thematic review

The application of computational methods to explore the diversity and structure of bacterial fatty acid synthase

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Abstract **Acyl carrier protein (ACP) is a central element in the bacterial, type II dissociated fatty acid synthase (FAS II) system. ACP delivers the fatty acyl intermediates to a variety of enzymes with different biochemical functions and 3-dimensional (3-D) structures. Computational techniques have proved invaluable in guiding the experimental designs that have uncovered the recognition helix on ACP and the common features on its target enzymes responsible for specific protein•protein interactions. *Escherichia coli* has been the model organism for the study of FAS II, but the availability of complete genomic sequences of a growing number of bacteria coupled with computational bioinformatics has led to new discoveries on the mechanisms that regulate *E. coli* FAS II and allowed the differences between the *E. coli* paradigm and major groups of pathogens to be identified and experimentally addressed. Computational methods facilitated the discovery of the *E. coli* fatty acid synthesis transcriptional regulator, FabR, and led to the identification of novel bacterial FAS II proteins in Gram-positive pathogens, including enoyl-ACP reductases (FabK and FabL) and *trans*-2-*cis*-3-decenoyl-ACP isomerase FabM.** **As more genomic sequences and 3-D coordinates are added to the databases, the power and resolution of the computational approaches will increase to offer deeper insight into the structure, diversity and function of lipid metabolic pathways.**—Zhang, Y-M., H. Marrakchi, S. W. White, and C. O. Rock. **The application of computational methods to explore the diversity and structure of bacterial fatty acid synthase. *J. Lipid Res.* 2003.** 44: **1–10.**

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BACTERIAL FATTY ACID SYNTHESIS

Fatty acid metabolism is a fundamental component of the cellular metabolic network. Fatty acids are the essential building blocks for membrane phospholipid formation. Most bacteria synthesize fatty acids using a series of discrete monofunctional proteins, each catalyzing one reaction in the pathway [reviewed in ref. (1–3)]. The bacterial system, also known as the dissociated, type II fatty acid synthase (FAS II), contrasts with the yeast and animal type I fatty acid synthases (FAS I). The type II system is a collection of individual enzymes encoded by separate genes, whereas the type I system is a polypeptide about 260 kDa in size with multiple active sites that perform all the catalytic reactions in the pathway. Although the structural organizations of FAS I and FAS II are different, the chemical reactions and the catalytic mechanisms for fatty acid synthesis are essentially the same.

Escherichia coli FAS II has been extensively studied and the biochemical properties of the individual enzymes are the paradigm for type II fatty acid synthase (1). **Figure 1** outlines the major steps in the bacterial FAS II. Acetyl-CoA carboxylase catalyzes the first committed reaction of fatty acid biosynthesis. The product of the reaction is malonyl-CoA and the malonate group is then transferred to ACP by malonyl-CoA:ACP transacylase (FabD) to form malonyl-ACP. Fatty acid synthesis is initiated by the Claisen condensation of malonyl-ACP with acetyl-CoA catalyzed by β-ketoacyl-ACP synthase III (FabH) to form β-ketobutyryl-ACP. Four enzymes catalyze each cycle of elongation. The β-keto group is reduced by the NADPH-dependent β-ketoacyl-ACP reductase (FabG), and the resulting β-hydroxy intermediate is dehydrated by the β-hydroxyacyl-ACP dehydratase (FabA or FabZ) to an enoyl-ACP. Next, the reduction of the enoyl chain by the NAD(P)H-dependent enoyl-ACP reductase (FabI, FabK, or FabL) produces an

Abbreviations: ACP, acyl carrier protein; AcpM, mycobacterial ACP; AcpS, holo-ACP synthase; FAS I, type I fatty acid synthase; FAS II, type II fatty acid synthase; UFA, unsaturated fatty acid; FabA, β-hydroxydecanoyl-ACP dehydratase/isomerase; FabB, β-ketoacyl-ACP synthase I; FabF, β-ketoacyl-ACP synthase II; FabG, β-ketoacyl-ACP reductase; FabH, β-ketoacyl-ACP synthase III; FabI, enoyl-ACP reductase I; FabK, enoyl-ACP reductase II; FabL, enoyl-ACP reductase III; FabZ, β-hydroxyacyl-ACP dehydratase; FabD, malonyl-CoA:ACP transacylase; FadR, fatty acid degradation regulator; and FabR, fatty acid biosynthesis regulator.

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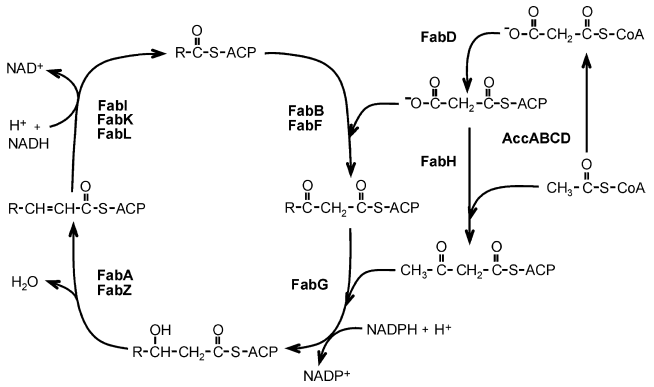

Fig. 1. Bacterial (FAS II) fatty acid synthesis pathway. Acetyl-CoA carboxylase (AccABCD) catalyzes the formation of malonyl-CoA from acetyl-CoA and the malonyl moiety is transferred to ACP by malonyl-CoA:ACP transacylase (FabD). The initial condensation reaction is catalyzed by β-ketoacyl-ACP synthase III (FabH), which condenses acetyl-CoA with malonyl-ACP to form acetoacetyl-ACP. The next reaction in the elongation cycle is the NADPH-dependent β-ketoacyl-ACP reductase (FabG). The resulting β-hydroxyacyl-ACP is dehydrated by β-hydroxyacyl-ACP dehydratase FabA or FabZ. The final step in the cycle is catalyzed by enoyl-ACP reductases I (FabI), II (FabK), or III (FabL). The resulting acyl-ACP can by elongated further by β-ketoacyl-ACP synthases I (FabB) and/or II (FabF).

acyl-ACP. Additional cycles of elongation are initiated by the β-ketoacyl-ACP synthase (FabB or FabF), which elongates the acyl-ACP by two carbons to form a β-ketoacyl-ACP. Elongation ends when the fatty acyl chain reaches the appropriate length that can be used for membrane phospholipid or lipopolysaccharide synthesis.

