Opinion

Plastids with or without galactoglycerolipids

Cyrille Y. Botté[1] and Eric Maréchal[2]

[1] ApicoLipid Group, Laboratoire Adapation et Pathogenie des Microorganismes; CNRS, Université de Grenoble-Alpes, UMR 5163, Institut Jean Roget, F-38042 Grenoble, France
[2] Laboratoire de Physiologie Cellulaire et Végétale; CNRS, CEA, INRA, Université de Grenoble-Alpes, UMR 5168, Institut de Recherches en Sciences et Technologies pour le Vivant, CEA Grenoble, F-38054 Grenoble, France

In structural, functional, and evolutionary terms, galactoglycerolipids are signature lipids of chloroplasts. Their presence in nongreen plastids has been demonstrated in angiosperms and diatoms. Thus, galactoglycerolipids are considered as a landmark of green and nongreen plastids, deriving from either a primary or secondary endosymbiosis. The discovery of a plastid in *Plasmodium falciparum*, the causative agent of malaria, fueled the search for galactoglycerolipids as possible targets for treatments. However, recent data have provided evidence that the *Plasmodium* plastid does not contain any galactoglycerolipids. In this opinion article, we discuss questions raised by the loss of galactoglycerolipids during evolution: how have galactoglycerolipids been lost? How does the *Plasmodium* plastid maintain four membranes without these lipids? What are the main constituents instead of galactoglycerolipids?

What are galactoglycerolipids?

All photosynthetic membranes (thylakoids) analyzed to date from cyanobacteria to chloroplasts of land plants are characterized by a low proportion of phospholipids, mainly phosphatidylglycerol (PG), and high levels of three nonphosphated glycoglycerolipids [i.e., the negatively charged sulfoquinovosyldiacylglycerol (SQDG) and the neutral mono- and digalactosyldiacylglycerol (MGDG and DGDG, respectively)] [1,2] (Figure 1). These galactoglycerolipids constitute up to 80% of thylakoid lipids [3]. Therefore, based on the natural abundance of photosynthetic organisms, galactoglycerolipids constitute the most profuse lipid class on Earth [4].

Galactoglycerolipid function

Galactoglycerolipids are not only building blocks for membranes. Structural studies have shown specific interactions with core functional systems for growth and development, such as subunits of photosystems [5] and the chloroplast import machinery [6,7]. Based on crystallographic analyses and characterization of lipids from purified protein–lipid complexes [5,8], galactoglycerolipid interactions are more important at the level of components of photosystem II (PSII) [5]. Genetic studies in the cyanobacterial model, *Synechocystis*, and the angiosperm model, *Arabidopsis* (*Arabidopsis thaliana*), enabled the functional characterization of mutants deficient in MGDG or DGDG content. *Arabidopsis* containing less MGDG was obtained by knocking down or knocking out (KO) *MGD1*, one of the three genes encoding MGDG synthases [9–12]. Levels of MGDG could also be altered by chemical treatment with galvestine-1, a specific inhibitor of MGDG synthases [13,14]. The *mgd1-1* mutant exhibited a severe growth phenotype and a disrupted chloroplast biogenesis [9], whereas the *mgd1-2* KO was unable to grow unless supplemented with sucrose. *Arabidopsis* treated with galvestine-1 showed a similar phenotype [13,14]. Thus, MGDG content is directly linked to thylakoid development, as would be expected for such an abundant lipid. Functional analyses of PSII in the *mgd1-1* mutant suggested that the remaining MGDG (40% of wild type level) is sufficient to maintain the function of this complex in normal light exposure [15]. Nevertheless, the *mgd1-1* mutant suffered from increased PSII photoinhibition, an inefficient thermal dissipation of excess light energy during short-term high-light stress [16,17].

