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Review

# Phosphatidylcholine synthesis and its catabolism by yeast neuropathy target esterase 1

J. Pedro Fernández-Murray, Christopher R. McMaster *

*Atlantic Research Centre, Departments of Pediatrics and Biochemistry and Molecular Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7*

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**Abstract**

Phosphatidylcholine (PtdCho) is the major phospholipid component of eukaryotic membranes and deciphering the molecular mechanisms regulating PtdCho homeostasis is necessary to fully understand many pathophysiological situations where PtdCho metabolism is altered. This concept is illustrated in this review by summarizing recent evidence on Nte1p, a yeast endoplasmic reticulum resident phospholipase B that deacylates PtdCho producing intracellular glycerophosphocholine. The mammalian and *Drosophila* homologues, neuropathy target esterase and *swiss cheese*, respectively, have been implicated in normal brain development with increased intracytoplasmic vesicularization and multilayered membrane stacks as cytological signatures of their absence. Consistent with a role in lipid and membrane homeostasis, Nte1p-mediated PtdCho deacylation is strongly affected by Sec14p, a component of the yeast secretory machinery characterized by its ability to interface between lipid metabolism and vesicular trafficking. The preference of Nte1p toward PtdCho produced through the CDP–choline pathway and the downstream production of choline by the Gde1p glycerophosphodiesterase for resynthesis of PtdCho by the CDP–choline pathway are also highlighted.


*Keywords:* Phospholipase; Metabolism; Phosphatidylcholine; Neuropathy target esterase; *Saccharomyces cerevisiae*

## 1. Phospholipid homeostasis

Lipid homeostasis refers to a dynamic situation where lipid synthesis, trafficking and degradation are coordinately orchestrated to provide and sustain the characteristic lipid compositions found among different membranous subcellular structures. Furthermore, proper lipid composition at defined membrane locations contributes to vesicle formation, trafficking and function, protein folding and maturation, and lipid signalling. Lipid biosynthesis and its regulation are quite well understood but much less is known about their intracellular trafficking and degradation, as well as the molecular mechanisms that coordinate these processes toward lipid homeostasis. Identification of the components involved in such complex cellular function and deciphering their roles are necessary steps to fully understand the molecular basis of many pathophysiological situations where lipid homeostasis is altered. *Saccharomyces cerevisiae* has proven to be a formidable experimental system to address such an important biological question. Besides its known tractability in terms of classical and molecular genetics, in recent years, the development of genomic and proteomic analysis technologies for *S. cerevisiae* are producing some of the most integrated views of the cellular physiology.

Phosphatidylcholine (PtdCho) is the major phospholipid in the membranes of eukaryotic cells where it is synthesized by methylation of phosphatidylethanolamine (PtdEtn) (methylation pathway) or by the CDP–choline (CDP–Cho) (or Kennedy) pathway [1,2]. In addition to its structural role in cellular membranes, PtdCho is a source of several lipid molecules involved in cell signalling [3]. In the present review, we summarize PtdCho synthesis in yeast and treat in depth its turnover by Nte1p, an endoplasmic reticulum (ER) phospholipase B that apparently deacylates PtdCho derived only from the CDP–Cho pathway.

## 2. Overview of phosphatidylcholine synthesis

In yeast, PtdCho constitutes around 30% to 50% of total cellular phospholipid depending on the genetic background and

* Corresponding author. Atlantic Research Centre, 5849 University Avenue, Room C302, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.
*E-mail address:* christopher.mcmaster@dal.ca (C.R. McMaster).

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on the availability of the aqueous soluble lipid precursors in the growth medium. Yeast synthesizes de novo PtdCho through the PtdEtn methylation pathway. PtdEtn is sequentially methylated producing PtdCho by Cho2p, which catalyzes the first methylation step, with the second and third methylations catalyzed by Opi3p. Alternatively, PtdCho is synthesized through the CDP–Cho pathway where choline (Cho) molecules are phosphorylated by choline kinase (Cki1p) to produce phosphocholine. CTP: phosphocholine cytidylyltransferase (Pct1p) is the rate-limiting step in the pathway and uses CTP to further activate phosphocholine to produce CDP–Cho for condensation with diacylglycerol (DAG) to produce PtdCho by 1,2-diacylglycerol cholinephosphotransferase (Cpt1p) (Fig. 1) [1,2].

## 3. Phosphatidylcholine turnover by phospholipase Bs

Different types of phospholipases have been described in yeast with activity against PtdCho. Phospholipase B deacylates PtdCho producing glycerophosphocholine (GroPCho) and free fatty acids. Three different genes coding for phospholipase B activities (*PLB1-3*) have been identified in *S. cerevisiae* with their protein products located at the plasma membrane within the periplasmic space [4–7]. None of the three genes are essential, since the triple mutant strain is viable. Plb1p is the main activity responsible for PtdCho deacylation at the plasma membrane with its production of GroPCho released into the extracellular medium. Besides their potential roles in phospholipid homeostasis, recent data suggest a function for these phospholipases in the utilization of exogenous phospholipids, via glycerophosphoalcohol formation, as alternate sources of phosphate [8–10]. Recently, an endoplasmic reticulum (ER) resident phospholipase in yeast, Nte1p, was discovered.

