PROSPECTS
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Structure and Function of Mycobacterium Glycopeptidolipids From Comparative Genomics Perspective

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ABSTRACT

Glycopeptidolipids (GPLs) attached to the outer surface of the greasy cell envelope, are a class of important glycolipids synthesized by several non-tuberculosis mycobacteria. The deletion or structure change of GPLs confers several phenotypical changes including colony morphology, hydrophobicity, aggregation, sliding motility, and biofilm formation. In addition, GPLs, particular serovar specific GPLs, are important immunomodulators. This review aims to summarize the advance on the structure, function and biosynthesis of mycobacterium GPLs. J. Cell. Biochem. 114: 1705–1713, 2013. © 2013 Wiley Periodicals, Inc.

KEY WORDS: GLYCOPEPTIDOLIPIDS; MYCOBACTERIA; BIOSYNTHESIS; BIOFILM; COLONY MORPHOLOGY

Mycobacteria can synthesize lipid-rich cell walls which confer these bacteria with many unique features such as proliferation in host macrophage phagolysosome, antibiotics resistance, and biofilm developing. The mycobacteria cell walls include peptidoglycan–arabinogalactan core coated by a lipid bilayer. The interior of the lipid bilayer consists of mycolic acids covalently attached to arabinogalactan, and the free glycolipids and phospholipids form the exterior of the lipid bilayer. The outer layer glycolipids usually confer various mycobacterium distinct surface features. Glycopeptidolipids (GPLs) are relatively important glycolipids produced by non-tuberculosis mycobacteria (NTM), including *Mycobacterium avium* complex (MAC), *M. abscessus*, *M. chelonae*, *M. smegmatis*, *M. fortuitum*, *M. porcinum*, *M. senegalense*, *M. xenopi*, etc. Due to their important role in mycobacterium physiology and pathogenesis, GPLs have been studied since 1960s. This review will summarize the structure, function, biosynthesis, and regulation of mycobacterium GPLs.

GPLs STRUCTURE

GPLs can be produced by nearly all NTM. These GPLs share a lipopeptide core of C_{26-34} fatty acids amidated with D-Phe-D-*allo*Thr-D-Ala-L-alaninol. The *allo* Thr residue of this core is glycosylated with 6-deoxy-α-L-talose (dTal) and the alaninol residue is glycosidically attached to α-L-rhamnose (Rha), while the C3 of the fatty acids is usually hydroxylated or methoxylated as depicted in Figure 1. These diglycosylated GPLs constitute the apolar GPLs or

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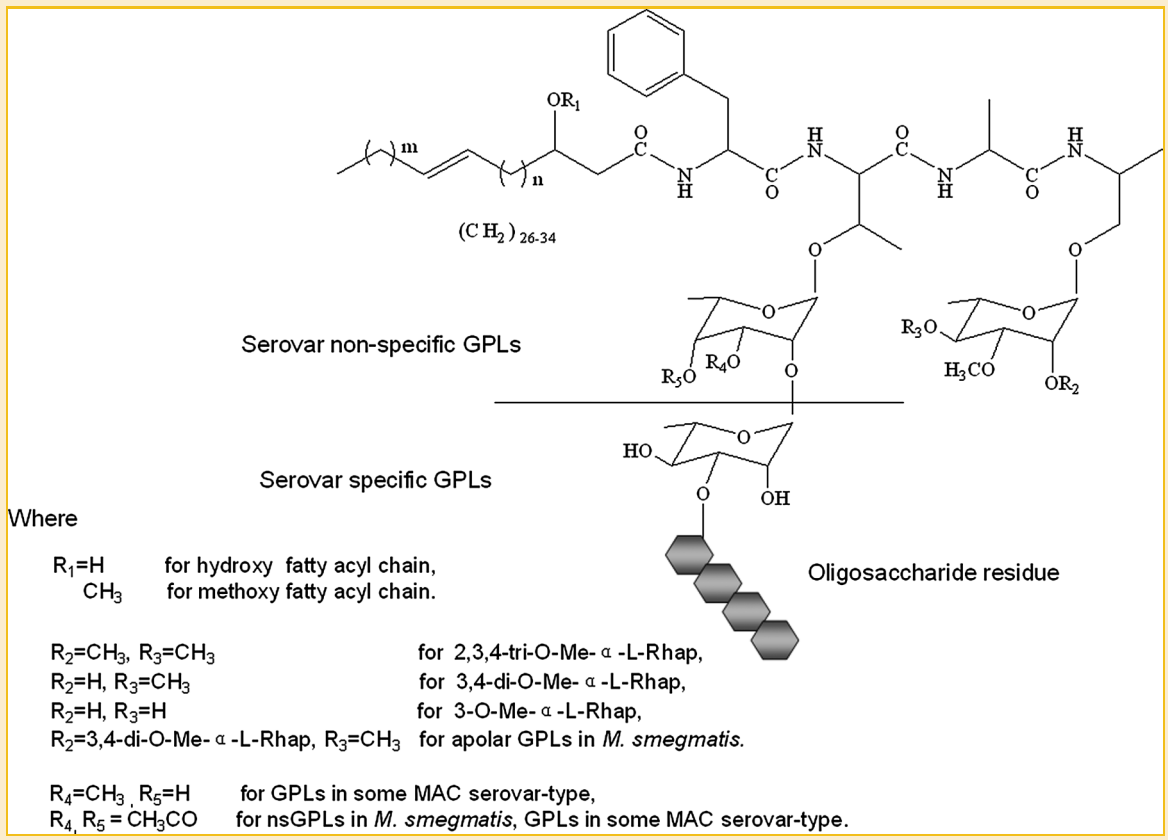

