Biological functions of glucolipids in *Bacillus subtilis*

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Glyceroglycolipids are very important in Gram-positive bacteria and cyanobacteria. In *Bacillus subtilis*, a model organism for the Gram-positive bacteria, the *ugtP* mutant, which lacks glyceroglucolipids, shows abnormal morphology. Lack of glucolipids has many consequences: abnormal localization of the cytoskeletal protein MreB and activation of some extracytoplasmic function (ECF) sigma factors (σ^M, σ^V and σ^X) in the log phase are two examples. Conversely, the expression of monoglycylglycerol (MGlcDG) by 1,2-diacylglycerol 3-glucosyltransferase from *Acholeplasma laidlawii* (alMGS) almost completely suppresses the *ugtP* disruptant phenotype. Activation of ECF sigmas in the *ugtP* mutant is decreased by alMGS expression, and is suppressed to low levels by MgSO_4 addition. When alMGS and alDGS (A. laidlawii 1,2-diacylglycerol-3-glucose (1-2)-glucosyltransferase producing diglycylglycerol (DGlcDG)) are simultaneously expressed, σ^X activation is repressed to wild type level. These observations suggest that MGlcDG molecules are required for maintenance of *B. subtilis* cell shape and regulation of ECF sigmas, and that DGlcDG regulates σ^X activity. The activation of ECF sigmas is not accompanied by proteolysis of anti-σ. Thus, glyceroglucolipids may have the specific role of helping membrane proteins function by acting in the manner of chaperones.

**Key words:** *Bacillus subtilis*, glucolipid, *ugtP*, ECF sigma factor, cell wall maintenance

**INTRODUCTION**

All living organisms have membranes that compartmentalize cells from the outer environment and from each other. A lipid bilayer structure for these membranes was proposed by Singer and Nicolson in 1972. In this model, membrane proteins are floating in a “sea of lipids” and can move freely in the membrane. Lipid molecules are uniformly distributed in the membrane. This model is almost correct; however, recent studies have demonstrated the existence of specific lipid-protein domains, which come about by lipid molecules and membrane proteins forming domain structures, so-called lipid rafts, in the membrane (Raghunathan et al., 1990). In bacteria, the existence of lipid rafts remains uncertain, although some researchers have reported evidence pointing to lipid rafts as detergent-resistant membrane fractions (Donovan and Bramkamp, 2009). On the other hand, localizations of lipid molecules have been reported. The presence of cardiolipin (CL) or anionic phospholipids in specific regions is required for many biological functions such as the localization of ClsA (Kusaka et al., 2016) and the Min system (Ishikawa et al., 2017) in *Bacillus subtilis*. In cyanobacteria, galactolipids play an important role in photosynthesis as they are included in the thylakoid membranes, which are the sites of oxygenic photosynthesis (Mizusawa and Wada, 2012); the crystal structures of the photosystem I (PSI) and PSII complexes from the thermophilic cyanobacterium *Synechococcus elongatus* reveal that PSI contains one molecule of monogalactosyldiacylglycerol (Jordan et al., 2001), while PSII contains six monogalactosyldiacylglycerol and five digalactosyldiacylglycerol molecules (Umema et al., 2011). It is difficult to uncover the biological functions of membrane lipid molecules because these are products resulting from enzyme reactions, not directly encoded in genes, and there are only few probes that detect specific lipid molecules. To reveal the biological functions of membrane lipids, genes that encode lipid synthesis enzymes have been inactivated, and observations of the resulting phenotypes have been performed in an attempt to gain clues. Shibuya, Dowhan and their colleagues have revealed gene regulation and physiological roles of membrane lipids in *Escherichia coli* using molecular genetics (Shibuya, 1992; Mileykovskaya...
and Dowhan, 2005). However, the knowledge based on E. coli studies cannot be naively applied to B. subtilis.

In this outline, lipid synthesis pathways, cell surface structures and biological functions of membrane lipids in B. subtilis are reviewed. The biological functions of glucolipids are then examined.

