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The chimeric origin of the cardiolipin biosynthetic pathway in the Eukarya domain

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ABSTRACT

Cardiolipin (CL) and phosphatidylglycerol (PG) are the main anionic phospholipids present in the Eukarya and Bacteria domains. They participate in energy transduction by activating and stabilizing the components of the oxidative phosphorylation machinery. Experimental evidence shows that they are synthesized by two different mechanisms which indicate that both pathways evolved convergently. Former studies on the lipid composition of archaeal membranes showed the absence of CL in these organisms, consequently, restricting it to the Eukarya and Bacteria domains. Interestingly, recent studies have demonstrated that both CL and PG are present as constitutive components of membranes of the haloarchaea group. However, this scenario complicates the analysis of the evolutionary origin of this biosynthetic pathway. Here I suggest that a phospholipid biosynthetic pathway in Eukarya probably arose from a chimeric event between bacterial and archaeal enzymes during the endosymbiosis event. Phylogenetic analyses support the different evolutionary origin of the enzymes comprising this pathway in bacteria and Eukarya. Based on protein domain analyses, orthologous proteins in the Archaea domain were identified. An integrative analysis of the proteins found demonstrates that CL biosynthesis in major clades of the Eukarya domain originated by chimerism between the bacteria and archaea pathways. Moreover, primary and secondary endosymbiontic events in plants and chromoalveolata respectively, reshaped this pathway again. The implications and advantages that these new enzymatic orders conferred to the Eukarya domain are discussed.

1. Introduction

Anionic phospholipids such as phosphatidylglycerol (PG) and cardiolipin (CL) are key components of cellular membranes, where they have important roles in several cellular processes such as energy transduction, stress response, participation in the mechanism of translation coupled to transcription (transertion) in bacteria and the stabilization, maintenance and segregation of mitochondrial DNA [1–4]. CL was believed to be restricted to the Bacteria and Eukarya domains because early evidence ruled out the existence of this phospholipid in archaeal membranes. Since PG has been demonstrated to be present in the three domains of life, it was believed that CL spread through eukaryotic cells during the endosymbiotic process from the bacterial endosymbiont [5]. This idea is being challenged based on the more recent discovery of CL in several archaeal species [6].

Apparently, PG and CL biosynthesis evolved convergently in bacteria and eukaryotes since they are synthesized by two different enzymatic pathways (see Fig. 1). In the bacterial pathway, glycerol 3-phosphate is condensed to a molecule of CDP-DAG, producing phosphatidyglycerol phosphate (PGP), which is dephosphorylated to produce PG. It is then condensed to another molecule of PG to produce CL. On other hand, in the eukaryotic pathway, glycerol 3-phosphate is transferred to a CDP-DAG molecule to produce PGP, as in bacteria. However, after dephosphorylation of PGP, PG is condensed to another CDP-DAG molecule producing CL, thus differing in this step in the bacterial substrates used for synthesis [7]. As stated before, the enzymatic mechanisms differ between domains: in bacteria, the first enzyme, phosphatidylglycerol phosphate synthase (Pgs_T), belong to the transferase class (E.C. 2.7.8.5), whilst in Eukarya the enzyme is a hydrolase (E.C.3.1.4.4) from the phospholipase D (PLD) family (Pgs_D). Conversely, cardiolipin synthase (Cls_D) activity in bacteria belongs to the hydrolase class (PLD) whilst the eukaryote (Cls_T) enzyme belongs to the transferase class. These differences in the position of each enzymatic step indicate that CL biosynthesis could have evolved independently in each domain. Interestingly, several versions of this pathway have been observed in eukaryotes: whilst in Kinetoplastid and Apicomplexan organisms both enzymes are phospholipases D, in plants both enzymes are CDP transferases. Moreover, recent evidence *in silico* has demonstrated the presence of a bacterial-like phosphatidylglycerol phosphate synthase together with evidence of CL in several species of archaeas [8–10]. This intricate diversity of pathways for CL biosynthesis has been poorly studied; the only study was restricted to uncovering the evolution of Cls and CL-remodeling enzymes (a process restricted to the Eukarya

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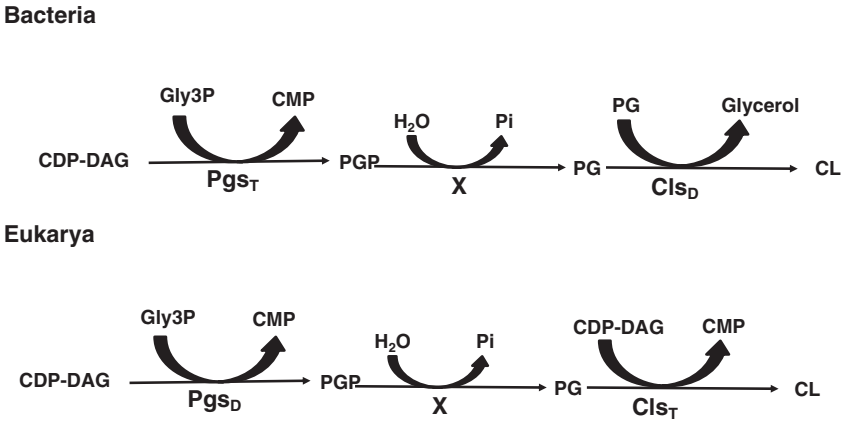