Fig. 1. The general structure of GPLs in non-tuberculosis mycobacteria. The additional oligosaccharide appendage linked to the 6-deoxytalose differentiates serovar specific (polar) GPLs found in MAC from non-serovar specific (apolar) GPLs. Though they share the lipopeptide core, there are slight differences between nsGPLs of MAC and *M. smegmatis* in the modification of the Rha and dTal. *M. smegmatis* sometimes produces the polar GPL with the diglycosylation at the alaninol.

non-serovar specific GPLs (nsGPLs), and further glycosylation of dTal is responsible for the formation of the polar GPLs or serovar specific (ssGPLs) with high immunogenicity. *M. smegmatis* synthesizes nsGPLs whose dTal is often acetylated and Rha is 3,4-di-*O*-methylated or 2,3,4-tri-*O*-methylated [Billman-Jacobe, 2004]. MAC produce both nsGPLs and ssGPLs whose dTal contains 3-*O*-Me sometimes and the terminal Rha is usually 3-*O*-methylated or 3,4-di-*O*-methylated. Different oligosaccharide residues of ssGPLs are chemical basis of various serotypes of MAC. Serovar 1-specific GPL with α-L-Rha-(1 → 2)-α-L-dTal is the simplest among the 31 ssGPLs identified so far [Chatterjee and Khoo, 2001].

Some mycobacteria species have distinct GPLs. *M. xenopi*, for example, synthesizes serine-containing GPLs whose peptide core is L-Ser-L-Ser-L-Phe-D-*allo*Thr-COOMe, fatty acyl group is dodecanoyl instead of the C_{32}–C_{35} acyl in MAC GPLs, and a 3-*O*-Me-α-L-dTal substituent is linked to the N-terminal serine residue [Chatterjee and Khoo, 2001]. A recent study indicated that there was a substitution of D-valine for the D-phenylalanine of the peptide core in a new GPL produced by MAC [Matsunaga et al., 2012]. A novel family of GPLs, whose lipopeptide core consists of a tripeptidyl amino-alcohol with a di-O-acetyl-6-dTal substituting for the *allo* Thr and a 2-succinyl-3,4-di-*O*-Me-Rha attached to the distal alaninol, was found in *M. smegmatis* [Villeneuve, 2003]. Furthermore *M. smegmatis* expresses the polar GPL with a hyperglycosylation of the terminal Rha under carbon starvation [Ojha et al., 2002; Mukherjee et al., 2005]. *M. fortuitum* biovar. *Peregrinum* also has a kind of special GPL whose oligosaccharide residue is linked to the alaninol unit and a 3-O-Me-α-L-Rha instead of the dTal is attached to the *allo* Thr [López Marin et al., 1991]. The presence of these new GPLs structures might result from the bacterial survival adaption to their habitats or the stresses.

