and CCT activity that is more elaborate than this. CCT is a lipid-activated phosphoenzyme that becomes dephosphorylated upon activation in the cell[1]. The kinetic effect of the binding of lipids to CCT is a significant decrease in the K_m for CTP (Ref. 27). Moreover, the phosphorylation state of CCT affects lipid binding: the dephosphoenzyme has a higher affinity for lipids than does the phosphorylated enzyme[28]. The phosphorylation state of the enzyme, therefore, can affect the K_m for CTP 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.

Phosphatidylcholine 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 phospholipid-transfer protein (Sec14p) and PLD (Pld1p). *In vitro*, Sec14p catalyses the transfer of PtdIns and PtdCho between membrane surfaces[29]. *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 (*cki1*), CCT (*cct1*) and choline phosphotransferase (*cpt1*) – the *cki1 sec14* double mutant, for example, is viable[29]. 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 CCT activity (Fig. 4)[30] and/or by preventing consumption of the diacylglycerol used for PtdCho synthesis through the CDP-choline pathway[31]. The enzymes in the PtdEtn-methylation pathway do not appear to be involved with Sec14p function: mutations in this pathway do not bypass its essential function[29].

Recent work by Henry and co-workers[10,11] 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 phenotype[10]. This phenotype depends on *PLD1*/*SPO14*-encoded-PLD-mediated turnover of PtdCho synthesized by PtdEtn methylation, and the inability of cells to reincorporate free choline into PtdCho through the CDP-choline pathway[10,11]. The choline-excretion phenotype is exacerbated if mutations in genes for CDP-choline-pathway enzymes are combined with a temperature-sensitive *sec14* (*sec14*^{ts}) mutation (when grown at the restrictive temperature)[10]. The increase in choline excretion in these mutants requires activation of PLD (Ref. 11). Furthermore, such mutations fail to bypass the *sec14*^{ts} phenotype if the gene that encodes PLD is also deleted, and secretion is not restored in these triple mutants[11]. 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-workers[10,11] have argued that phosphatidate derived from the PLD-mediated turnover of PtdCho in the *cki1 sec14*^{ts} double mutant is the basis for the suppression of the *sec14* mutation. This hypothesis is supported by the work of Bankaitis and co-workers[32], 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 function[31]. They now propose that PLD activity yields sufficient phosphatidate for phosphatidate-phosphatase enzymes to provide a diacylglycerol pool that mediates protein secretion[32]. However, a specific requirement for

