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Mammalian cardiolipin biosynthesis

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ABSTRACT

Cardiolipin is a major phospholipid in mitochondria and is involved in the generation of cellular energy in the form of ATP. In mammalian and eukaryotic cells it is synthesized *via* the cytidine-5′-diphosphate-1,2-diacyl-*sn*-glycerol phosphate pathway. This brief review will describe some of the more recent studies on mammalian cardiolipin biosynthesis and provide an overview of regulation of cardiolipin biosynthesis. In addition, the important role that this key phospholipid plays in disease processes including heart failure, diabetes, thyroid hormone disease and the genetic disease Barth Syndrome will be discussed.

1. Introduction

Phospholipids serve important structural and functional roles in the body. They can act as an energy storage source, as signaling molecules and most importantly, as the main structural and functional components of cellular membranes (van Meer et al., 2008). The major polyglycerophospholipid in mammalian tissues is bis-(1,2-diacyl-sn-glycero-3-phospho)-1′-3′-sn-glycerol or cardiolipin (CL). Normally, most phospholipids are synthesized in the endoplasmic reticulum (ER), and some are then imported into the mitochondria for use (reviewed in Hatch, 2004). CL is unique in that it is synthesized exclusively in the mitochondria. It is predominantly found in the inner and, to a lesser extent, the outer membranes of mitochondria (Hatch, 2004; Houtkooper and Vaz, 2008). CL is most abundant in cardiac tissues as it makes up 15–20% of the total phospholipid phosphorus mass of the heart (Hostetler, 1982). Given its high abundance in the heart, CL has been proposed to be important for ATP production *via* the oxidative phosphorylation (OXPHOS) system embedded in the inner mitochondrial membrane (IMM) (reviewed in Claypool and Koehler, 2012). Indeed, studies have shown that CL is critical for the optimal activity of several enzymes involved in OXPHOS (reviewed in Hatch, 2004; Houtkooper and Vaz, 2008). This is particularly evident in a recent report by Arnarez et al. (2013) in which a molecular dynamic simulation model of cytochrome c oxidase complex (complex IV) was used to show that there are precise CL binding sites at the entrance to the proton channels on the matrix side of the complex. With the ability of CL to trap protons, these authors suggested that CL may also actively take part in the proton transport across complex IV to the intermembrane space. CL is also believed to act as the "glue" that holds the mitochondrial complexes together in the supercomplex formation to ensure efficient electron flow and proton transport (Arnarez et al., 2013; Zhang et al., 2002). These data support the initial observation of Lange et al. (2001) in which CL was proposed to ensure the structural integrity of the proton-conducting protein environment and takes part directly in proton uptake. Therefore, maintenance of the appropriate levels of CL in mitochondria is essential for proper cellular function. CL was first isolated from beef heart by Pangborn (1942) and its mammalian biosynthesis was first described in rat liver by Hostetler et al. (1971).

2. Biosynthesis of cardiolipin

The biosynthesis of phospholipids is highly conserved from yeast to mammals (reviewed in Schlame, 2008). In mammalian tissues, more specifically in the heart, the *de novo* synthesis of

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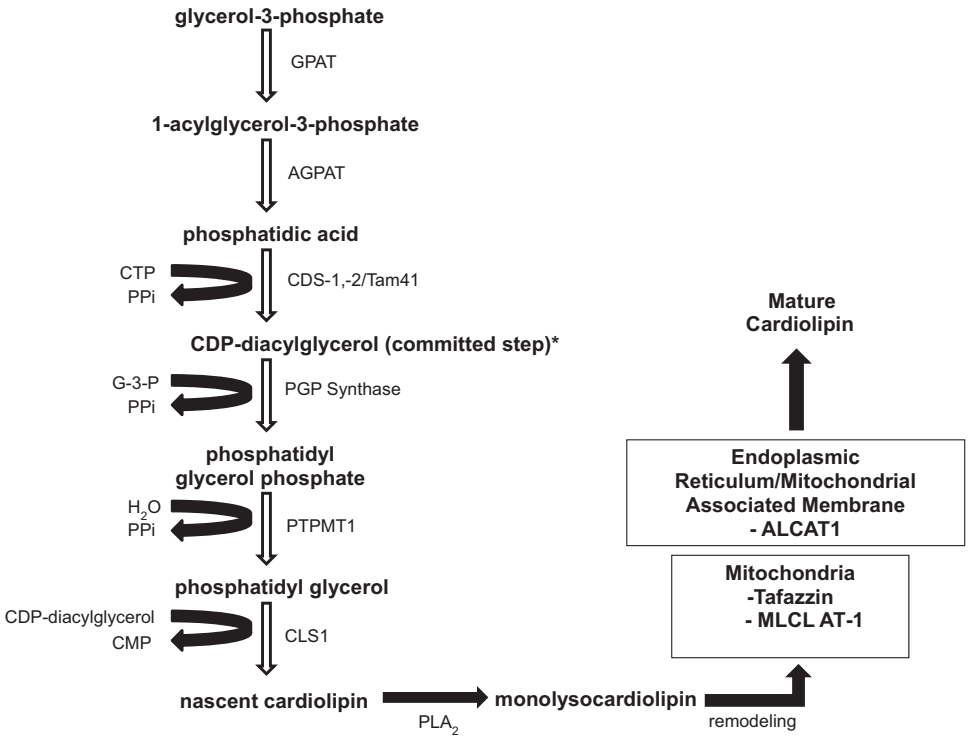

Fig. 1. Mammalian CL *de novo* biosynthesis and remodeling pathway: recent studies have identified new proteins such as Tam41 and PTPMT1 that are involved in the *de novo* synthesis of CL. (*At this point, the pathway can branch-off and CDP-DAG may also be incorporated into phosphatidylinositol.) See text for abbreviations.

