Versatile polyketide enzymatic machinery for the biosynthesis of complex mycobacterial lipids

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The cell envelope of *Mycobacterium tuberculosis* (Mtb) is a treasure house of a variety of biologically active molecules with fascinating architectures. The decoding of the genetic blueprint of Mtb in recent years has provided the impetus for dissecting the metabolic pathways involved in the biosynthesis of lipidic metabolites. The focus of this *Highlight* is to emphasize the functional role of polyketide synthase (PKS) proteins in the biosynthesis of complex mycobacterial lipids. The catalytic as well as mechanistic versatility of PKSs in generating metabolic diversity and the significance of recently discovered fatty acyl-AMP ligases in establishing "biochemical crosstalk" between fatty acid synthases (FASs) and PKSs is described. The phenotypic heterogeneity and remodeling of the mycobacterial cell wall in its aetiopathogenesis is discussed.

1 Introduction

Microorganisms produce a wide variety of biologically active metabolites with tremendous chemical diversity. Unexpectedly, genome sequencing efforts have revealed a far lower number of genes dedicated to secondary metabolism than would be anticipated based on the abundance of natural product structures. The cell envelope which constitutes 40% of the dry weight of the bacteria contains many unusual compounds.[1–3] While structures of many of these molecules have been known for years, the molecular mechanisms underlying their biosynthesis have been unclear. Genome sequencing projects have revolutionized efforts to reconstruct metabolic pathways by providing an inventory of all the genes. One of the striking features of the Mtb genome is the presence of a number of genes homologous to polyketide synthases (PKSs).[4] PKSs are structurally and mechanistically related to fatty acid synthases (FASs).[5,6] Whereas FASs catalyze the biosynthesis of fatty acids which are primary metabolites and components of cellular membranes in all living systems, PKSs primarily catalyze the formation of polyketide natural products, which are secondary metabolites and have been a rich source of commercially important antibiotics and other therapeutics.[7,8] Traditionally, the biosynthetic machineries for fatty acids and polyketides have been studied independently, but recent evidence suggests that these enzyme systems may regularly interact in many organisms to generate hybrid molecules of fatty acids and polyketides.[9–12]

Polyketide synthases function by carrying out Claisen-like condensations of small to long chain carboxylic acid moieties with acetate or branched chain acetate units commonly derived from malonyl-CoA or methylmalonyl-CoA.[13–15] PKSs are classified as Type I, II and III according to their architecture. Type I PKSs can be subdivided into iterative, which use the same set of catalytic sites in a repetitive manner[16,17] and modular, where a specific set of catalytic sites exist for each round of chain elongation.[18] Type II PKSs constitute the next class, where each catalytic site is present on a separate polypeptide and various polypeptides work by forming non-covalent complexes.[19–21] Type III or chalcone synthase (CHS)-like PKS enzymes maintain a simple architecture consisting of a homodimer that performs consecutive elongation reactions at two independent active sites.[22–26]

In this *Highlight*, we focus on clever ways in which mycobacteria utilize polyketide enzymatic machinery to produce diverse lipidic metabolites.

2 Esoteric cell wall lipids from *Mycobacterium tuberculosis*

For years attempts have been made to correlate the virulence of mycobacteria with the morphological features of the bacilli.[27] The study of mycobacterial surface lipids was initiated about 80 years ago, and resulted in the identification of most of the significant substances.[28] The development of reproducible culturing conditions for mycobacteria boosted the systematic characterization of mycobacterial cell wall lipids.[29] Phenomenal development of mass spectrometry in recent years now makes it possible to characterize even low abundant lipidic constituents from mycobacteria.[30] The Mtb cell envelope consists of a chemically dense network of sugars and lipids (Fig. 1).[1,3,31,32] The base of the mycobacterial cell wall is the plasma membrane which anchors the principal lipopolysaccharides, lipomannan (LM) and lipoarabinomannan (LAM)[33] through phosphatidyl inositol mannosides (PIMs).[34] The plasma membrane also interacts with a peptidoglycan (PG) layer which is connected to the arabinogalactan through phosphodiester

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Born and brought up in New Delhi, India, Priti Saxena completed her Bachelors degree in Life Sciences and her Masters degree in Microbiology from Barkatullah University, Bhopal, India. During her MSc she joined Dr Rajesh S. Gokhale at the National Institute of Immunology (NII), New Delhi and worked on isolation and biochemical characterization of mycobacterial polyketide synthases. She gained further experience in molecular methods coupled with proteomics, mass spectrometry and metabolite identification. Presently, pursuing a PhD in chemical biology under Dr Gokhale, Priti is engaged in dissecting the mechanistic and structural aspects of type III polyketide synthases and investigating the molecular basis of the novel modes adopted by these proteins for generating chemical and functional diversity.

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linkages by using a linker unit consisting of L-rhamanose-D-*N*-acetylglucosamine.[35] This assembly forms the core of the mycobacterial cell wall, on which various lipids are tethered. Mycolic acids (**V**), one of the major constituents of mycobacterial lipids, are esterified to the arabinose sugar of arabinogalactan to form mycolyl arabinogalactan (MAG).[36,37] These acids are also acylated to trehalose units to form trehalose monomycolate (TMM) and trehalose dimycolate (TDM).[38,39] Other lipids such as sulfolipids (SL, **I**), polyacyl trehaloses (PAT, **II**), phthiocerol dimycocerosate (DIM, **III**), mannosyl-β-1-phosphomycoketides (MPMs, **IV**) and diacyl trehalose (DAT) interact extensively with MAG and are sometimes shed out of the cell wall.[30,40]

Mtb is evidently a store house of esoteric metabolites. In an ongoing quest to delineate the biology of mycobacteria, the last decade has revealed a number of classes of enzymes, other than FAS, involved in lipid metabolism.[4] The biochemical functions of some of the multiple paralogs of enzymes, such as PKSs, FadDs (fatty acid degradation), FadEs, FadBs, FadAs, AccDs (Acyl-CoA carboxylase) and AccAs are being elucidated.[4] While the significance of this apparent redundancy is not clear, several gene inactivation studies and high throughput experimental procedures have highlighted their importance in the biology of mycobacteria.[12,41–43]

