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Preface

Tuberculosis was one of the earliest organisms associated with disease in humans, along with leprosy or Hansen's disease, and anthrax. Koch demonstrated that a specific disease, tuberculosis, could be caused by *Mycobacterium tuberculosis* using guinea pigs in the 1880s. The resulting host-bacteria system was used to develop Koch's postulates, one of the foundations of infectious diseases research, illustrating the importance of understanding both sides of the disease process for infections, the host and pathogen. Prior to the late 1980s, the study of the pathogen that causes tuberculosis was very limited due to the inability to genetically manipulate the organism, but a little over 100 years after Koch's discovery, molecular genetics became possible in *M. tuberculosis*, opening a vast array of new studies on these bacteria along with endless possibilities to create novel interventions. Many of the chapters in this book come out of having new abilities to manipulate tuberculosis host-pathogen interactions beyond those previously possible.

Our aim is to present some of these more recent findings and novel technologies that have come out of research in host-pathogen interactions. In this book, we aim to provide insight into many aspects of tuberculosis host-pathogen interactions, though we would not even attempt to be comprehensive, since the breadth of knowledge and the pace of advancement in the field are great. Attempting to be comprehensive in the tuberculosis field is no longer possible, mostly because that would require many volumes of reviews, rather than a single volume. We requested authors to provide their thoughts on topics within host-pathogen interactions from their own perspective and believe that the resulting chapters give insight into the breadth of the field currently. We cover many individual components of the bacteria that are involved in host-pathogen interactions as well as aspects of the host and comorbidities in disease. In some cases, it is obvious there remains a great deal more to do and examination of much biology in mycobacteria remains to be explored. Latent infections, in particular, represent a problematic issue that is difficult to model and gain insight into the actual situation in humans. This problem is partially due to the difficulty of tracking and study of very few bacteria directly in humans. New technologies on the horizon in imaging may help to contribute to our understanding of the bacteria without having to isolate them, and the state of the art in this area is presented.

The combination of the ability to track very few bacteria during infections as well as monitor the host response during all aspects of disease could ultimately provide a more comprehensive picture of tuberculosis host-pathogen interactions. The understanding of mesenchymal, myeloid, and T cells during all stages of disease, including latency, combined with understanding epigenetic changes could provide much of the necessary information regarding how different hosts respond to tuberculosis that is sorely needed. We present information on these cells and would suggest that the ability to evaluate these populations of cells and their ability to respond in patients may be key to the development of intervention strategies that may be used to improve vaccine and therapeutic technologies.

Overall, the information provided in these chapters is only a taste of the tuberculosis field, and we would suggest interested readers look to a number of additional resources that are available such as the extensively informative source of B. R. Bloom (*Tuberculosis: Pathogenesis, Protection, and Control*, 653 p., ASM Press, 1994), the important general molecular overview of Hatfull and Jacobs (*Molecular Genetics of Mycobacteria*, 363 p., ASM Press, 2000), the genetics methods compendium of Jacobs et al. (Genetic Systems of Mycobacteria in *Methods in Enzymology Vol. 204 Bacterial Genetic Systems*, J.H. Miller (Ed.), 537–555 p., Academic Press, 1991), the valuable overview by Grosset and Chaisson (*Handbook of Tuberculosis*, 221 p., Springer, 2017), and the key overview by Shinnick (*Tuberculosis*, 307 p., Springer, 1996), just to name a few of the available resources. We provide a supplement and update to these prior publications with a focus on how each area impacts interactions of *M. tuberculosis* with its human host.

Areas that are covered include surface molecules, lipids, and cell wall, with how they play a role in direct modulation of the host and how tuberculosis can affect the host and the role of these modulatory effects on HIV. Specific cell types play an important role in tuberculosis, such as stem cells, myeloid cells, and T cells, all of which have been examined in overviews that are included. Latent infections can persist for the life of a host, reactivating at any time, and aspects of the bacterial interactions are covered in multiple chapters to emphasize their importance and the complexity of the issues involved. We are also on the cusp of new key discoveries through exploration of new research directions, including the use of imaging technologies and examination of the role of epigenetic relationships in disease processes that are explored in two other chapters. Thus, although not at all comprehensive, this overview summarizes several key areas of tuberculosis host-pathogen interactions with the goal of stimulating additional enthusiasm in readers that will translate into further advances that will contribute in the fight against this devastating disease.