Concerning DGDG, *Synechocystis* mutants were obtained by knocking out the *dgdA* gene (the cyanobacterial DGDG synthase gene) [18–20]. Growth of *dgdA* mutants was not affected in low-light conditions, and neither was photosynthesis. Under high-light or high-temperature conditions, DGDG was required to shape the PSII structure through the binding of extrinsic proteins [18,19]. *Arabidopsis* mutants affected in their DGDG composition were obtained by disruption of *DGD1* and *DGD2* [21–23]. The *dgd1* mutant was pale green, contained structurally altered chloroplasts and exhibited a decreased PSII:PSI ratio [21]. Stoichiometry of pigments and pigment-binding apoproteins was also impaired and an increased number of peripheral light-harvesting complex II subunits, relative to the inner antenna and PSII core complexes, was detected [22]. The *dgd1* × *dgd2* mutant contained traces of DGDG, produced by an alternative pathway (a galactolipid:galactolipid galactosyltransferase producing DGDG with a different sugar anomery) and had a more severe phenotype [23]. Functional analyses of photosynthesis in *mgd1-1*, *dgd1*, and *dgd1* × *dgd2* mutants were consistent with

Corresponding author: Maréchal, E. (eric.marechal@cea.fr).
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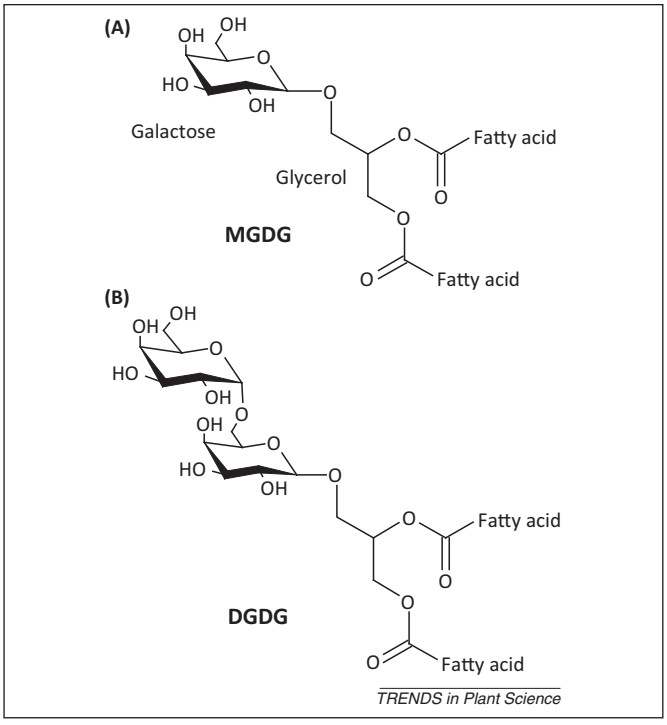

Figure 1. Plastid galactoglycerolipids. The structure of **(A)** monogalactosyldiacylglycerol (MGDG) and **(B)** digalactosyldiacylglycerol (DGDG) is based on a glycerol backbone, esterified by two fatty acids and harboring a polar head containing one or two galactosyl residues. Fatty acids differ in carbon chain length and desaturation levels depending on the species, the physiological and developmental statuses, and their position on the glycerol backbone. The first galactose residue is bound by a β-glycosidic bond to diacylglycerol, whereas the second is linked by a (1→6) α-glycosidic bond. MGDG is synthesized by the action of MGDG synthases (three enzymes in *Arabidopsis*, called MGD1, MGD2, and MGD3). DGDG is synthesized by the action of DGDG synthases (two enzymes in *Arabidopsis*, DGD1 and DGD2). These two lipids have been identified in all photosynthetic membranes analyzed to date.

the binding of the small fraction of DGDG molecules left in the thylakoid membranes to PSII [15]. Altogether, mutant analyses showed a critical role of DGDG in the structure and function of PSII from cyanobacteria to angiosperms. Therefore, in structural, functional, and evolutionary terms, galactoglycerolipids are considered signature lipids in all photosynthetic plastids [24].

