How Bacterial Pathogens Eat Host Lipids: Implications for the Development of Fatty Acid Synthesis Therapeutics

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Running Title: Exogenous fatty acid incorporation in bacteria

Bacterial type II fatty acid synthesis (FASII) is a target for the development of novel therapeutics. Bacteria incorporate extracellular fatty acids into membrane lipids raising the question of whether pathogens use host fatty acids to bypass FASII and defeat FASII therapeutics. Some pathogens suppress FASII when exogenous fatty acids are present to bypass FASII therapeutics. FASII inhibition cannot be bypassed in many bacteria because essential fatty acids cannot be obtained from the host. FASII antibiotics may not be effective against all bacteria, but a broad-spectrum of Gram-negative and -positive pathogens can be effectively treated with FASII inhibitors.

The Biological Problem

The discovery of antibiotics for the treatment of infectious bacterial diseases has led to significant improvements in human health. The success of these broad-spectrum “miracle drugs” is reflected in the dearth of new antibiotic classes introduced in the last 30 years (1). However, the steady increase in bacterial antibiotic resistance to all antimicrobials in clinical use (2,3) have caused infectious bacterial diseases to re-emerge as a serious threat to human health. These resistant bacteria are a clear and present danger that will require the discovery of new antibiotic targets and drugs to combat (4). Fatty acid synthesis is a vital facet of bacterial physiology and its essentiality for membrane formation makes it an attractive target for drug discovery (5). Nature has exploited this dependence to produce a variety of natural products that target bacterial fatty acid synthesis (5-7). Selective targeting of the bacterial pathway is possible due to the significant differences in the structure of eukaryotic and bacterial fatty acid synthesis systems. Mammalian type I fatty acid synthase is a large, multifunctional peptide that elongates acetyl-CoA to produce a single product, palmitic acid (8). In the bacterial type II system (FASII), each enzymatic step is carried out by a discrete, monofunctional enzyme (Fig. 1) (9). Many inhibitors targeting FASII enzymes have been made in the past two decades, and several of these inhibitors are effective antibiotics both in vitro and in animal models (10-13). Two of these compounds, AFN-1252 and CG400549, have proven efficacy in human clinical trials providing direct evidence that targeting FASII components can result in effective therapeutic agents (14,15).

Most bacteria are able to incorporate extracellular fatty acids into their membrane phospholipids, leading to the important question of whether this property will allow them to circumvent FASII inhibitors by acquiring the fatty acids they need from the host. Brinster et.
al. (16) argued that FASII is not an antibacterial target in Gram-positive bacteria due to the ability of *Streptococcus agalactiae* to circumvent FASII inhibitors when supplied with exogenous host-derived fatty acids. However, the situation is more complex because not all Gram-positive bacteria have the same fatty acid structures as mammals and the conclusion is not consistent with the experimental evidence showing the efficacy of FASII therapeutics against the Gram-positive pathogen *Staphylococcus aureus* in animal models (10-12). Parsons and coworkers showed that endogenous fatty acid synthesis is essential for the Gram-positive pathogen *S. aureus*, and that the results with *S. agalactiae* are not representative of all Gram-positive bacteria (17). One of the main gaps in our understanding of this important biological problem is that the mechanism for the incorporation of exogenous fatty acids into phospholipids of Gram-positive bacteria has only recently been revealed (18,19). The goal of this review is to cover the diversity of pathways used by bacteria for host fatty acid utilization, explain how these pathways are deployed in major groups of pathogens, and to discuss how understanding these biochemical pathways informs the development of FASII inhibitors as therapeutics.

**Drug targets in FASII**

An overview of the core enzyme set in bacterial type II fatty acid synthesis is illustrated in Fig. 1. In principle, each of these enzymes is essential and would therefore be a target for antibacterial drug discovery. In practice, drug discovery efforts have primarily focused on four enzymes that are of regulatory significance (6,20). Acetyl-CoA carboxylase (AccABCD) supplies malonyl-CoA for fatty acid elongation, and β-ketoacyl-ACP synthase III (FabH) is responsible for the initiation of new acyl chains and determines how many fatty acids are made. In addition, there are two enzymes in the elongation cycle that have received attention. These are: 1) the enoyl-ACP reductase (FabI), which is responsible for pulling cycles of elongation to completion in the *E. coli* model; and 2) the elongation condensing enzymes (FabF/B) which start each new round of elongation. Nature has also produced a collection of natural product antimicrobial FASII inhibitors, and each of these molecules target one of these enzyme systems (6). These natural products have been recently reviewed (5), and a discussion of their properties is beyond the scope of this review. The existence of multiple anti-FASII natural products supports the conclusion that FASII is a viable target for antibacterial drug discovery. However, it is important to point out that the major drawback to the use of these natural products as antibacterial therapeutics lies in their poor pharmacokinetic properties that make them poor drug candidates.