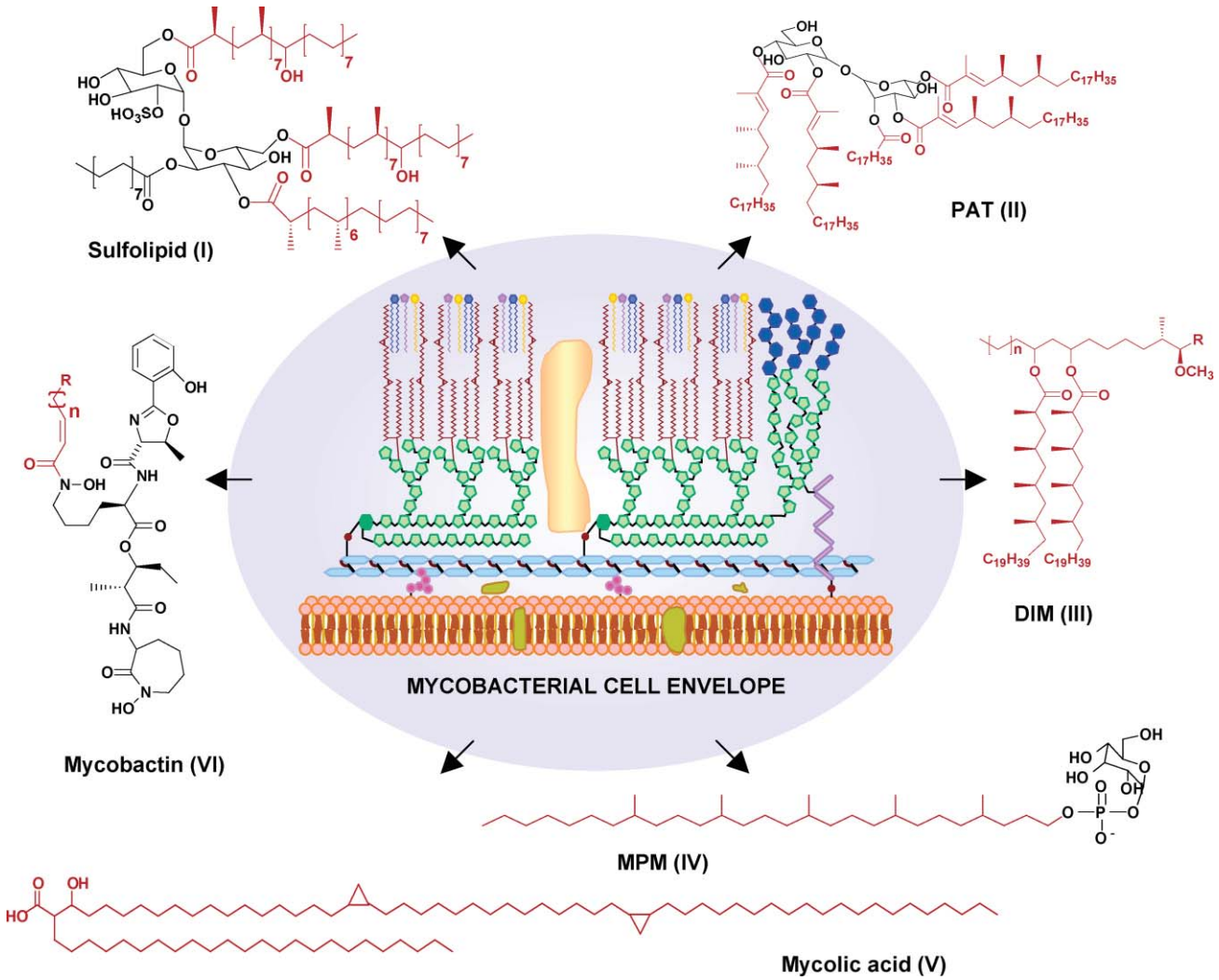

Fig. 1 Schematic representation of the mycobacterial cell envelope depicting some of the essential polyketide derived lipids.

3 Polyketide synthases facilitate biosynthesis of complex mycobacterial lipids

The polyketide biosynthetic machinery paradigm has been primarily restricted to the biosynthesis of natural products that are not essential for the survival of the organism. The first report of the presence of polyketide synthases in mycobacteria appeared from Kolattukudy and coworkers[44] in which they suggested that erythromycin-like modular PKSs are involved in the biosynthesis of phenolic glycolipids (PGLs). The genome sequence of Mtb revealed an even greater number of PKS-like genes.[4] This finding was rather remarkable as Mtb is not known to produce any secondary metabolites. *M. ulcerans*, an extracellular pathogen known to cause Buruli ulcers produces mycolactone, which is a polyketide derived metabolite.[45] In the last decade, the development of new techniques for genetic inactivation in mycobacteria[46–49] as well as the ability to express large multifunctional proteins in heterologous hosts,[50,51] in conjunction with rapid advances in mass spectrometry,[52,53] have helped to describe the functional significance of many PKSs in the biology of mycobacteria. In the following sections we discuss the role of mycobacterial PKSs.

3.1 Modular assembly-line PKS enzymology in the biosynthesis of dimycocerosate esters

Dimycocerosate esters (DIMs, **III**) are a family of compounds that show heterogeneity in their chemical structures.[54,55] These compounds promote Mtb virulence and are found primarily in pathogenic mycobacteria.[56–58] Essentially the molecules consist of di-, tri- or tetramethyl-branched fatty acyl chains called mycocerosic acids that are esterified to the diol component of phthiocerol or *p*-glycosyl phenolphthiocerol moieties. Phenolic glycolipid variants are proposed to be the causative agents of leprosy and were also recently characterized from hypervirulent strains of Mtb.[58,59] Based on a retrobiosynthetic analysis, the strain should contain machinery to produce both multi-methylated long-chain fatty acids and 3-methoxy-4-methyl-9,11-dihydroxy long-chain glycols. Kolattukudy's group first demonstrated biosynthesis of methylated fatty acids by a multifunctional protein, mycocerosic acid synthase (Mas).[60] Mycocerosic acids are produced by an iterative mechanism analogous to vertebrate fatty acid synthesis, except that the acyl transferase (AT) domain from Mas shows specificity for methylmalonyl-CoA.