A central feature of the bacterial FAS II is that all the fatty acyl intermediates are covalently attached to a small, acidic, and highly conserved ACP. The intermediates are covalently attached to ACP by a thioester bond to the sulfhydryl of the 4′-phosphopantetheine prosthetic group. The prosthetic group is in turn covalently attached to an invariant serine residue via a phosphodiester linkage. Apo-ACP is inactive in fatty acid synthesis, and is converted to the active protein by holo-ACP synthase (AcpS), which transfers the 4′-phosphopantetheine from CoA to apo-ACP (4). On the other hand, the prosthetic group is cleaved from ACP by [ACP]phosphodiesterase, although not much is known about this enzyme (5, 6). There are over a hundred ACP sequences deposited in the molecular sequence databases. Multiple sequence alignment analysis reveals a high degree of homology for ACPs across species centered around the serine where 4′-phosphopantetheine is attached (**Fig. 2A**).

PROTEIN•ACP INTERACTIONS IN FAS II

ACP structure and function has received considerable attention over the past several decades in light of its central role in fatty acid and polyketide synthesis. ACP also supplies acyl groups for the synthesis of lipid A (7), glycerolipids (2), and quorum sensing compounds (8). Expression of ACPs from a variety of bacteria, plants, and animals in an *E. coli* host strain produces the respective ACP proteins in three forms, apo-ACP, holo-ACP, and acyl-ACP (9–13). These observations, along with numerous in vitro enzymatic experiments, illustrate that ACPs from highly diverse organisms function interchangeably in the *E. coli* FAS II system. This conclusion predicts that the protein•ACP interactions are achieved through a conserved set of electrostatic and/or hydrophobic contacts. This hypothesis is also supported by the fact that domains of ACPs from distantly related organisms (*E. coli* and Rhizobium) can be interchanged without affecting the protein's conformation and function (14). Consistent with this, the primary sequence analysis of the ACPs identifies a motif that is conserved in ACP family members (Fig. 2A). Several amino acids in this region of the protein, Asp35, Ser36, Leu37, Glu41, and Glu47 (*E. coli* numbering), are completely conserved in the over 50 ACP sequences analyzed in preparation for this article. The 3-dimensional (3-D) structures of several bacterial ACPs clearly define the similar structural characteristics within the protein family. The ACPs are asymmetric monomers consisting of four α-helices packed into a bundle held together by interhelical hydrophobic interactions (15–19). The conserved acidic residues (Fig. 2B, C) are arrayed along helix II with Ser36, the site of prosthetic group attachment, located on a loop at the N-terminus of helix II. This conserved region of the protein is postulated to represent a "recognition helix" that accounts for the ability of the ACPs from widely diverse organisms to interact with target enzymes and to function interchangeably in FAS II systems.

As the carrier of intermediates in FAS II, ACP must interact with the proteins in the pathway to specifically deliver the appropriate substrate to each enzyme with a different biochemical function. At the same time, protein/ACP interactions must be transient (and relatively weak) to allow rapid on and off rates for the substrates and products. Unlike ACP itself (Fig. 2A), and many groups of enzymes that bind a common substrate, ACP-interacting proteins do not share a primary signature sequence that defines an ACP binding motif. Instead, recent experiments support the hypothesis that the enzymes of the type II system share 3-D surface features that account for their specific recognition of ACP and its thioesters.

This hypothesis was first explored with FabH, the condensing enzyme that catalyzes the initial step in the elongation of fatty acids. Its two substrates, acetyl-CoA and malonyl-ACP, bind sequentially in a ping-pong enzyme mechanism to produce acetoacetyl-ACP (20–22). The study of *E. coli* FabH•ACP interactions was guided by an automated protein•protein docking program, SurfDock (23), which was used to predict the FabH•ACP complex using the X-ray structure of FabH and the NMR structure of ACP (24). The predicted interaction surface on FabH is located adjacent to the active site tunnel. It consists of a pattern of positively charged residues imbedded in a hydrophobic patch (**Fig. 3B**), a pattern observed in several other protein•protein interfaces (25, 26). Significantly, the computational predictions identified helix II of ACP