GPLs BIOSYNTHESIS

GPLs biosynthesis is a fascinating topic. The genes involved in the biosynthesis of GPLs might be new targets for the drugs against mycobacteria. The traditional method to identify these genes is to screen the interested mutants from transposon insertion mutant libraries. Bioinformatics prediction contributes to exploring GPLs synthetic pathway as diverse mycobacterium genomes were sequenced. Nearly 30 genes organized in clusters and conserved in NTM, participate in GPLs biosynthesis. These genes, many in operons, are responsible for the synthesis of peptide core and fatty acid acyl chain, the modification of GPLs core (glycosylation, methylation, acetylation), the assembly of various synthesizing enzymes on the cell membrane and the export of GPLs, respectively.

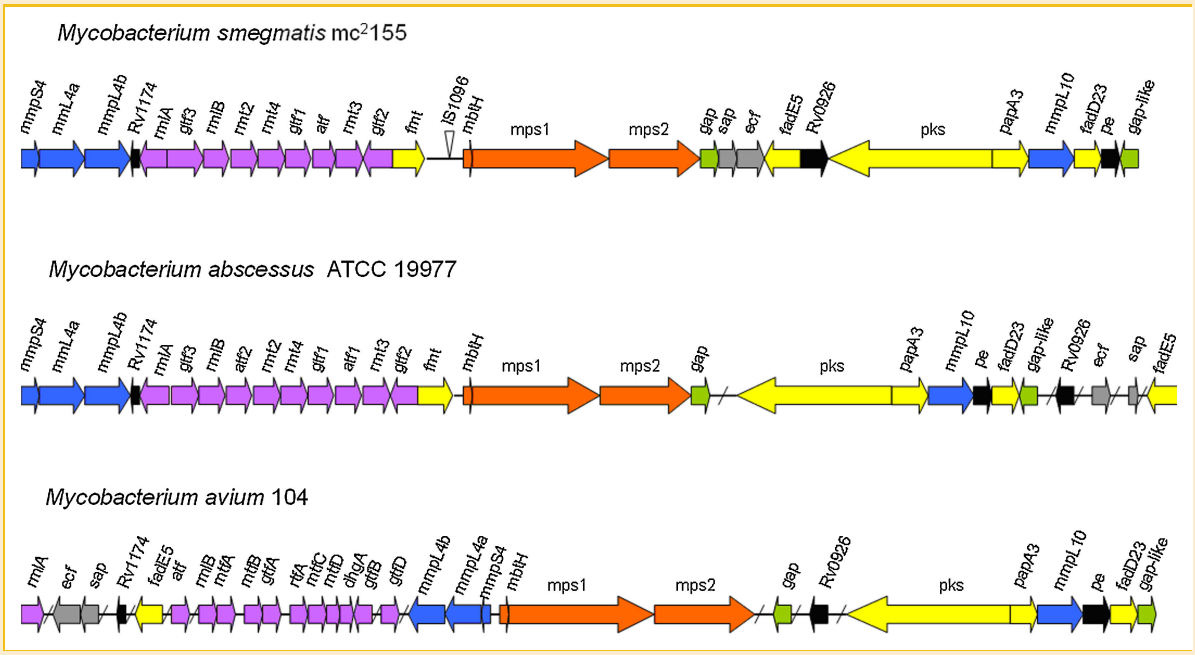

Fig. 2. Genetic structure of the GPL biosynthesis locus in *M. smegmatis*, *M. abscessus*, and MAC serovar 1. Arrows represent the ORFs not drawn to scale. Color code: blue: members of mmpSL family; purple: saccharides biosynthesis, transfer and modifications; red: tripeptidly amino alcohol biosynthesis; green: GPL export to the cell surface; yellow: fatty acyl chain biosynthesis, modification and transfer; grey: possible regulatory genes [Ripoll et al., 2007]; black: unknown.

GPLs locus is around 65 kb in *M. smegmatis* [Ripoll et al., 2007; Mukherjee and Chatterji, 2012]. *M. abscessus* and *M. chelonae* have the GPLs locus similar to that of *M. smegmatis*, but the genes involved in synthesis of fatty acid chain are comparatively scattered. These genes are distant to those for peptide core synthesis. MAC genomes contain many additional genes coding ssGPLs modification enzymes. It can be hypothesized that the compact architecture of GPLs locus in *M. smegmatis* represents an ancestral form [Ripoll et al., 2007] evolved to generate diverse GPLs locus found in other NTM genomes as illustrated in Figure 2. There are some homologus proteins of GPLs biosynthases found in *M. tuberculosis* genome (Table I), but they are more scattered and some have already been reported to be involved in the synthesis of other components, for example, Rv2952 (ortholog of MSMEG_0393) and Rv0101 (ortholog of MSMEG_0402) both participate phthiocerol dimycocerosates (PDIM) synthesis. *M. tuberculosis* genome has the vestige of GPLs biosynthesis capability. Unlike *M. smegmatis*, *M. tuberculosis* might adopt different evolution to produce components (like PDIM) instead of GPLs to fit its niche.