CL occurs in the IMM *via* the cytidine-5′-diphosphate-1,2-diacyl-*sn*-glycerol pathway (Hatch, 1994) (Fig. 1). The pathway begins with the addition of an acyl group from acyl-CoA to the sn-1 position of glycerol-3-phosphate (G3P) by glycerol-3-phosphate acyltransferase (GPAT) to form 1-acyl-*sn*-glycerol-3-phosphate (or lysophosphatidic acid) (Schlame, 2008). As GPAT preferentially uses saturated fatty acids as substrates, nutritional intake can thus regulate its activity (Coleman et al., 2000). GPAT is not only involved in the formation of glycerophospholipids, but is also required for the *de novo* synthesis of neutral lipids such as triglycerides (Gimeno and Cao, 2008). Mammals have been reported to have at least four GPAT isoforms (Gimeno and Cao, 2008). GPAT1 and GPAT2 predominantly reside in the mitochondria, where GPAT1 is the major isoform. In the microsomal fractions, GPAT3 and GPAT4 predominate. The expression of these GPAT enzymes also varies greatly in different tissues. For example, GPAT1 and GPAT2 were reported to express mainly in skeletal tissues (Park et al., 2002), while the microsomal isoforms were highly expressed in the heart (Swanton and Saggerson, 1997; Lewin et al., 2008) and in the brain (Fitzpatrick et al., 1982).

The 1-acyl-*sn*-glycerol-3-phosphate product of GPAT is then acylated at the *sn*-2 position to form 1, 2-diacyl-*sn*-glycerol-3-phosphate (or phosphatidic acid) by 1-acyl-*sn*-glycerol-3-phosphate acyltransferase. Subsequently, a condensation reaction catalyzed by cytidinediphosphate-1,2-diacyl-*sn*-glycerol (CDP-DAG) synthetase (CDS), occurs between PA and CTP to form the high energy intermediate phosphatidyl-CMP (or CDP-DAG) (Schlame, 2008). Studies suggest that PA conversion to CDP-DAG is a rate-limiting step of phosphatidylglycerol and CL biosynthesis (Cheng and Hatch, 1995; Hatch, 1994). As both 1-acyl-*sn*-glycerol-3-phosphate and PA are synthesized on the outer surface of the mitochondrial outer membranes, PA has to be transported into the IMM for CL synthesis (Chakraborty et al., 1999), as importing CDP-DAG into the IMM has been shown to be significantly less efficient (Tamura et al., 2013). PA lies at a branching point as it can be used not only in the formation of CDP-DAG for mitochondrial polyglycerolphospholipid synthesis or phosphatidylinositol biosynthesis, but can also be utilized to form 1,2-diacyl-*sn*-glycerol for the synthesis of phosphatidylcholine, phosphatidylethanolamine and triacylglycerols (Hatch, 2004). Mammalian CDS is believed to exist in both the mitochondria and the ER. In humans, two isoforms (Cds1 and Cds2) have been cloned and sequenced (Halford et al., 1998). Since Cds1 was shown to account for more than 90% of the synthase activity in yeast (Shen et al., 1996) and its enzymatic activity was present in both the mitochondria and the ER (Kuchler et al., 1986), it was thought to be mainly responsible for CDP-DAG synthesis in the mitochondria. However, the fact that 90–95% of CDS activity was reported to occur in the microsomal fractions (Vance and Vance, 2004) and that Cds1 was recently shown by Tamura et al. (2013) to localize exclusively in the ER in yeast, argues against this view and suggests that there must exist another CDS in the mitochondria. To further support this concept, Tamura et al. (2013) showed that depletion of Cds1 in yeast did not change the CDP-DAG levels in the mitochondria, but did cause a significant reduction in CDP-DAG in the ER fractions. Interestingly, they were able to identify Tam41, an IMM protein originally described as a maintenance protein for the TIM 23 complex (Tamura et al., 2006), to also have CDP-DAG synthase function. The role of Tam41 for mitochondrial CDP-DAG production is also supported by a previous study by Kutik et al. (2008), who showed that yeast lacking Tam41(tam41Δ) could not produce CL and PG and had an accumulation of PA in the mitochondria. Furthermore, these defects were effectively rescued when Tam41 was re-introduced into these cells. Essentially, the CDS responsible for CDP-DAG synthesis in the mitochondria may now be assigned to Tam41.

The committed step in the CL synthesis pathway is the transfer of the activated phosphatidyl group from CDP-DAG to the sn-1 position of *sn*-glycerol-3-phosphate by phosphatidyl glycerol phosphate (PGP) synthase to yield PGP (Hatch, 2004). Data from our