Fig. 1. Cardiolipin biosynthesis in the Bacteria and Eukarya domain. Although the first enzyme common for both domains uses the same substrates, the enzymatic mechanism varies between them. The bacterial enzyme is a CDP-DAG transferase whilst the eukaryote enzyme is a PLD. On the other hand, the Cls is a PLD in bacterial whilst a CDP-DAG transferase in Eukarya. The archeal pathway is believed to be identical to the bacterial. Pgs, phosphatidylglycerol phosphate synthase; Cls, cardiolipin synthase; CDP-DAG, cytidine diphosphate-diacylglycerol; PGP, phosphatidylglycerol phosphate; PG, phosphatidylglycerol; CMP, cytidine monophosphate. The X symbol represents a poorly conserved enzyme necessary for the hydrolysis of the ester bond between glycerol and phosphate from the head group.

domain). This study concluded that eukaryal Cls activity evolved from two endosymbiotic events, one giving rise the first eukaryotic common ancestor (FECA) with a PLD activity and the second one from a secondary endosymbiont replacing the FECA Cls with a transferase enzyme [5].

In this report, the evolution of cardiolipin biosynthesis was reanalyzed together with orthologous proteins found in the Archaea. I suggest that CL biosynthesis in the Eukarya domain rose by a chimeric mechanism involving both archaeal and bacterial proteins. Subsequent endosymbiotic events provided the basis to further diversify this pathway into the Eukarya domain. This evolutionary exchange had important repercussions in the subsequent evolution and specialization of several mitochondrial features.

2. Methods

2.1. Identification of sequences from CL synthase and PGP synthase

A phylogenetic distribution of the four enzymes through the three domains was retrieved from STRING v.9.1 [11]. Aminoacid sequences for phosphatidylglycerol phosphate synthase (Pgs) and cardiolipin synthase (Cls) were identified by Domain Enhanced Look up Time accelerated (DELTA)—BLAST from the NCBI server using *Saccharomyces cerevisiae* and *Escherichia coli* sequences as queries. All proteins selected had a maximum *E*-value $< 10^{-15}$. Same results were obtained using *Bacillus subtilis and Homo sapiens* sequences as queries. For clarity, a subscript D means that the enzyme has a phospholipase D mechanism whilst a C it is a transferase. 143 non-redundant sequences for phospholipase D enzymes (Pgs_D and Cls_D) and 140 for transferase enzymes (Pgs_T and Cls_T) were selected for phylogenetic reconstruction.

Ancestral sequences of the Cls_T and Pgs_T groups were acquired after phylogenetic reconstruction of the respective tree using the ancestral reconstruction function implemented in Mega 6 software [12]. Multiple alignments between those sequences and the Cls from *S. coelicolor* were aligned with ClustalW.

Transmembrane helix topology of PLD sequences was performed with the toppred program implemented in the Mobyle 1.5 portal developed by the Institute Pasteur Biology IT Center and the *Ressource Parisienneen Bioinformatique Structurale* (mobyle.pasteur.fr/cgi-bin/portal.py#welcome) [13].

2.2. Phylogenetic analysis

Selected sequences were aligned with MUSCLE (v3.7) without curation. Sequences causing aberrant alignments were removed from posterior analysis. Trees were inferred using the maximum likelihood method with the Mega6 program. The Jones–Taylor–Thornton method was used as substitution matrix. Statistical significance was calculated by the bootstrapping method using 100-pseudoreplicas [12].

3. Results

3.1. Taxonomical distribution of CL biosynthetic enzymes

A first search for the occurrence of all Pgs_D, Pgs_T, Cls_D and Cls_T sequences showed that Pgs_T was the only enzyme confidently distributed between, Bacteria and Archaea domains, but absent in Eukarya. All other enzymes were present in just one domain. For Archaea, proteins homologous to Cls_D and Pgs_D present homology to phospholipases D with unknown functions (Table 1). Interestingly, eukaryote Pgs_D presented poor similitude to bacterial Cls_D. Since these preliminary results show that Pgs_D was absent in Bacteria, questions arise: How did this enzyme appear in Eukarya? Would it be possible for Pgs_D to be related to archaeal phospholipases D? A refined search based on conserved domains was performed to answer these questions. Each enzymatic activity was analyzed individually.

3.2. CDP-DAG transferases

Previous *in silico* studies identified several proteins in methanoarchaea as homologous to bacterial Pgs_T [14]. However, experimental evidence has not corroborated the catalytic activity of those proteins as with other members of this family, including the archeal phosphatidylinositol synthase (Pis) [15]. Thus, Cls_T from *S. cerevisiae* and *H. sapiens*, and Pgs_T from *E. coli* and *Methanosarcina barkeri* (an archaea) were used as queries.

Table 1
Domain distribution of the enzymes involved in CL biosynthesis. Phosphatidylglycerol phosphate synthase (Pgs) and CL synthase (Cls).

Protein	Bacteria	Achaea	Eukarya
PgsD	○	?*	●
PgsT	●	●	○
ClsD	●	?*	○
ClsT	○	?	●

Note: D—phospholipase D mechanism, C—CDP-DAG transferase mechanism, ○—absent, ●—present, ?—unknown. *—Protein similar to phospholipase D. Pgs, phosphatidylglycerol phosphate synthase. Cls, cardiolipin synthase.