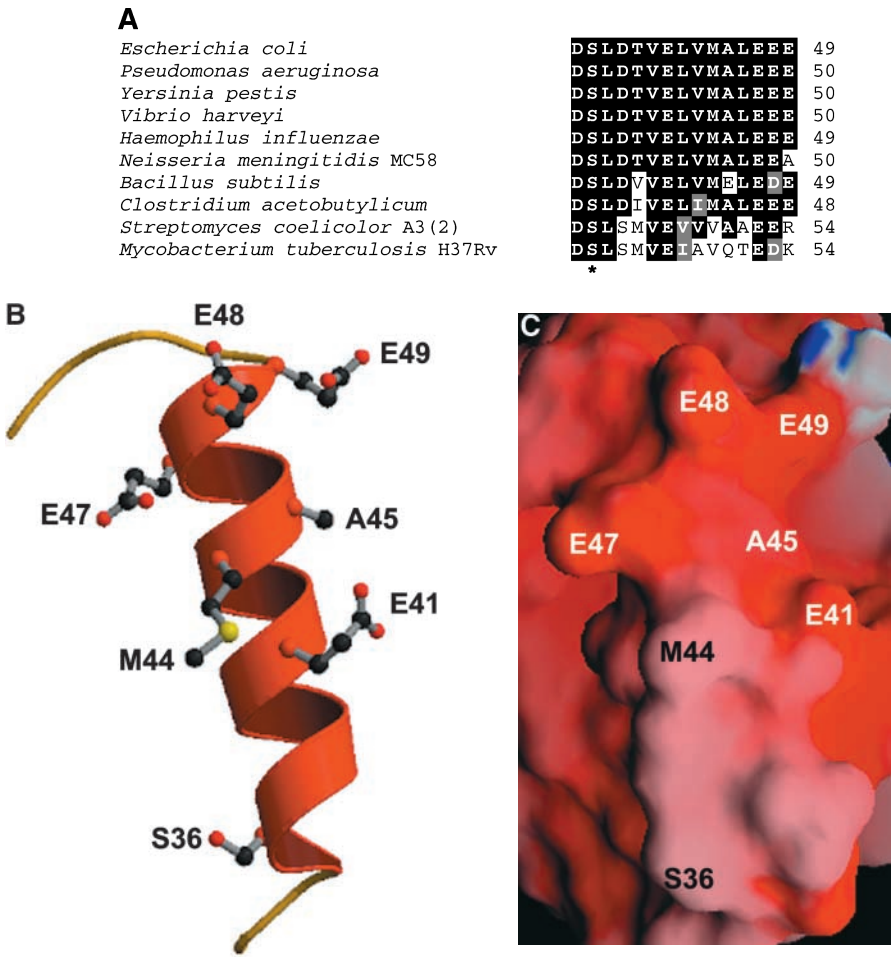

Fig. 2. Highly conserved negatively charged and hydrophobic residues in the helix II of ACP are important for protein recognition. A: Multiple alignment of ACP amino acid sequences within the helix II region. Helix II is predicted to be the recognition site on ACP in protein•ACP complexes. Primary sequences in the helix II region of various bacterial ACPs are compared using the program Clustal W (67). The completely conserved residues are highlighted in black and residues that are conservatively substituted are highlighted in gray. The serine residue to which the prosthetic group is attached is indicated with an asterisk. The numbers refer to the portions of the last amino acids in the sequences. B: A ribbon diagram of the helix II of the *E. coli* ACP. Twenty-one residues (Ala34 to Ile54) were used to generate the structure using the program MOLSCRIPT (68), and the graph was rendered with Raster 3D (69). The side chains of the conserved negatively charged residues, E41, E47, E48, and E49 (shown in CPK style), protrude from the surface along helix II to interact with positively charged residues on the complementary proteins. Highly conserved hydrophobic residues that are important for FabH/ACP interactions, M44 and A45, are also indicated. C: Electrostatic potential surface of the *E. coli* ACP helix II region. Red indicates negative charge, blue indicates positive charge, and white is hydrophobic. Negatively charged and hydrophobic residues corresponding to those shown in B are labeled. The extreme ranges of red and blue represent electrostatic potential of <-12 to $>+3$ k_bT, where k_b is the Boltzmann constant and the T is the temperature. The figure was calculated using the GRASP program (70).

(Fig. 3A) as the partner with FabH, which placed the prosthetic group attachment site at the entrance of the active site tunnel. The computational predictions were verified experimentally by site-directed mutagenesis of FabH. All of the positively charged residues adjacent to the active site tunnel contribute to ACP binding, but Arg249 on FabH provides the most important electrostatic interaction, possibly with Glu41 on ACP (24). The FabH from *Mycobacterium tuberculosis* (mtFabH) has a markedly different substrate specificity than *E. coli* FabH (ecFabH), but it is similar to its *E. coli* counterpart in terms of topology (27). There are only a few amino acid residue alterations in the active site pocket that are postulated to account for the differences in substrate specificity between the two proteins. Like ecFabH, the surface of mtFabH is highly electronegative with the exception of the electropositive/hydrophobic patch adjacent to the active site tunnel (Fig. 3D). This surface feature of mtFabH is complementary to the electronegative/hydrophobic surface of the mycobacterial acyl carrier protein, AcpM, along helix II (Fig. 3C). A survey of six other 3-D structures of *E. coli* FAS II enzymes indicates that they all contain a region adjacent to their active entrances that resembles the electropositive/hydrophobic FabH•ACP binding surface (24). These similarities between the helix IIs of ACPs and the surface features of the FabH enzymes explain the ability of both ACP and AcpM to interchangeably interact with ecFabH and mtFabH. In fact, all of the FAS II components of known structure have a electropositive/hydrophobic feature adjacent to their active sites, which likely represents the ACP binding sites on these