A putative cluster of genes that could biosynthesize phthiocerol was also predicted based on its homology to the erythromycin modular polyketide synthases.[12,44,61] Recent cell-free reconstitution studies of these PKSs demonstrated the step-wise two-carbon chemistry that dictates the site-specific incorporation of diols.[51] The first step in the biosynthesis of phthiocerol involves the transfer of a long-chain fatty acid on protein PpsA by a homologue of a newly discovered family of fatty acyl-AMP ligases (FAALs), FadD26[62] (Fig. 2a). Once this starter unit is loaded at the active cysteine residue of the ketosynthase (KS) domain and the acyl transferase domain transfers a malonyl group onto the phosphopantetheine arm of the acyl carrier protein (ACP) domain, a decarboxylative condensation occurs that extends the long-chain fatty acid by a two-carbon unit. The presence of a ketoreductase (KR) domain in the PpsA protein reduces the β-keto group to a hydroxyl group. This chain is then covalently transferred from the ACP of PpsA to the active site KS domain of PpsB through an acyl transfer reaction. The PpsB protein again utilizes another molecule of malonyl-CoA to add a two-carbon unit and generates another hydroxyl group. Similar rounds of chain transfer and extension continue with PpsC and PpsD proteins, which convert the β-keto groups into completely saturated carbons due to the presence of additional dehydratase (DH) and enoyl reductase (ER) domains. The AT domain of the PpsE protein has broad substrate specificity and can therefore utilize both malonyl- as well as methylmalonyl-CoA extender units, which produces diversity in DIMs (**III**). Biosynthesis of phthiocerol thus requires at least 24 catalytic activities present on five large multifunctional modular type I PKS proteins.[51] The specificity of hand-to-hand transfer of this chain from one Pps protein to another is dictated by the linkers (also referred to as docking domains) present on the C- and N-terminus of these proteins respectively.[50,63–66]

The assembly and transport of DIMs require several other accessory proteins that are briefly described here. Further details can be found in two recent excellent reviews.[55,67] Transesterification of mycocerosic acids with the diol component of phthiocerol is brought about by PapA5, a gene located mid way between PpsA–E and Mas genes in the Pps cluster.[51] The crystal structure of PapA5 reveals structural homology with the condensation domain of non-ribosomal peptide synthetases (NRPSs) and the domain interacts with the ACP domain of Mas to directly transfer covalently acylated chains onto the phthiocerol moiety.[68] The methoxy group at the 3-position of phthiocerol derives from reduction of a keto group by a stand-alone ketoreductase protein,[69] which is followed by transfer of the methyl group by an *O*-methyl transferase protein Mtf2.[70] The mechanism of release of the phthiocerol chain from PpsE is presently unknown. The TesA protein from the Pps cluster is proposed to specifically interact with the PpsE protein to release the covalently acylated phthiocerol chain.[71] However, our unpublished results suggest that TesA

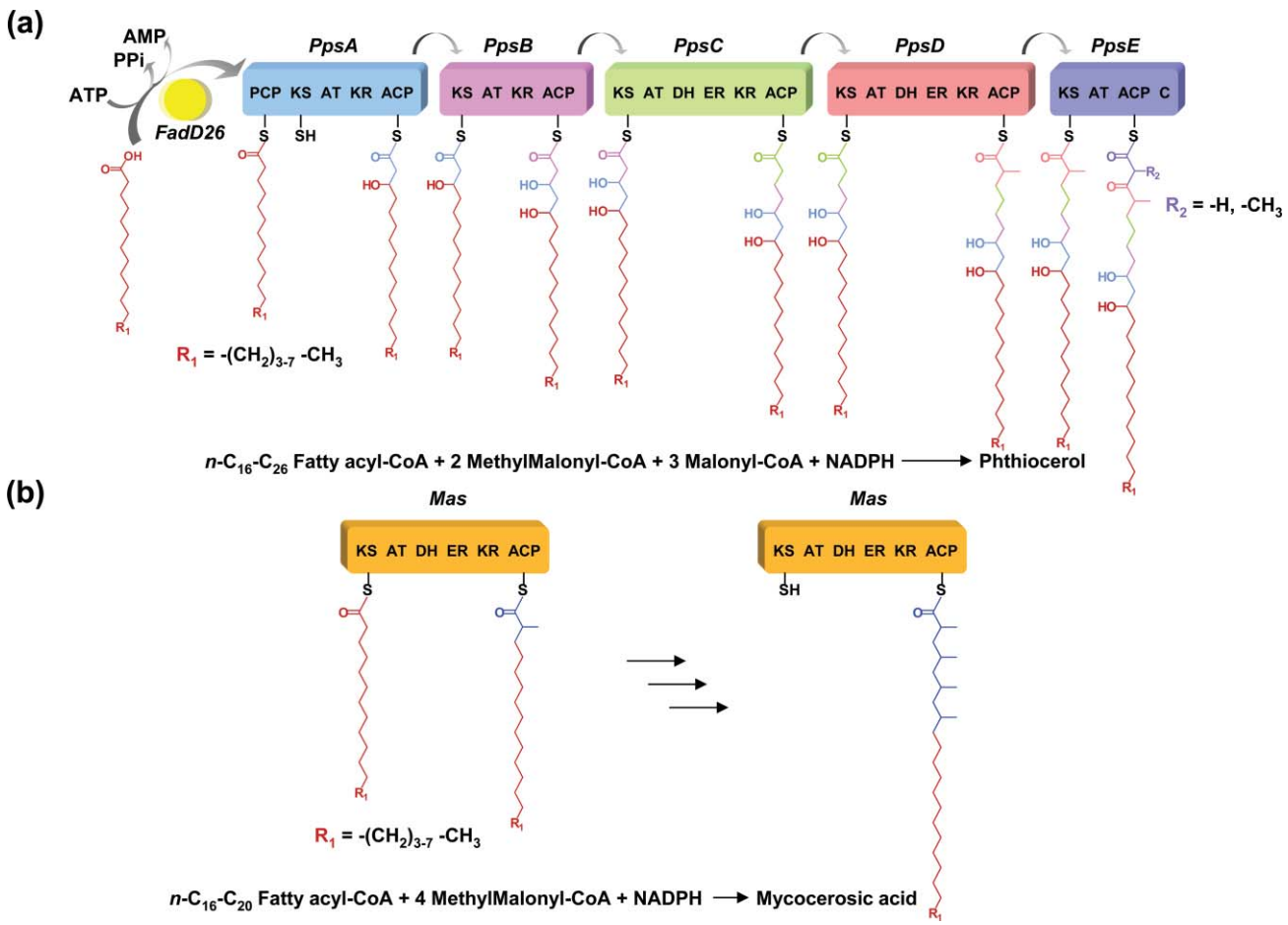

Fig. 2 DIM biosynthetic machinery. (a) Modular organization of Pps proteins showing stepwise chain elongation and functional group modification of the growing polyketide. FadD26 provides precursors for phthiocerol synthesis. (b) Mycocerosic acids are synthesized by Mas, a type I iterative PKS.