The branch points in unsaturated fatty acid synthesis will not be considered in this discussion because in all bacteria that produce unsaturated fatty acids, the requirement for this fatty acid can be met by providing extracellular unsaturated fatty acid supplements (21). Readily available and abundant host unsaturated fatty acids, like oleate, can circumvent inactivating mutations in these pathways, making unsaturated fatty acid synthesis a target that is unlikely to receive much attention in the drug discovery field.

**Exogenous fatty acid metabolism by Gram-negative bacteria**

For many years, *Escherichia coli* was considered the presentative organism for all bacteria. Therefore, the fatty acid synthesis and exogenous fatty acid incorporation pathways in *E. coli* were the first to be fully characterized (Fig. 2) (22). Gram-negative FASII generates two products that become essential components of the bacterial membrane: acyl-ACP and β-hydroxyacyl-ACP. Two acyl-ACP molecules are used by the sn-glycerol-3-phosphate acyltransferase (PlsB) and 1-acyl-sn-glycerol-3-phosphate acyltransferase (PlsC) to generate phosphatidic acid (23-25), the precursor to all cytoplasmic membrane phospho- and glycolipids. Gram-negative bacteria have an outer membrane surrounding the cytoplasmic membrane that is composed primarily of phosphatidylethanolamine and lipopolysaccharide (26,27). β-Hydroxyacyl-ACP molecules are substrates for the acyltransferases that catalyze the initial steps in the biosynthesis of lipid A, the core lipid component of lipopolysaccharide (28). Both phospholipids and lipopolysaccharides are
essential for *E. coli* survival (29,30), and FASII inhibition stops *E. coli* growth by blocking these two essential pathways.

In *E. coli*, the pathway for extracellular fatty acid utilization begins with the conversion of the fatty acid to an acyl-CoA thioester by FadD, an acyl-CoA synthetase (25,31) (Fig. 2A). *E. coli* PlsB and PlsC acyltransferases use acyl-CoAs as substrates (23,24), permitting exogenous fatty acids to be directly used for phospholipid biosynthesis. The second fate for acyl-CoA is their utilization as a carbon and energy source via \( \beta \)-oxidation (32,33). The key feature of most Gram-negative bacteria is that they lack both an acyl-ACP synthetase and/or an acyl-CoA:ACP transacylase and cannot convert fatty acids or acyl-CoAs to acyl-ACP (34). Thus, entry of exogenous fatty acids into FASII cannot occur. This means that the \( \beta \)-hydroxy-fatty acids produced by FASII for incorporation into lipopolysaccharide via the acyl-ACP-specific acyltransferases cannot be made from extracellular fatty acid sources. One confusing piece of metabolism is that exogenous fatty acids produced by FASII for incorporation into lipopolysaccharide via the acyl-ACP-specific acyltransferases cannot be made from extracellular fatty acid sources. One confusing piece of metabolism is that exogenous fatty acids can be converted into acyl-ACP by the bifunctional 2-acylglycerophosphoethanolamine acyltransferase enzyme, which was called acyl-ACP synthetase (aas) based on the ability to detect this activity in vitro (35) (Fig. 2B). In the first step, fatty acids are activated by the synthetase domain of the membrane-associated enzyme and ligated to a bound ACP subunit. The acyltransferase domain then transfers the acyl group attached to the ACP subunit to lysophospholipids that arise as a byproduct of lipoprotein synthesis (36). Although the acyl-ACP synthetase half-reaction can be demonstrated in vitro and has found use in the synthesis of these key intermediates, acyl-ACP is not liberated from the enzyme complex in vivo (37-39) (Fig. 2B). Thus, an acyl-ACP synthetase reaction does occur in these bacteria, but the acyl-ACP is not available to the acyltransferases of phospholipid and lipopolysaccharide synthesis. There are of course exceptions to this rule. *Vibrio harveyi* is capable of elongating exogenous fatty acids (40). This property is attributed to the presence of a soluble acyl-ACP synthetase (41,42). It is not clear how widespread the acyl-ACP synthetase activation pathway is in bacteria, because this enzyme has not been characterized in medically relevant pathogens.

