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Cardiolipin-dependent formation of mitochondrial respiratory supercomplexes

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ABSTRACT

The organization of individual respiratory Complexes I, III, and IV (mammalian cells) or III and IV (yeast) of the mitochondria into higher order supercomplexes (SCs) is generally accepted. However, the factors that regulate SC formation and the functional significance of SCs are not well understood. The mitochondrial signature phospholipid cardiolipin (CL) plays a central role in formation and stability of respiratory SCs from yeast to man. Studies in yeast mutants in which the CL level can be regulated displayed a direct correlation between CL levels and SC formation. Disease states in which CL levels are reduced also show defects in SC formation. Three-dimensional density maps of yeast and bovine SCs by electron cryo-microscopy show gaps between the transmembrane-localized interfaces of individual complexes consistent with the large excess of CL in SCs over that integrated into the structure of individual respiratory complexes. Finally, the yeast SC composed of Complex III and two Complexes IV was reconstituted in liposomes from purified individual complexes containing integrated CLs. Reconstitution was wholly dependent on inclusion of additional CL in the liposomes. Therefore, non-integral CL molecules play an important role in SC formation and may be involved in regulation of SC stability under metabolic conditions where CL levels fluctuate.

1. Introduction

The anionic phospholipid cardiolipin (CL), also called diphosphatidylglycerol, (1,3-*bis*(*sn*-3′-phosphatidyl)-*sn*-glycerol), is uniquely localized to energy-transducing membranes, which couple generation of an electrochemical potential with ATP synthesis and substrate transport. In eukaryotes CL is a signature phospholipid of mitochondria. The unique structure of CL is composed of two phosphates, four fatty acids, three chiral centers and a free central hydroxyl (Fig. 1), which has been suggested to serve as a proton sink. In animals and higher plants the majority of acyl chains of CL contain polyunsaturated fatty acids with 18 carbons, while in *Saccharomyces cerevisiae* (hereafter referred to as yeast) the fatty acids are 16 and 18 carbon monounsaturated chains (Schlame and Ren, 2009). CL interacts with many membrane proteins affecting their activity, stability, level of aggregation, and compartmentalization. In this review we will focus on the specific role CL plays in organization and function of the mitochondrial respiratory chain.

2. Synthesis of CL

The function and synthesis of mitochondrial lipids in mammalian cells and yeast are highly homologous. Yeast cells have a distinct research advantage over higher eukaryotes in ease of growth and genetic manipulation coupled with viability in the face of dramatic alterations in mitochondrial phospholipid composition. In yeast, CL and its precursor phosphatidylglycerol (PG) are synthesized from the common precursor phosphatidic acid (Fig. 1) by mitochondrial-localized enzymes that are encoded by nuclear genes, synthesized on cytoplasmic ribosomes and imported into the inner mitochondrial membrane. The fatty acid composition of newly synthesized CL is remodeled to its unique composition by transacylation reactions in which the *CLD1* and *TAZ1* gene products are involved. All genes of yeast involved in biosynthesis of CL have been identified and cloned with mutants available in each step of synthesis.

Abbreviations: CL, cardiolipin; CI, Complex I; CIII, Complex III; CIV, Complex IV; BN-PAGE, blue-native polyacrylamide gel electrophoresis; SC, supercomplex; CN-PAGE, colorless-native polyacrylamide gel electrophoresis; PE, phosphatidylethanolamine; PC, phosphatidylcholine; IMS, intermembrane space; MA, matrix; CGMD, coarse-grained molecular dynamics.

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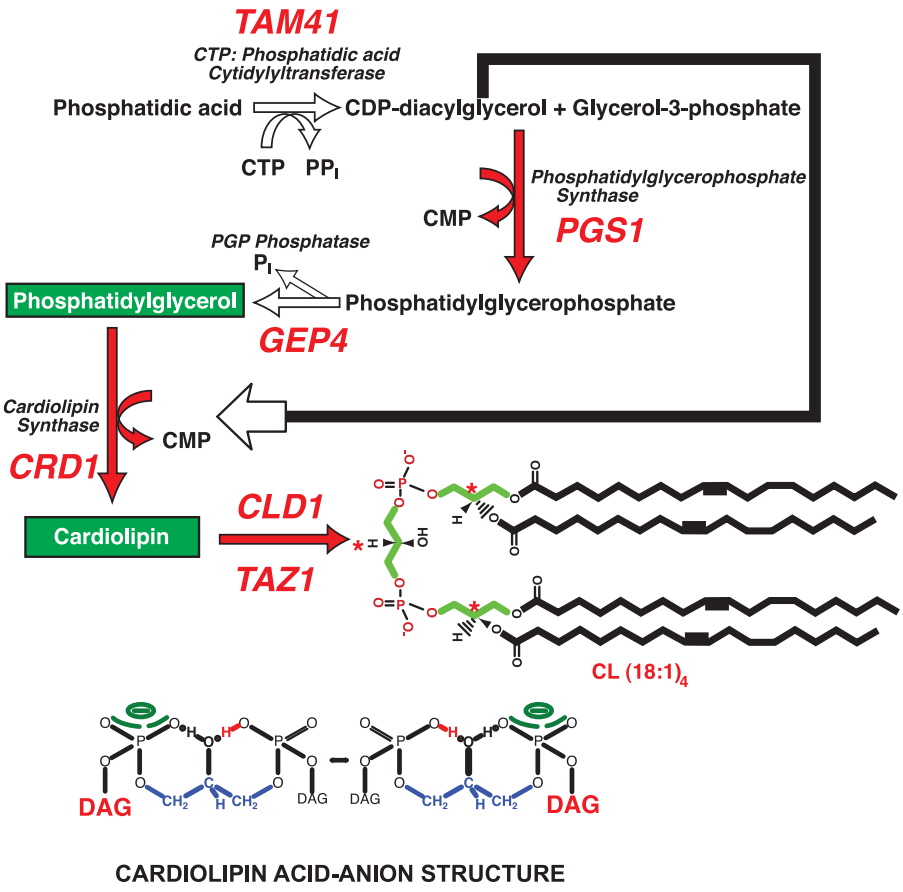

