An unusual phosphatidylethanolamine-utilizing cardiolipin synthase is discovered in bacteria

George M. Carman[1]

Department of Food Science and Rutgers Center for Lipid Research, Rutgers University, New Brunswick, NJ 08901

Cardiolipin (also known as diphosphatidylglycerol) is a four-chained anionic membrane phospholipid composed of two phosphatidyl moieties joined by a glycerol link (Fig. 1). It was first isolated from beef heart as a serologically active phospholipid (1), hence the name cardiolipin. In animals, plants, and lower eukaryotic organisms (e.g., yeast), cardiolipin is a component of the inner mitochondrial membrane bilayer, where it plays an important role in the function of enzyme complexes involved in energy transduction and ATP synthesis (2, 3). It is found in the membranes of Gram-positive and Gram-negative bacteria, where it also functions in energy production (2, 3). The nature (i.e., chain length and degree of saturation) of the four acyl groups and the hydrophilic head group of this phospholipid have an impact on mitochondrial membrane structure and function, and, by extension, the roles this organelle plays in cell physiology (2, 4). Thus, how cardiolipin is synthesized, remodeled, and metabolized are important areas of investigation. The importance of cardiolipin in mitochondrial function is further emphasized by the fact that defects in tafazzin-mediated remodeling of the fatty acyl composition of cardiolipin is the molecular basis for human Barth syndrome, a disease characterized by heart failure, myopathy, neutropenia, and growth retardation in children (3, 4).

In all organisms, cardiolipin is synthesized from phosphatidylglycerol, a two-chained anionic phospholipid derived from the liponucleotide CDP-diacylglycerol (Fig. 1). How phosphatidylglycerol is converted to cardiolipin differs depending on whether cells are prokaryotic or eukaryotic. In prokaryotic cells, the cardiolipin synthase reaction involves the transfer of the phosphatidyl moiety of phosphatidylglycerol to a second molecule of phosphatidylglycerol (5), whereas, in eukaryotic cells, CDP-diacylglycerol donates its phosphatidyl moiety to phosphatidylglycerol to form cardiolipin (6, 7) (Fig. 1). In PNAS, Tan et al. (8) use a combination of genetic and biochemical approaches to discover an unusual cardiolipin synthase in the bacterium *Escherichia coli* by which phosphatidylethanolamine, a zwitterionic membrane phospholipid, provides the phosphatidyl group to phosphatidylglycerol to form cardiolipin (Fig. 1).