We thank all of the authors for their patience and tireless effort as we completed this project. Everyone included did their part quickly and without complaint, despite all of our busy schedules, making the project very enjoyable and easier than expected. We look forward to working with everyone well into the future in our own efforts to make more rapid progress in the tuberculosis field.

Bryan, TX, USA Jeffrey D. Cirillo

Contents

Cell Wall Biosynthesis and Latency During Tuberculosis Infections

Michio Kurosu

Abstract *Mycobacterium tuberculosis* (Mtb) uses a wide range of mechanisms to survive the host immune system. Mtb can persist in host tissues for months to decades without replicating, however, non-replicating (or dormant) Mtb has the ability to resume growth at any time. Persons with latent TB infection do not have typical TB symptoms, and dormant forms of Mtb are not considered transmissible. Several factors (e.g. HIV infection, cancers, and immunosuppressive drug therapy) alter the course of latent TB and are thought to have the potential to cause reactivation, leading to active TB. Treatment regimens for latent TB infection require long durations in order to prevent relapse. It is difficult to eradicate latent forms of the relatively drug resistant Mtb in short periods of time with the currently available TB therapies. Thus, it is very important to develop new drugs for the treatment of latent or persistent forms of Mtb that reduce treatment time required for TB patients. Mycobacterial cell walls consist of complex mixtures of polysaccharides and mycolic acids, and they play an important role in escaping from the host immune systems and in surviving within granulomas that form in response to the bacteria. This chapter reviews the potential drug targets that exist in cell wall biosynthesis for non-replicating (or dormant) Mtb based on bioinformatics, genomic and proteomic analyses and *in vitro* data with replicating and non-replicating bacilli.

Keywords Cell wall biosynthesis · Non-replicating *Mycobacterium tuberculosis* · Dormant bacilli · Latent tuberculosis infections · Granuloma · Anti-TB drugs · Mycolic acid biosynthesis inhibitors · Bacterial phosphotransferase inhibitors · Arabinogalactan biosynthesis inhibitors · Peptidoglycan biosynthesis inhibitors

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Introduction

The non-replicating (or dormant state) of *Mycobacterium tuberculosis* (Mtb) is characterized by non-dividing or slowly replicating bacilli with a low metabolic state as well as resistance to standard anti-tuberculosis (anti-TB) agents [1]. The majority of Mtb infections are thought to occur in alveolar macrophages [2, 3]. Chemotactic cytokines produced by alveolar macrophages stimulate inflammatory cells (e.g. neutrophils, monocyte derived macrophages, NK cells, and T-cells) that further promote inflammation and tissue remodelling, establishing granulomas [4]. Granulomas are a cluster of immune cells including infected and uninfected macrophages, differentiated macrophages, epithelioid cells, and giant cells. Granuloma formation is a well-known pathological characteristic of TB [5]. In most healthy individuals, Mtb infection causes few problems in health and display no symptoms due to control of infection by the innate immune response. Mtb infected alveolar macrophages frequently escape from the early immune response [6]. In a small number of cases (~10% of infected people), the infection spreads via macrophages and dendritic cells to the draining lymph nodes and other organs, leading to clinically significant disease [7].

Mtb is a facultative intracellular bacterium. Entry of Mtb into alveolar macrophages is facilitated by several mechanisms, including specific receptor-mediated and signal transduction pathways. During the last decade, mycobacterial genetic analyses of *ex-vivo* and *in vivo* gene expression has allowed elucidation of a number of components that contribute to survival and replication within macrophages [8–12]. Because it is commonly believed that the dormant form of Mtb is non-replicating bacteria within granulomas, cell wall biosynthesis is often not considered a favourable drug target to treat dormant TB. However, recent studies have demonstrated that several cell wall biosynthesis enzymes are essential to maintain viability of dormant Mtb in granulomas and macrophages [13–16]. We summarize in this chapter many of the unique metabolic pathways associated with cell wall biosynthesis and the potential drug targets that are effective against non-replicating (or dormant) Mtb.