Fig. 1. Biosynthesis and structure of CL. The pathway, genes (*red*) and gene products responsible for CL synthesis in yeast mitochondria are shown (Henry et al., 2012; Tamura et al., 2013). The pathway in higher eukaryotes is essentially the same and is carried out by homologous genes and gene products. The *CLD1* gene product (CL specific deacylase) initiates the remodeling of CL fatty acid chain composition in yeast by forming monolyso-CL (Beranek et al., 2009); higher eukaryotes utilize multiple deacylases (Baile et al., 2013). The *TAZ* gene product in both yeast and mammalian cells is responsible for completing the remodeling of nascent CL by transferring fatty acids to monolyso-CL from other phospholipids (Schlame et al., 2012). The result is highly unsaturated forms of CL as represented by one of the structures found in yeast and mammalian cells. The (*) in *red* indicates the three chiral centers of naturally occurring CLs; carbon of the central glycerol is only a chiral center if the two adjacent phosphatidyl moieties have different fatty acid compositions. The glycerol backbone (*green*) is indicated. The lower figure depicts the hydrogen-bonding between the central free hydroxyl of CL and the phosphate residues creating a proton sink in the lipid bilayer and raising the pKa of one phosphate near neutrality (Haines, 2009). *DAG* denotes the diacylglycerol lipid domain. (For interpretation of references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Mitochondrial respiratory chain organization and function

In mammalian mitochondria the respiratory chain is composed of four multi-subunit electron transfer protein complexes: Complex I (CI, NADH:ubiquinone oxidoreductase), Complex II (succinate:ubiquinone oxidoreductase), Complex III[1] or cytochrome bc_1 complex (CIII, ubiqunol:cytochrome *c* oxidoreductase), Complex IV (CIV, cytochrome *c* oxidase) and two small electron carriers, which transfer electrons from CI or Complex II to CIII (lipid-soluble ubiquinone (CoQ)), or from CIII to CIV (water-soluble cytochrome *c*). Yeast lacks CI and utilizes peripheral membrane NADH dehydrogenases lacking proton-pumping ability. Electron transfer is coupled with proton pumping by CI, CIII and CIV from the mitochondrial matrix to the mitochondrial intermembrane space (IMS) resulting in an electrochemical proton gradient across the inner mitochondrial membrane, which F_1F_0-ATPase (Complex V) uses for ATP synthesis (for most recent review and references see (Sun et al., 2013)

Organization of the respiratory chain as one structural and functional unit, now termed a respirasome (Schagger and Pfeiffer, 2000), was originally formulated by Chance and Williams (1955). Once active individual respiratory complexes were purified and reconstituted into liposomes, this model was substituted by the random collision model (Hackenbrock et al., 1986), in which the respiratory chain is composed of individual complexes independently imbedded in the lipid bilayer and connected by randomly diffusing CoQ and cytochrome *c*. Mild solubilization of mitochondrial membranes with digitonin and development of blue-native and colorless-native polyacrylamide gel electrophoresis (BN-PAGE and CN-PAGE), demonstrated active stoichiometric assemblies of individual complexes in mitochondria from yeast to mammals. Association of CI with CIII (I_1III_2) or CI with CIII and one to four copies of CIV ($I_1III_2IV_{n=1-4}$, termed "respirasome") resulting in multiple supercomplexes (SCs) was demonstrated in mammalian mitochondria. In yeast two SCs, one composed of III_2IV_1, and another one of III_2IV_2, were found (Schagger, 2001; Schagger and Pfeiffer, 2000; Stuart, 2008).

Flux control analysis of electron transfer through the respiratory chain showed substrate channeling of CoQ in bovine heart mitochondria and of both, CoQ and cytochrome *c*, in mitochondria of potato tuber, thus demonstrating the existence of functional respiratory SCs (for review and refs see (Genova and Lenaz,

[1] Complex III (CIII) is a structural and functional homodimer of two cytochrome bc_1 monomeric complexes and when in a supercomplex (SC) is referred to as III_2.