**Synthesis of membrane lipids in B. subtilis**  The biosynthesis pathways of phospholipids are shown in Fig. 1. The pathway in B. subtilis is almost the same as that in E. coli. The main phospholipid molecules of E. coli are phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and CL. They make up about 70%, 25% and 5%, respectively, of phospholipids in the cell membrane. In B. subtilis, PE, PG and CL constitute 30%, 40% and 5% of the cell membrane lipids, respectively. In addition to these phospholipids, diacylglycerol (DG), glucolipids and lysylphosphatidylglycerol (LysylPG) make up about 5% and 10% each, respectively, in B. subtilis cell membranes (Matsuoka et al., 2011b; Hashimoto et al., 2013). The B. subtilis cell contains three kinds of glucolipids, monoglucosyldiacylglycerol (MGlcDG), diglucosyldiacylglycerol (DGlcDG) and triglucosyldiacylglycerol (TGlcDG), which amount to 1.2%, 9.8% and 0.3% of total membrane lipids (Kawai et al., 2006). These are synthesized processively by UgtP, which transfers glucose to diacylglycerol from UDP-glucose (Jorasch et al., 1998). In these pathways, phosphatidic acid (PA) is synthesized from glycerol-3-phosphate (G3P), but the first reactions to lysophosphatidic acid (LPA) synthesis are different between B. subtilis and E. coli (Hara et al., 2008). The gene cdsA, which encodes CDP-diglycerol synthase, is an essential gene in both organisms. Conditional knockout mutants lacking either PE or acidic phospholipids (PG and CL) can be constructed in E. coli. By contrast, pssA, which encodes the key enzyme to synthesize PE, can easily be deactivated without obvious defective phenotype, and pgsA, which encodes the key enzyme to synthesize PG, is indispensable in B. subtilis (Salzberg and Helmann, 2008; Hashimoto et al., 2009). DgkB, a diacylglycerol kinase of B. subtilis, is also essential (Kobayashi et al., 2003). In the absence of LtaS homolog genes, dgkB is dispensable because accumulation of DG, which is toxic for cells, does not occur (Matsuoka et al., 2011b).

**Cell surface and membrane lipids of B. subtilis**  The cell walls of B. subtilis, a model microorganism representative of the Gram-positive bacteria, consist of thick multiple layers of peptidoglycan, which is the major physical and chemical defense against deadly agents. The envelope of Gram-positive bacteria contains further components: polymers of glycerolphosphate, wall teichoic acids covalently linked with peptidoglycan, and lipoteichoic acids (LTA) linked with DGlcDG on the membrane (Schirner et al., 2009). In Staphylococcus aureus, LTA is essential for normal cell growth (Gründling and Schneewind, 2009). In Staphylococcus aureus, LTA is essential for normal cell growth (Gründling and Schneewind, 2009).
Biological functions of glucolipids in *B. subtilis* Based on many genetic studies using *E. coli* cells, the physical and chemical properties of membrane lipids are unquestionably important for their functions. In other words, their "acidity" and capacity to form nonbilayers are essential for them to function in the membrane. However, recent studies imply that lipid molecules have other specific roles that go beyond providing material with certain simple chemical properties. In *B. subtilis*, PG is essential for growth (Hashimoto et al., 2009), whereas in *E. coli* it is not. This suggests that the repertoire of functions a lipid performs is specific in each organism, and not a universal aspect of the substance itself.

Although *ugtP* in *B. subtilis*, encoding glucosyltransferase, is dispensable, a *ugtP* mutant shows abnormal morphology. The phenotype of cells with *ugtP* disruption was first reported to be shorter and rounder by Price et al. (1997). The *ugtP* mutant cells have also been reported to show strikingly abnormal cell morphology (Lazarevic et al., 2005). In addition, the GFP fusion of UgtP, which is predicted to be a cytoplasmic protein with no transmembrane segment, is localized in the septal region in the form of an open-ring similar to the FtsZ-ring (Nishibori et al., 2005). Weart et al. (2007) have proposed that UgtP regulates the timing of cell division by adjusting the assembly of FtsZ protein, based on their finding of dissociation in vitro of FtsZ polymer in the presence of UgtP. Disruption of *pgcA* and *gtaB*, which are involved in UDP-glucose synthesis, leads to a cell shape that is known to occur as a consequence of *ugtP* disruption. Since these mutants lack glucolipids, the abnormal morphology of the *ugtP* mutant is presumably caused by the lack of glucolipids (Matsuoka et al., 2016).