Could galactoglycerolipids be absent in nongreen plastids?

This question has been addressed in plastids found in nonphotosynthetic organs and tissues of angiosperms or when plants are grown in the dark. Nongreen plastids include proplastids (in meristems) [25], etioplasts (in etiolated tissues) [26], amyloplasts (in storage tissues, filled with starch) [27], and more exotic forms, globally called leucoplasts, including elaioplasts (in some floral parts, with platoglobules containing sterols) [28] (Figure 2). The chromoplasts (filled with carotenoid-rich plastoglobules) [29] can contain thylakoids and are here considered to be 'green'. All nongreen plastids analyzed to date in vascular plants contained high levels of MGDG and DGDG [26]. The persistence of a galactoglycerolipid-rich envelope during the interconversion of plastids (Figure 2) is considered as one of the features that characterize the uniqueness of plastids in their diversity. Thus, they are considered landmarks of all types of plastid, green or nongreen.

The presence of galactoglycerolipids in nongreen plastids indicates a physiological role that is not directly linked to photosynthesis. On the one hand, when plants are subjected to phosphate (Pi) deprivation, galactoglycerolipids synthesis increases [30] and DGDG is exported to various extraplastidial membranes [31–33], where it can substitute phospholipids [1,31,34,35]. This phenomenon has been observed in roots (containing nongreen plastids) and green tissues (containing chloroplasts). Thus, in response to some environmental changes, galactoglycerolipids become critical for the biogenesis of nonplastidial membranes. On the other hand, analysis of pollen treated with galvestine-1 also highlighted a novel role of galactoglycerolipid for pollen tube elongation [36]. Based on genetic analyses, galactoglycerolipids also have a role in embryogenesis and development, although the underlying processes have not yet been fully elucidated [10,37].

Based on the presence of galactoglycerolipids in all plastids studied so far, their occurrence could be hypothesized in the plastid discovered two decades ago in the cells of *Plasmodium falciparum*, the causative agent of malaria [38,39]. Have galactoglycerolipids been conserved or lost in this parasitic organism? If lost, how would the *Plasmodium* plastid maintain four membranes without this class of lipid? What would be the main constituents instead of galactoglycerolipids? What would a loss of this class of lipid in the Apicomplexa phylum inform on the role of galactoglycerolipids in eukaryotes?

Evolution of galactoglycerolipid biosynthesis

The biosynthesis of MGDG, DGDG, SQDG, and PG, before their incorporation into photosynthetic membranes, occurs in the membranes that delineate cyanobacteria or chloroplasts. A chloroplast bound by two membranes is the most basic structure for this category of organelle, which is well known from algae to plants (the Archaeplastida kingdom). Such plastids are called 'primary plastids' and the two limiting membranes are known as the 'plastid envelope' [2]. Molecular evidence reveals that primary plastids originated from a single event of endosymbiosis (Figure 3) [40]. The envelope derives from the two limiting membranes of the cyanobacterial ancestor [40].

Three lineages have evolved from this initial endosymbiosis. The green lineage, in which chlorophyll *a* and *b* are associated, includes green algae (Chlorophyta) and plants (Charophyta and Embryophyta); the red lineage, where chlorophyll *a* is coupled to phycobilin, comprises the red algae (Rhodophyta); and the blue lineage, where chlorophyll *a* is associated with phycocyanin and allophycocyanin, is a small group (Glaucocystophytes), in which chloroplasts still contain a peptidoglycan cell wall [40].

The localization of galactoglycerolipid synthetic enzymes in primary plastids has been best characterized in angiosperms, using appropriate models for membrane fractionation, that is, pea (*Pisum sativum*) [41], cucumber (*Cucumis sativus*) [42], and spinach (*Spinacia olearacea*) [43]. The localization in *Arabidopsis* has benefitted from the improvement of methods to purify chloroplast envelope membranes [44], advances in proteomic techniques [45],