can in fact release acylated chains from several PKSs and thus may function as an editing enzyme. The transmembrane protein MmpL7 is proposed to couple the synthesis of DIM with its export outside the cell envelope, by specifically interacting with PpsE.[72] Recently, it was found that the LppX protein was required for the translocation of DIM across the plasma membrane. The three-dimensional structure of this protein has revealed a fold similar to the periplasmic chaperone of Gram-negative bacteria, with a large cavity suitable to accommodate a single DIM molecule.[73]

3.2 Novel condensase activity of PKS13 in the biosynthesis of mycolic acids

Since the early fifties, mycolic acids (**V**) have been defined as high molecular weight fatty acids with a hydroxyl group at the β-positions and a long alkyl chain at the α-positions.[32] It is now clear that they exist as a homologous series of fatty acids differing by 28 atomic mass units (a two-carbon unit). Mtb mycolic acids are very hydrophobic, consisting of C_{54} to C_{63} fatty acids with C_{22} to C_{24} α-side chains. The long fatty acid contains cyclopropyl groups that apparently play an important role in mycobacterial virulence.[74–76] There are at least three different forms of structural variants, which have been described in recent outstanding reviews.[77,78]

The biosynthesis of mycolic acids involves various multienzyme complexes. It has been clear since the 1970s that two types of FAS system coordinate to produce long-chain fatty acids. The initial C_{18}/C_{20} chains are produced by the type I FAS system and are further extended by the dissociated type II FAS to produce a very-long chain component of mycolic acids, also referred to as meroacid.[79] The mechanism of incorporation of unsaturation in meroacid is not clear. However, oxidoreductases and methyltransferases involved in producing methoxy-, keto- and cyclopropanone-forms of meroacid have been characterized recently.[80–83] The α-alkyl chain of mycolic acid is assumed to be synthesized by a type I FAS, which is then converted to its coenzyme A thioester. The antimycobacterial drug isoniazid targets this essential mycolic acid biosynthetic pathway.[84,85] This pro-drug probably interferes with multiple components of the type II FAS enzymatic machinery with 2-*trans*-enoyl-ACP reductase (InhA) being one of the most important targets.[86–88]

For many years, the mechanism to couple these two fatty acid components to make mycolic acids was not known. Recently, PKS13 was identified as being involved in the final assembly of mycolic acid in *M. tuberculosis*.[89] PKS13 is a minimal module consisting of KS, AT ACP and TE domains (Fig. 3). At its N-terminus, PKS13 contains another ACP_N domain that accepts acyl-adenylate starter substrates from the FadD32 protein.[62] Based on the final structure of mycolic acid, it can be speculated that the fatty acyl-AMP ligase (FadD32) activates meroacid and transfers it on to PKS13 to make mero-S-ACP_N. The α-alkyl chain, C_{26}-CoA, is carboxylated by acyl-CoA carboxylases (AccD4 and AccD5 along with α-subunit AccA3),[89–91] and is then transferred to the phosphopantetheine thiol group of the ACP domain of PKS13 by the AT domain. This unusual AT domain specificity, however, needs to be investigated. The transfer is followed by a decarboxylative Claisen-type condensation. The formation of mature α-mycolate would

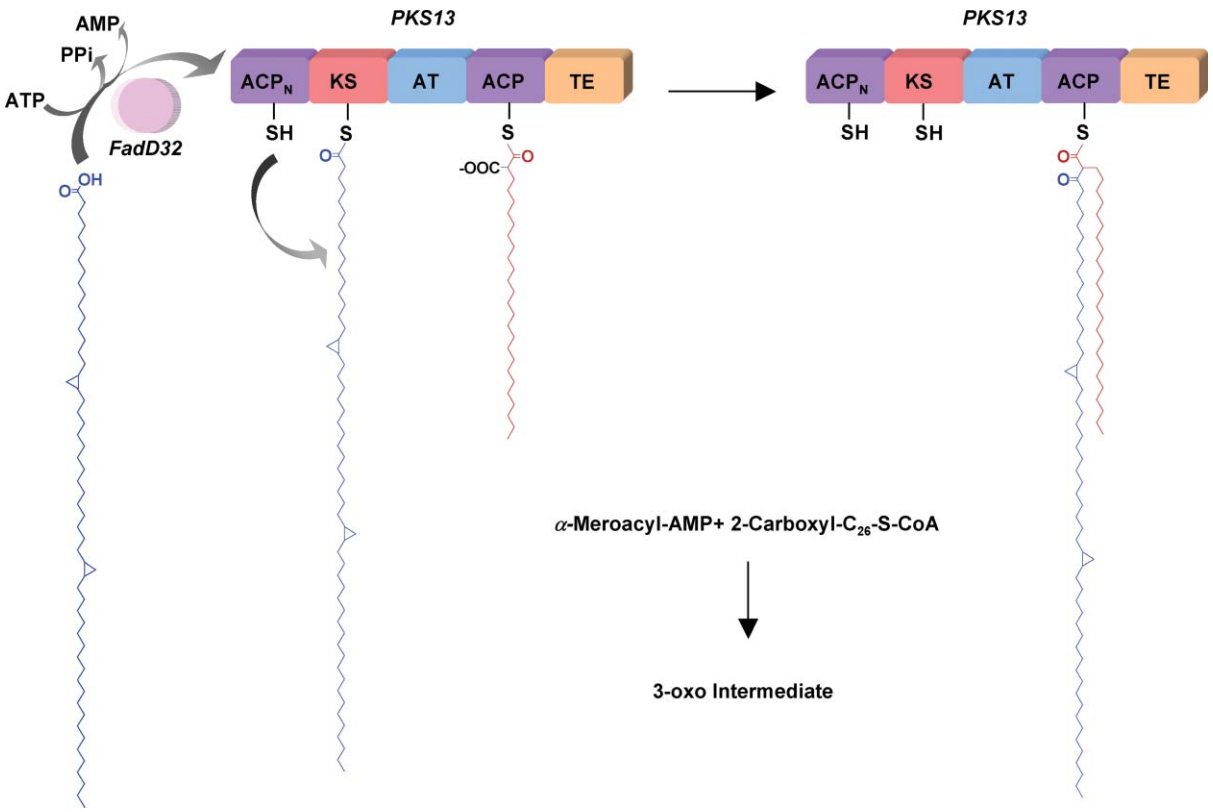

Fig. 3 PKS13 condenses two FAS derived long chain precursors in the final steps of mycolic acid biosynthesis. FadD32 loads activated substrate onto the N-terminal ACP (ACP_N) of this minimal PKS module.

