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Roles of multiple KASIII homologues of *Shewanella oneidensis* in initiation of fatty acid synthesis and in cerulenin resistance

Qiu Meng, Huihui Liang, Haichun Gao*

Institute of Microbiology and College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China

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ABSTRACT

It is fully established that the condensing reaction for the initiation of fatty acid synthesis is essential for viability of many bacteria. In model bacteria such as *Escherichia coli*, this reaction is exclusively catalyzed by β-ketoacyl-ACP synthase (KAS) III (encoded by *fabH*) and the FabH loss results in a fatty acid auxotroph. However, such a notion has been under the challenge of recent findings. In an attempt to resolve the conflicting results, in this study, we examined the physiological role of multiple KASIII enzyme homologues in *Shewanella oneidensis*, an excellent model for researching type II fatty acid synthesis (FASII) and its regulation. We demonstrated that FabH1 and temperature-responsive FabH2 are primarily responsible for initiating synthesis of straight- and branched-chain fatty acids respectively, whereas FabH3 and OleA are dispensable. Cells lacking all these enzymes as a set are viable but carry severe defects in growth. Further analyses revealed that in the absence of *KAS*III either of FabB (KASI) and FabF2 (KASII) is able to support growth, suggesting that they could initiate FASII. Strikingly, KASIII enzymes and OleA together confer *S. oneidensis* cells resistance to cerulenin, a selective inhibitor of FabF and FabB. Along with our previous finding that *S. oneidensis* FabF1 and FabB are fully equivalent with respect to their physiological impacts, these results imply that physiological function promiscuity of bacterial KAS enzymes could be more extensive than previously expected.

1. Introduction

The de novo fatty acid (FA) synthetic (FAS) pathway (Fig. 1), named type II (FASII), is the predominant, if not exclusive, route for endogenous production of fatty acids in bacteria, the current knowledge of which derives mainly from model organism *Escherichia coli* [1]. FASII, consisting of the initiation and elongation modules, produces not only fatty acids for phospholipids but also a diversity of intermediates for cellular metabolism [2,3]. The initiation module functions to produce the primers and the building blocks for the elongation module, acyl-CoA and malonyl-ACP (β-ketoacyl-Acyl Carrier Protein), respectively [3]. The elongation module is a cycle responsible for extension of the primer by two carbons from malonyl-ACP with the completion of each turn of the cycle until the chain of the resulting acyl-ACP reaches the requirement.

Central to the bacterial FASII are reactions catalyzed by β-ketoacyl-ACP synthases (KAS), including *KAS*I (FabB), KASII (FabF), and KASIII (FabH), members of the thiolase superfamily [2,4]. Both FabB and FabF function in the elongation module, responsible for the condensation of malonyl-ACP with the growing acyl chain. Although they are exchangeable in elongation of saturated intermediates, each catalyzes a reaction within the unsaturated branch that the other cannot, or at least much less effectively [3] (Fig. 1). FabB is essential to UFA biosynthesis because it exclusively catalyzes the elongation of the *cis*-3-decenoyl-ACP (*cis*-3-10:1-ACP) [5]. In contrast, FabF is not fully required although FabF but not FabB when in excess induces growth inhibition and viability loss by blocking fatty-acid-chain elongation [6–8]. As the last enzyme in the initiation module, FabH accounts for condensation of an acyl-CoA unit and a malonyl-ACP unit [9]. In *E. coli*, FabH preferentially utilizes acetyl-CoA to synthesize straight-chain fatty acids (SCFA) whereas its counterparts in some other bacteria, such as *Bacillus subtilis*, catalyze selectively branched-chain acyl-CoA intermediates from metabolism of branched-chain amino acids, leading to production of branched-chain fatty acids (BCFAs) [10,11]. Because of substrate specificity, KASIII enzymes underlie the diversity in the fatty acid structure produced by different bacteria and dictate the physiological architecture of the envelope and therefore membrane fluidity, in response to environmental changes such as temperature [12,13].

Abbreviations: ACP, acyl carrier protein; CoA, coenzyme A; FAS, fatty acid synthesis/synthetic; KAS, β-ketoacyl-ACP synthase; SCFA, straight-chain fatty acid; BCFA, branched-chain fatty acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid

* Corresponding author.
E-mail address: haichung@zju.edu.cn (H. Gao).

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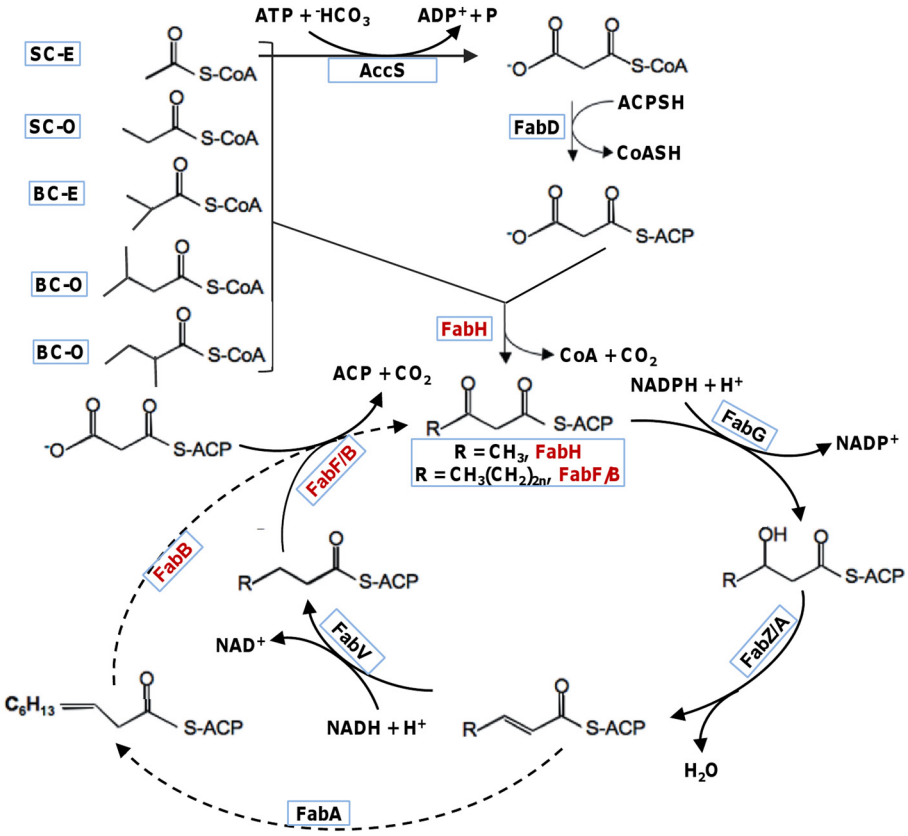

