CellPress

Review

New Insights into the Mycolate-Containing Compound Biosynthesis and Transport in Mycobacteria

Annaïk Quémard[1,*]

Mycolic acids are extremely-long-chain fatty acids that compose a large family of mycolate-containing compounds, major envelope lipid components and critical pathogenicity factors of *Mycobacterium tuberculosis*. In recent years there have been major advances in understanding their metabolic pathway. Unknown enzymes of the fatty acid synthase type II elongation system and the condensation system that builds the mycolic acid scaffold were identified. Missing links with the mycolate-containing compound biosynthesis–such as the mechanisms of transfer onto trehalose and of translocation through the inner membrane–were deciphered, while recycling processes have emerged. Beyond the more accurate picture of the biosynthesis and translocation pathways dedicated to these unique molecules, major issues that should be addressed in the future are also discussed.

A High Diversity of Mycolate-Containing Compounds Dedicated to an Array of Biological Functions

The story of the mycolic acids (MAs) started 80 years ago when they were isolated as the major lipid components of the tubercle bacillus envelope. These α-alkylated β-hydroxylated molecules–which typify the Corynebacteriales order, including the causative agents of many diseases such as tuberculosis (TB; *Mycobacterium tuberculosis* or *Mtb*), leprosy (*Mycobacterium leprae*) and diphtheria (*Corynebacterium diphtheriae*)–are the longest fatty acids ever found in nature. Mycobacteria produce specimens among the longest ones (C_{60}–C_{90}) (Figure 1), but even longer molecules, called 'ultra/extra long-chain mycolic acids' (XL-MAs), were recently discovered in the *Segniliparus* genus and certain mycobacterial species [1–4]. Various types of chemical function (cyclopropane ring, double bond, methyl branch and oxygenated functions) on the main 'meromycolic' chain define different MA subclasses (Figure 1). The anchoring of the MAs in the outer membrane or mycomembrane, where they are believed to adopt singular foldings such as the 'W-shape' conformation (Figure 1) [5–8], gives them a strategic position within the envelope, at the interface between the cell wall and the protective outer layer [9]. The MAs compose a broad array of mycolate-containing compounds, where they are linked either to the arabinogalactan forming the cell wall mycolyl-arabinogalactan-peptidoglycan (mAGP) complex or to diverse polyol molecules such as trehalose (Figure 1). Large amounts of free MAs are also present in the extracellular matrix of mycobacteria grown as biofilms, and are required for this type of growth [10,11]. MAs are essential to the survival of mycobacteria and crucial for their physiology and fitness [12–22]. Furthermore, they play a role in the virulence and the persistence of *Mtb* within infected organisms, strongly potentiating the immune response to infection [4,13,18,20–23]. The functionality of the free MAs and mycolate-containing lipids is critically

Trends

HadAB and HadBC enzymes of the mycobacterial FAS-II system are a novel type of bacterial monofunctional 3-hydroxyacyl-ACP dehydratases.

The mycolic condensation system is at least composed of FadD32, Pks13, CmrA, and AccA3-AccD4, all with unique properties dedicated to the very long chain molecules that form the mycolic scaffold.

The transfer of unreduced mycoloyl chains onto trehalose, a pivotal step of the mycolate-containing compound biosynthesis, is catalyzed by the Pks13 thioesterase-like domain following a new lipid transfer mechanism.

The RND superfamily transporter MmpL3 exports the mycoloyl chains carried by trehalose to the envelope for the mycomembrane biogenesis, and the extracellular matrix formation during biofilm growth. Other (mero) mycolic acid derivatives are likely exported by MmpL11.

Extracellular free mycolic acids and trehalose enzymatically released from mycolate-containing compounds are likely recycled via ABC transporters.

[1]Département Tuberculose & Biologie des Infections, Institut de Pharmacologie et de Biologie Structurale, UMR5089, Université de Toulouse, CNRS, UPS, France

*Correspondence:
annaik.quemard@ipbs.fr (A. Quémard).