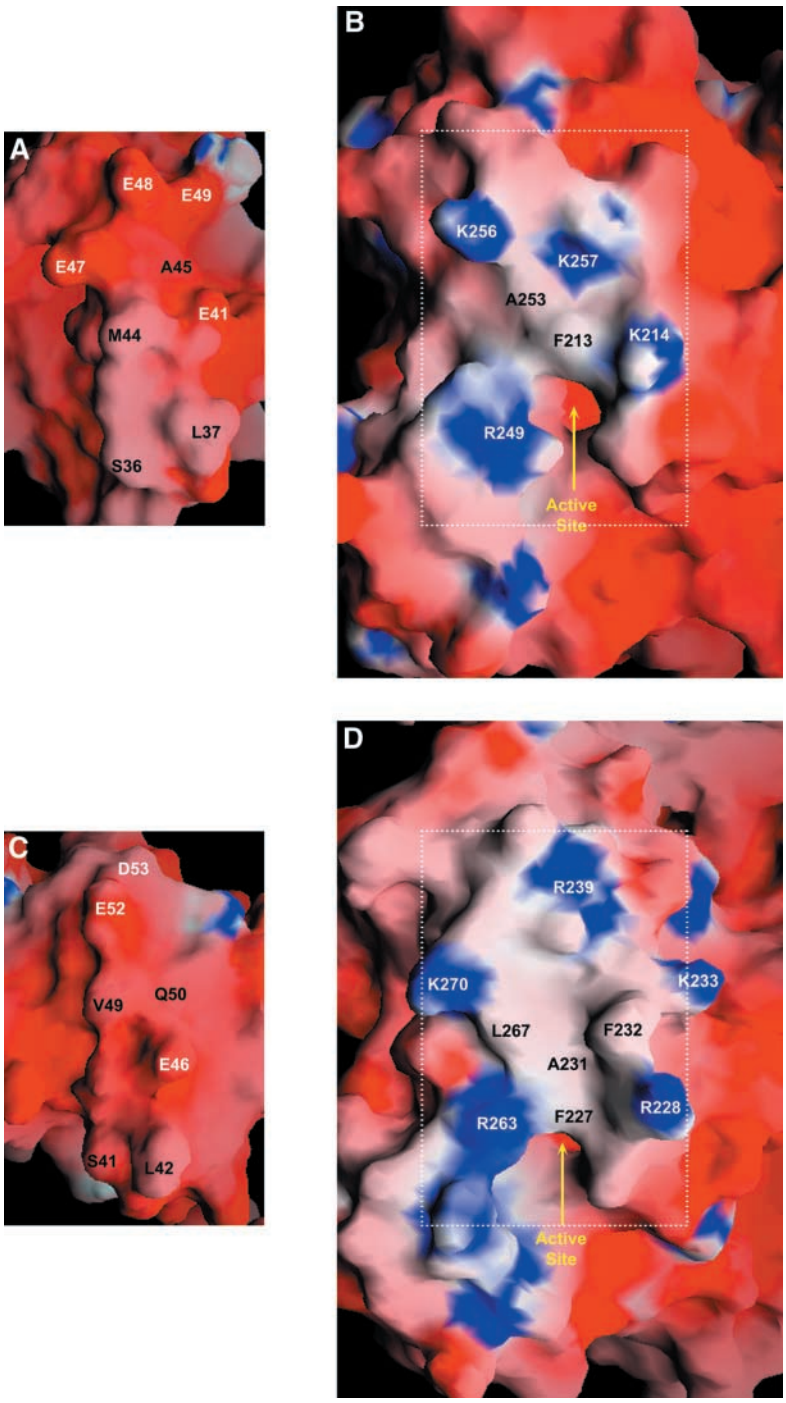

Fig. 3. The positively charged/hydrophobic region of FabH that is predicted to be the binding site for ACP. A: The surface electrostatic potential of the *E. coli* ACP shown with the recognition helix (helix II) facing the reader. The amino acids proposed to be important for FabH/ACP interactions are indicated. B: The predicted ACP-binding site on ecFabH. The surface of the *E. coli* FabH is generally negatively charged. The predicted ACP-binding site is a positively charged and hydrophobic area located adjacent to the active site tunnel entrance. Mutagenesis data show that all of the four positively charged residues contribute to FabH•ACP interaction, with R249 providing the most important electrostatic interaction. Hydrophobic residues thought to be important for FabH•ACP interaction are also indicated. C: The surface potential of helix II of *M. tuberculosis* AcpM illustrating the similarities to helix II of *E. coli* ACP. D: The putative AcpM binding site on *M. tuberculosis* FabH. The structure of mtFabH is very similar to *E. coli* FabH. An electropositive/hydrophobic patch is also found next to the active site. Residues that are predicted to interact with AcpM are shown. The extremes of red (negative) and blue (positive) represent the electrostatic potentials described in Fig. 2 and were calculated using the GRASP program (70). The surface of FabH complementary to ACP is indicated with the dashed rectangle.

proteins and accounts for their ability to interact with ACP from multiple species (24).

Despite considerable effort, there are no cocrystal structures of complexes between FAS II enzymes and ACP, perhaps due to the low affinity of the complexes (μM). However, AcpS forms a high-affinity complex with ACP, and the recent structure determination of the *Bacillus subtilis* AcpS•ACP binary complex provides strong support for the idea that ACP-binding proteins interact with helix II on ACP (28). The interactions between AcpS and ACP are predominately electrostatic with two hydrophobic contacts. The interacting residues are located on ACP helix II, which contacts a complementary helix on AcpS. As noted in the primary sequence alignment (Fig. 2A), the amino acids that contact AcpS are highly conserved along helix II of ACP, including Asp35, Glu41, and Glu47 (*E. coli* numbering). The side chains of the negatively charged residues (Glu or Asp) situated along ACP helix II protrude from the surface to interact with positively charged Arg residues on a corresponding helix of AcpS (28). These data support a general role for helix II as the recognition helix that dictates the specific binding interactions between ACP and its complementary enzymes. However, considerably more research is needed to validate this hy-

pothesis and it is anticipated that NMR spectroscopy will play an important role in forwarding this area of investigation, since this technique is particularly well suited to study weak protein•protein interactions.