Mycobacterial Cell Wall

The complexity of mycobacterial cell wall is relatively unusual as compared to other Gram-positive and -negative bacteria (Fig. 1). The core structure of the mycobacterial cell wall is commonly termed mycoly-arabinogalactan-peptidoglycan complex. The biosynthesis of peptidoglycan (PG) of *E. coli* has been discussed extensively in reviews by van Heijenoort [17]. Most of the genes involved in peptidoglycan biosynthesis in *E. coli* are known, and orthologues have been identified in Gram-positive genomes. However, some of the genes responsible for the unique features found in mycobacterial peptidoglycan remain unknown. Detailed analyses

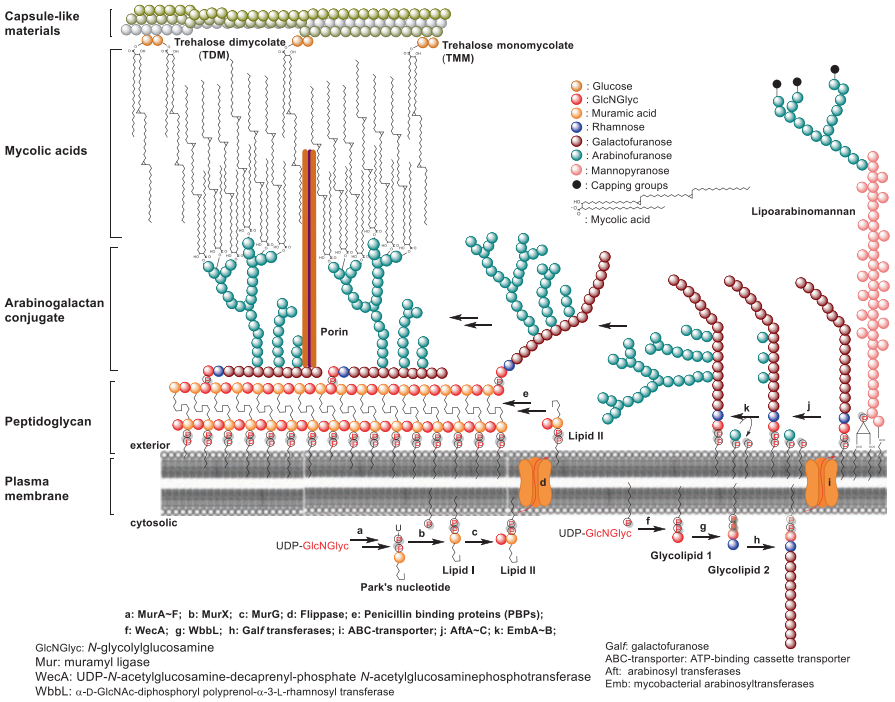

Fig. 1 *Mycobacterium tuberculosis* cell wall and cell wall biosynthesis pathways

of the components of mycobacterial PG revealed that it contains a variety of modified molecules including *N*-glycolyl (NGlyc) in addition to *N*-acetyl (NAc) groups on muramic acid (Mur), amidation of the carboxylic acids, and additional glycine or serine residues [18, 19]. Although the precise functions of these modified molecules have not yet been elucidated, it is speculated that the additional coordinated groups participate in electrostatic interactions contributing to reinforcement of the PG layer [20].

Lipid II, a precursor of PG, is synthesized in the cytosol initially and then in the inner portion of the plasma membrane from UDP-GlcNGlyc (and UDP-GlcNAc) by Mur enzymes (MurA~F, MurX, and MurG). Lipid II is required to flip-flop so that it can localize to the outer plasma membrane where penicillin-binding proteins (*e.g.* PBPa and PBPb) polymerize lipid II to form PG. Approximately 10–12% of the C6-hydroxy groups of muramic acid form phosphodiester bonds with the L-α-rhamnopyranosyl-(1→3)-D-α-GlcNGlyc-(1→P) unit (Fig. 2), which serves as linker to conjugate arabinogalactan (AG) [16].

