Configuration of the sugar head of glycolipids in thylakoid membranes

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Running Head: Heterologous glycolipids in thylakoid membranes

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Abstract

Glycolipids constitute the majority of membrane components in oxygenic photosynthetic organisms, whereas they are minor lipids in other organisms. In cyanobacteria, three glycolipids comprise ~90 mol% of the total lipids in thylakoid membranes, where photosynthetic electron transport occurs. Among these glycolipids, 80 mol% are galactolipids (monogalactosyldiacylglycerol and digalactosyldiacylglycerol). Galactolipids are well conserved in oxygenic photosynthetic organisms and are believed to be essential for the integrity of the membrane system. It remains unclear, however, which part(s) of the galactolipid structure is the key factor for their function, e.g., the sugar moiety and/or the anomeric configuration. To address this issue, several bacterial membrane glycolipid synthase genes have been introduced into cyanobacteria to test for complementation of knocked-out genes involved in galactolipid biosynthesis. In this review, we summarize recent advances in the analyses of sugar species and configurations of glycolipids heterologously synthesized in the thylakoid membrane and discuss their functional importance.
INTRODUCTION

The galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are the major constituents of photosynthetic membranes in photosynthetic organisms. These galactolipids are highly conserved, i.e., from the thylakoid membranes of cyanobacteria to the chloroplasts of algae and land plants (Boudière et al., 2014; Hori et al., 2016). MGDG and DGDG comprise up to 50 mol% and 30 mol%, respectively, of thylakoid membrane lipids of these photosynthetic organisms. The remaining major classes of lipids are sulfoquinovosyldiacylglycerol (SQDG) and a phospholipid, phosphatidylglycerol (PG), and each of these lipids comprises 10 mol% of lipids in the membrane system, indicating that the glycolipids MGDG, DGDG, and SQDG constitute ~90 mol% of thylakoid membrane lipids. These glycolipids not only function as constituents of the membrane system but also integrate into photosynthetic protein complexes and are essential for photosynthetic electron transport reactions (Kern et al., 2010). Among them, MGDG and DGDG are conserved in all oxygenic phototrophs and are believed to have pivotal roles in those organisms. However, the reason why these organisms utilize galactolipids is still unclear.
In this review, we first introduce the structure and biosynthetic pathways of galactolipids in the thylakoid membrane. We also describe the physiological relevance of galactolipids and the attempts that have been made to complement the functions of these lipids by introducing heterologous glycolipid synthesis genes into photosynthetic organisms, especially cyanobacteria. For further details about these related topics, please refer to the following reviews: evolution of galactolipid synthesis in photosynthetic organisms (Petroutsos et al., 2014; Sato and Awai, 2016); biogenesis of thylakoid membranes (Rast et al., 2015; Awai, 2016); glycolipids in plants and bacteria (Hölzl and Dörmann, 2007).