In contrast to *E. coli*, some bacteria have additional specialized ACPs. For example, there are four different ACPs in Rhizobia, AcpP, NodF, RkpF, and AcpXL (29, 30). *M. tuberculosis* has a biosynthetic AcpM and two other putative ACPs (11). *Streptomyces coelicolor* A3(2) contains the regular ACP for FAS II and two other ACPs involved in polyketide synthesis (9). Although these ACPs show a lower level of homology in their overall amino acid sequences, the amino terminal 4-helix bundle and the primary sequence along helix II are highly conserved. The working hypothesis is that these ACPs have acquired additional novel structural features, outside the protein recognition site, to accommodate the different types of acyl chains they carry. For example, the C-terminal half of Rhizobial NodF may be specialized for sequestering polyunsaturated fatty acyl chains (14). The extended carboxy-terminus in *M. tuberculosis* AcpM is proposed to protect the extremely long-chain fatty acyl chains in mycolic acid biosynthesis from the hydrophilic environment (10). These hypotheses await experimental validation through the structural determination of these acyl-ACP derivatives, and/or their complexes with FAS II enzymes.

In addition to shedding light on the biochemical mechanism of FAS II, these computational predictions and structural studies may have practical value in the development of new drugs. ACP is an integral feature of the multifunctional subunit of vertebrate FAS I (31), in contrast to the dissociable ACPs in the bacterial FAS II systems. An exciting idea for future development of the protein•ACP interaction study is to design compounds that bind to the recognition helix of ACP and block its interaction with target enzymes. Compounds with this property represent candidate molecules toward the development of novel broad-spectrum antimicrobials. Recently, there has been success in the design and synthesis of molecules, which are capable of blocking protein•protein interactions between protease and proteinaceous inhibitors (32).

In addition to predicting protein•protein interactions, computational docking programs are widely used to analyze protein•ligand interactions (33–35). An application of this technology to FAS II drug discovery was the use of the flexible docking program FlexX (36) to model the binding of thiolactomycin (TLM) to *E. coli* FabB (37). TLM is a broad-spectrum antimicrobial that reversibly inhibits FAS II but not FAS I. It binds to the active site of the condensing enzymes by mimicking the substrate malonyl-ACP. The FabB/TLM complex modeled by the docking program is essentially identical to the crystal structure of the FabB•TLM binary complex (**Fig. 4**). Mutant FabB[F390V] is resistant to TLM inhibition and the computational analysis provides a structural rationale for the acquisition of resistance by the enzyme (38). With databases containing structures of thousands of readily available small molecules, virtual screening of these databases is accelerating the search for ligands that can potentially bind either the active sites or the ACP binding sites of bacterial FAS II enzymes. The future will see more intensive application of this technique to drug discovery.

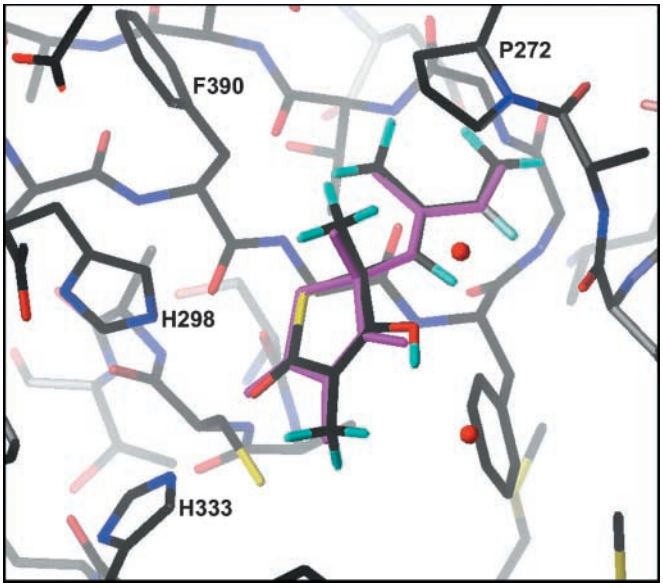

Fig. 4. Superposition of TLM from the FabB/TLM binary crystal structure (TLM shown in magenta) with the FabB/TLM structure calculated using FlexX. TLM forms hydrogen bonds with the two active site histidines of FabB, H298, and H333, and to a network of water molecules (red spheres). TLM is further stabilized by the stacking interactions between its isoprenoid side chain and P272. The side chain of F390 points away from the C11 methyl group of TLM. In the TLM-resistant mutant, FabB[F390V], there is a steric clash between the valine side chain and the C11 methyl group of TLM, pointing to steric incompatibility as the structural explanation for the resistance of the mutant protein to TLM.

BIOINFORMATICS AND BACTERIAL GENOMES

With the increasing number of bacterial genome sequences being completed, experimental biologists are applying new methods to translate the mountains of sequence data into useful knowledge. The application of these techniques to the analysis of bacterial FAS II systems has resulted not only in the discovery of novel enzymes and synthetic mechanisms in Gram-positive pathogens, but surprisingly, also in the identification of new components of the FAS II system of *E. coli*. Sequence homology programs, such as BLAST (39), are widely used to extend experimental knowledge of protein function to new sequence information and many of the new sequences are assigned a function in this way. In general, the genes encoding the FAS II components in organisms other than *E. coli* are significantly conserved, and their identity and function in the pathway is easily recognized using BLAST. However, there are a number of instances where some of the FAS II components cannot be identified by this straightforward bioinformatics approach. In these cases, the "missing enzymes" are products of convergent evolution and their sequences are not homologous to their *E. coli* counterparts. More powerful genome-wide methods,

such as phylogenetic profile analysis and gene neighbor approaches (40), are employed to focus experimental attention on the potential new players in FAS II. Phylogenetic profiles describe the pattern of presence and absence of a particular protein in all surveyed genomes. Proteins with the identical and similar phylogenetic profiles are likely to be involved in a common pathway (40). Functional linkages of proteins can also be revealed by the gene neighbor method, if the genes encoding the proteins are neighbors (operon-cluster like) in several different genomes (40). The results obtained with these in silico approaches provide clues to establish functional linkages between novel proteins and the core FAS II system and serve to prioritize experimental efforts to accelerate the process of discovery.