AG biosynthesis commences with WecA (polyprenyl phosphate-GlcNGlyc-1-phosphate transferase)-catalysed synthesis of glycolipid-1 (D-GlcNGlyc-P-P-C_{50} and D-GlcNAc-P-P-C_{50}) with UDP-GlcNGlyc (and UDP-GlcNAc) and decaprenylphosphate (C_{50}-P) (Fig. 3). Glycolipid-1 is anchored into the intracellular plasma membrane where WbbL (a rhamnosyltransferase) transfers L-Rha*p* to the

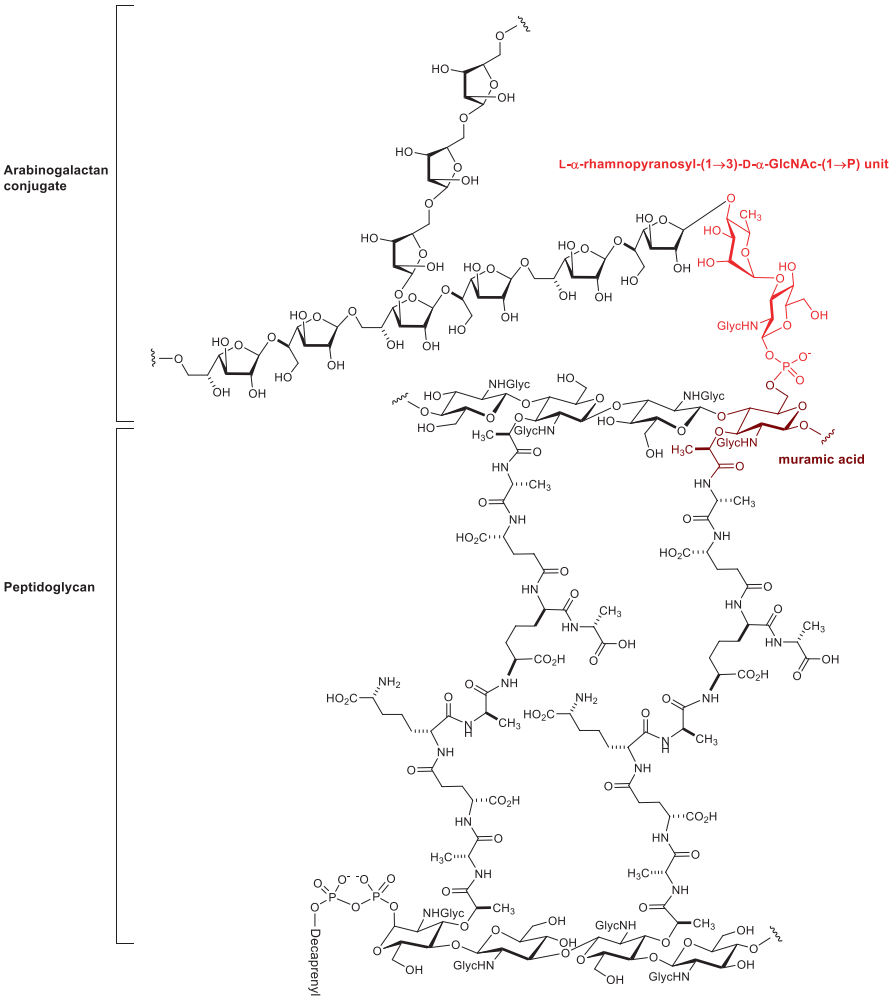

Fig. 2 Stereochemistries of the carbohydrate and peptide portions of mycobacterial cell wall, L-α-rhamnopyranosyl-(1→3)-D-α-GlcNAc-(1→P) unit is highlighted

GlcNGlyc with dTDP-rhamnose, furnishing glycolipid-2 (L-Rha*p*-(α1→3)-D-GlcNGlyc-P-P-C_{50}).