require conversion of the 3-oxo group to a secondary alcohol, however, the enzyme involved in this function has not been characterized. It is believed that the function of TE domain from PKS13 may not only be restricted to release of mycolic acids, but may also play a role in the transfer of mycolic acids to mannopyranosyl-1-phosphoheptaprenol. While the biological significance of mycolyl mannopyranosyl-1-phosphoheptaprenol is not clear, it may function as an intermediate carrier. TE domains that possess hydrolytic as well as transferase activity have been characterized from multifunctional NRPSs.[92–94] Interestingly, all of these enzymes involved in mycolic acid biosynthesis have been proposed to form a large supramolecular assembly; however many of the purified proteins *in vitro* do not show strong protein–protein affinities.[95,96]

3.3 Iterative PKS2 required for sulfolipid biosynthesis

Sulfolipids (SLs) (**I**) constitute one of the important classes of sulfated metabolites thought to be present exclusively in human pathogen Mtb.[97] The sulfolipid family (SL I, II and III) of acyltrehaloses was first detected while searching for the origin of adsorption of cationic neutral–red on Mtb.[98] SL–I, the most studied sulfolipid, has a sulfated trehalose core that is tetra-acylated with fatty acids. While one of the positions contains *n*-fatty acids (palmitate and stearate primarily), the other three sites are occupied by long-chain methylated fatty acids called phthioceronic acids and hydroxyphthioceronic acids. The positions of the acylation sites on the trehalose moiety are interchanged in a number of isolated variants of SL–I.[99,100] Gene inactivation of PKS2 revealed its role in the formation of phthioceronic acids and hydroxyphthioceronic acids.[101] PKS2 has a complete set of catalytic domains required for the formation of phthioceronic acids (Fig. 4). The hydroxyl moiety on the carbon α- to the first methyl branch in the case of hydroxyphthioceronic acids could in principle be produced after the biosynthesis of the complete chain by an independent P450 hydroxylase, or it could be synthesized by PKS2 by skipping the DH and ER domains during the first cycle of condensation. Such remarkable selectivity in utilization of ancillary reductive and methyl transferase domains has been observed for fungal PKSs during iterative biosynthesis.[102] The AT domain of PKS2 shows specificity for methylmalonyl-CoA and the starter unit could be loaded through another FAAL homologue FadD23 (Chopra and Gokhale; unpublished results). The acyl transferases PapA1 and PapA2 have been shown to be essential in the production of sulfolipid.

The primary step in the biosynthesis of SL-1 has been shown to be the sulfation of trehalose by a mycobacterial sulfotransferase (stf0) to form trehalose-2-sulfate (T2S).[103] Whether the transfer of acyl units on T2S proceeds inside the cytoplasm or on the cell membrane is still unclear. Two independent studies involving disruption of MmpL8 resulted in the isolation of an immature form of SL–I. Both SL_{1278}[104] and SL–N[105] are diacylated intermediates. It is possible that SLs, unlike DIMs are translocated at an intermediate stage in their biosynthesis and the complete esterification of trehalose may be an extracytoplasmic event. The other possibility is that these proteins form a large supramolecular complex and only mature SL is transported efficiently after completion of the synthesis.

3.4 Biosynthesis of mannosyl-β-1-phosphomycoketides requires the unusual multifunctional PKS12 protein

Recently, novel phospholipid MPMs (**IV**) were isolated from Mtb and other pathogenic mycobacteria.[30] MPMs are found in low abundance in the cell envelope and were detected as antigens that could be presented by host CD1d cells. MPMs consist of a mannosyl-β-1-phosphate moiety identical to that found in mannosyl-β-1-phosphodolichols from mammalian cells. The alkyl chain in MPMs is a 4, 8, 12, 16, 20-pentamethylheptacosyl moiety,

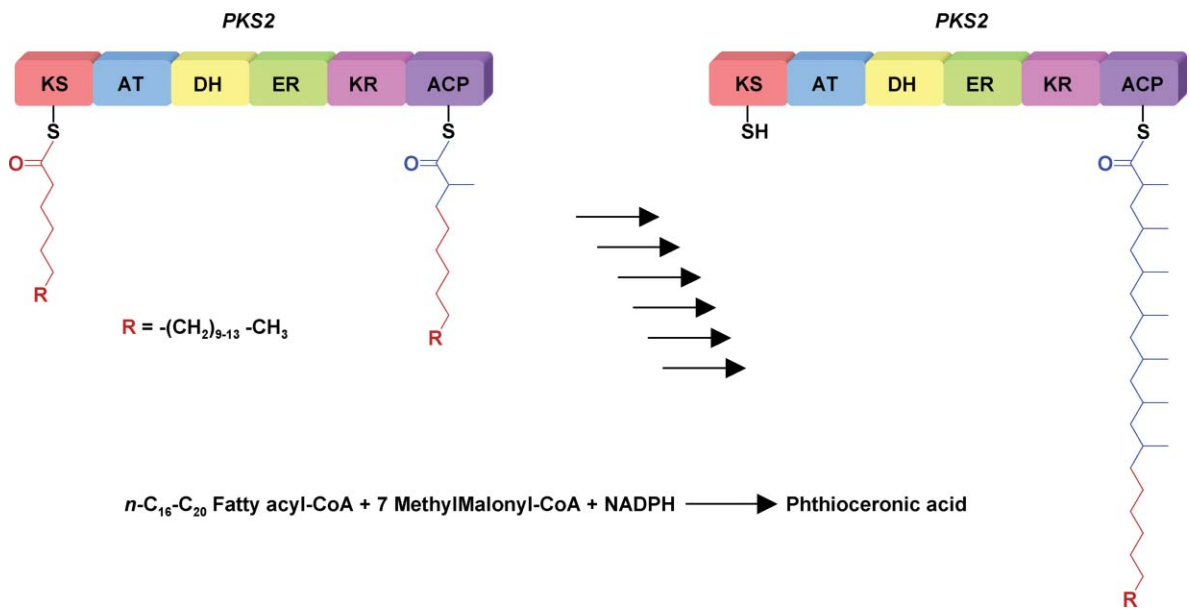

Fig. 4 The Type I iterative machinery of PKS2 protein is required for the synthesis of phthioceronic acids and hydroxyphthioceronic acids, the major components of mycobacterial sulfolipids.

