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Review

# Phosphatidic acid synthesis in bacteria ☆

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ABSTRACT

Membrane phospholipid synthesis is a vital facet of bacterial physiology. Although the spectrum of phospholipid headgroup structures produced by bacteria is large, the key precursor to all of these molecules is phosphatidic acid (PtdOH). Glycerol-3-phosphate derived from the glycolysis via glycerol-phosphate synthase is the universal source for the glycerol backbone of PtdOH. There are two distinct families of enzymes responsible for the acylation of the 1-position of glycerol-3-phosphate. The PlsB acyltransferase was discovered in *Escherichia coli*, and homologs are present in many eukaryotes. This protein family primarily uses acyl–acyl carrier protein (ACP) endproducts of fatty acid synthesis as acyl donors, but may also use acyl-CoA derived from exogenous fatty acids. The second protein family, PlsY, is more widely distributed in bacteria and utilizes the unique acyl donor, acyl-phosphate, which is produced from acyl-ACP by the enzyme PlsX. The acylation of the 2-position is carried out by members of the PlsC protein family. All PlsCs use acyl-ACP as the acyl donor, although the PlsCs of the γ-proteobacteria also may use acyl-CoA. Phospholipid headgroups are precursors in the biosynthesis of other membrane-associated molecules and the diacylglycerol product of these reactions is converted to PtdOH by one of two distinct families of lipid kinases. The central importance of the *de novo* and recycling pathways to PtdOH in cell physiology suggest that these enzymes are suitable targets for the development of antibacterial therapeutics in Gram-positive pathogens. This article is part of a Special Issue entitled Phospholipids and Phospholipid Metabolism.



## 1. Introduction

Bacteria produce a bewildering variety of phospholipids that play critical roles in the adaptation to the environment. Phosphatidic acid (PtdOH) (Fig. 1) is the universal precursor required for the production of these molecules. In contrast to the variety of enzymes involved in producing the broad spectrum of bacterial phospholipid structures, there are only a limited number of enzymes required for the formation of PtdOH, the key intermediate in their synthesis. This review covers the enzymes and pathways responsible for the *de novo* formation of PtdOH and the recycling enzymes that produce PtdOH from the diacylglycerol formed from the utilization of phospholipids in the biosynthesis of other molecules. In the early days of research on bacterial lipid metabolism, the field thought that understanding these pathways would provide important insight into how all cells construct their phospholipids. Indeed, the elucidation of the formation of *sn*-glycerol-3-phosphate (G3P) and the consecutive acylation of the 1-position followed by the 2-position of G3P in *Escherichia coli* identified enzymes and genes that have homologous sequences and functions in mammalian systems. More recently, it has become apparent that a primary bacterial enzyme for the acylation of the 1-position of G3P in many human pathogens has no mammalian homologs and uses a different acyl donor that other acyltransferases in biology. This review covers the current knowledge and future directions for research on these two primary acyltransferase pathways to PtdOH and the kinases involved in PtdOH metabolism in bacteria.

## 2. Glycerol-3-phosphate

PtdOH is the biosynthetic product of the esterification of two fatty acids onto the two hydroxyl groups of *sn*-glycerol-3-phosphate (G3P). The formation of G3P from the reduction of dihydroxyacetone phosphate by the G3P synthase (GpsA) is the only *de novo* pathway to G3P in bacteria [1–4] (Fig. 1). Dihydroxyacetone phosphate is diverted from the glycolytic pathway, so GpsA links intermediary and lipid metabolism. GpsA is different from GlpD, the aerobic G3P dehydrogenase, which breaks down G3P for energy production [5]. The inactivation of the *gpsA* gene in *E. coli* and *Staphylococcus aureus* gives rise to glycerol or G3P auxotrophs illustrating that GpsA is required for the biosynthesis of G3P *in vivo* [1,6]. *E. coli* GpsA is a soluble enzyme that is strongly inhibited by its product, G3P. The stringent regulation of the production of G3P buffers the intracellular G3P concentration to ensure a steady supply of G3P for lipid biosynthesis. However, the experimental manipulation of the intracellular G3P concentration showed that it does not have a role in regulating phospholipid formation [7].