Two galactofuranosyltransferases (Gal*f*Ts) are responsible for completion of galactan biosynthesis. The first and second transfers of Gal*f* residues are catalysed by GalT1 (a GT-A superfamily) using UDP-Gal*f*, forming glycolipid-4 (D-Gal*f*-(α1→4)-D-Gal*f*-(α1→4)-L-Rha-(α1→3)-D-GlcNGlyc-P-P-C_{50}). Glycosylations of ~30 Gal*f* residues with alternating β(1→5) and β(1→6) linkages are catalysed by GalT2, which possesses dual enzymatic functions of UDP-Gal*f*: β-D-(1→5) GalT and UDP-Gal*f*: β-D-(1→6) GalT activities. These decaprenyl-diphosphate linked-polysaccharides, D-Gal*f*-(α1→5)-[D-Gal*f*-(α1→6)-D-Gal*f*-(α1→5)]$_n$-D-

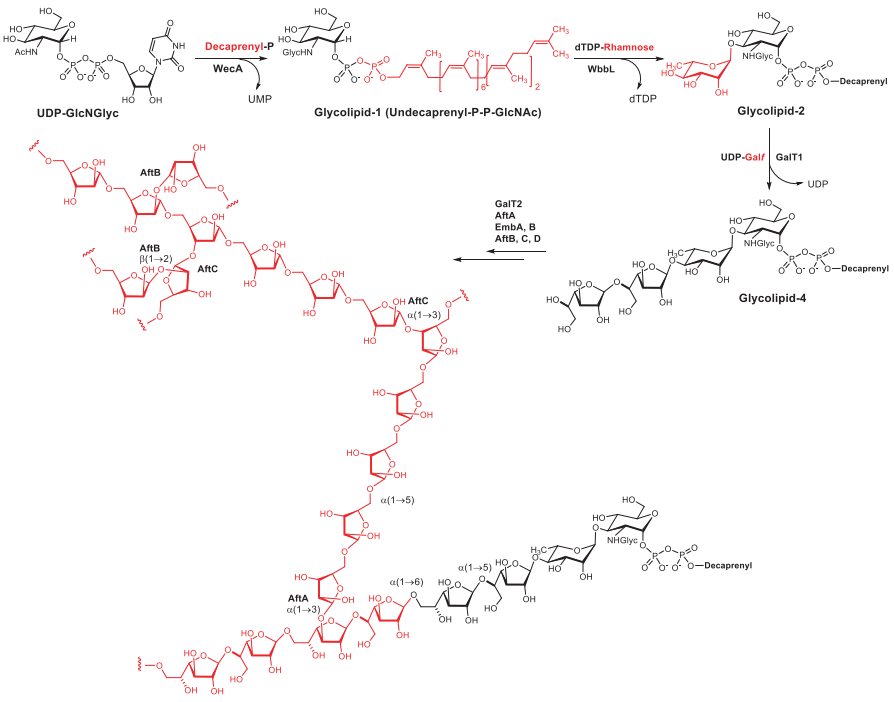

Fig. 3 Biosyntehsis of arabinogalactan

Gal*f*-(α1→4)-D-Gal*f*-(α1→4)-L-Rha-(α1→3)-D-GlcNAc-P-P-C_{50} are transported across the cytoplasmic membrane by the ABC (ATP-binding cassette)-transporter [21]. The translocated galactans are further modified with the arabinofuranoses by arabinosyltransferases with decaprenylphosphoryl-D-arabinofuranose, forming the AG conjugate. Several mycobacterial arabinosyltransferases such as EmbA, EmbB, AftA, AftB, AftC, and AftD have been characterized and implicated to be involved in arabinosylations of the galactan moiety. A number of distinct types of 22 arabinofuranose (Ara*f*) residues with alternating α(1→5), α(1→3), and β(1→2) linkages have been characterized in AG in *Mycobacterium spp*. [22].