which is believed to be the antigenic determinant of MPMs, since MPD with the same glycosylation pattern is non antigenic. Based on genetic studies, PKS12 is proposed to biosynthesize the lipid chain of MPMs, which then undergoes offloading from the protein, followed by reduction, phosphorylation and finally glycosylation by an unknown mechanism.[30]

PKS12 is the largest open reading frame in the Mtb genome and contains two complete sets of modules each consisting of six catalytic domains (Fig. 5). The lipid could be biosynthesized by five repetitive cycles of condensation of methylmalonyl and malonyl units in an alternate fashion by PKS12. The AT domain of the first module shows signature sequence that could incorporate methylmalonyl-CoA whereas, the AT domain from the second module appears to be specific for malonyl CoA. The iterative process would yield a completely saturated chain with branching at every alternate ketide unit. The iterative mechanism of biosynthesis would require transfer of growing chains from the ACP of the second module to the KS active site of the first module. Interestingly, all known examples of iterative PKSs isolated from fungi consist of one module. PKS12 raises interesting issues with regard to its novel mechanism of iterative biosynthesis. The other enzymes involved in the biosynthesis of MPMs have not been identified. The computational analysis of this region in the Mtb genome suggests few putative enzymes that could be involved in the complete biosynthesis of MPMs. Rv2047c protein shows homology with domains that have recently been characterized to reductively release non-ribosomally synthesized peptide chains from multifunctional non-ribosomal peptide synthetases. Located upstream of PKS12 is Ppm1, encoding a polyprenol monophosphomannose synthase that could mannosylate the phosphorylated alkyl chain. The chemical structures of mycoketides suggest that the molecule may function as a mannose carrier with a hydrophobic alkyl chain aiding the transfer of the sugar outside the cell membrane through a flipping action.

3.5 Plant-like type III PKSs produce long-chain metabolites

The Mtb genome revealed three genes homologous to type III PKSs;[4] PKS10 and PKS11 constitute a large PKS cluster in the genome, whereas PKS18 is not flanked by other PKS-related genes. Proteins encoded by these genes are small, iterative homodimers that belong to the chalcone synthase (CHS) superfamily of enzymes, which have been extensively studied from plants for almost three decades.[106,107] Biochemical reconstitution studies have provided some insight into the biological function of these Mtb proteins. The PKS18 protein displays remarkable substrate specificity for incorporating C_2–C_{26} acyl-CoA units (Fig. 6).[108] By utilizing malonyl-CoA as substrate, this protein efficiently catalyzes the formation of long-chain tri- and tetraketide α-pyrones. Crystallographic investigations of PKS18 protein revealed an unusual 20 Å long substrate binding tunnel that extends from the active site to the surface of the protein.[109] Despite their simpler protein architecture, Type III PKSs can perform typical two-carbon sequential condensations. The long-chain acyl-CoA starter substrate is covalently acylated to the active site Cys, whereas the extender substrates are non-covalently sequestered, unlike other PKS systems. The repetitive Claisen-like condensation produces poly β-keto intermediates, which are then cyclized in the active site pocket.

Demonstration of the *in vivo* functional relevance of type III PKS mycobacterial proteins is still awaited. In a recent study on type III PKSs from *Azotobacter vinelandii*, long-chain alkyl-resorcinols and alkyl-pyrones have been identified as major chemical components of the protective cyst coat. These cysts are metabolically dormant states that confer resistance to various chemical and physical agents. Both resorcinol as well as pyrone can be formed from the same tetraketide intermediate by two different mechanisms of cyclization[110] (Fig. 6). The notable preference of mycobacterial type III PKSs for long-chain acyl-CoA thioesters and the presence of a remarkable array of long-chain and very

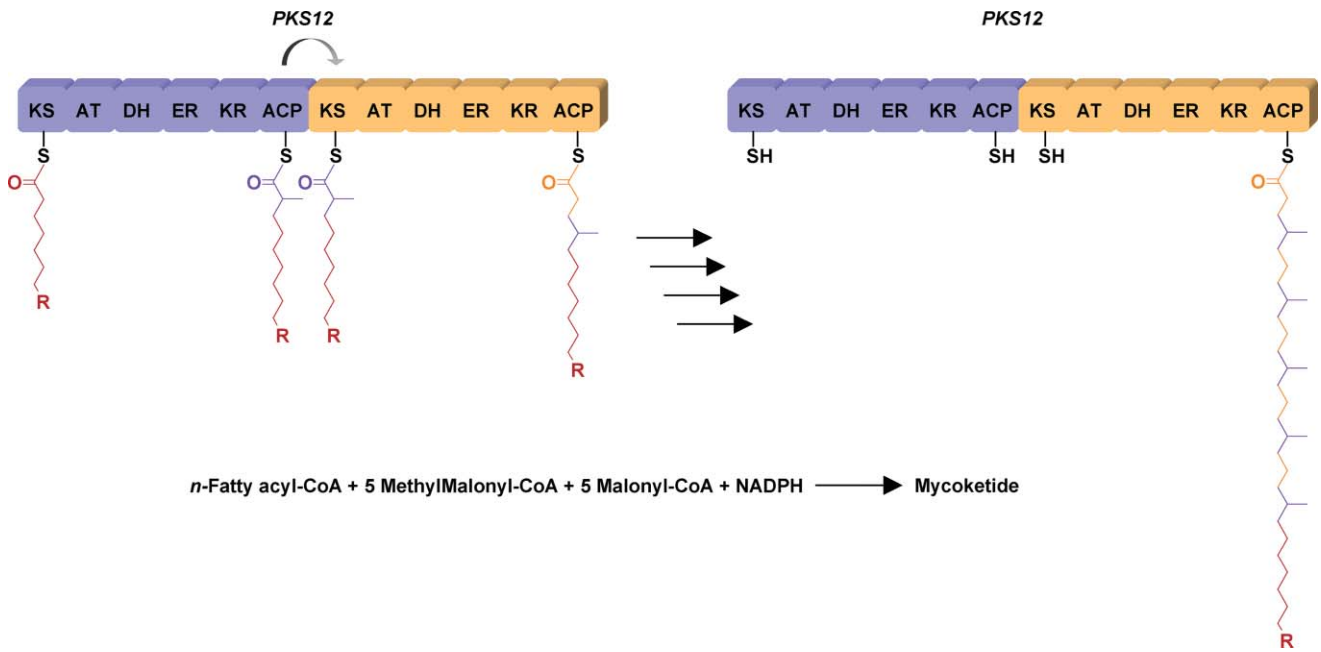

Fig. 5 Bimodular organization of PKS12 generates mycoketides with methyl groups at alternate ketide units.

