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COMMENTARY

Mycobacterium tuberculosis Cholesterol Catabolism Requires a New Class of Acyl Coenzyme A Dehydrogenase

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In this month's issue of the *Journal of Bacteriology*, Wipperman et al. establish a new category of acyl coenzyme A (acyl-CoA) dehydrogenases (ACADs) that form unique $\alpha_2\beta_2$ heterotetrameric complexes required to catalyze unsaturation reactions in β-oxidation of steroid-CoA substrates (1). The described $\alpha_2\beta_2$ ACADs allow *Mycobacterium tuberculosis*, and perhaps other pathogens, to take advantage of ubiquitous cholesterol as a readily available carbon source.

The *M. tuberculosis* genome contains six sets of ACAD genes (*fadE* genes) that are regulated by cholesterol, with each set of genes found adjacent to each other within the same operon (1). The current study provides compelling evidence that all of these adjacent cholesterol-regulated genes encode members of a new $\alpha_2\beta_2$ heterotetrameric ACAD class. The Sampson laboratory previously demonstrated that two of these genes, *chsE1* and *chsE2*, encode an $\alpha_2\beta_2$ ACAD required for the dehydrogenation of the cholesterol side chain (2). The current study extends the initial characterization of ChsE1/2 to five additional sets of $\alpha_2\beta_2$ ACADs. Of the six $\alpha_2\beta_2$ ACAD sets, five are induced by cholesterol and likely important for cholesterol catabolism, while one set is repressed by cholesterol and is not likely involved in cholesterol catabolism.

Acyl-CoA dehydrogenases were first characterized in the 1950s by Beinert and colleagues as enzymes that catalyze the αβ unsaturation of acyl-CoA thioesters in β-oxidation (3, 3a). Prior to the recent studies by the Sampson laboratory, ACADs had only been characterized that catalyze the αβ unsaturation of short-, medium-, and long-chain acyl-CoA thioesters, and all were homotetrameric or homodimeric in structure (4). Thus, the recent report on ChsE1/E2 was the first characterization of an $\alpha_2\beta_2$ heterotetrameric ACAD (2). Homotetrameric ACADs have four active sites with four flavin adenine dinucleotide (FAD) cofactors. In five of the six $\alpha_2\beta_2$ ACADs, only one of the adjacent ACAD subunits contains the catalytic residue required for deprotonation of the acyl-CoA substrate. The authors demonstrate that the $\alpha_2\beta_2$ ACADs have one active site and one FAD per heterodimer instead of two. Five of the six genome adjacent *fadE* pairs formed only heteromeric complexes when expressed *in vitro*, while the sixth set, FadE17/18, did not form soluble protein. A control pair of *fadE* genes that are transcribed in the same operon, but are not adjacent pairs, formed typical homomeric complexes only when expressed *in vitro* and did not have characteristics of the $\alpha_2\beta_2$ ACAD class. Thus, the $\alpha_2\beta_2$ ACADs are formed from two heterodimers in which each specific monomer is required to form a unique active site.

The *M. tuberculosis* genome contains a disproportionally large number of lipid and fatty acid metabolic genes including 35 members of the *fadE* family (5). This apparent redundancy in the genome has long been a mystery. The study by Wipperman et al. has unraveled a piece of this mystery by establishing a role for 11 *fadE* genes that encode six highly specific ACAD complexes. It has also become clear that *in vivo*, *M. tuberculosis* prefers to consume fatty acids and lipids; thus, it has preserved the ability to metabolize complex lipids such as cholesterol. Mutants with defects in the glyoxylate, methylcitrate, and gluconeogenic pathways, important for even- and odd-chain fatty acid metabolism, are all severely attenuated in mouse models of tuberculosis (TB) infection (6–9). *M. tuberculosis* isolated from murine lungs readily metabolize fatty acids but not carbohydrates (10). A variety of lipids are available to *M. tuberculosis* during different stages of infection, including cholesterol, an abundant and essential component of animal cell membranes. Catabolism of cholesterol provides a carbon source for energy production and *M. tuberculosis* lipid synthesis (11). Cholesterol is broken down to acetyl-CoA for the tricarboxylic acid (TCA) cycle, propionyl-CoA for the methylcitrate cycle or lipid synthesis, and pyruvate for the generation of acetyl-CoA, or potentially to drive gluconeogenesis.