**Biosynthesis of galactolipids in oxygenic phototrophs**

Thylakoid galactolipids are composed of a galactose moiety attached to the \( sn \)-3 position of the glycerol backbone and two acyl chains esterified at positions \( sn \)-1 and \( sn \)-2 (Fig. 1). The galactose of MGDG is in the \( \beta \) configuration within the 1,2-diacyl-3-O-(\( \beta \)-D-galactopyranosyl)-\( sn \)-glycerol structure, whereas the second galactose of DGDG is in the \( \alpha \) configuration within the 1,2-diacyl-3-O-(\( \alpha \)-D-galactopyranosyl-\((1\rightarrow6)\)-O-\( \beta \)-D-galactopyranosyl)-\( sn \)-glycerol.
structure (Carter et al., 1956; Boudière et al., 2014; Petroutsos et al., 2014). Although chloroplasts directly synthesize MGDG from diacylglycerol and UDP-galactose (Shimojima et al., 1997), cyanobacteria first convert diacylglycerol and UDP-glucose into 1,2-diacyl-3-O-(β-D-glucopyranosyl)-sn-glycerol (GlcDG), and then its glucose moiety is epimerized to galactose to form MGDG (Feige et al., 1980; Sato and Murata, 1982b; Awai, 2016). These two steps of the cyanobacterial MGDG synthetic pathway involve two different enzymes. The first step is catalyzed by monoglucosyldiacylglycerol synthase (MgdA) (Sato and Murata, 1982a), the gene for which was identified by comparative genomic analysis in two different types of cyanobacteria—unicellular Synechocystis sp. PCC 6803 (hereafter Synechocystis) and filamentous Anabaena sp. PCC 7120 (hereafter Anabaena) (Awai et al., 2006). Candidate genes for MgdA were screened on the basis of conservation of the enzyme activity in both Synechocystis and Anabaena. The unknown genes encoding the glycosyltransferase motif were selected and further extracted using the following criteria: (i) found only in cyanobacteria, and (ii) significant similarity of amino acid sequence in both Synechocystis and Anabaena. mgdA was identified among several candidate genes expressed in Escherichia coli by monitoring the accumulation of GlcDG.
Epimerase, encoded by *mgdE*, is the other enzyme involved in the MGDG synthetic pathway in cyanobacteria. Epimerase is responsible for converting GlcDG into MGDG (Awai et al., 2014) by isomerizing glucose into galactose at the C4 hydroxyl group (Sato, 2015; Sato et al., 2016). A comparative genomic analysis also identified *mgdE* (Awai et al., 2014). The photosynthetic cercozoan *Paulinella chromatophora* was found to possess all the genes for membrane lipid synthesis as cyanobacterial homologs including *mgdA*; thus, *mgdE* was also expected to be conserved in the genome of the chromatophore (a chloroplast-like organelle). Candidate genes were selected from among the unknown genes using the following criteria: (i) widely conserved in most cyanobacteria and in the chromatophore genome of *P. chromatophora*, and (ii) encoding the oxidoreductase motif that triggers the epimerase reaction of glucose into galactose. Three candidate genes were knocked out in *Synechocystis*, which contains a plant-type MGDG synthase from cucumber to prevent a possible lethal effect of the gene disruption (Awai et al., 2006; Shimojima et al., 2009). A knockout mutant of one gene resulted in accumulation of GlcDG. The epimerase activity was further confirmed by co-expressing the candidate gene with *mgdA* in *E. coli* and assessing MGDG accumulation in *E. coli* membranes (Awai et al., 2014).
In contrast with the MGDG synthetic pathway, the DGDG synthetic pathway does not utilize the epimerization reaction. Instead it directly transfers a galactose moiety to MGDG in both plants and cyanobacteria. In plants, a gene that encodes a galactosyltransferase (DGDG synthase, DGD1) was identified by forward genetics using the model plant *Arabidopsis thaliana* (Dörmann et al., 1999). Although DGDG is conserved in both plants and cyanobacteria, there is no homolog of the gene for DGD1 in the genomes of cyanobacteria or primitive unicellular red algae, such as *Cyanidioschyzon merolae* (Matsuzaki et al., 2004). The corresponding gene (*dgdA*) was identified by comparative genomic analysis of glycosyltransferases conserved in cyanobacteria and *C. merolae* (Awai et al., 2007; Sakurai et al., 2007). These studies identified candidate genes in the glycosyltransferase family that produce α-glycosidic linkages because the second galactose moiety of DGDG is linked in the α configuration. DGDG synthase activity was further confirmed by co-expression of *dgdA* with cucumber MGDG synthase cDNA (*csMGD1*) in *E. coli* (Shimojima et al., 1997) because *E. coli* does not have MGDG—the substrate for DGDG synthase. At the same time, glycosyltransferase activity of the enzyme encoded by this gene was also confirmed by disruption of *dgdA* in the *Synechocystis* genome. As expected, the DGDG content was undetectable in the mutant cells of *Synechocystis*, strongly suggesting that
the candidate gene corresponds to DGDG synthase in cyanobacteria (Awai et al., 2007; Sakurai et al., 2007).