SYNTHESIS OF THE LIPOPEPTIDE CORE

It is believed that an Acyl-CoA dehydrogenase (FadE5) incorporates an unsaturation into the fatty acyl chain generated by the fatty acid synthase system in mycobacteria (FAS I and FAS II), then this fatty acyl chain is extended and hydroxylated by the polyketide synthase Pks [Trivedi et al., 2004; Mukherjee and Chatterji, 2012]. The hydroxylated fatty acyl chain transferred from Pks to the peptide synthase (Mps), with the help of the polyketide synthase associated protein (PapA3), is attached to the tripeptidyl amino-alcohol [Trivedi et al., 2004; Mukherjee and Chatterji, 2012; Tatham et al., 2012]. Mps identified by Billman-Jacobe and his colleagues in 1999 from the Tn insertion mutant library of *M. smegmatis* consists of four function modules responsible for the formation of tripeptidyl amino alcohol skeleton. The first three modules with racemase activity generate the D configuration of the target amino acids and add these D form amino acids to the fatty acyl chain. The module IV without racemase activity incorporates a L-alanine into the tripeptide D-Phe-D-*allo*Thr-D-Ala and further reduces this L-alanine to a L-alaninol [Billman-Jacobe et al., 1999; Mukherjee and Chatterji, 2012]. The above enzymes synergize to form the lipopeptide core. In the following studies, *mps* was annotated three ORFs, namely *mbtH*, *mps1*, *mps2* [Sondén et al., 2005], and the last two might be responsible for the synthesis of the dipeptides and the amino alcohol, respectively [Ripoll et al., 2007]. A recent report revealed that *mbtH*-like gene (*MSMEG_0399*) played an important role in GPLs biosynthesis though its concrete function remained unknown [Tatham et al., 2012]. *mbtH* may affect the formation of the lipopeptide core due to its invariably adjacent position to *mps1* in many mycobacteria GPLs loci. Being Mps homologous proteins, PstA and PstB in MAC are essential for peptide core formation [Freeman et al., 2006].

GLYCOSYLATION OF GPLs

In *M. smegmatis*, the glycosyltransferases encoded by *gtf1* and *gtf2* are responsible for the transfer of dTal and Rha to the *allo*-threonine and the alaninol respectively, while the production of the polar GPLs

TABLE I. Genes of *M. smegmatis* GPL Locus and Their Orthologs Found in the Genomes of *M. abscessus*, *M. avium*, and *M. tuberculosis*