Mycolic acids (MAs) are a hallmark of the cell wall of *Mycobacterium spp*. [23] They exist as esters of trehalose and/or glycerol and of the terminal penta-arabinofuranosyl moiety of AG. The former MA-esters are not linked with the cell wall components via covalent bond, but comprise the cell wall via electrostatic interaction. MAs play a critical role in localization of Mtb in the lung tissue and formation of granulomas. A large number of long-chained fatty acids have been characterized from different bacteria. The total carbon chain of C_{66}–C_{90} of MAs have been determined in fatty acid analyses of Mtb. MAs display a large diversity of chemical structures (Fig. 4). The general structures of MAs are referred to as α-branched β-hydroxy long-chain fatty acids. The α-branched moiety is composed of the C_{22}–C_{26} fatty acids. The main carbon chain where the long-alkyl group is

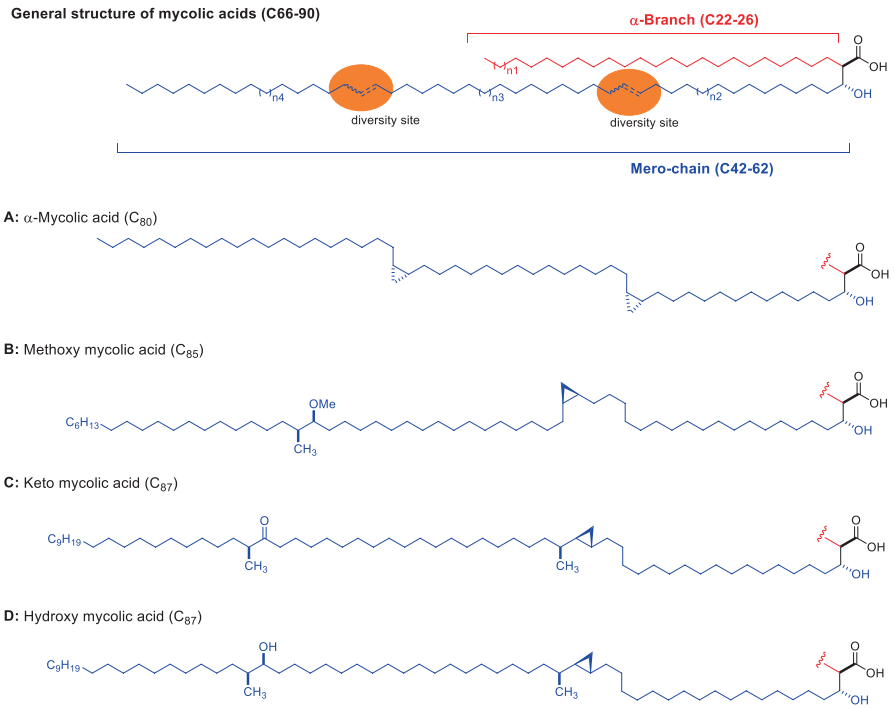

Fig. 4 Structure of mycolic acids

attached at the C3 (or β)-position is designated mero-mycolic chain composed of ω-42 to -62 fatty acids. The degree of unsaturation (*cis* or *trans* geometry or cyclopropyl) of the mero-mycolic chain is generally one or two. In some Mtb strains, a longer mero-mycolic chain and higher degree of unsaturation have been identified. Additional functional modifications observed are the carbonyl (ketone), hydroxy, methoxy, epoxy, methyl, and ester groups in the mero-mycolic chain [24]. The biosynthesis of MAs is achieved by fatty acid synthases FAS-I, FAS-II, Fab, and several modification enzymes. Unlike the enzymes in FAS-I, the enzymes of FAS-II and FabH and are not found in humans. Fatty acid biosynthesis has been reviewed in a number of scientific articles and books (Fig. 5) [23, 25]. In Mtb, β-ketoacyl-(acyl-carrier-protein) synthase III (designated MtFabH) compensates the gap in the mycobacterial fatty acid biosynthesis via FAS-I and FAS-II pathways. FAS-I produces C_{16}–C_{18} and C_{24}–C_{26} acyl CoAs. FAS-II elongates C_{12}–C_{16} fatty acids to furnish C_{18}–C_{30} acyl carrier proteins (ACPs) for the synthesis of meromycolic acid. MtFabH carries out the initial coupling (Claisen-type) reaction of fatty acid biosynthesis with malonyl-ACP and acetyl-CoA, producing short-chain fatty acid primers [24]. The generated β-ketoacyl-ACPs are reduced by NADPH-dependent β-ketoacyl-ACP reductase (MabA). The resulting β-hydroxy ACPs are dehydrated by dehydrases (HadAB, HadBC, or HadABC complexes) and saturations of the double bond(s) are accomplished by NADH-dependent trans-2-enoyl-ACP reductase