Physiological functions of MGDG in thylakoid membranes

Disruption of mgdA is lethal in Synechocystis, probably because MgdA is involved in the synthesis of both MGDG and DGDG (Awai et al., 2006; Shimojima et al., 2009). Knockout mutants of mgdE in Synechocystis cannot produce MGDG or DGDG but instead accumulate GlcDG (Awai et al., 2014). However, these mutants seem to maintain a proper thylakoid membrane structure and can carry out photosynthesis sufficiently to grow photoautotrophically. This finding suggests that galactolipids are not necessary for thylakoid membrane biogenesis or for photosynthesis in Synechocystis. Furthermore, it proved that GlcDG can replace the functions of galactolipids as a bulk constituent of thylakoid membranes (Awai et al., 2014; Awai, 2016). Several cyanobacteria species such as Gloeobacter violaceus, Thermosynechococcus elongatus, and Acaryochloris marina do not possess mgdE. It is reported that these species possess mgdA and also contain GlcDG (Sato, 2015). GlcDG epimerization activity was confirmed in G. violaceus by pulse-chase experiments (Sato, 2015). These results
suggest that these cyanobacteria have GlcDG epimerase types other than MgdE (Sato, 2015). Although GlcDG can substitute for MGDG and DGDG as a building block of thylakoid membranes, Yuzawa et al. (2014) reported that GlcDG is not necessary in *Synechocystis*; they disrupted the endogenous MGDG synthetic pathway via *mgdA* knockout and replaced it with a plant-type MGDG synthetic pathway from Arabidopsis. The consequent absence of GlcDG in *Synechocystis* did not affect its growth rate or photosynthetic activity as long as sufficient MGDG was supplied. The mutant cells also had healthy thylakoid membranes, normal chlorophyll content, and similar lipid composition compared with wild type, i.e., even without GlcDG. We conducted the same *mgdA* knockout experiments in *Synechococcus elongatus* PCC 7942 (hereafter *Synechococcus*) and obtained similar results, indicating that the particular synthetic pathway of MGDG is not important, and GlcDG is not necessary for *Synechococcus* at least under optimal growth conditions (Inoue and Awai, unpublished).

**Physiological functions of DGDG in thylakoid membranes**

As described above, knockout mutants of *dgdA* have been successfully isolated in *Synechocystis*, indicating that the gene, encoded protein, and the reaction product
DGDG are not essential, at least in *Synechocystis* under optimal growth conditions (Awai et al., 2007; Sakurai et al., 2007). However, DGDG is suspected to enhance the growth rate under phosphate-deficient conditions; under phosphate deficiency, the growth of mutant *Synechocystis* cells was inhibited compared with that of the wild type (Awai et al., 2007). The mutant cells also showed a significant decrease in oxygen-evolving activity because of impairment of the photosystem II complex. PsbU, PsbV, and PsbO, which have specific roles in stabilizing the oxygen-evolving complex of photosystem II, were dissociated from the complex in the mutant cells. Although the bulk of DGDG could be replaced by MGDG in *dgdA* knockout *Synechocystis* cells, the specific function of DGDG in stabilizing the oxygen-evolving complex of photosystem II through the binding of extrinsic proteins could not be compensated by other membrane lipids (Sakurai et al., 2007).

Other physiological functions of DGDG in *Synechocystis* have also been defined in different studies. DGDG prevents photoinhibition, as growth of *dgdA* mutants is retarded under intense light (Mizusawa et al., 2009b). This growth phenotype could be complemented to the wild-type level when exogenous DGDG was added to the medium. Growth of *dgdA* mutants was impaired under Ca\(^{2+}\) and/or Cl\(^{-}\) deficiency and exogenous
DGDG also helped the mutant cells recover normal growth capability under Ca\(^{2+}\)-
and/or Cl\(^{-}\)-deficient conditions (Mizusawa et al., 2009b). These results indicate that
DGDG deficiency leads to an imbalance between the photodamage and repair processes
of photosynthesis, which increases sensitivity to photoinhibition (Mizusawa et al.,
2009b). Besides its role in preventing photoinhibition, DGDG is also important for
preventing stress-induced damage at high temperatures (Mizusawa et al., 2009a). The
growth rate of \textit{dgdA} mutants has been compared with that of the wild type at both 30°C
and 38°C and at different light intensities (low light, moderate light, and high light; 10,
40, and 200 μmol photons m\(^{-2}\) s\(^{-1}\), respectively) in solid and liquid media. Wild-type
cells were able to maintain growth under all conditions, whereas the growth of \textit{dgdA}
mutant cells was significantly inhibited under intense light. Even greater inhibition was
observed when the temperature was changed to 38°C, both in solid and liquid media.
Exogenous DGDG restored the stress-induced damage even under intense light and high
temperatures, indicating that, in \textit{Synechocystis}, DGDG prevents stresses not only from
photoinhibition but also from high temperature.