M. smegmatis			*M. abscessus*		*M. avium* 104		*M. tuberculosis* H37Rv	
Gene	Gene symbol	Proposed function	Gene symbol	%[a]	Gene symbol	%[a]	Gene symbol	%[a]
mmps4	MSMEG_0380	Members of the MmpS family. Required for assembly of GPLs synthesis enzymes in cell membrane[+]	MAB_4117c	78	MAV_3247	59	Rv0451c	60
mmpL4a	MSMEG_0381	Members of the MmpL family. Required for assembly of GPLs biosynthases in cell membrane[+]	MAB_4116c	78	MAV_3248	67	Rv0450c	64
mmpL4b	MSMEG_0382	Members of the MmpL family. Required for assembly of GPLs biosynthases in cell membrane[+]	MAB_4115c	76	MAV_3249	65	Rv0450c	63
Rv1174	MSMEG_0383	None	MAB_4114	48	MAV_3362	54	Rv1174c	100
rmlA	MSMEG_0384	Glucose-1-phosphate thymidylyltransferase[*]	MAB_4113	85	MAV_4820	80	Rv0334	79
gtf3	MSMEG_0385	D-rhamnose rhamnosyltransferase[+]	MAB_4112c	70			Rv1524	57
							Rv1526c	52
rmlB	MSMEG_0386	dTDP glucose 4,6 dehydrogenase[*]	MAB_4111c	78	MAV_3269	76	Rv0536	33
			MAB_4110c (*atf2*)		MAV_3268 (*mtfA*)			
rmt2	MSMEG_0387	Rhamnose 2-*O*-methyltransferase[+]	MAB_4109c	72				
rmt4	MSMEG_0388	Rhamnose 4-*O*-methyltransferase[+]	MAB_4108c	83	MAV_3266 (*mtfB*)	78		
					MAV_3261 (*mtfC*)	80		
					MAV_3262 (*rtfA*)			
gtf1	MSMEG_0389	D-allo-threonine 6-deoxytalosyltransferase[+]	MAB_4107c	76	MAV_3265 (*gtfA*)	75	Rv1524	58
							Rv1526c	51
atf	MSMEG_0390	6-deoxytalose 3,4-*O*-acetyltransferase[+]	MAB_4106c (*atf1*)	72	MAV_3274	61		
rmt3	MSMEG_0391	Rhamnose 3-*O*-methyltransferase[+]	MAB_4105c	82	MAV_3260 (*mtfD*)	82		
					MAV_3259 (*dhgA*)			
gtf2	MSMEG_0392	L-alaninol rhamnosyltransferase[+]	MAB_4104	67	MAV_3258 (*gtfB*)	65	Rv1524	58
							Rv1526c	52
					MAV_3253 (*gtfD*)			
fmt	MSMEG_0393	Fatty acid *O*-methyltransferase[+]	MAB_4103c	68			Rv2952	56
							Rv1523	54
mbtH	MSMEG_0399	None	MAB_4100c	91	MAV_3245	81	Rv2377c	76
mps1	MSMEG_0400/0401	Peptide synthase. Synthesis of the dipeptide[*]	MAB_4099c	69	MAV_3244	67	Rv0101	44
							Rv2379c	34
mps2	MSMEG_0402	Peptide synthase. Synthesis of the amino acid alcohol[*]	MAB_4098c	72	MAV_3243	72	Rv0101	64
							Rv2379c	37
gap	MSMEG_0403	Integral membrane protein. Required for GPL export[+]	MAB_4097c	58	MAV_3059	35	Rv1517	32
							Rv3821	30
sap	MSMEG_0404	Sigma associated protein[*]	MAB_4454c	31	MAV_4518	42		
ecf	MSMEG_0405	Sigma factor of the ECF family[*]	MAB_4459c	48	MAV_4519	44	Rv1189	30
fadE5	MSMEG_0406	Fatty acid dehydrogenase[*]	MAB_4437	78	MAV_3309	66	Rv0244c	81
Rv0926	MSMEG_0407	None	MAB_4633	36	MAV_2461	39	Rv0926c	39
pks	MSMEG_0408	Fatty acid synthesis[+]	MAB_0939	79	MAV_1763	76	Rv3825c	43
							Rv2048c	41
papA3	MSMEG_0409	Transfer of the fatty acid from Pks to the peptide synthase[+]	MAB_0938c	77	MAV_1762	76	Rv1182	55
mmpL10	MSMEG_0410	Members of the MmpL family[*]	MAB_0937c	76	MAV_1761	67	Rv1183	57
fadD23	MSMEG_0411	acyl-CoA synthase[*]	MAB_0935c	73	MAV_1759	71	Rv1185c	61
pe	MSMEG_0412	None	MAB_0936c	64	MAV_1760	67	Rv1184c	45
gap-like	MSMEG_0413	Integral membrane protein[*]	MAB_0934	55	MAV_1758	57	Rv3821	44
							Rv1517	30

+, Experimentally validated function; *, predicted function.
[a]Percentage of identity between *M. abscessus*, *M. avium* or *M. tuberculosis* genes and *M. smegmatis* genes.