Because acyl-CoA molecules made from exogenous fatty acids of the appropriate length can be substituted for acyl-ACP in making phosphatidic acid, exogenous fatty acids bypass the effects of FASII inhibition on phospholipid synthesis. However, there is no pathway for exogenous fatty acids to become \( \beta \)-hydroxyacyl-ACP required for lipid A biosynthesis because acyl-CoA molecules cannot be converted to acyl-ACP and enter FASII. Therefore, the block on lipopolysaccharide synthesis from FASII inhibition cannot be bypassed by exogenous fatty acids. Even if the exogenous fatty acids were activated by an acyl-ACP synthetase and enter the FASII pathway, the acyl-ACP still cannot be converted to \( \beta \)-hydroxyacyl-ACP if the FASII system is chemically inhibited. The essentiality of the cell wall to Gram-negative bacteria means that exogenous fatty acids cannot rescue these organisms from FASII therapeutics. The exogenous fatty acid incorporation pathway of *E. coli* is likely conserved in most \( \gamma \)-proteobacteria, which includes medically important bacteria groups such as *Enterobacteriaceae* and *Pseudomonas*. However, Gram-negative bacterial diversity is large, and organisms more distantly related to *E. coli* need to be characterized to validate that these conclusions hold for all members of this group.

**Exogenous fatty acid metabolism by Gram-positive bacteria**

Gram-positive bacteria do not have an outer membrane and therefore use fatty acids derived from FASII primarily for membrane phospholipid formation. These bacteria use the same basic FASII pathway, but have a distinct acyltransferase system (Fig. 3). In these bacteria, long chain acyl-ACPs produced from FASII are converted into a novel activated fatty acid, an acyl-phosphate (acyl-PO\(_\alpha\)). Acyl-PO\(_\alpha\) is formed by PlsX, an acyl-ACP:PO\(_\alpha\) transacylase (43). The acyl-PO\(_\alpha\) is used to acylate the 1-position of sn-glycerol-3-phosphate by PlsY. The second step in phosphatidic acid formation is catalyzed by PlsC, but unlike Gram-negative PlsC, the Gram-positive isoforms only utilize exclusively acyl-ACP as substrate (Fig. 3). Acyl-CoA has no known role in Gram-positive lipid metabolism (18). Rather, the first step in the incorporation
of exogenous fatty acids is their activation by fatty acid kinase to generate acyl-\(\text{PO}_4\). The acyl-\(\text{PO}_4\) can be used by PlsY to initiate phospholipid synthesis or be converted to acyl-ACP by PlsX (Fig. 3). The acyl-ACP is either used by PlsC or can enter FASII for elongation. Fatty acid kinase is composed of two dissociable subunits (19). The kinase domain protein (FakA) phosphorylates a fatty acid tightly bound to the fatty acid binding protein component (FakB). FakB is capable of exchanging the bound acyl-\(\text{PO}_4\) with a membrane bound fatty acid releasing the acyl-\(\text{PO}_4\) into the membrane for utilization by PlsY or PlsX and picking up another fatty acid substrate for FakA. The repetitive cycle of phosphorylation and exchange accounts for fatty acid incorporation into the membrane lipids of Gram-positive bacteria (Fig. 3).