Studies with mice demonstrate that high dietary cholesterol enhances TB infection (12). The mere fact that *M. tuberculosis*, an obligate human pathogen, has maintained over 80 genes involved in cholesterol metabolism points to the importance of cholesterol to the pathogen. *In vivo* experiments have produced mixed results in demonstrating an essential role for cholesterol metabolism. Experiments with strains with deletions of genes coding for specific parts of the cholesterol degradative pathway exhibited attenuated phenotypes during infection of mice and guinea pigs that were attributed to accumulation of toxic intermediates (13–15). Deletion of the *mce4* cholesterol transport locus resulted in decreased growth on cholesterol and decreased virulence in activated macrophages and murine infection (16), supporting an important role for cholesterol catabolism in *M. tuberculosis* pathogenesis. However, deletion of *M. tuberculosis Rv1106c*, which encodes the first step in cholesterol catabolism and is necessary for *in vitro* growth on cholesterol, did not result in attenuation in macrophage or guinea pig infections (17), indicating that cholesterol catabolism may not be essential for infection. Nevertheless, the buildup of toxic intermediates upon interruption of cholesterol degradation and the resulting bacteriostasis or bacillus death make the catabolic enzymes of cholesterol degradation attractive drug targets (18).

Cholesterol is made up of an 8-carbon side chain and a polycyclic steroid ring moiety with A, B, and C cyclohexane rings and a cyclopentane D ring. *M. tuberculosis* cholesterol catabolism

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starts at the side chain while it is still connected to the steroid rings, thus, the requirement for the $\alpha_2\beta_2$ ACAD ChsE1/2 to catalyze the dehydrogenation reaction of the attached side chain (2). ChsE1/2 also has a preference for the four-ring steroid skeleton form over the two-ring form and does not have activity against short-chain substrates, indicating that the side chain is metabolized before degradation of the polycyclic skeleton (2). The current study shows that a second $\alpha_2\beta_2$ ACAD set (FadE26/27) catalyzes the dehydrogenation of 3β-hydroxy-chol-5-en-24-oyl-CoA, indicating it is also involved in side chain degradation (1, 2). Cholesterol catabolism appears to be primarily controlled by two transcriptional regulators, KstR and KstR2 (18). The KstR and KstR2 regulons contain genes for four of the six $\alpha_2\beta_2$ ACAD pairs. Both ChsE1/2 and FadE26/27 are part of the KstR regulon (18), which is consistent with their role in side chain degradation. KstR2 regulation of the triplet cluster *fadE31-fadE32-fadE33* suggests that the $\alpha_2\beta_2$ ACADs formed from combinations of FadE31/32/33 are likely involved in the degradation of the C/D rings, but this is yet to be confirmed (19). One set of *fadE* genes (*fadE17* and *fadE18*) is induced by cholesterol but is controlled by Mce3R (20). The *mce* loci are important for virulence, and *fadE17* and *fadE18* genes are not found in nonpathogenic mycobacteria, which suggest a connection between pathogenesis and cholesterol metabolism. Many of the specific steps of cholesterol catabolism are yet to be elucidated, but it is clear that the $\alpha_2\beta_2$ ACADs play a pivotal role in this important metabolic pathway.

One set of genes that encode an $\alpha_2\beta_2$ ACAD, *fadE23* and *fadE24*, is repressed by cholesterol and thus unlikely involved in cholesterol catabolism. Instead, it is partially controlled by sigma E (21) and is highly induced upon several conditions that cause cell envelope damage including SDS exposure (21), oxidative and nitrosative stress (22), and two antimycobacterial drugs that target cell wall biosynthesis, isoniazid (23) and ethambutol (M. I. Voskuil, unpublished results). It has been suggested that induction of *fadE23* and *fadE24* by isoniazid indicates that FadE23/24 may be involved in recycling of *M. tuberculosis* fatty acids (23). The fact that induction occurs under several conditions that are all known to damage the cell envelope further implicates FadE23/24 in catabolism of *M. tuberculosis* cell wall mycolic acids or another complex fatty acid-containing molecule. Interestingly, the cholesterol-binding antifungal agent amphotericin B binds as well to mycolic acids as to cholesterol. In addition, antibodies often cross-react with cholesterol and mycolic acids (8). These findings indicate that cholesterol and mycolic acids may share a three-dimensional structure that necessitates an $\alpha_2\beta_2$ ACAD for degradation.

Prokaryotic ACADs are not extensively characterized, but their eukaryotic counterparts are divided into seven classes that have fatty acid substrate specificity and four classes that are involved in amino acid degradation (24). In a search to identify other organisms with the $\alpha_2\beta_2$ ACAD class, Wipperman et al. found $\alpha_2\beta_2$ ACAD homologs in actinobacteria and some members of the *Proteobacteria* and *Firmicutes* phyla. Interestingly, the genes for these homologs were always paired in the genome with the same homologous partner found in *M. tuberculosis* (1). Most genomes with paired $\alpha_2\beta_2$ ACAD genes also contained key genes for the first step in cholesterol catabolism and for oxidation of the steroid A ring, further supporting their role in αβ unsaturation of steroid-CoA substrates. The *Proteobacteria* in which $\alpha_2\beta_2$ ACAD genes were identified include *Pseudomonas*, *Burkholderia*, and *Comamonas*, genera that all contain bacteria that are opportunistic pathogens in humans. It is tempting to speculate that their ability to metabolize cholesterol provides a general benefit to animal pathogens.

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