depends on Gtf3 which, under carbon starvation, transfers a 3-*O*-Me-Rha or 3,4-di-*O*-Me-Rha to the 3,4-di-*O*-Me-Rha residue linked to L-alaninol. The expression of *gtf3* regulated by environmental factors such as the nutrient condition or sigma factor is usually repressed [Miyamoto et al., 2006]. *rmlA* and *rmlB* found in the GPLs locus of *M. smegmtis* encode the putative glucose-1-phosphate thymidylyl transferase and dTDP glucose 4,6 dehydrogenase, respectively. These two enzymes might be involved in the synthesis of some substrates for GPLs biosynthesis like deoxyhexoses, Tal and Rha [Billman-Jacobe, 2004]. In the case of *M. avium*, GtfA and GtfB share the function of *M. smegmatis* Gtf1 and Gtf2 [Eckstein et al., 2003]. In 1991, Belisle et al. isolated from *M. avium* serovar 2 strain TMC 724 the *ser2* gene cluster responsible for the biosynthesis of oligosaccharide residues attached to the dTal [Belisle et al., 1991]. This cluster consists of *ser2A-ser2D* four functional regions among which *ser2A* encodes the rhamnosyltransferase, *ser2B* and *ser2D* encode methyltransferases required for the methylation of the fucose at C3 and C2, respectively. The fucosyltransferase is encoded by *ser2C* or *ser2D*, both are involved in the synthesis of fucose [Mills et al., 1994]. The *ser2A* locus contains three ORFs, one of which designated *rtfA* encodes the rhamnosyltransferase required specifically to transfer a Rha to the 6dTal instead of the alaninol [Eckstein et al., 1998; Maslow et al., 2003]. The incorporation of the fucose in serovar 2 GPL is dependent on *gtfD*, and the adjacent genes *mdhtA* and *merA* are responsible for the formation of fucose [Miyamoto et al., 2007]. In MAC serovar 8 strain, *gtfTB* encodes the glucosyltransferase essential for serovar 8-specific GPL [Miyamoto et al., 2008]. In the serovar 4 strain frequently isolated from HIV AIDs patients, there is a 4-*O*-Me-Rha via 1 → 4 linkage to the fucose in ssGPL. A rhamnosyltransferase encoded by *hlpA* transfers this

rhamnose residue to the fucose residue of serovar2-specific GPL to form serovar4-specific GPL [Miyamoto et al., 2010]. There were some studies on the glycosylation of other ssGPL such as serovar 7-specific GPL [Fujiwara et al., 2006] and serovar 12-specific GPL [Nakata et al., 2008].

METHYLATION AND ACETYLATION OF GPLs

In the *M. smegmatis* methyltransferase involved in the biosynthesis of GPLs, the 3-*O*-methyltransferase encoded by *rmt3* (formerly designated *mtf1*) can methylate the terminal rhamnose at C3 position [Patterson et al., 2000], whereas the methylations at C2 and C4 are executed by the products of *rmt2* (formerly designated *mtf4*) and *rmt4* (formerly designated *mtf3*) [Jeevarajah et al., 2004]. Fmt (formerly designated Mtf2) catalyses the conversion of the fatty acid chain from the 3-hydroxyl form to the 3-*O*-methyl form [Jeevarajah et al., 2002]. In *M. avium*, the 3-*O*-methylatransferase (the product of *mtfD*) and 4-*O*-methyltransferases (encoded by *MtfB* and *MtfC* respectively) can modify the rhamnose linked to the alaninol. MtfA might be involved in the methylation of the distal fucose in ssGPLs [Jeevarajah et al., 2004]. In MAC serotype 12 strain, there are two novel ORFs (*orfA* and *orfB*) encoding methyltransferases which catalyze *O*-methylations at the C4 in the Rha residue next to the distal hexose and at the C3 in the terminal hexose respectively. The *O*-methylations at these positions can distinguish the serotype 12 GPL from the serotype 7 GPL [Nakata et al., 2008]. The function of OrfB is absent in serotype 13 strain, so the difference between serovar 13 GPL and serovar 12 GPL is the absence of methylation at the terminal hexose [Naka et al., 2011]. *M. avium* genome lacks *fmt*, therefore the methylated fatty acid chain is not found in its GPLs [Jeevarajah et al., 2004]. The dTal in *M. smegmtis* GPLs is usually acetylated, and *atf1* encodes the enzyme required for the acetylation at these positions. It was shown that the acetylation of the dTal residue occurred independently of the methylation of the Rha residue in *M. smegmatis* [Recht and Kolter, 2001]. The presence of Atf1 homolog in *M. avium* genome implies that *M. avium* GPL dTal might be acetylated in its native pattern. The proposed biosynthesis pathways of GPLs in *M. smegmatis* and *M. avium* 104 stain are illustrated in Figure 3.