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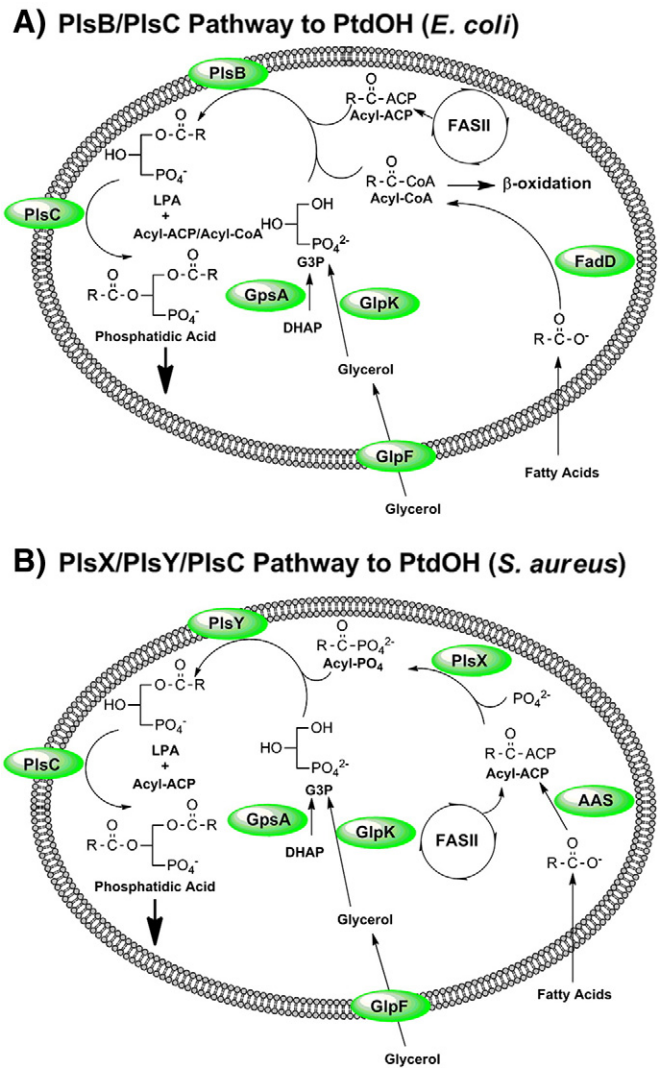


**Fig. 1.** Pathways for the biosynthesis of PtdOH in bacteria. (A) PtdOH metabolism in *E. coli* is representative of the bacteria that utilize the PlsB/PlsC acyltransferase pathway to PtdOH. These acyltransferases use either acyl-ACP substrates produced by type II fatty acid synthesis (FASII) or acyl-CoA thioesters generated by the activation of exogenous fatty acids by acyl-CoA synthetase (FadD). The PlsB pathway is largely confined to the γ-proteobacteria. (B) PtdOH metabolism in *S. aureus* is representative of bacteria that utilize the PlsX/PlsY/PlsC acyltransferase pathway to PtdOH. Acyl-ACP generated by FASII is either used by PlsC to acylate the 2-position of LPA or is converted by PlsX to acyl-$PO_4$ for incorporation into the 1-position by PlsY. This is the only pathway present in most Gram-positive pathogens. In both schemes, the G3P backbone is produced by GpsA, but may also be obtained from the environment by the GlpF/GlpK pathway. Many bacteria also have a transport system for G3P (not shown). Exogenous fatty acids are incorporated into phospholipid following their ligation to ACP by acyl-ACP synthetases (AAS). PlsB, PlsC, PlsY and GlpF are intrinsic membrane proteins. AAS, FadD and PlsX are soluble proteins that are thought to interact with the membrane interface.

In contrast, *Bacillus subtilis* GpsA is not feedback inhibited by G3P, possibly due to Gram positive bacteria requiring more G3P units for cell wall biosynthesis than for phospholipid formation [8]. A major Gram-positive cell wall component lipotechoic acid contains 14–33 G3P units [9,10]. The increased metabolic demand for G3P correlates with the relaxed regulation of the GpsA in Gram-positive bacteria.