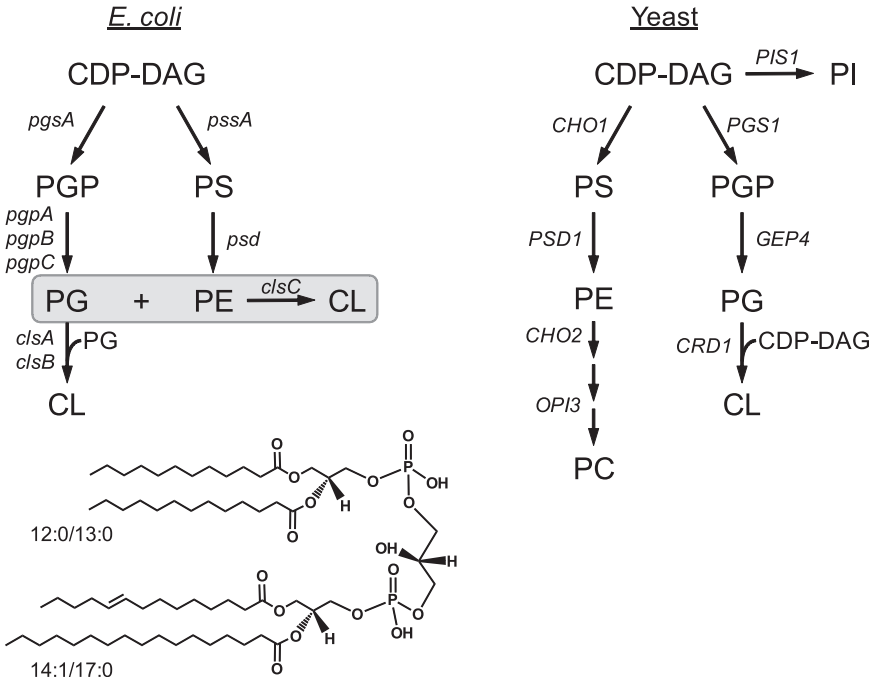

Fig. 1. Pathways for the synthesis of cardiolipin in *E. coli* and in yeast. In all organisms, cardiolipin is synthesized by pathways emanating from the liponucleotide CDP-diacylglycerol. In prokaryotic cells (e.g., the bacterium *E. coli*, *Left*), the final step in cardiolipin synthesis terminates with the joining of two phosphatidylglycerol molecules, but in eukaryotic cells (e.g., yeast, *Right*), cardiolipin is made from phosphatidylglycerol and a second molecule of CDP-diacylglycerol. In the PNAS article by Tan et al. (8), cardiolipin in *E. coli* is also shown to be synthesized from phosphatidylglycerol and phosphatidylethanolamine by an unusual cardiolipin synthase encoded by the *clsC* gene (highlighted in gray). The diagram shows the names of the genes encoding enzymes catalyzing each step in the phospholipid synthesis pathways. The structure of cardiolipin is shown with the synthetic fatty acyl groups that were used to identify the substrates of the *clsC*-encoded cardiolipin synthase. *CL*, cardiolipin; *CDP-DAG*, CDP-diacylglycerol; *PC*, phosphatidylcholine; *PE*, phosphatidylethanolamine; *PG*, phosphatidylglycerol; *PGP*, phosphatidylglycerophosphate; *PI*, phosphatidylinositol; *PS*, phosphatidylserine.

Two genes, *clsA* (9, 10) and *clsB* (11), encode phosphatidylglycerol-dependent cardiolipin synthases (ClsA and ClsB, respectively) in *E. coli*. Although cardiolipin is undetectable in exponential phase Δ*clsA* Δ*clsB* double mutant cells, the formation of the lipid is apparent in stationary phase cells indicating a growth phase-dependent formation of cardiolipin by the newly discovered *clsC* gene product ClsC. This gene–enzyme relationship was identified by informatics, and confirmation was obtained through biochemical experiments that included mutagenesis of the enzyme active site (e.g., HKD phospholipase D motifs characteristic of prokaryotic cardiolipin synthases). Maximum expression of *clsC*-encoded activity was also dependent on the product of its neighboring gene, *ymdB*, but only when both are transcribed from the same operon. The mechanism by which *ymdB* expression stimulates ClsC activity is unclear, but is thus far unique among cardiolipin synthases.

To identify the phosphatidylethanolamine-dependent nature of this unusual enzyme, Tan et al. (8) carry out a series of elegant biochemical experiments by using synthetic phospholipids with unique fatty acyl moieties and extracts of cells expressing plasmid-born *clsC-ymdB* but lacking the ability to synthesize phosphatidylglycerol (e.g., a Δ*pgsA* mutation) and cardiolipin (e.g., Δ*clsA* Δ*clsB* Δ*clsC*

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[1]E-mail: carman@aesop.rutgers.edu.

Δ*ymdB* mutations). By using state-of-the-art collision-induced dissociation dual MS to analyze reaction products, Tan et al. (8) rule out the involvement of CDP-diacylglycerol- and phosphatidate-dependent reactions and confirmed that the product cardiolipin was composed of fatty acyl groups derived from synthetic phosphatidylglycerol (e.g., 12:0 and 13:0 acyl chains) and synthetic phosphatidylethanolamine (e.g., 14:1 and 17:0 acyl chains). Thus, the power of MS in defining structure and reaction mechanism was central in making this discovery.

Although the physiological significant of this phosphatidylethanolamine-dependent cardiolipin synthase has yet to be defined, there are significant implications that might be considered. For example, in exponential-phase *E. coli* cells, ample phosphatidylglycerol is most likely available via energy dependent formation for utilization by ClsA and ClsB to make cardiolipin. However, in stationary phase, in which cardiolipin levels rise while de novo synthesis of phosphatidylglycerol stops, ClsC can use the ample supply of phosphatidylethanolamine as the donor of a phosphatidyl moiety to phosphatidylglycerol, thus expanding the available pool of cardiolipin.

A similar scenario might hold for eukaryotic cells (e.g., yeast) in which cardiolipin synthesis is limited by the amounts of phosphatidylglycerol and CDP-diacylglycerol. In particular, CDP-diacylglycerol is a minor phospholipid because it is used by four phospholipid synthesis enzymes (e.g., phosphatidylglycerophosphate synthase, cardiolipin synthase, phosphatidylserine synthase, and phosphatidylinositol synthase, encoded by *PGS1*, *CRD1*, *CHO1*, and *PIS1*, respectively; Fig. 1). Thus, a phosphatidylethanolamine-dependent reaction would allow cardiolipin synthesis under conditions in which CDP-diacylglycerol is limited given that phosphatidylethanolamine is an abundant phospholipid. In fact, a physiological connection between phosphatidylethanolamine and cardiolipin has been established in regulating mitochondrial function in yeast because loss of phosphatidylethanolamine synthesis (e.g., *psd1*Δ mutation) is synthetically lethal with the loss of cardiolipin synthesis (*crd1*Δ mutation) (12). Whether the basis for this connection is because phosphatidylethanolamine may be used to synthesize cardiolipin is not known, but this observation provides impetus for examining a phosphatidylethanolamine-dependent cardiolipin synthase in yeast and in higher eukaryotes.

Tan et al. use a combination of genetic and biochemical approaches to discover an unusual cardiolipin synthase in the bacterium *Escherichia coli*.

Finally, the work of Tan et al. (8) also points to a need to reexamine the mode of cardiolipin synthesis in the many bacteria with multiple *cls* homologues identified mainly by informatics and assayed in crude extracts assuming involvement of two phosphatidylglycerol molecules.

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