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Review

Biogenesis of thylakoid membranes ☆

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ABSTRACT

Thylakoids mediate photosynthetic electron transfer and represent one of the most elaborate energy-transducing membrane systems. Despite our detailed knowledge of its structure and function, much remains to be learned about how the machinery is put together. The concerted synthesis and assembly of lipids, proteins and low-molecular-weight cofactors like pigments and transition metal ions require a high level of spatiotemporal coordination. While increasing numbers of assembly factors are being functionally characterized, the principles that govern how thylakoid membrane maturation is organized in space are just starting to emerge. In both cyanobacteria and chloroplasts, distinct production lines for the fabrication of photosynthetic complexes, in particular photosystem II, have been identified. This article is part of a Special Issue entitled: Chloroplast Biogenesis.

1. Introduction

The origin of oxygenic photosynthesis dates back more than 2.4 billion years. At that time, primordial cyanobacteria first succeeded in harnessing solar energy to drive the extraction of electrons from water; these are then fed into a transmembrane photosynthetic electron transport chain (PET; [1]). PET takes place across specialized membranes named thylakoids, which represent one of the most elaborate bioenergetic machines known to date [2]. Thylakoids form an intracellular membrane system which harbors most of the constituents involved in the harvesting and utilization of light energy. As a consequence of PET, the chemical energy carriers NADPH and ATP are generated, and utilized for the synthesis of carbohydrates from CO_2. Photosynthesis thus forms the basis of nature's food chains.

The main protein components of thylakoids include the mobile, low-molecular-weight carriers plastoquinone and plastocyanin, which shuttle electrons between huge multisubunit protein/pigment complexes, i.e., photosystem II (PSII) and photosystem I (PSI), via the cytochrome b_6f (Cytb_6f) complex. Finally, an ATPase complex utilizes the energy stored in the proton gradient established across the thylakoid membrane by PET to synthesize ATP. This core molecular machinery mediating PET is highly conserved from cyanobacteria to plants [3], although the light-collecting systems are more diverse, varying from soluble phycobilisomes in cyanobacteria and red algae to membrane-integrated light-harvesting (LHC) systems in plants [4]. The ultrastructure of these various components has been determined at close to atomic resolution in recent years, providing a detailed picture of the structure and function of the PET chain [5,6].

However, despite this in-depth knowledge of their structure, the processes that guide the construction of the various photosynthetic complexes during thylakoid membrane biogenesis are only beginning to emerge. As the available structural data imply, dozens of integral and peripheral protein subunits and hundreds of organic cofactors including chlorophylls, carotenoids, cytochromes and quinones, as well as metals and other ions, have to be brought together and properly assembled. And of course these diverse building materials must be synthesized and supplied in amounts reflecting at least approximately their final stoichiometry. Then the components must be brought together in a lipid environment and assembled in a strictly determined and stepwise sequence. Thus, the entire process must be highly ordered both in space and in time.

The current status of our understanding of the biogenesis of particular complexes is reviewed in detail in Chapters 11–14 [7–10] of this special issue. Therefore, we focus here on how different biosynthetic pathways for thylakoid membrane constituents might be interconnected to enable coordination of biogenesis in the temporal domain. In

Abbreviations: PET, photosynthetic electron transport; NADPH, nicotinamide adenine dinucleotide phosphate; ATP, adenosine triphosphate; CO_2, carbon dioxide; Cytb_6f, cytochrome b_6f complex; PSI, photosystem I; PSII, photosystem II; LHC, light-harvesting complex; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; PG, phosphatidylglycerol; PDC, pyruvate dehydrogenase complex; ACCase, acetyl-CoA carboxylase; PA, phosphatidic acid; DAG, diacylglycerol; MGS, monoglucosyldiacylglycerol synthase; CURT1, CURVATURE THYLAKOID1; THF1, thylakoid formation 1; POR, protochlorophyllide oxidoreductase; ChlG, chlorophyll synthase; CTM, chloroplast translation membrane

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addition, the cytological aspects of the whole process are emphasized by reviewing current findings regarding the spatial organization of thylakoid membrane biogenesis and architecture. Despite differing significantly in structural organization, the functions of thylakoids have been highly conserved throughout evolution. Hence, the biogenesis process will be discussed in the light of recent findings in organisms ranging from cyanobacteria to vascular plants.

2. Forming the matrix of thylakoid membranes — the lipids

2.1. Composition and function of the thylakoid lipidome

Thylakoids represent remarkably conserved energy-transducing membranes with a high protein content that can reach up to 80% in stacked grana regions of eukaryotic chloroplasts accumulating PSII [2]. Like the photosynthetic protein/pigment complexes that account for this high protein/lipid ratio, the lipophilic matrix in which they are embedded is unique, and has been conserved during the course of evolution. This suggests that a specific lipid environment is required to ensure the stability and activity of photosynthetic complexes [11].