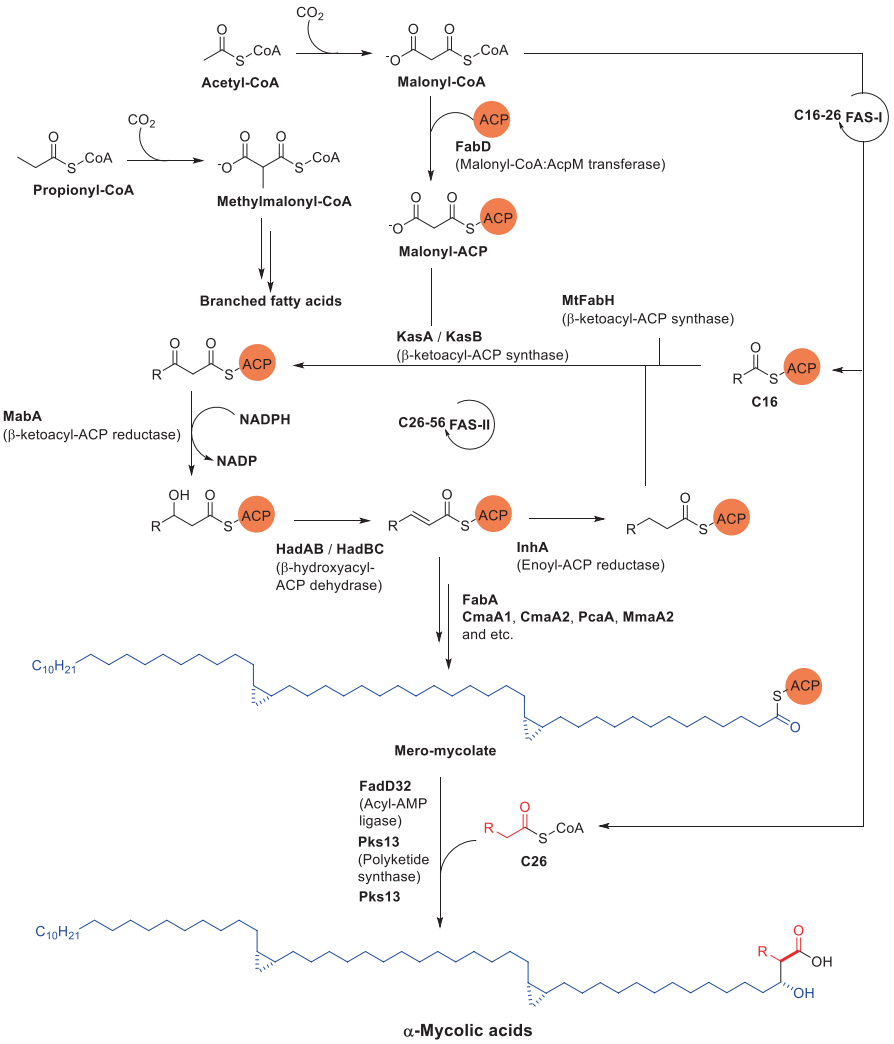

Fig. 5 Mycolic acid biosynthesis

(InhA), leading to saturated acyl-ACPs [26]. The other carbon-chain elongations are performed by β-ketoacyl-ACP synthases (KasA and KasB). KasA elongates C16-CoA to monounsaturated fatty acids with the average length of ~C40. In the presence of KasA, KasB produces a longer chain with multiunsaturated hydrocarbons with the average length of ~C54. These β-ketoacyl-ACP synthases are essential for the synthesis of mero-mycolic acids. Biochemical analyses of the Mtb ΔkasB mutant strain revealed that it synthesizes mycolic acids with shorter carbon-chain lengths. In addition, the loss of acid-fastness and ketomycolic acids and a reduction of trans-cyclopropanated mycolic acids and methoxymycolic acids were observed.