The Order Lactobacillales, which contain bacteria like *Streptococcus*, *Lactococcus*, *Clostridia*, etc. have a unique mechanism to regulate FASII in the presence of exogenous fatty acids. These bacteria completely suppress FASII activity when exogenous fatty acids are present, and if sufficient fatty acid supplement is available, extracellular fatty acids are exclusively incorporated into their membrane lipids (16,17). In this group of bacteria, the FASII genes are organized into a single locus on the chromosome and are coordinately regulated by FabT, a transcriptional repressor (44). FabT binding to long-chain acyl-ACP increases its affinity for the repressor binding sites (45). Therefore, exogenous fatty acids are converted to acyl-\(\text{PO}_4\) and acyl-ACP, which then binds to FabT to severely repress the transcription of the genes encoding the essential core enzymes of FASII (17). However, this stringent transcriptional regulation does not account for the suppression of FASII by exogenous fatty acids because *fabT* deletion strains that possess constitutively high levels of FASII enzymes still completely suppress FASII activity in the presence of extracellular fatty acids (17). The reduction in malonyl-CoA in fatty acid treated cells suggests the control point is acetyl-CoA carboxylase (17). It seems reasonable to think that acyl-ACP is the negative regulator of acetyl-CoA carboxylase in light of its role in regulating this enzyme in *E. coli* (46), but biochemical validation of this regulatory circuit is required to verify this conclusion. This stringent biochemical regulation of FASII by exogenous fatty acids means that these organisms are refractory to FASII inhibitors when cultured in the laboratory with a fatty acid supplement like human serum (16,17).

*Lactobacillus johnsonii* is a member of this bacterial Order that resides in the human gut and is fatty acid auxotroph (47). *L. johnsonii* lacks the genes to carry out FASII and requires exogenous fatty acids from either the host or the gut microbial community for membrane lipid synthesis (48). Therefore, *L. johnsonii* would be expected to be refractory to FASII inhibitors. The genome of *L. johnsonii* encodes a FakA, FakB, ACP, PlsX, PlsY, and PlsC. Therefore, this organism has the minimal gene set for the incorporation of exogenous fatty acids via the fatty acid kinase pathway (Fig. 3). Many of the gut bacteria have complex nutrient requirements, and other residents may also be fatty acid auxotrophs (49). These species are usually considered beneficial for human health (50,51), so the lack of inhibition of these microbes by fatty acid synthesis inhibitors may prove to be beneficial in maintaining the gut microbiome during antibiotic therapy. An important area for future research will be to precisely determine the nutritional requirements and importance of FASII in bacteria that constitute the gut microbiome. This research is complicated by the inability to culture many of these bacteria in the lab and the lack of full genome sequences that would permit a bioinformatic analysis of fatty acid and phospholipid metabolism.

An important point is that the members of the Lactobacillales Order are not representative of all Gram-positive bacteria, and conclusions made using these organisms cannot be extended to all pathogens. The Gram-positive Order Bacillales contains the very relevant human pathogen *Staphylococcus aureus*, which does not have the same mechanism for the regulation of FASII as representative Lactobacillales (i.e. *S. pneumoniae*). The Bacillales use fatty acid kinase to activate exogenous fatty acids, but the regulation of FASII in response to exogenous fatty acids is very different. The FASII genes in *S. aureus* are spread throughout the chromosome and are controlled by the FapR repressor that is released from its DNA binding sites following
the binding of malonyl-CoA (52,53). Most importantly, the level of malonyl-CoA is maintained in cells exposed to exogenous fatty acids (17). This means that the activity of acetyl-CoA carboxylase is not efficiently inhibited, and FASII initiation and elongation is not completely repressed by exogenous fatty acids. This seemingly small regulatory nuance explains the effectiveness of FASII inhibitors against S. aureus (17). AFN-1252 is an inhibitor of the enoyl-ACP reductase (FabI) of FASII, and blocks the elongation of acyl-ACP (54,55). In AFN-1252 treated S. aureus, short chain acyl-ACP accumulates at the blocked FabI step leading to the severe depletion of free ACP (56,57). Because all of the ACP is tied up at the blocked FabI step, there is no ACP available for the synthesis of acyl-ACP from exogenous fatty acids via PlsX (Fig. 3). Therefore, the PlsC acyltransferase reaction, which requires acyl-ACP, cannot occur and phospholipid synthesis halts. Thus, the inability of S. aureus to down regulate FASII when exogenous fatty acids are present accounts for the inability of fatty acids to overcome the effect of FASII inhibitors.