With minor deviations, thylakoids from cyanobacteria to plants contain four major lipid types, i.e., the galactoglycerolipids MGDG (monogalactosyldiacylglycerol) and DGDG (digalactosyldiacylglycerol), the sulfolipid SQDG (sulfoquinovosyldiacylglycerol) and the phospholipid PG (phosphatidylglycerol). The glycolipids comprise more than 70% of thylakoid lipids, with MGDG accounting for >50% of the total. The relative proportions of the lipid classes are stable, underlining their defined structural/functional roles which are dependent on their physicochemical properties [12,13]. These properties include the presence of negatively charged headgroups in only SQDG and PG, and the small size of MGDG's headgroup, which allows it to form inverse hexagonal structures (the so-called HII phase), unlike DGDG, SQDG and PG [14,15]. As a functional consequence, DGDG forms and stabilizes membrane bilayers, whereas MGDG is likely to favor curvature of thylakoids. This idea is supported by the finding that highly stacked grana regions with a high content of curved membrane regions exhibit higher MGDG/DGDG ratios than do stroma lamellae [16]. Recent analyses support the critical role of the MGDG/DGDG ratio for membrane phase transitions and highlight the function of DGDG for membrane stacking via the formation of hydrogen bonds between the headgroups of adjacent bilayers [17]. In addition to their matrix role, all glycerolipid classes have been shown to interact closely with photosynthetic protein complexes, in particular with PSII, as deduced from X-ray crystallographic analyses [18–20]. This suggests that individual lipids may also play critical roles for the structure and function of the protein complexes. Indeed, genetic studies of mutants with defects in glycerolipid accumulation in plants and cyanobacteria have confirmed that MGDG is essential for thylakoid formation generally [21], while DGDG and PG are important for the function of PSII in particular [11,22,23].

2.2. Synthesis of lipids

The biosynthesis of thylakoid lipids follows a complex pathway which requires the production and combination of fatty acids and polar head precursors [11,24]. Fatty acid synthesis begins in the chloroplast stroma with the conversion of pyruvate into acetyl-CoA by the pyruvate dehydrogenase complex (PDC). Subsequently, acetyl-CoA is carboxylated to malonyl-CoA by the acetyl-CoA carboxylase (ACCase), which is considered to be the rate-limiting "committed step" in fatty acid synthesis [24]. Interestingly, recent studies of the regulation of chloroplast gene expression have revealed that the E2 dihydrolipoyltransferase subunit of the PDC acts as a regulator of the synthesis of the D1 protein of the PSII reaction center by localizing its mRNA to specialized biogenic membrane regions in *Chlamydomonas reinhardtii* as outlined below (Fig. 2B, Section 3.2 and [25]). Thus, the moonlighting activity of a metabolic enzyme appears to provide for reciprocal regulation of plastid fatty acid synthesis and protein synthesis. This kind of crosstalk in the initial phase of thylakoid membrane biogenesis would guarantee that lipid and protein syntheses are harmonized from the beginning to avoid unbalanced accumulation of the major classes of thylakoid constituents during chloroplast biogenesis [25]. In line with this, transcriptomic studies in *Arabidopsis thaliana* have recently revealed tight coordination between the expression of nuclear genes involved in later steps of both lipid and chlorophyll syntheses and genes coding for photosynthetic proteins [26]. Whether similar regulatory principles also operate in cyanobacteria is currently unknown.

Fatty acid synthesis is completed within the chloroplast, and thylakoid lipids are then synthesized via the resident prokaryotic pathway in the chloroplast or the extra-organellar eukaryotic route, which requires the export of fatty acids into the cytoplasm, their assembly into lipid precursors in the ER and subsequent reimport into plastids [27]. Irrespective of their precise derivation, the lipid precursors phosphatidic acid (PA) and diacylglycerol (DAG) are conveyed to the inner envelope, and further processed by the key enzymes MGD1 and DGD1, which catalyze the formation of the most abundant lipids MGDG and DGDG, respectively. While MGD1 is localized to the inner envelope membrane, DGDG is synthesized at the outer envelope, as revealed by proteomic approaches following membrane fractionation [27–29]. Interestingly, the MGDG/DGDG ratio of membranes has a direct influence on the association of MGD1 with the membrane and its self-organization into reticulated structures; this in turn suggests the existence of specialized membranous microdomains for plastid galactolipid synthesis [30].

Interestingly, recent membrane fractionation experiments have revealed that galactolipid synthesis in cyanobacteria seems to occur at both the plasma membrane and the thylakoids [31]. Formation of MGDG in cyanobacteria differs from that in chloroplasts insofar as the former lack MGD1 activity. Instead, DAG is first converted into monoglucosyldiacylglycerol by the essential enzyme monoglucosyldiacylglycerol synthase (MGS) and then further processed by an epimerase to yield MGDG [32]. In agreement with a broader distribution of lipid synthesis, MGS was found to localize to both plasma membrane and thylakoids [31].

2.3. Trafficking of lipids

One crucial but still unanswered question is how lipids are transferred from the plastid envelope to the internal thylakoid membrane system during its maturation. In principle, three forms of trafficking can be envisaged, based on (i) lateral fusions between inner envelope and thylakoids, (ii) vesicular transport, or (iii) soluble lipid carriers (Fig. 1 and [33]).

In algal chloroplasts and proplastids of vascular plants, thylakoids appear to develop via local invaginations of the inner envelope membrane [34,35]. Less frequently, connections between envelope and thylakoids have been observed in mature chloroplasts also, suggesting that intraplastidic lipid transfer occurs via lateral diffusion (Fig. 1A and [36]). In support of this scenario, in the *A. thaliana mgd1* mutant, which does not form photosynthetically active thylakoids, invaginations of the inner envelope membrane are observed [37].

However, ultrastructural evidence for vesicle-based transfer processes has also been repeatedly obtained (Fig. 1B and [34,38–40]). Furthermore, inhibitors of the cytoplasmic secretory pathway similarly affect vesicle formation in isolated chloroplasts, suggesting that related systems exist in both cellular compartments [40–42]. More recently, comprehensive bioinformatic studies have provided evidence for chloroplast localization for several proteins that share high sequence similarity with known components of the vesicle-based secretory pathway in the cytoplasm [43–45]. Based on these analyses, it seems likely that a complete vesicle transport system which resembles the cytosolic COPII system is active in the chloroplast, whereas COPI and clathrin-

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