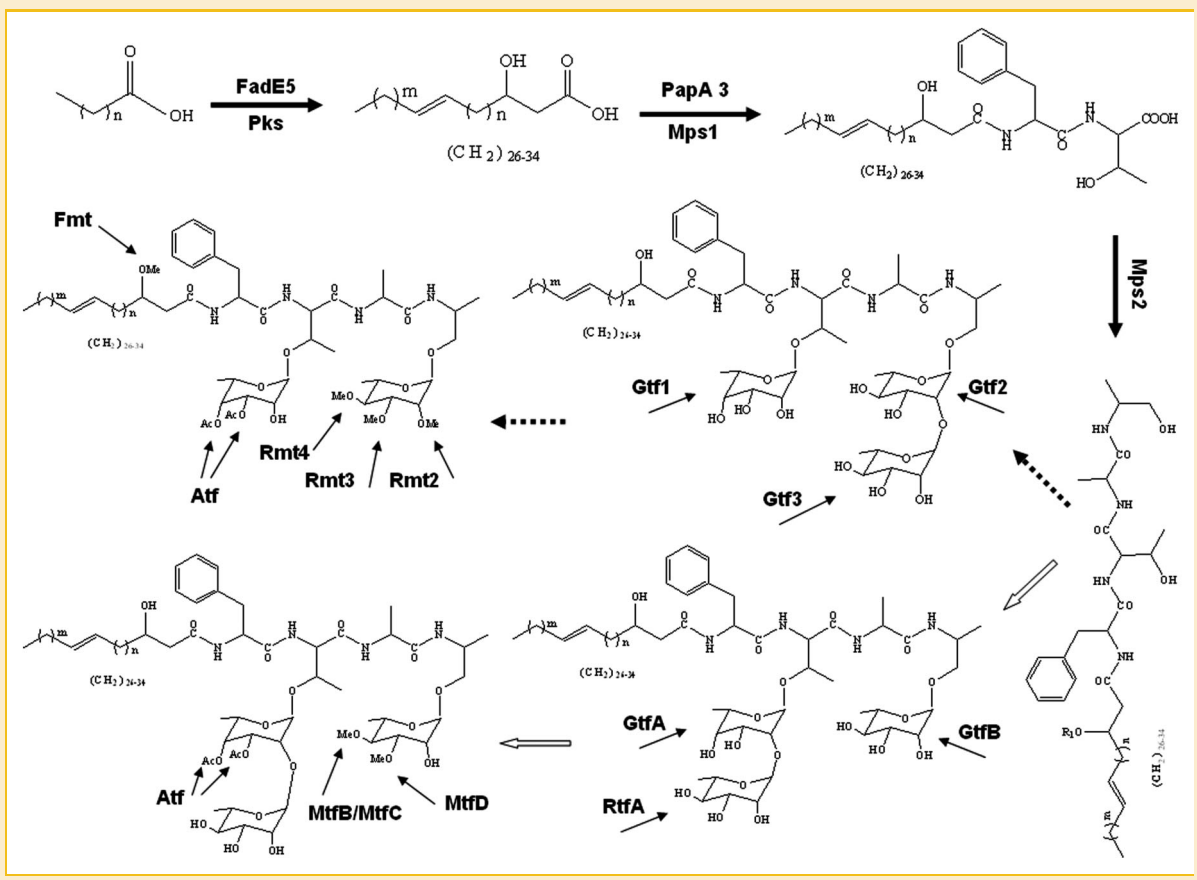

Fig. 3. The proposed pathway of GPLs biosynthesis. Major steps include (1) fatty acyl chain extension, unsaturation and hydroxylation by Pks and FadE5, (2) tripeptidyl amino-alcohol synthesis by Mps1 and Mps2, (3) glycosylation of allo-threonine and alaninol by various glycosyltransferases, (4) methylation and acetylation of the sugars added to the lipopeptide core by the enzymes for the modification of GPL core. The processes following the lipopeptide core formation occurring in *M. smegmatis* is shown through the dotted arrows, while the counterparts in MAC is represented through the hollow arrows. (Pks, polyketide synthase; FadE5, fatty acyl desaturase; Mps, mycobacterium peptide synthetase; PapA3, Pks associated protein; Gtf, glycosyltransferase; Rmt, rhamnosyl methyltransferase; Fmt, fatty acyl methyltransferase; Atf, acetyltransferase; Rtf, rhamnosyltransferase).