**Fatty acid metabolism by intracellular bacterial pathogens**

Intracellular pathogens characteristically obtain many of their nutrients from the host cell, but in some cases FASII remains important and in others it does not. *Chlamydia trachomatis* is a Gram-negative, obligate intracellular parasite (58) where the operation of FASII is critical for proliferation (59). *C. trachomatis* has a reduced genome devoid of many biosynthetic pathways and depends on the host for a multitude of molecules. However, the *C. trachomatis* genome encodes for all FASII and phospholipid biosynthetic genes needed for de novo fatty acid and phospholipid synthesis (60). Furthermore, bacterial made, branched-chain fatty acids are found at the 2-position of the *C. trachomatis* phospholipids (61). Inhibiting *C. trachomatis* FASII at the FabI step blocks the replication of *C. trachomatis* in HeLa cells through inhibiting fatty acid and phospholipid synthesis (59). Given that fatty acids are made and available inside of the HeLa cells, this result shows that exogenous fatty acids cannot rescue FASII inhibition in *Chlamydia trachomatis*. A requirement for lipopolysaccharide synthesis in *C. trachomatis* appears relevant, but the specific inhibition of lipopolysaccharide formation does not stop bacterial replication like a FASII inhibitor (62), indicating that FASII provides fatty acids for more than just lipopolysaccharide biosynthesis. The extent to which *C. trachomatis* may utilize host fatty acids remains an important unanswered question, but it is clear that de novo fatty acid synthesis is required for a vital cellular process. In contrast, *Mycoplasma pneumoniae* is an example of an intracellular pathogen that lacks FASII. This organism uses the FakA, FakB, ACP, PlsX, PlsY, and PlsC genes to introduce fatty acids into the phospholipid biosynthetic pathway (Fig. 3). One would presume that FASII inhibitors would not be effective against bacteria like these which lack FASII and depend on exogenous host fatty acids for membrane lipid synthesis.

**Phospholipid structure and host fatty acids**

The acyl chains attached to the phospholipids determine the fluidity of the membrane, and proper membrane function requires that the acyl chain composition be controlled (63). In mammals and some bacteria, membrane fluidity is controlled by the ratio of saturated to unsaturated fatty acids in the membrane phospholipids. *S. pneumoniae* and *E. coli* are examples of Gram-positive and negative bacteria that contain saturated and monounsaturated fatty acids with similar structures and physical properties to the most abundant host fatty acids. However, many bacteria, notably *S. aureus*, regulate fluidity by altering the ratios of straight-chain, iso and anteiso branched-chain saturated fatty acids (63). Branched-chain fatty acids are not made by the mammalian host, and so phospholipids with branched-chain fatty acids cannot be made if *S. aureus* depends exclusively on the host for fatty acids. The importance of branched-chain fatty acids in these organisms is demonstrated in the lab by the increased susceptibility to stress of a *S. aureus* strain with decreased levels of branched-chain fatty acids (64). Most importantly, *S. aureus* acetyl-CoA carboxylase knockouts are fatty acid auxotrophs and are easily maintained in the lab on rich medium supplemented with branched-chain fatty acids (65). Feeding this strain saturated and unsaturated mammalian fatty acids leads to
severe growth defects once the membrane phospholipids become devoid of branched-chain fatty acids. Accordingly, these \textit{S. aureus} acetyl-CoA carboxylase knockouts do not proliferate in a mouse infection model, illustrating that host fatty acids cannot support their growth. Based on this limited information, one would predict that exogenous fatty acids should not be able to bypass FASII inhibition in Gram-positive human pathogens such as \textit{Staphylococcus}, \textit{Bacillus}, \textit{Listeria}, and \textit{Propionibacterium} (66). However, direct experimental evidence for this conclusion is required.