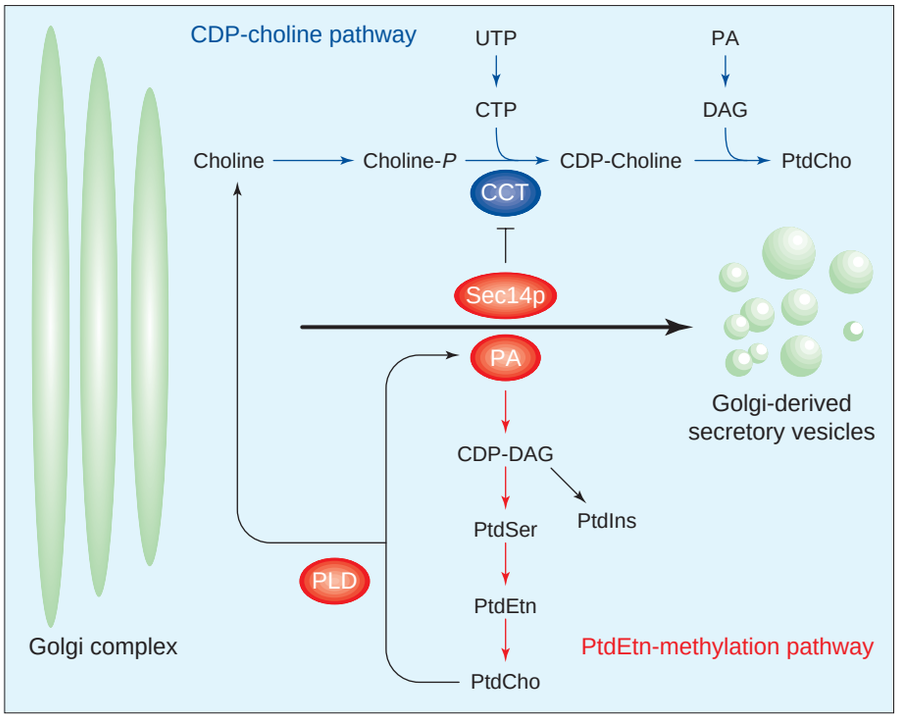

Figure 4

Involvement of phosphatidylcholine (PtdCho) metabolism with CTP synthesis and the secretory pathway. The choline used for the synthesis of PtdCho through the CDP-choline pathway (shown in blue) is derived from transport of exogenous choline into the cell or the phospholipase D (PLD)-mediated turnover of PtdCho. The phosphatidate (PA) derived from PtdCho turnover is recycled back into PtdCho, and other phospholipids such as phosphatidylinositol (PtdIns), through CDP-diacylglycerol (CDP-DAG). The synthesis of CTP is essential to PtdCho synthesis by the CDP-choline and phosphatidylethanolamine (PtdEtn)-methylation pathways. Elevated CTP synthesis favors increased use of the CDP-choline pathway. However, this might be detrimental to cell physiology. In yeast, synthesis of PtdCho by the CDP-choline pathway is lethal in the absence of a functional Sec14p. Sec14p, a PtdIns/PtdCho transfer protein, is essential for cell viability and the formation of Golgi-derived secretory vesicles. Sec14p could down-regulate choline-phosphate (choline-*P*) cytidylyltransferase activity, the rate-limiting step in the CDP-choline pathway[37,38]. PLD also plays an essential role in the secretory pathway. Data indicate that the PA derived from the PLD-mediated turnover of PtdCho mediates secretory-vesicle budding. CCT, choline-*P* cytidylyltransferase.

phosphatidate rather than diacylglycerol is further supported by studies in mammalian systems[33,34] (see below).

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[35,36], 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[33,34]. Degradation of PtdCho by PLD would then necessitate PtdCho biosynthesis to achieve homeostasis. CCT is activated in response to PtdCho degradation[37,38], presumably because its lipid-binding domain is sensitive to the composition of PtdCho-deficient membranes. It is not unreasonable, therefore, to expect activation of CCT in response to endocytosis-associated 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 CCT[39–43]. TAP/ p115 is necessary for vesicle transport within the Golgi apparatus[43], for transcytosis[42] and possibly for post-mitotic vesicle fusion[44]. The effect of the binding of TAP/p115 to CCT on the activities of the two proteins is not yet clear.

It might be difficult to envision a truly physiological role for interactions between CCT and either secretory vesicles or TAP/p115, because CCT is nuclear in many situations[1] whereas secretory vesicles and TAP/p115 are cytoplasmic. Recently, however, Lykidis and co-workers[45] discovered another isoform of CCT, CCTβ. This new isoform is similar to the nuclear CCT in its catalytic and regulatory domains, but lacks the nuclear-targeting sequence and appears to be cytoplasmic[45]. Although interactions between CCTβ and TAP/p115 or phospholipid-transfer proteins have not yet been investigated, we suspect that CCTβ plays a role in connecting vesicle traffic with PtdCho metabolism.

Conclusions and outlook

Studies in yeast and mammalian systems have revealed complex interrelationships among PtdCho-synthesis pathways, CTP-synthesis enzymes, PLD and Sec14p. A unifying theme is evident: although the CDP-choline pathway plays an important role in PtdCho synthesis, its misregulation is detrimental to cell physiology. Figure 4 summarizes the conclusions that can be drawn from the studies discussed here. The processes shown are an oversimplification of the complex regulation of PtdCho metabolism, CTP synthesis and the secretory pathway. Clearly, pathways for metabolism of other phospholipids, particularly PtdIns and its derivatives, play roles in metabolic regulation and cellular trafficking; these are discussed elsewhere[29].

Several questions require further examination. The CDP-choline and PtdEtn-methylation pathways appear to be regulated coordinately through the actions 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[4]. 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.

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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 compartments during loading of major histocompatibility complex (MHC) class II molecules, specialized peptide receptors that are expressed by antigen-presenting cells of the immune system.

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 complex (MHC) class II molecules at the cell surface[1]. The foreign peptide cargo can be recognized by T lymphocytes, which are activated, produce cytokines and provide help for activation and differentiation of antibody-producing B cells.

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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[2]. 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 fulfil some structural requirements. (1) 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. This requirement is different among the 50–100 allelic variants of the MHC class II molecule, owing to the high degree of variation in the antigenic-peptide-binding groove. (2) They must be at least 13–15 residues in length. Consequently, proteolysis of antigenic proteins has to be limited in order to generate and preserve such peptides.

How can this be achieved in a compartment that has an extremely high proteolytic activity that normally cleaves proteins to di- and tri-peptides and amino acids? One possibility is that the processing compartments contain specialized peptide shuttles that bind intermediary oligopeptides, prevent their terminal degradation and deliver them to MHC class II molecules. In order to serve all types of MHC class II variants, the specificity of any such protective molecules would have to be rather broad. Repeatedly, investigators have postulated that such peptide-binding molecules interfere with antigen processing in the endocytic system[3], but no proof of their involvement has been obtained.

An alternative, and more direct, way to capture antigenic fragments before quantitative proteolysis is for MHC class II molecules themselves to be present in proteolytic compartments; this is in fact the case in APCs. Consequently, binding

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