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Will the Initiator of Fatty Acid Synthesis in *Pseudomonas aeruginosa* Please Stand Up?

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Type II fatty acid synthesis (FASII) is vital for bacterial membrane biogenesis. The first condensation step in the pathway is carried out by β-ketoacyl-acyl carrier protein (ACP) synthase III, or FabH, and subsequent condensation reactions are executed by the FabB/F family of enzymes. The prototypical FabH activity of *Escherichia coli* catalyzes the condensation of acetyl coenzyme A (acetyl-CoA) with malonyl-ACP to form the first β-ketoacyl-ACP intermediate (7). Variations on this theme are Gram-positive bacteria that use 5-carbon branched-chain acyl coenzymes A (acyl-CoAs) to initiate fatty acid synthesis (1) and mycobacteria where long-chain acyl-CoAs are used by FabH to initiate mycolic acid synthesis (2). Most bacteria have a single, essential *fabH* gene (9). However, the situation with *Pseudomonas aeruginosa* is far more complex, because there are at least three candidates for the FabH initiator of FASII and four isoforms of the FabB/F elongation condensing enzymes (Fig. 1). In this issue, two accompanying papers, "Fatty acid biosynthesis in *Pseudomonas aeruginosa* is initiated by the FabY class of β-ketoacyl acyl carrier protein synthases" and "*Pseudomonas aeruginosa* directly shunts β-oxidation degradation intermediates into *de novo* fatty acid biosynthesis", both by Yuan and colleagues (15, 16), have unraveled the complexity in *fabH* gene functions in *Pseudomonas aeruginosa*. They reach the surprising conclusion that none of the *fabH* genes are responsible for the initiation of fatty acid synthesis in *P. aeruginosa*! Rather, initiation of FASII is carried out by a unique gene named FabY that is more closely related to the elongation condensing enzymes (FabB/F). Furthermore, one of the *fabH* homologs encoded by PA3286 plays a unique role in channeling 8-carbon acyl-CoA intermediates arising from fatty acid β-oxidation into the FASII elongation cycle. This is the first example of an enzyme that allows β-oxidation intermediates to be utilized by the FASII pathway.

Two classes of condensing enzymes. The condensing enzymes of FASII belong to the α/β-hydrolase superfamily and are divided into two classes based on their active site triads (Fig. 1). The FabH class possesses a Cys-His-Asn catalytic triad and condenses an acyl-CoA with malonyl-ACP to initiate FASII. The FabB/F class of elongation condensing enzymes carries out all subsequent condensation reactions between the elongating acyl-ACP and malonyl-ACP using a Cys-His-His configuration. All biochemically characterized condensing enzymes have fallen so far into one of these two classes based on the active site triad leading to confident functional predictions. Although the FabH enzymes (FabHs) use acyl-CoA and FabB/F use acyl-ACP, the condensation reaction occurs after the acyl chain has been transferred to the active site cysteine and the subsequent binding of malonyl-ACP. The active site chemistry performed by both of these enzymes appears identical, so it has never been clear why initiation condensing enzymes have one type of active site and the elongation enzymes have another type. The discoveries of Yuan and colleagues (15, 16) mean that we can no longer confidently assign roles to a condensing enzyme based on the presence of a Cys-His-Asn or Cys-His-His active site triad.

FabY, a FabB/F homolog that functions as a FabH. Deleting each of the 3 *fabH* candidates to determine which gene was involved in FASII eventually led to the generation of a triple knockout strain that had no overt growth phenotype. Yuan et al. (16) then identified PA5174 as an open reading frame capable of initiating FASII, using a *P. aeruginosa* cosmid library to select for genes that cured the growth defect in an *E. coli* strain with downregulated *fabH* expression. Biochemical characterization of purified FabY showed that the enzyme efficiently catalyzed the condensation of acetyl-CoA with malonyl-ACP. It is not known whether FabY possesses FabB/F-like activity using acyl-ACP substrates. The *fabY* knockout exhibited a distinct growth defect and was also deficient in the formation of numerous secondary metabolites, quorum-sensing signaling molecules, and siderophores. All of these phenotypes are restored to normal by the expression of *E. coli fabH*. Thus, FabY is a condensing enzyme that performs a task usually attributed to FabH but uses a Cys-His-His active site of FabB/F enzymes.