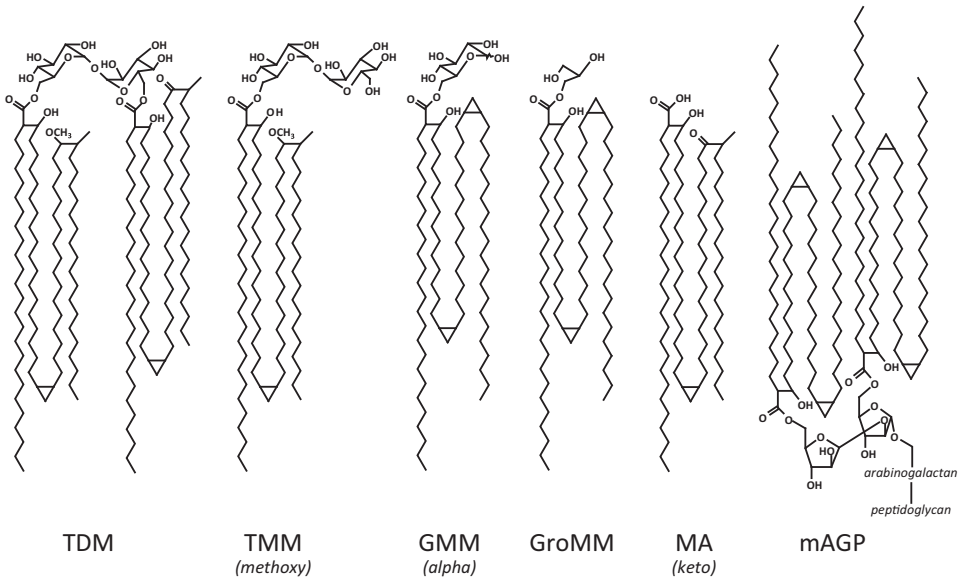

Figure 1. Structures of Mycolic Acids and Mycolate-Containing Compounds. The represented mycolates correspond to major types (mentioned in brackets) of *Mycobacterium tuberculosis*; they are drawn in fully folded 'W-shapes'; α- and methoxymycolates can also adopt a range of more extended conformations [7,8]. Abbreviations: TDM, trehalose dimycolate; TMM, trehalose monomycolate; GMM, glucose monomycolate; GroMM, glycerol monomycolate; mAGP, mycolyl-arabinogalactan-peptidoglycan complex. Only a portion of the mycolylated arabinogalactan ends is displayed.

influenced by the length of the mycoloyl chains, the nature and stereochemistry of the two functional groups in the meromycolic chain, and the nature of the hydrophilic head [13,24].

MAs are the products of a mixed fatty acid synthase (FAS)/polyketide synthase (PKS) biosynthesis pathway (Figure 2). They result from the condensation between a very long meromycolic chain and a shorter carboxylated fatty acyl chain that generates the α-chain; this step is catalyzed by the so-called 'mycolic condensation system'. Mycobacteria have the unique property of possessing two FAS systems [25]. The eukaryote-type FAS-I, made of a large multidomain polypeptide, displays a bimodal product profile corresponding to the precursors of the meromycolic chains, that is, C_{16}–C_{18} acyl-CoAs, and to the α-chain, that is, C_{22}–C_{26} acyl-CoAs (Figure 2). The FAS type II (FAS-II) system takes over to synthesize the meromycolic chains via iterative elongation cycles. Thereafter, I will focus on the recent advances made in the field of the mycolate-containing compound biosynthesis and transport in mycobacteria.

A New Type of Dehydratases in the Mycobacterial FAS-II System

The FAS-II systems are made of discrete monofunctional proteins [25]. In contrast to the standard FAS-II systems that perform *de novo* biosynthesis, the mycobacterial FAS-II has the unique property of elongating standard-sized fatty-acid precursors (C_{16}–C_{18}) into unusually long-chain fatty acids (C_{36}–C_{72}), the meroMAs (Figure 2). The iterative elongation cycles include four main catalytic steps dependent on a mycobacterial acyl carrier protein (ACP), AcpM. Three of these steps are catalyzed by enzymes homologous to bacterial FAS-II components: the β-ketoacyl-ACP synthetases KasA and KasB, the β-ketoacyl-ACP reductase MabA, and the *trans*-2-enoyl-ACP reductase InhA [21]. In contrast, no (3*R*)-hydroxyacyl-ACP dehydratase (HAD) could be identified in the mycobacterial genomes by simple sequence homology with the FabZ (dehydratase) or FabA (dehydratase–isomerase) enzymes that classically bear this function in bacterial FAS-II systems [26]. A thorough bioinformatic analysis of the