In contrast, in the cyanobacterium \textit{Synechococcus}, \textit{dgdA} is essential and the
knockout is tolerated only when a DGDG synthase gene is introduced from another
cyanobacteria or a plant (Maida and Awai, 2016). This study suggests that the particular synthetic pathway of DGDG is not important, but the lipid product DGDG is necessary in *Synechococcus*. Whereas DGDG is essential in *Synechococcus* but not in *Synechocystis*, the reverse is true for the requirement of SQDG—the other glycolipid in thylakoid membranes. This anionic glycolipid is essential in *Synechocystis* (Aoki et al., 2004), whereas the genes required for SQDG synthesis can be knocked out in *Synechococcus* without effect under optimal conditions (Güler et al., 1996, 2000). The reason for the difference in the SQDG requirement between *Synechocystis* and *Synechococcus* is not known. The requirement for the glycolipids in thylakoid membranes is summarized in Table 1.

**Molecular species and the configuration of the sugar head in MGDGs**

MGDG in thylakoid membranes has a galactose moiety attached to diacylglycerol in the β configuration. In this section, we discuss the molecular species and the configuration of the sugar head; hereafter, we refer to MGDG as βMGDG (also GlcDG as βGlcDG, and DGDG as αβDGDG, see Fig. 2 and 3). What happens to the membranes if the sugar species and/or configuration differs? Knockout mutants of *mgdE* in *Synechocystis*
accumulated βGlcDG instead of βMGDG. These mutant cells showed retarded growth, decreased chlorophyll content, and impaired photosynthetic activity. However, these cells were still capable of growing photoautotrophically (Awai et al., 2014). These results indicate that, at least in *Synechocystis*, βMGDG can be complemented by βGlcDG. The chemical difference between βMGDG and βGlcDG is the sugar species attached to diacylglycerol, namely galactose or glucose. Thus, the particular sugar species in monoglycosyldiacylglycerol is not critical in *Synechocystis*. To determine the effect of the configuration (α or β), Hölzl et al. (2005b) expressed 1,2-diacyl-3-O-(α-D-glucopyranosyl)-sn-glycerol (αGlcDG) synthase from *Acholeplasma laidlawii* (Berg et al., 2001), *Deinococcus radiodurans* (dr1225), or *Thermotoga maritima* (tm0744) in *Synechococcus*. Although accumulation of αGlcDG was expected in the transformants, αMGDG also accumulated. The investigators presumed that this conversion was attributable to the activity of MgdE, which converts βGlcDG into βMGDG. To test this hypothesis, we first co-expressed the gene for αGlcDG synthase from *A. laidlawii* (*AlMGS*) and *mgdE* from *Synechocystis* in *E. coli* and found accumulation of αMGDG (Inoue and Awai, unpublished). Thus, we concluded that the conversion of αGlcDG into αMGDG is attributable to MgdE activity. We then further expressed *AlMGS* in *Synechococcus* to test whether βMGDG can be
replaced by αMGDG and/or αGlcDG by knocking out mgdA in the transformants. The transformants expressing AlMGS accumulated αMGDG; however, no knockout mutant of mgdA was segregated, implying that the functions of βMGDG cannot be complemented by αMGDG, namely that the configuration of monoglycosylacylglycerol in thylakoid membranes does matter in Synechococcus. It is possible that the amount of αMGDG was not sufficient to constitute the membrane system. In Synechococcus, as described above, αβDGDG is essential. αβDGDG synthase in cyanobacteria (DgdA) has strict substrate specificity and utilizes only βMGDG (Awai, 2016), suggesting that αMGDG cannot be a substrate for DgdA. Thus, it is also possible that αMGDG could not complement the mgdA mutation because of the substrate specificity of DGDG synthase. We are now trying to express AlMGS in Synechocystis to test whether αMGDG can complement the mgdA mutation, i.e., given that DGDG is not essential in Synechocystis (Awai et al., 2007; Sakurai et al., 2007).