Glucolipids are important for the localization of MreB, especially during the mid-log phase. MreB had been believed to localize helically along the cell axis with MreBH and Mbl in wild type cells (Jones et al., 2001; Graumann, 2007; Kawai et al., 2009a; Kawai et al., 2009b). This apparently helical pattern has recently been shown to stem from a misinterpretation owing to the increased depth of field (Domínguez-Escobar et al., 2011; Garner et al., 2011). In another study of cells lacking glucolipids, discrete dots of GFP-MreB failed to appear. Instead, faint dots of fluorescence were scattered all over whole cells and the MreB amount was labile in the absence of glucolipids (Matsuoka et al., 2011a). Glucolipids may be required, directly or indirectly, for construction of the MreB cytoskeletal structure.

Glucolipids may also be required for maintenance of cell surface structure, as it has been observed that the *ugtP* mutant shows abnormal morphology. Therefore, it is believed, the extracytoplasmic function (ECF) sigmas are activated in glucolipid-lacking cells. The ECF sigmas σ^V, σ^X and σ^M were found to be activated in the *ugtP* mutant cells (Matsuoka et al., 2011a). Analysis of the influence of glucolipids produced by UgpT on the activity of ECF sigmas in an *E. coli* heterologous expression system also suggested that glucolipids alone are sufficient to strongly affect the activity of σ^M and σ^X (Seki et al., 2015). Furthermore, production of heterologous MGLcDG in the *ugtP* mutant suppressed the abnormal morphology and repressed activation of σ^M, σ^X and σ^M. Moreover, when heterologous glucolipid synthases (alMGS and alDGS) from *Acholeplasma laidlawii* were expressed simultaneously, the σ^X activity was specifically suppressed to wild type level (Matsuoka et al., 2016). This indicates that MGLcDG is required for maintaining normal cell shape and controls the activities of a number of ECF sigmas via membrane-anchored anti-σ factors. Addition of magnesium ion allows the cell membrane to attain a condition similar to the condition it has when containing glucolipids. We come to the tentative conclusion that DGlcDG and/or LTA may be involved in the regulation of σ^X. Thus, each glucolipid species plays many different roles in *B. subtilis* cells.

The ECF sigmas that are activated in *ugtP* mutants are concerned with cell envelope maintenance (Cao et al., 2002; Asai et al., 2003). Activation of ECF sigmas in the *ugtP* mutant is not accompanied by regulated intramembrane proteolysis. In the case of σ^V activation, no proteolysis of RsiV was observed in *ugtP* mutant cells (Seki et al., 2017). It has been suggested that an appropriate length of transmembrane region is required for proper integration of anti-σ into the membrane (Yano et al., 2011). Thus, glucolipids seem to have specific functions, and aid the function of membrane proteins by acting as "lipid chaperones" (Fig. 2). In the absence of glucolipids, membrane proteins do not fold normally and their dysfunctionality may lead, in particular, to release of sigma factors. The lack of glucolipids affects cell functions in multiple ways.
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Fig. 2. A model for the roles of glucolipids in the B. subtilis membrane. In wild type cells, ECF sigma is tethered by its cognate membrane anti-$\sigma$, and the activity of ECF sigma is repressed (left). In the absence of glucolipids (in the ugtP mutant), ECF sigma is released because anti-$\sigma$ is misfolded and thus dysfunctional. The regulon genes of sigma are then expressed (right). Glucolipids control the folding and functionality of anti-$\sigma$ by acting like chaperones.
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