FATTY ACID BIOSYNTHESIS REGULATOR

Unsaturated fatty acid (UFA) synthesis is essential for the maintenance of membrane structure and function in many groups of bacteria that embrace the anaerobic life style. In eukaryotes, olefin formation requires molecular oxygen, and double bonds are introduced into the fatty acids following the completion of their synthesis via the type I, multifunctional fatty acid synthase. In contrast, double bonds are directly introduced into the growing acyl chain by FAS II, and molecular oxygen is not required. The key players in UFA synthesis were first defined by the isolation and characterization of UFA-auxotrophs (41). In the type II system, the double bond is introduced anaerobically into the growing acyl chain at the 10-carbon intermediate by β-hydroxydecanoyl-ACP dehydratase, FabA (42). FabA is capable of both the removal of water to generate *trans*-2-decenoyl-ACP and the isomerization of this intermediate to the *cis*-3-decenoyl-ACP (1, 43). However, FabA is not the only protein that is required for the introduction of the double bond, and it does not catalyze the rate-limiting step in UFA formation (44). A second unsaturated fatty acid auxotroph was isolated that corresponds to the *fabB* gene, which encodes β-ketoacyl-ACP synthase I. In *fabB* mutants, saturated fatty acid synthesis persists due to the presence of the other elongation condensing enzyme in *E. coli*, FabF (45, 46). Although FabF readily elongates 16:1 to 18:1 (46), the inability to support UFA synthesis in *fabB* mutants leads to the conclusion that FabF cannot elongate a key intermediate in UFA biosynthesis (1, 2). The analysis of *fabB* and *fabF* mutants, coupled with the catalytic properties of FabB and FabF in vitro supports a function for FabB in UFA synthesis and a role for FabF in the thermal modulation of membrane fatty acid composition (47).

Expression of the two essential genes for *E. coli* UFA synthesis, *fabA* and *fabB*, are regulated by the transcription factor FadR. FadR was discovered through the analysis of a mutation that results in the constitutive induction of the β-oxidation enzymes, and led to its characterization as a repressor of fatty acid β-oxidation genes (48). FadR is released from its DNA binding sites by long-chain acyl-CoAs, which bind to the carboxy terminus of the protein and release the amino terminal winged helix domain from the DNA (49, 50). An interesting twist in the FadR story began with the observation that *fabA*(Ts) *fadR* double mutants were unable to grow at the permissive temperature without a UFA supplement (51). This suggested a positive effect of FadR on UFA synthesis, and it was soon demonstrated that FadR is a transcriptional activator that binds to the −40 region of the *fabA* gene, a site common for activators of σ^{70}-responsive promoters (52, 53). FadR is also a positive regulator of the *fabB* gene, although the changes in *fabB* expression in *fadR* mutants are not as great as with *fabA* (54). Thus, FadR acts as a repressor of β-oxidation genes and an activator of the two genes required for unsaturated fatty acid synthesis (48).

The FadR binding site is not the only region of palindromic sequence similarity between the *fabB* and *fabA* promoter regions, suggesting the existence of a second regulatory factor. The advance in this area was triggered by the application of a computational "phylogenetic footprinting" method by McCue et al. (55), which was developed to identify transcription factor binding sites in bacterial genomes. The predicted transcription factor binding sites are available to the research community on the web (http://www.wadsworth.org/resnres/bioinfo/). Their technique located a strongly predicted second transcription factor binding site in the promoter regions of the *fabA* and *fabB* genes, and subsequent studies show that a protein, YijC, specifically binds to a DNA affinity column carrying the predicted transcription factor palindromic sequence (55). Based on the location of these binding sites, McCue et al. (55) proposed renaming the *yijC* gene *fabR* for fatty acid biosynthesis regulator.

These strong bioinformatics predictions stimulated the investigation of the *fabR* gene to experimentally establish its function in *E. coli* FAS II. The *fabR* deletion strains contained significantly elevated level of UFAs, especially *cis*-vaccenate, in the membrane phospholipids (56). Both the FabB mRNA and enzyme activity levels increase significantly in the *fabR* deletion strains, illustrating that FabR is a potent repressor of *fabB*. Similar results were obtained for the *fabA* gene except that FabR is a weaker repressor for *fabA* (56). Thus, the UFA synthesis in *E. coli* is coordinately regulated by FadR and FabR. The application of the phylogenetic footprinting approach identified FabR homologs and DNA binding motifs in Gram-negative bacteria that contain both the *fabA* and *fabB* genes (55), indicating that most Gram-negative organisms utilize a mechanism for UFA synthesis and regulation similar to that in *E. coli*.

FabR is a member of the TetR superfamily [Pfam00440; (57)] of bacterial regulatory proteins, and by analogy to the known mechanisms used by these factors, it seems reasonable to postulate that a regulatory ligand binds to the carboxy terminal domain of the protein to release the amino terminal winged helix DNA-binding domain from its target sequence. Thus, the search is on to iden-

tify the cellular ligand that regulates the FabR/DNA interaction, which in turn governs the product distribution of the pathway and the physical properties of the membrane bilayer.