Fig. 1. Proposed type II fatty acid (FA) synthesis in *S. oneidensis*. FA types: SC, straight-chain; BC, branched-chain. Carbon atoms of FA: E, even number; O, odd number. In *S. oneidensis*, homologues of two *E. coli* enzymes, AccABCD and FabI, are not found and replaced by AccS (SO_0840) and FabV (as in *V. cholerae* and *P. aeruginosa*), respectively. Dash line represents the steps specific to UFA synthesis. Enzymes in red are KAS.

Although all KAS enzymes catalyze decarboxylative Claisen condensation reactions between an acyl donor and malonyl-ACP and have many similarities, there are critical mechanistic differences between KASIII and other two types of KAS enzymes [3]. For example, FabH possesses a Cys-His-Asn (CHN) catalytic triad whereas the catalytic triad of FabB/F is Cys-His-His (CHH) [9]. As a consequence, it is conceivable that *E. coli* FabH could not function as a replacement for FabB/F, and vice versa, because of inability to work with substrates of the other side [9]. Accordingly, KASIII is essential for fatty acid biosynthesis and viability across a diverse spectrum of both Gram-positive and Gram-negative bacteria, including *E. coli*, *Streptomyces coelicolor*, and *Lactococcus lactis* [14–16]. In parallel, FASIII differs from other KAS enzymes in antibiotic susceptibility. For example, cerulenin is an inhibitor of FabB and FabF but not of FabH [17]. However, these notions have been under the challenge of recent findings: FabH is not essential in *E. coli* [18] and cerulenin is also effective against *Staphylococcus aureus* FabH [19].

Shewanella, ubiquitous in natural environments, comprise a group of facultative bacteria renowned for respiratory versatility, having great potential for bioremediation of toxic elements and for bioelectricity [20,21]. In addition, *Shewanella* are increasingly being implicated as fish spoilage bacteria, even in iced products because many species can grow at 4 °C or lower, and human pathogens in marine niches where most of the species thrive [22].

Our previous studies have revealed that *S. oneidensis KASI* and KASII enzymes carry many features distinct from their studied counterparts in other bacteria [23–25]. Unlike *E. coli*, which has only one enzyme for each KAS, *S. oneidensis* possesses one *KASI* (FabB), two KASII (FabF1 and FabF2), and three KASIII (FabH1, FabH2, and FabH3) [26]. Because of the presence of the aerobic pathway for UFA synthesis, which relies on a single desaturase DesA, FabB is not essential for UFA biosynthesis and viability [23,24]. Intriguingly, this FabB differs from its *E. coli* counterpart in that it dictates elongation of C_{14}-ACP, both $C_{14:0}$ and $C_{14:1}$, to C_{16}-ACP; as a consequence, its loss results in accumulation of C14 fatty acid species whereas its overproduction at modest levels promotes production of C_{18}-ACP and longer chain-ACP and at further increased levels induces growth inhibition and viability loss [24]. Under normal conditions, FabF2 is a functioning KASII; its loss slightly impairs growth and it is synthetic lethal with FabB. However, unlike *E. coli* FabF, FabF2 in overproduction hardly impairs growth or viability [25]. In contrast, FabF1 is negligible because it is barely produced. But by forced production it could fully complement the FabB loss but, unlike FabB, is not detrimental in excess [25]. Given the novel features of *S. oneidensis KASI* and KASII, we reasoned that there would be great merits to elucidate physiological roles of KASIII enzymes. Therefore, we undertook a detailed mutational analysis of KASIII homologues to assess their impacts on growth, fatty acid composition, and cerulenin resistance, which is the subject of this study.

2. Materials and methods

2.1. Bacterial strains, plasmids, and culture conditions

All bacterial strains and plasmids used in this study were listed in Table 1 and information for primers used was available upon request. All chemicals were acquired from Sigma Co. (Shanghai, China) unless specifically noted. For genetic manipulation, *E. coli* and *S. oneidensis* strains under aerobic conditions were grown in Lysogeny broth (LB) at 37 and 30 °C, respectively. When needed, the growth medium was supplemented with chemicals at the following concentrations: 2,6-diaminopimelic acid (DAP), 0.3 mM; ampicillin, 50 μg/ml; kanamycin, 50 μg/ml; and gentamycin, 15 μg/ml.

2.2. In-frame deletion mutagenesis and genetic complementation

In-frame deletion strains were constructed according to the *att*-based Fusion PCR method described previously [27]. In brief, two fragments flanking the gene of interest were amplified with primers