\textbf{FASII is not just for fatty acids}  
An important side effect of blocking fatty acid synthesis is that it interferes with the synthesis of other cellular components with a role in metabolism and cell-cell communication that are important to virulence. The multifunctional eukaryotic type I fatty acid synthase is very efficient, but it only produces palmitic acid (8). In contrast, the dissociated FASII system allows enzymes in other pathways to access FASII intermediates to synthesize important molecules. Lipoic acid (67) and biotin (68) are two important cofactors that require FASII intermediates to initiate their synthesis. Lipoic acid is central for energy generation in many bacteria, and although an organism may obtain fatty acids from the host, they may not be able to acquire sufficient lipoate to support growth in animals. While the inability of \textit{S. aureus} acetyl-CoA carboxylase knockouts to proliferate in mice is most likely due to the inability of the bacteria to fulfill their branched-chain fatty acid requirement (65), they are also lipoate auxotrophs and it is possible that there is insufficient lipoate present for the bacteria to establish an infection. FASII intermediates are also used to produce signaling molecules like homoserine lactones and quinolones that control bacterial social behavior (69), (70), and fatty acid modification is important for the function of virulence factors such as staphyloxyanthin (71) and hemolysin (72). Many of these molecules are implicated in survival or virulence in infection models, suggesting that the inhibition of FASII may be therapeutically beneficial even if the fatty acid requirement for membrane phospholipid synthesis is met by incorporation from the host. These considerations emphasize the importance of in vivo experiments to make a final decision on whether FASII inhibitors would be effective in vivo because it is not reasonable to think that laboratory media accurately mimics the nutritional mix found in the niches colonized by invasive pathogens.

\textbf{Summary and future questions}  
Recent years have seen solid advances in identifying the mechanisms that bacteria use to incorporate host fatty acids into their membrane phospholipids. However, many aspects of our current models remain to be tested to determine the relevance of these pathways to the efficacy of FASII therapeutics. The key first step in fatty acid incorporation is catalyzed by one of two activation enzymes: acyl-CoA synthetase or fatty acid kinase that produce the correct activated forms to feed the two major acyltransferase systems in bacteria (Figs. 2 and 3). A bacterial acyl-ACP synthetase is known from \textit{Vibrio harveyi}, and this enzyme is capable of activating medium-chain fatty acids that are capable of entering FASII when the enzyme is expressed in heterologous \textit{E. coli} system (42,73). Cyanobacteria also have functional acyl-ACP synthetases (74), but more research is needed to determine if this activation pathway has any relevance to the acquisition of host fatty acids in important bacterial pathogens. In bacteria that lack FASII, fatty acid kinase appears to be the main route for fatty acid utilization in membrane formation. The enormous amount of energy expended in the synthesis of fatty acids provides a rationale for understanding why these uptake pathways are wide-spread in bacteria. Despite their prevalence, it is clear that many important human pathogens including Gram-negative bacteria (\textit{E. coli}, \textit{Enterobacteriaceae}, \textit{Salmonella}, \textit{Shigella}, etc.) and some Gram-positive bacteria (exemplified by \textit{S. aureus}) can be effectively treated using FASII inhibitors. Laboratory results suggest that bacteria like \textit{S. pneumoniae} (Order: Lactobacillales) may be refractory to FASII inhibition due to their efficient utilization of extracellular fatty acids. However, this conclusion is not solid because it has not been experimentally tested in animal infection models. Such experiments are essential to establish this point due to the role of FASII intermediates in the synthesis of...
cofactors and signaling molecules that are central to energy production and virulence. A relevant proof of principle experiment would be to compare the virulence of wild-type and acetyl-CoA carboxylase knockout strains in animal models. If the Lactobacillales are proven to be refractory to FASII inhibitors, then there are potentially important medical consequences of deploying FASII therapeutics against other sensitive pathogens. Members of the Lactobacillales are major beneficial members of the gut microbiome community. There are serious consequences that arise from the elimination of these bacteria when broad-spectrum antibiotics are deployed (75-77). However, FASII inhibitors may not affect this community, making these agents desirable for the treatment of susceptible pathogens such as S. aureus. Given the difficulties in discovering new, truly broad-spectrum antibiotics, the paradigm of designing narrower-spectrum antibiotics that effectively target a specific pathogen with reduced collateral damage to the human gut microbiome should be more enthusiastically considered. Finally, understanding how de novo FASII is biochemically regulated by exogenous fatty acids will be critical to determining the extent of host fatty acid utilization. It is clear that there are major differences in the stringency of regulation in groups of Gram-positive pathogens; however, the biochemical mechanism(s) that account for the rapid, and in some cases, nearly complete inhibition of FASII by exogenous fatty acids remains unknown. Current thinking suggests acyl-ACP regulation of acetyl-CoA carboxylase (Fig. 3), but experimental validation of this hypothesis is not available.