## 4. Nte1p, yeast homologue of neuropathy target esterase

In 2001 Patton-Vogt and coworkers published a seminal paper reporting a PtdCho deacylating activity responsible for the production of intracellular GroPCho. This activity was induced by the addition of Cho to the culture medium. A further increase in GroPCho levels was observed when the growth temperature was raised to 37 °C concurrent with Cho supplementation. It was shown that the stimulating effects of the addition of Cho and of the elevation of temperature on the deacylating activity were dependent on an active CDP–Cho pathway for PtdCho biosynthesis. Interestingly, the rate of PtdCho synthesis through the CDP–Cho pathway was 2-fold higher at 37 °C than at 30 °C, whereas the rate of synthesis by the methylation pathway remained essentially unaffected upon temperature shift, suggesting a homeostatic mechanism linking increased PtdCho synthesis via the CDP–choline pathway with increased PtdCho deacylation [11].

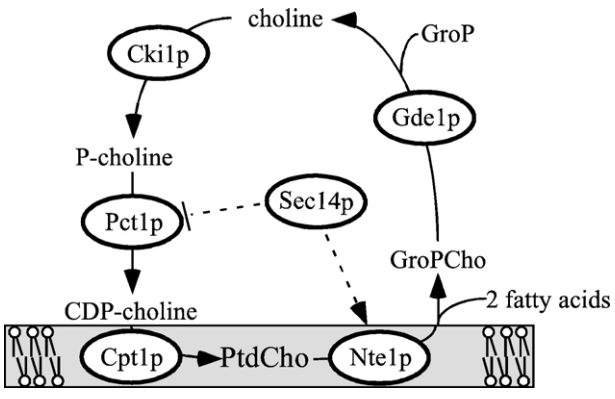


Fig. 1. Model of the regulation of the CDP–choline pathway for phosphatidylcholine synthesis and its turnover by Nte1p. Sec14p negatively regulates PC synthesis by inhibiting Pct1p, and positively regulates PC catabolism by activating Nte1p mediated PC deacylation. Cho molecules formed by Gde1p are released into the cytoplasm where they can be used for resynthesis of PC by the CDP–Cho pathway. Solid lines indicate metabolic steps and dotted lines regulatory interactions.

Mammalian Neuropathy Target Esterase (NTE) was originally identified through studies aimed to uncover the molecular basis of a neuropathy triggered by toxic organophosphorus esters (reviewed in [12,13]). NTE exhibited in vitro esterase activity against phenolic esters and was inhibited upon covalent reaction with certain organophosphorus esters. Based on the protein sequence data, a human NTE cDNA was isolated with the predicted protein containing four potential transmembrane stretches and a C-terminal motif found in the active site of many members of the serine hydrolase family. Homologues from *Drosophila*, *C. elegans* and yeast were identified that shared the highly conserved C-terminal region [14]. Metabolic labelling studies demonstrated conclusively that the yeast homologue of NTE, encoded by the open reading frame Yml059c and named *NTE1*, bears the activity previously characterized by the Patton-Vogt lab in that it deacylates PtdCho synthesized through the CDP–Cho pathway producing GroPCho (Fig. 1) [15]. Nte1p resides at the ER and is a serine hydrolase with similar sensitivities against organophosphorus esters as its mammalian homologue. Confirming the functional similarities between these two proteins, it was shown in COS cells that the levels of intracellular GroPCho varied as the amount of active NTE was manipulated by its overexpression or its reduction through siRNA and organophosphorus ester inhibition [15].

Whereas *nte1* null mutant yeast cells are viable under all conditions analyzed, and does not exhibit any detectable phenotype beyond the lack of GroPCho formation, the absence of NTE activity has severe effects in some cell types of metazoan organisms. Homozygous $NTE^{-/-}$ mice die at embryonic day nine [16]. The embryo exhibits growth retardation associated with impaired placental development and blood vessel growth, followed by massive apoptosis and death. Cells derived from the mutant mice could be cultivated for more than a week and differentiated into several cell types, indicating that the cause of death is not a general defect in cellular proliferation but it is probably due to poor nourishment and exchange of compounds [16]. Mice with brain specific deletion of NTE exhibit neurodegeneration with prominent lesions in the hippocampus, thalamus and cerebellum. Disruption of the ER, vacuolation of nerve cell bodies, abnormal reticular aggregates and reduced dendritic trees are associated with the absence of NTE. These mice only exhibited a mild motor disorder [17].

Homozygous inactivation of the *Drosophila* homologue of NTE, the *swiss cheese* gene, resulted in age dependent