Molecular species and configuration of the sugar head in diglycosylacylglycerols

αβDGDG is conserved in thylakoid membranes of oxygenic photosynthetic organisms (Fig. 3). In the dgd1-1 mutant of Arabidopsis, which has a stop codon mutation in the
glycosyltransferase motif of DGD1, the αβDGDG content decreased from 16.0 mol% to 1.2 mol% of the total lipid content (Dörmann et al., 1995). This led to various defects in the mutant, especially the dwarf phenotype, mainly because of overproduction of oxylipin and lignification of the phloem cap (Lin et al., 2016). These phenomena were partially complemented by introducing Chloroflexus aurantiacus glycosyltransferase (β-GlcT) (Hölzl et al., 2006, 2009), which synthesizes 1,2-diacyl-3-O-(β-D-glucopyranosyl-(1→6)-O-β-D-galactopyranosyl)-sn-glycerol (ββGlcGDG) with βMGDG as a substrate. Thus, ββGlcGDG can complement at least some functions of αβDGDG in Arabidopsis. However, this is not the case in cyanobacteria. In Synechococcus, accumulation of ββGlcGDG by introducing β-GlcT could not complement the knockout of dgdA (Maida and Awai, 2016). Considering that ββGlcGDG can partially complement photosynthetic efficiency with increased susceptibility of photosystem II to photodamage (Hölzl et al., 2009) and that dgdA mutants of Synechocystis have a similar phenotype (Mizusawa et al., 2009a, 2009b), this result implies that αβDGDG has specific roles in the recovery from photodamage.

SFR2 is the processive glycolipid synthase that synthesizes di-, tri- and tetra-galactosyldiacylglycerol all in the β configuration using MGDG as a substrate (van
Besouw and Wintermans, 1978). In plants, SFR2 encodes an enzyme with the β-galactosidase motif and mainly synthesizes 1,2-diacyl-3-O-(β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl)-sn-glycerol (ββDGDG) and 1,2-diacyl-3-O-(β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl)-sn-glycerol (βββTriGDG) (Moellering et al., 2010). We expressed SFR2 in Synechocystis and Synechococcus, but no accumulation of new glycolipids was detected (Nakaya and Awai, unpublished). It is possible that SFR2 only synthesizes ββDGDG in cyanobacteria, and it co-chromatographs with αβDGDG (Hölzl et al., 2005a). We are now disrupting internal dgdA in the transformants of Synechocystis that overexpress SFR2 to test whether the introduced SFR2 can, at a minimum, synthesize ββDGDG.

Expression of YpfP/UgtP in photosynthetic organisms

ypfP/ugtP encodes the glycolipid synthase that synthesizes mono-, di-, tri-, tetra-glucosyldiacylglycerol by processive reactions (Jorasch et al., 1998). A knockout mutant of this gene in Bacillus subtilis had a round phenotype (Price et al., 1997;
Matsuoka et al., 2011). Detailed regulatory mechanisms of this phenotype have been reported (Seki et al., 2015; Matsuoka et al., 2016) and summarized (Matsuoka, 2018). Hölzl et al. (2006) expressed ypfP/ugtP from B. subtilis and Staphylococcus aureus in Arabidopsis but could not detect any accumulation of newly synthesized glycolipid. Introduction of ypfP/ugtP of S. aureus (ugt106B1) into Synechococcus was also attempted, but no transformants were obtained (Hölzl et al., 2005b). We expressed B. subtilis ypfP/ugtP in Synechococcus and found the accumulation of a newly synthesized glycolipid, but the result was ambiguous. In the cyanobacterial transformants expressing ypfP/ugtP, we detected only one new glycolipid, which chromatographed close to αβDGDG even though YpfP/UgtP is a processive enzyme. The accumulated lipid was presumed to be 1,2-diacyl-3-O-(β-d-glucopyranosyl-(1→6)-O-β-d-glucopyranosyl)-sn-glycerol (ββDGlcDG). However, the glycolipid did not co-chromatograph with either αβDGDG or ββDGlcDG. We have not yet analyzed the structure of the accumulated lipid, but we believe there are two possibilities: (i) MgdE epimerizes the first or second glucose into galactose, or (ii) YpfP/UgtP utilizes βMGDG as a substrate. In the first case, the accumulated lipid will be ββGlcGDG or 1,2-diacyl-3-O-(β-d-galactopyranosyl-(1→6)-O-β-d-glucopyranosyl)-sn-glycerol.
(ββGlcDG) (Fig. 3). In the second case, it will be ββGlcGDG. We are now co-expressing ypfP/ugtP with either mgdE or CsMGD1 in E. coli to determine the mechanisms of synthesis and the structure of the accumulated lipid.