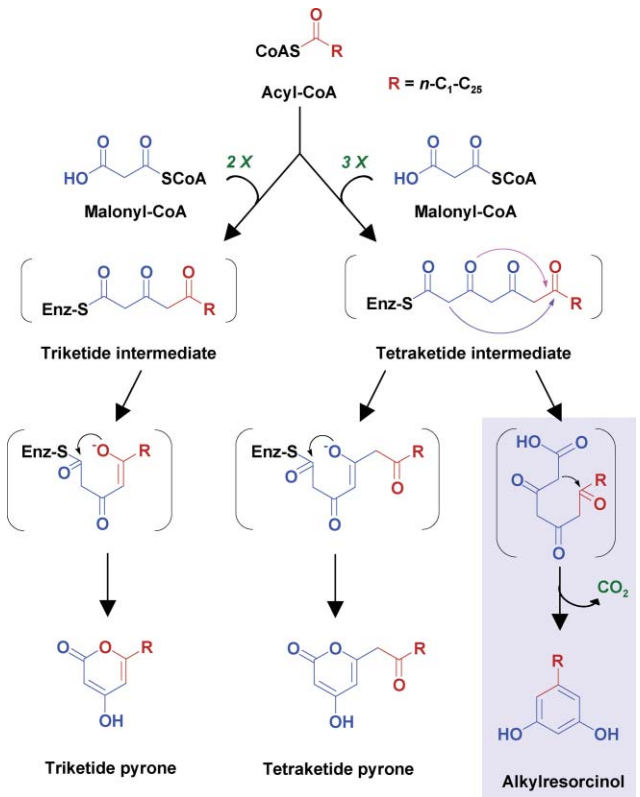

Fig. 6 Reactions catalyzed by PKS18 for the generation of pyrone products. Highlighted region depicts a possible *in vivo* scenario.

long-chain metabolites in Mtb, suggest that similar molecules may indeed be present in mycobacteria. It is possible that these metabolites are either produced in low abundance or are not expressed under standard laboratory culture conditions.

4 FAAL proteins establish biochemical crosstalk between FASs and PKSs

It is now clear that mycobacteria exploit assembly-line enzymatic machinery to produce complex lipids, wherein the biosynthetic intermediates are covalently sequestered on FASs and PKSs.[51] The two FAS systems generate *n*-long-chain fatty acid precursors, which are further extended by PKSs that impart complexity and structural diversity. This mode of hand-to-hand acyl transfer may be the most efficient method to channel intermediates. It was therefore essential to identify an enzyme system that would be able to transfer fatty acid precursors onto PKSs. The commonly known mechanism of *n*-fatty acid activation involves conversion of fatty acids to their corresponding CoA-derivatives.[111,112] This reaction is universally catalyzed by a family of acyl-CoA synthetases (ACSs) or also referred to as FadD proteins (the *D* gene of the fatty acid degradation operon from *E. coli*).[113] Sequencing of the mycobacterial genome revealed an astounding 34 FadD homologues. Even more remarkable, was the fact that many of the homologues were present adjacent to PKS genes in the Mtb genome.[4] Systematic investigations of a number of these FadD proteins revealed new enzymatic activity. These proteins convert fatty acids to fatty acyl-adenylates, which are a reaction intermediate of acyl-CoA synthetase (ACS) enzymes, and do not catalyze formation of CoA thioesters.[62] Interestingly, the structures of FadD proteins puts them in a family of acyl-activating enzymes, which includes adenylation domains of NRPSs that convert amino acids to amino-acyl adenylates and transfer them onto the carrier proteins.[114,115] Even though the activation to fatty acyl-adenylate differed from the classical mechanism, the genetic knock out data and the enzymological activity of these proteins conclusively established that they comprise of a novel family of fatty acyl-AMP ligases (FAALs). Cell-free reconstitution studies of FAAL proteins with their cognate PKSs indeed demonstrated their biological functions[51] (Fig. 7). Many of the other FadD proteins are similar to ACS proteins and thus catalyze the synthesis of a variety of acyl-CoA thioesters.[116] Since FAAL proteins feed into PKSs to produce a number of crucial lipids, small molecule inhibitors of these proteins may have tremendous therapeutic potential.

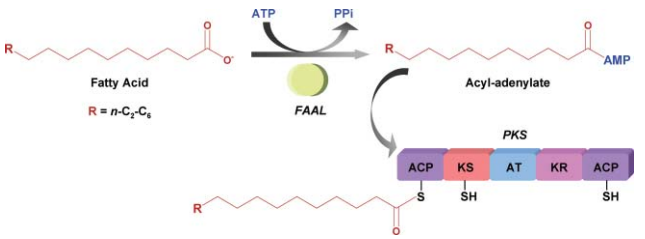

Fig. 7 FAAL mediated activation of fatty acids as acyl-adenylates and their transfer onto cognate PKS systems.

5 Metabolic repertoire and cell wall remodeling in Mtb infections

Mycobacterial lipids elicit a complex inflammatory immune response. It is therefore attractive to speculate that mycobacteria may be establishing their niche in the host by controlled alteration of the phenotypic expression of their metabolites. The control of expression of these molecules could be elegantly calibrated to achieve a particular equilibrium in both recruitment and activation of inflammatory cells in the mycobacterial granuloma. Such a situation would aid the creation of a state of chronicity, which is permissive for both the bacilli and the host. In order to understand and recreate the cell wall remodeling process of Mtb in a laboratory, a library of chemical mutants of Mtb could be engineered that could mimic different clinical isolates. Simultaneously, the catalytic versatility of mycobacterial enzymes involved in lipid biosynthetic pathways could be explored to catalog their biosynthetic potential. In a recent study, the promiscuous specificity of Mtb fatty acyl-CoA ligase (FACL) protein was investigated. The remarkably relaxed substrate specificity resulted in the biosynthesis of a variety of fatty acyl-CoA substrates, which are precursors of lipid biosynthesis.[116] Furthermore, *in situ* coupling of acyl-thioesters with PKS18 and Mas PKSs, produced a variety of hydroxyl-, unsaturated and methyl-modified pyrones and fatty acids (Fig. 8). This study emphasized the vast potential of mycobacterial proteins in generating metabolic diversity. Clearly, alterations of metabolic fluxes *in vivo* could dictate the biosynthesis of different products.