Bacteria also obtain G3P directly from the environment (GlpT) or through the uptake and phosphorylation of glycerol by GlpF and GlpK. *E. coli* can use G3P and glycerol as the sole carbon source through the utilization of the genes encoded in the *glp* regulon, spread over 5 operons that allow the import and metabolism of G3P, glycerol, and glycerophosphodiesters [11,12]. Expression of the *glp* regulon is controlled at two levels. At the global level, the expression of the *glp* genes is suppressed when preferred carbon sources, such as glucose, are present through the regulation by the cAMP-CRP complex as a part of global catabolite repression [13]. At the local level, *glp* regulon expression is controlled by the *glp* represser, GlpR [14]. GlpR is a tetrameric protein that binds to the operators of the *glp* operons to prevent transcription [11,12]. G3P induces the expression of the regulon by binding to GlpR and decreasing the affinity of GlpR for the operators. The *glp* operons have differential sensitivity to the repressor, with *glpFK* operon approximately 3 times more sensitive to repression than the *glpTQ* operon [14]. The regulatory mechanism of glycerol and G3P metabolism from *E. coli* is not representative of all other bacteria. *B. subtilis* glycerol and G3P utilizing genes are organized differently and expression of the *glp* regulon is controlled by the antiterminator protein GlpP [2,15]. Certain bacteria, such as *Streptococcus pneumonia* don't have the genes for glycerol or G3P metabolism and therefore can't metabolize exogenous G3P or glycerol at all. Most of the genes in the *glp* regulon are involved in breaking down G3P for energy, and therefore not the subject of this review. However, three gene products from the *glp* regulon, GlpT, GlpF, and GlpK, are involved in assimilating G3P from the environment and are important to understanding how *gpsA* mutants are used to study PtdOH metabolism.

The organophosphate:phosphate antiporter GlpT actively transports G3P into the cell using the energy from the efflux of phosphate [16–18]. *E. coli* GlpT is the best characterized family member and serves as a model for the GlpT from other bacteria [16]. *E. coli* GlpT consists of twelve transmembrane helices spanning the inner membrane. The crystal structure of *E. coli* GlpT has been solved to 3.3 Å [19,20]. GlpT operates via a single binding site mechanism. The antiporter alternates between two conformations: the $C_i$ conformation where the active site is accessible from the cytosol and the $C_o$ conformation where the active site is accessible from the periplasm. Inorganic phosphate binding to the $C_i$ conformation causes the transporter to adopt the $C_o$ conformation, transporting the phosphate across the membrane and allowing G3P to bind. G3P binding to the $C_o$ conformation causes the conformation to change back into the $C_i$ conformation, transporting G3P into the cytosol and allowing another cycle of transport.

The aquaglyceroporin GlpF facilitates the passive diffusion of glycerol through the cell membrane [21,22], and the glycerol kinase, GlpK, phosphorylates glycerol to trap G3P inside the cell [23,24]. GlpF mediated influx is rapid, highly selective for glycerol and other polyols, and essentially nonsaturable [25,26]. The crystal structure of GlpF shows that each GlpF monomer of the associated tetramer forms a glycerol conducting channel with two half-membrane-spanning and six transmembrane α helices [21]. The helices form a narrow amphipathic selectivity channel that is wide enough to accommodate a single CH – OH group that forces the glycerol hydroxyls to traverse the channel in single file. Intracellular glycerol is trapped by GlpK phosphorylation. *E. coli* GlpK is a soluble protein that associates as a homotetramer [24]. *E. coli* GlpK operates via an ordered mechanism where glycerol binds first to the enzyme followed by ATP. Fructose-1,6-bisphosphate allosterically inhibits *E. coli* GlpK, and the feedback inhibition prevents the overproduction of G3P when glucose is present in the media [27,28]. The growth of *E. coli* constitutively expressing a mutant GlpK that is refractory to fructose-1,6-bisphosphate regulation is inhibited by extracellular glycerol [29] illustrating that the overproduction of intracellular G3P causes growth stasis in *E. coli*.

## 3. Acyl donors

### 3.1. Acyl-ACP (acyl–acyl carrier protein)

ACP is the predominant acyl group carrier in bacterial fatty acid synthesis [30] (Fig. 1). ACP is a 9 kDa protein with a 4′-phosphopantetheine