**Perspectives**

Many bacteria reportedly synthesize glycolipids (Hölzl and Dörmann, 2007). However, not many bacterial genes have been reported to encode enzymes for the synthesis of galactolipids or glucolipids. It will be important to identify more genes encoding a galactolipid or glucolipid synthase, such as βαDGDG synthase, to verify the functions of galactolipids in thylakoid membranes. On the other hand, all the genes for galactolipid synthesis in plants and cyanobacteria are known. Also, there are cyanobacteria strains with different lipid requirements. Together with various types of glycolipid synthases, these resources will help us analyze the configurations, i.e., α or β, that are crucial for glycolipid functions in photosynthetic membranes.

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Table 1. Essentiality of thylakoid glycolipids in representative cyanobacteria and *Arabidopsis thaliana*

<table>
<thead>
<tr>
<th>Thylakoid glycolipids</th>
<th><em>Synechocystis</em> sp. PCC 6803</th>
<th><em>Synechococcus elongatus</em> PCC 7942</th>
<th><em>Arabidopsis thaliana</em></th>
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<tbody>
<tr>
<td>MGDG</td>
<td>Not essential&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>Essential&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GlcDG</td>
<td>Not essential&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Not essential&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>DGDG</td>
<td>Not essential&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Essential&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Not essential&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>SQDG</td>
<td>Essential&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Not essential&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Not essential&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ND: Not determined; -, not applicable.

<sup>a</sup>MGDG can be replaced by GlcDG (Awai et al., 2014).

<sup>b</sup>Knockout mutants of major MGDG synthase gene are lethal (Kobayashi et al., 2007).

<sup>c</sup>Cyanobacterial MGDG synthetic pathway can be replaced by the plant-type pathway (Yuzawa et al., 2014; Inoue and Awai, unpublished).
Knockout mutants of *dgdA* can grow photoautotrophically (Awai et al., 2007; Sakurai et al., 2007).

Knockout mutants of *dgdA* are lethal (Maida and Awai, 2016).

Double-knockout mutants of *DGD1* and *DGD2* can grow photoautotrophically (Kelly et al., 2003).

Knockout mutants of *sqdB* are lethal (Aoki et al., 2004).

Knockout mutants of *sqdB* and *sqdX* can grow under optimal conditions (Güler et al., 1996, 2000).

Knockout mutants of SQDG synthetic genes (*sqd1, sqd2, ugp3*) can grow photoautotrophically (Yu et al., 2002; Okazaki et al., 2009).
Figure legends

**Fig. 1. Chemical structures of MGDG and DGDG.** The galactolipids are composed of a galactose moiety attached to the \( sn-3 \) position of the glycerol backbone and two acyl chains esterified at positions \( sn-1 \) and \( sn-2 \). Typical lipid species in cyanobacteria are shown, i.e., oleic acid at the \( sn-1 \) and palmitic acid at the \( sn-2 \) position.

**Fig. 2. Schematic representation of monoglycosyldiacylglycerols.** All sugars are glucose or galactose in the \( \alpha \) or \( \beta \) configuration. DAG: diacylglycerol.

**Fig. 3. Schematic representation of diglycosyldiacylglycerols.** All sugars are glucose or galactose in the \( \alpha \) or \( \beta \) configuration. DAG: diacylglycerol.
Monogalactosyldiacylglycerol (MGDG)

Digalactosyldiacylglycerol (DGDG)

Apdila and Awai Figure 1
\( \alpha\beta\text{DG}_{\text{DG}} \)  
\( \beta\beta\text{DG}_{\text{DG}} \)  
\( \beta\beta\text{DG}_{\text{GlcDG}} \)  
\( \beta\beta\text{DG}_{\text{GlcDG}} \)  
\( \beta\beta\text{G}_{\text{GlcDG}} \)  

Apdila and Awai Figure 3