THE FAS II SYSTEM IN *STREPTOCOCCUS PNEUMONIAE*

The recent work with the Gram-positive pathogen *S. pneumoniae* illustrates the application of bioinformatics analysis to guide the discovery of novel FAS II components. Like *E. coli*, *S. pneumoniae* FAS II produces straight-chain, 16 and 18 carbon saturated and unsaturated fatty acids, and therefore is anticipated to possess a FAS II system identical to the *E. coli* paradigm. However, a comparison of the FAS II enzymes in the completed *S. pneumoniae* genomes to the *E. coli* protein set reveals that this pathogen lacks several key players in the biosynthetic pathway. *S. pneumoniae* does not contain a homolog of the FabI enoyl-ACP reductase, an enzyme that is essential for the completion of elongation cycles in *E. coli*. Furthermore, *S.*

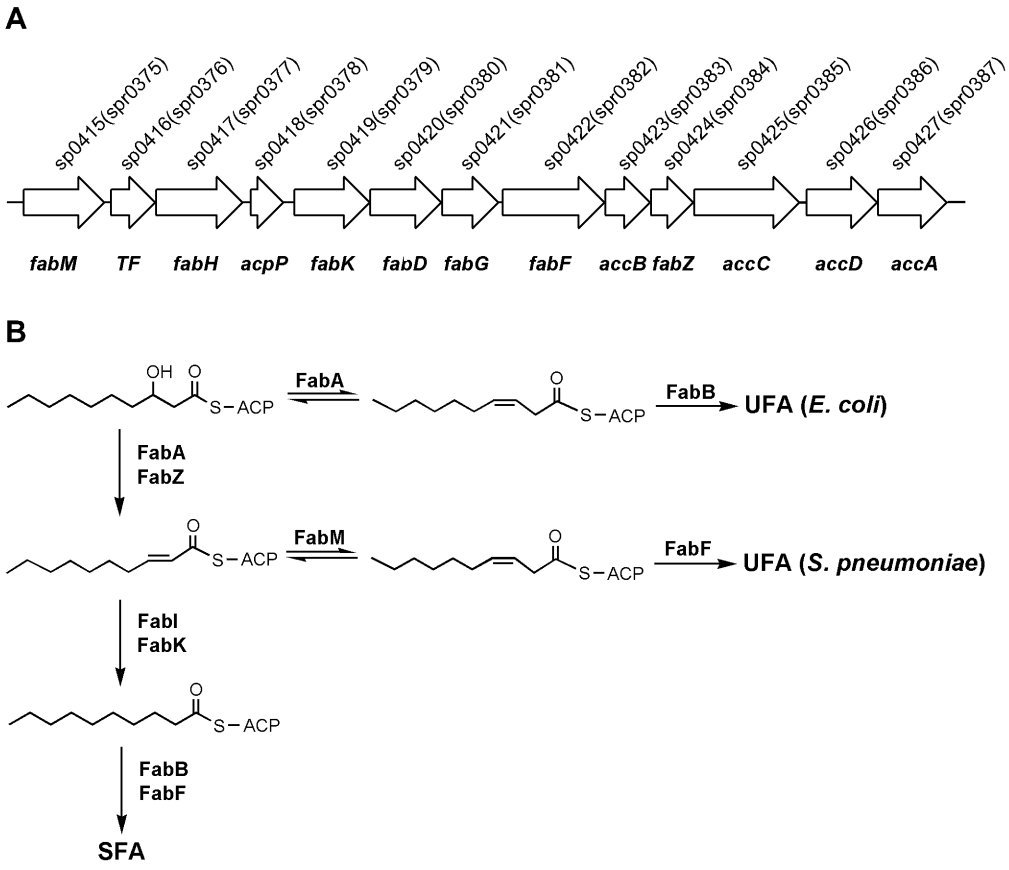

Fig. 5. Differences between the FAS II systems of *E. coli* and *S. pneumoniae*. A: the fatty acid biosynthetic gene cluster in *S. pneumoniae*. The genome sequences of two *S. pneumoniae* strains, namely TIGR4 and R6, are complete. All of the identified genes for fatty acid synthesis are located in a DNA fragment of about 10 kb in length. Each open arrow represents a gene or open reading frame. The labels above the arrows are the locus numbers for strain TIGR4, and the locus numbers of strain R6 are in the brackets. The gene annotations based on the functions are located below the arrows. Abbreviations: *fabM*, *trans*-2-*cis*-3-decenoyl-ACP isomerase; *TF*, an unknown transcriptional factor containing a helix-turn-helix (HTH) DNA binding motif; *fabH*, β-ketoacyl-ACP synthase III; *acpP*, acyl carrier protein; *fabK*, *trans*-2-enoyl-ACP reductase II; *fabD*, malonyl-CoA:ACP transacylase; *fabG*, β-ketoacyl-ACP reductase; *fabF*, β-ketoacyl-ACP synthase II; *accB*, acetyl-CoA carboxylase subunit; *fabZ*, β-hydroxyacyl-ACP dehydratase; *accC*, *accD*, and *accA*, subunits of acetyl-CoA carboxylase. B: Comparison of the unsaturated fatty acid synthesis in *E. coli* and *S. pneumoniae*. The branch point for *E. coli* UFA synthesis is at the β-hydroxydecanoyl-ACP stage. *E. coli* FabA not only dehydrates β-hydroxy-C10:0 intermediate to form *trans*-2-C10:1 but also isomerizes the double bond to its *cis*-3 isomer. The *cis*-3-decenoyl-ACP skips the FabI reaction and gets elongated by FabB to form UFAs. On the other hand, saturated fatty acid (SFA) synthesis proceeds by the reduction of the *trans*-2-decenoyl-ACP by FabI to produce decanoyl-ACP which can be elongated by either FabB or FabF. In contrast to the *E. coli* UFA synthesis, the branch point for *S. pneumoniae* UFA synthesis takes place at the *trans*-2-decenoyl-ACP stage. FabM isomerizes the double bond to form *cis*-3-decenoyl-ACP, which is elongated by FabF to produce UFA eventually. SFA synthesis in *S. pneumoniae* is essentially the same as *E. coli*, except that FabK is used to reduce the *trans*-2 intermediate and FabF is the only condensing enzyme.

pneumoniae lacks homologs of the *fabA* and *fabB* genes, two enzymes essential for UFA synthesis in *E. coli* (**Fig. 5** and above). These findings triggered a search for the novel FAS II components in *S. pneumoniae*, which was facilitated by genomic sequence analysis that pointed to specific genes that were potential FAS II components.