Emerging evidences suggest that Mtb alters its genetic programming during infection and may utilize alternative carbon sources for its survival.[117,118] It is clear that the relative composition of

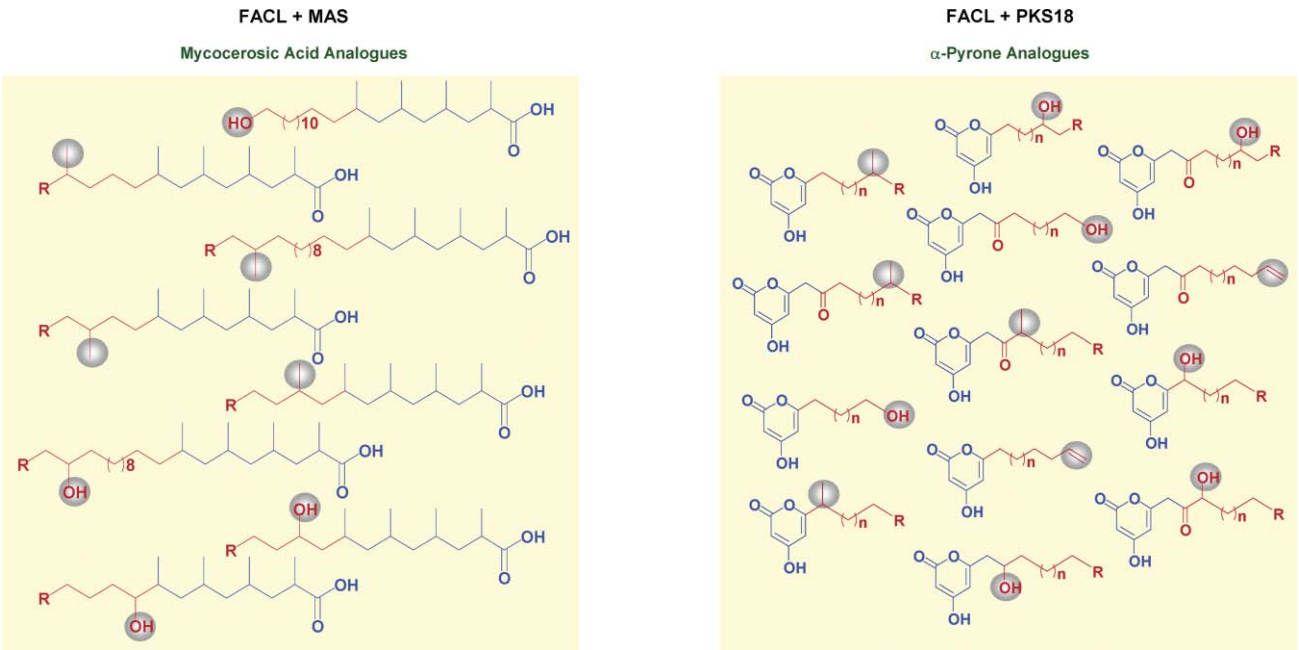

Fig. 8 *In situ* coupling of FACL protein, independently with Mas and PKS18 generates novel analogues of mycobacterial mycocerosic acids and α-pyrones.

mycolates or some of the apolar lipids can significantly influence the intramacrophage growth of Mtb. Thus it is not surprising that despite little genetic heterogeneity, many clinical isolates of Mtb show distinctive differences in the host immune responses they induce.[74,119] In a recent study, a hypervirulent strain HN878 was found to express a glycolipid PGL, which is not detected in the laboratory strain H37Rv.[58] PGL is universally produced by *M. leprae*, but in the H37Rv strain of Mtb there is a frameshift mutation in one of the biosynthetic genes (pks1/15) that provides an acyl *p*-hydroxy-benzoic acid precursor.[120] Genetic disruption strategies of generating mutants in the biosynthetic genes have made it feasible to describe biological effects of these metabolites in the hosts. Some of the recent studies interestingly suggest that even the 'fine' structural modifications of Mtb cell envelope lipids could have dramatic effects on the host immune cell activation during infection. For example, it has been shown that the two stereochemistries of the cyclopropane rings of mycolic acids in TDM have opposite effects on the immune response of mice. Whereas *trans*-cyclopropanation in TDM is a suppressor of Mtb-induced inflammation and virulence, the *cis*-cyclopropanation of TDM is reported to be a proinflammatory modification.[121,122] However, it is worthwhile to keep in mind that such modifications can also lead to overall changes in the fluidity of the cell wall and can result in improper translocation of other membrane-associated molecules.[123] An interesting example is of the DIMs of H37Rv, which are spontaneously lost during routine lab work, which result in 'DIMless' attenuated Mtb mutant. This DIMless phenotype has led to incorrect identification of some of the PKS proteins in DIM biosynthesis.[124–126]

6 Conclusions

The membrane interface between host and pathogen plays an important role in adaptation of bacteria to diverse environments. Along with complex lipids and polysaccharides, the bacterial cell envelope is associated with numerous proteins that influence cellular structure and its interactions with host cells. For example, Vi, a polymer of galacturonic acid with *N*- and *O*-acetyl modifications, is only present in *Salmonella typhi* and not in non-typhoid serovar *S. typhimurium.*[127] This molecule interacts with host cells through a recognition complex containing the prohibitin family of molecules which leads to downregulation of inflammatory responses in intestinal epithelial cells.[128] Recently, polyketide–peptide hybrid compounds have been reported from intestinal pathogenic *E. coli.* These molecules belong to a growing family of bacterial toxins and effectors that interfere with the eukaryotic cell cycle.[129] There is also an increasing realization that cell surface remodeling and dynamics may indeed be an important arsenal in the repertoire of bacterial pathogenesis.

Mycobacterium tuberculosis has clearly developed a remarkable outer coat and novel biochemical mechanisms that facilitate its survival under changing environmental conditions. Since PKSs are known to produce a vast array of natural products, in hindsight it is not surprising that mycobacteria utilize these proteins effectively to produce exotic lipids. The identification and characterization of molecular mechanisms that generate functional variety can significantly expand our understanding of how pathogens evolve their metabolic pathways to generate molecular diversity, and how they use this diversity to respond to the complex challenges thrown up by host immunity.

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