Connecting fatty acid oxidation and biosynthesis. The discovery of FabY begs the question: what are the roles of all those *fabH* genes? Yuan et al. (15) have defined the role of PA3286, the third FabH in *P. aeruginosa* (FabH3) (Fig. 1A). The authors observed that the growth defect of the Δ*fabY* strain was cured by supplementing the medium with decanoic acid. Experiments using deuterated decanoic acid feeding established that FabH3 shunts the octanoyl-CoA arising from the β-oxidation of fatty acids into the FASII pathway. Thus, FabH3 is an initiation condensing enzyme with the unique property of utilizing octanoyl-CoA derived from fatty acid degradation. Subtle differences in the 3-dimensional structures of the hydrophobic pockets that accommodate the acyl-enzyme intermediate account for the acyl chain specificity of the FabHs (5). Thus, PA3286 (FabH3) is predicted to have a hydrophobic tunnel that acts as a molecular ruler to selectively accept an octanoyl-CoA. This substrate specificity is the key to its function in *P. aeruginosa* lipid metabolism. Most obvious is the savings in energy to produce a fatty acid chain starting from an 8-carbon precursor compared to the standard 2-carbon primer provided by acetyl-CoA. However, the importance of introducing

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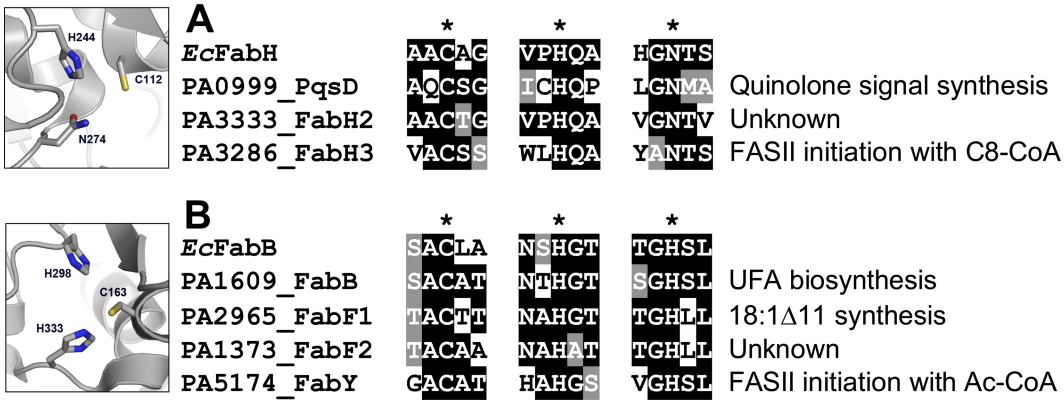

FIG 1 The condensing enzymes in *P. aeruginosa*. The active site structures, locus tags, gene symbols, blocks of homology surrounding the key catalytic residues, and the functional annotations of the genes are provided for the three FabH (A) and four FabB/F (B) homologs in *P. aeruginosa*. The sequence alignment blocks surrounding the Cys-His-Asn triad of the FabH homologs and Cys-His-His of the FabB/F homologs of *P. aeruginosa* are compared to the active site residues of the prototypical *E. coli* FabH (*Ec*FabH) and FabB (*Ec*FabB), respectively. PqsD is sometimes annotated as FabH1. Identical residues are shown on a black background, and conservatively substituted residues are shown on a gray background. The depicted active site structures are derived from the crystal structures of *E. coli* FabH (Protein Data Bank [PBD] accession no. 1EBL) (3) and *E. coli* FabB (PDB accession no. 1G5X) (17). UFA, unsaturated fatty acid; 18:1Δ11, *cis*-vaccenic acid; C8-CoA, octanoyl-CoA; Ac-CoA, acetyl-CoA.

the β-oxidation intermediates into FASII at the 8-carbon step is more clearly appreciated by understanding the critical importance of FASII intermediates in *P. aeruginosa* physiology. 3-Hydroxydecanoyl-ACP is the key intermediate in unsaturated fatty acid synthesis (20) and is also used for the synthesis of lipopolysaccharides (4), secreted rhamnolipids (21), and the poly-β-hydroxy-alkanoate storage lipids (13). Octanoyl-ACP is the precursor to lipoic acid (19). The 10- and 12-carbon β-ketoacyl-ACP intermediates are used in the biosynthesis of acyl-homoserine lactones (10) and alkyl-quinolone (12) signaling molecules. Thus, the reintroduction of acyl chains at the 8-carbon stage allows for the production of a spectrum of products derived from FASII.

Two enigmatic condensing enzymes remain. Two of the seven condensing enzymes in *P. aeruginosa* still lack a functional annotation (Fig. 1). PqsD (FabH1) catalyzes a condensation reaction in the synthesis of dihydroxy- and alkyl-quinolines (12, 18). The function of PA3333 (FabH2) is unknown, and it is located in an operon with an ACP gene that is induced in stationary phase (14). PA3286 is responsible for initiating FASII from octanoyl-CoA (15). PA1609 (FabB) is required for unsaturated fatty acid biosynthesis (6). Strains lacking PA2965 (FabF1) are deficient in *cis*-vaccenic acid (8) and are unable to swarm, twitch, or swim (11). There is no clue to the function of PA1373 (FabF2). The most divergent of the FabB/F enzymes is PA5174 (FabY), which is now known as the FASII initiator (16). Neither PA1373 nor PA3333 are essential for *P. aeruginosa* survival, so perhaps these enzymes support the formation of secondary metabolites in stationary phase, but this is just a guess at where to begin searching for their functions. Unraveling the transcriptional and biochemical regulation of the condensing enzymes of *P. aeruginosa* should prove interesting.

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