Bioinformatics tools located the FAS II components in *S. pneumoniae* within a single large cluster of genes (Fig. 5A). This cluster lacks a FabI homolog, but it does contain an unidentified open reading frame termed *fabK* (Fig. 5A). The location of *fabK* within this large FAS II gene cluster strongly suggested that it was a key component in the FAS II pathway. The subsequent expression and purification of the FabK protein, and the genetic complementation of *fabI* mutants, proved that it was a bone fide enoyl-ACP reductase (58). FabK and FabI do not share any sequence similarities. Unlike FabI, FabK has a centrally located flavin-binding domain and utilizes both FMN and NADH as cofactors. In addition, FabK is resistant to triclosan, a broad-spectrum antimicrobial that targets the FabI step in bacterial FAS II (59, 60).

The FabK story also provides a word of caution about a strict reliance on bioinformatics predictions. FabK is annotated in the database as a nitropropane dioxygenase based on its strong sequence similarity, and its true function could have been overlooked based on this definitive, but incorrect annotation. Similarly, a third enoyl-ACP reductase, FabL, was discovered in *Bacillus subtilis* by genomic sequence analysis followed by biochemical and genetic characterization (61). This protein has a relatively low similarity to FabI, but it contains the key catalytic residues of enoyl-ACP reductase (Tyr-X_6-Lys).

In *E. coli*, the branch point for UFA formation from the overall course of FAS II takes place when the growing acyl chain reaches the β-hydroxydecanoyl-ACP stage (Fig. 5B). Either FabA or FabZ catalyzes the dehydration reaction of the β-hydroxyacyl-ACP to *trans*-2-decenoyl-ACP, but FabA also isomerizes the *trans*-2-enoyl bond to form the *cis*-3 isomer, which is then elongated by FabB. Both *fabA* and *fabB* homologs are conspicuously absent from the FAS II gene cluster of *S. pneumoniae*. Only the FabZ dehydratase is present, and FabF is the only elongation condensing enzyme. Thus, this organism must utilize a different mechanism for UFA synthesis.

The initial search centered on identifying the *S. pneumoniae* gene that encodes an enzyme capable of isomerizing the *trans*-2 intermediate to its *cis*-3 isomer. There are two unidentified genes adjacent to the *S. pneumoniae* FAS II gene cluster (Fig. 5A). One gene (SP0416) encodes a protein containing a helix-turn-helix motif and it is homologous to the MarR family of transcriptional factors. The other gene (SP0415) is annotated as an enoyl-CoA hydratase/isomerase family protein. Members of this family participate in the isomerization reaction in fatty acid β-oxidation; however, the absence of any other fatty acid degradation genes from the complete genome sequence of *S. pneumoniae* TIGR4 indicates that this organism does not carry out β-oxidation. These observations led to the hypothesis that SP0415 encodes a novel *trans*-2-*cis*-3-enoyl-ACP isomerase, FabM. Again, biochemical and genetic analysis verified that FabM functions as an isomerase in the FAS II system (62). Thus, the branch point for *S. pneumoniae* UFA synthesis is one step further down the type II pathway in comparison to *E. coli* (Fig. 5B). The FabK enoyl-ACP reductase II competes with the FabM isomerase for the *trans*-2-decenoyl-ACP intermediate. Both of these reactions produce intermediates that are subsequently elongated to either saturated or unsaturated fatty acids by the single FabF elongation-condensing enzyme in *S. pneumoniae*. The differences between the *E. coli* and *S. pneumoniae* FAS II systems offer an explanation for the inability of *fabM* alone to complement *E. coli fabA*(Ts) mutants. In *E. coli*, FabA competes with FabI and FabZ, whereas UFA production in *S. pneumoniae* arises from FabM competition with FabK (Fig. 5B). FabM alone is not sufficiently active to effectively compete with FabI for the *trans*-2 intermediates when expressed in *E. coli*. However, when FabM is co-expressed with its partner FabK under an experimental condition where endogenous FabI activity is eliminated by treatment with triclosan, UFA synthesis is restored to *fabA*(Ts) mutants (62).

Genomic analysis gave the first indication that FabM and FabK are partners. A metabolic relationship between FabM and FabK was predicted by the identification of the same transcription factor binding site within the promoters of both the *fabM* and *fabK* genes in *S. pneumoniae*, pointing to the control of these two genes by the same transcriptional regulator. This leads to the hypothesis currently being tested that these two partners in UFA biosynthesis are coordinately regulated, reminiscent of the coordinate regulation of *fabA* and *fabB* in *E. coli* (see above).

CONCLUSIONS

There is a lot more diversity in bacterial FAS II systems that remain to be discovered. Understanding the differences between the *E. coli* paradigm and the FAS II mechanisms of major pathogens is important not only from a basic research viewpoint, but also because FAS II is emerging as a major target for the development of novel antibacterial agents (63–65). For example, fatty acid synthesis is a major focus for drug development in the fight against *Mycobacterium tuberculosis* (66). However, not all of the enzymes of FAS II have been identified in this organism, and this opens the possibility that functional differences exist between this organism and *E. coli*. Defining the species-specific differences in the pathway is also critical for determining the spectrum of activity of drugs developed against selected FAS II targets.

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