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**Figure Legends**

**FIGURE 1.** The core enzyme set in bacterial type II fatty acid synthesis (FASII). The acetyl-CoA carboxylase enzyme complex (AccABCD) converts acetyl-CoA into malonyl-CoA. Malonyl-CoA:ACP transacylase (FabD) converts malonyl-CoA into malonyl-ACP. The β-ketoacyl-ACP synthase III (FabH) initiates fatty acid synthesis by condensing malonyl-ACP with either 2-methylbutyryl-CoA for branched-chain anteiso fatty acid synthesis or acetyl-CoA for straight chain fatty acid synthesis to make β-ketoacyl-ACP. The β-ketoacyl-ACP reductase (FabG) reduces β-ketoacyl-ACP to make β-hydroxyacyl-ACP. The β-hydroxyacyl-ACP dehydratase (FabZ) dehydrates β-hydroxyacyl-ACP into trans-2-enoyl-ACP. The enoyl-ACP reductase (FabI) reduces trans-2-enoyl-ACP into acyl-ACP. Acyl-ACP of the appropriate length can be used for phospholipid biosynthesis while β-hydroxyacyl-ACP of the proper length is used for lipopolysaccharide synthesis in Gram-negative bacteria. The figure lists the multiple isoforms of several of these core enzymes that are expressed by different bacteria. The branch points in the pathway for unsaturated fatty acid synthesis are not illustrated. Please refer to a recent review for detailed information on the organism-specific nuances in the operation of FASII (9).

**FIGURE 2.** Exogenous fatty acid incorporation in *E. coli*: the Gram-negative paradigm. (A) Pathways for the incorporation of fatty acids into phospholipids. Acyl-ACP molecules with the appropriate acyl chain length generated in FASII are used to acylate sn-glycerol-3-phosphate (G3P) to make lysophosphatidic acid (LysoPA) by PlsB, and then phosphatidic acid (PA) by PlsC. Phosphatidic acid is the precursor to all phospholipid species. β-Hydroxyacyl-ACP of the appropriate acyl chain length is used to make lipid A, the lipid core of lipopolysaccharides. Exogenous fatty acids are converted into acyl-CoAs by acyl-CoA synthetase (FadD). Acyl-CoA molecules of the appropriate length can be used as substrates for the PlsB and PlsC acyltransferases. Otherwise, acyl-CoA is β-oxidized to generate acetyl-CoA and energy. Exogenous fatty acids cannot rescue FASII inhibition because β-hydroxyacyl-ACP made from FASII is essential. (B) Free fatty acids are also used to acylate lysophospholipids by the 2-acylglycerophosphoethanolamine acyltransferase of *E. coli* (Aas). The lysophospholipid is generated as a byproduct of lipoprotein synthesis. The Aas is a bifunctional protein containing acyl-ACP synthetase (STase) and acyltransferase (ATase) domains in addition to a tightly bound ACP subunit. The STase activates the fatty acid to the bound ACP subunit and the ATase domain transfers the acyl chain to the 1-position of the lysophospholipid. Acyl-ACP is not liberated from the enzyme complex during the catalytic cycle, so the acyl-ACP is not available for the synthesis of phospholipids or lipopolysaccharides.

**FIGURE 3.** Exogenous fatty acid incorporation in *S. aureus* and *S. pneumoniae*: the Gram-positive paradigm. Acyl-ACP molecules with the appropriate acyl chain length generated in FASII are used to acylate lysophosphatidic acid (lysoPA) by PlsC to make phosphatidic acid, or converted into acyl-phosphate (acyl-PO₄) by PlsX. Acyl-PO₄ is used by PlsY to acylate sn-glycerol-3-phosphate (G3P) to make lysophosphatidic acid (LysoPA). Phosphatidic acid is the precursor to all phospholipid species. Exogenous fatty acids are phosphorylated by the two component fatty acid kinase (FakA/B) to make acyl-PO₄. The resulting acyl-PO₄ is used by PlsY, or converted to acyl-ACP by PlsX. The acyl-ACP can be used by the PlsC acyltransferase or re-enter the FASII elongation cycle. Bacteria that utilize exogenous fatty acids for phospholipid synthesis exhibit a concomitant decrease in FASII activity. Current thinking suggests regulation of acetyl-CoA carboxylase (AccABCD) by acyl-ACP is responsible for this phenomenon, but this proposed biochemical mechanism has not been experimentally validated.
FIGURE 1

FIGURE 2
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