ASSEMBLY OF GPLs SYNTHASES AND EXPORT OF GPLs

GPLs are biosynthesized in the cytoplasm, while their ultimate location is the outmost layer of the cell wall. Which protein transports GPLs across the cell membrane? The members of the mycobacterium membrane proteins (MmpSL) family including MmpS proteins and MmpL proteins contribute to the transportation of many molecules, but not GPLs. The mycobacterium specific integral membrane protein Gap was reported to be responsible for the export of GPLs. Different from the members of MmpSL family, Gap is a new transport protein for small molecules in mycobacteria [Sondén et al., 2005]. Before the function of Gap was reported, Recht et al. found that *tmtpC* (designated *MmpL4b* now) disrupted *M. smegmatis* failed to produce GPLs. Sequence alignment suggested that TmtpC might be responsible for the export of GPLs to the surface of the cell wall [Recht et al., 2000]. A recent report showed that MmpS4 promoted GPLs synthesis and export by acting as a scaffold with MmpL4 for the assembly of GPLs biosynthases on the membrane [Deshayes et al., 2010]. This might underlie the indirect role of TmtpC in GPLs export. Elucidation the function of ORFs in GPLs locus will give more details for the biosynthesis of GPLs.

ABILITY TO AFFECT MYCOBACTERIUM SURFACE PROPERTIES

The hydrophobic fatty acyl chains and the hydrophilic carbohydrate groups make GPLs amphipathic molecules. The head-tail orientation of GPLs on the cell wall remains unknown. GPLs might be linked to the mycolic acids via fatty acyl chains, thereby exposing the polysaccharides [Chatterjee and Khoo, 2001; Mukherjee and Chatterji, 2012]. It also might be true that the hydrophobic tails (composed of the fatty acids chains) exposed outside boost the hydrophobicity of mycobacteria cell walls [Recht et al., 2000; Recht and Kolter, 2001].

The cell wall located GPL is reasonably able to alter the property of the cell wall and phenotypes such as colony morphology, hydrophobicity of cell wall and aggregation of bacteria. It is well known that members of MAC normally exhibit different colony morphologies including smooth transparent (SmT), rough transparent (RgT), smooth opaque (SmO), rough (Rg) ones [Kansal et al., 1998; Torrelles et al., 2002]. Irreversible spontaneous conversion from a smooth strain to a rough strain can accidentally occur. *M. abscessus* also has smooth and rough strains, but the conversion between these two different strains is reversible [Howard et al., 2006]. It is generally thought that the conversion from the smooth strain to the rough stain results from the deletion or structural change of GPLs. *M. smegmatis* displays Rg strain due to the mutation of genes involved in GPLs biosynthesis like *mps*, *gtf3*, or *atf1* [Recht and Kolter, 2001; Deshayes et al., 2005]. In *M. avium*, the deletion of *ser2* genes cluster or *gtfA* results in the absence of GPL and appearance of Rg strain [Belisle et al., 1993; Eckstein et al., 2003]. *M. abscessus* Rg strain is devoid of GPLs as well [Byrd and Lyons, 1999]. The GPLs-deficient strain usually exhibits the increase of the cell hydrophobicity and the cellular aggregation [Etienne et al., 2002], which supports the head-tail orientation of GPLs referred in some literatures [Chatterjee and Khoo, 2001; Mukherjee and Chatterji, 2012]. Interestingly *gtf3* overexpressed *M. smegmatis* displays Rg colony morphology identical to that done by GPL-deficient strain, and the cell hydrophobicity and aggregation of this strain also increase [Deshayes et al., 2005].

SUSCEPTIBILITY TO ANTIBIOTICS AND MYCOBACTERIUM PHAGES

GPLs might play a role in the resistance to antibiotics. In *M. smegmatis*, the absence of GPLs increases the uptake of chenodeoxycholate by the cell wall, indicating GPLs may work as a permeability barrier [Etienne et al., 2002], but the mutant increases moderately the sensitivity only to cephalosporins among many kinds of antibiotics like isoniazid, streptomycin, erythromycin, rifampicin, ethambutol, quinolones, when GPLs of this strain decreases significantly due to the deletion of *mbtH*-like gene *gplH* [Tatham et al., 2012]. We also found that GPL-deficient strain had the same susceptibility to rifampicin, streptomycin, isoniazid, and capreomycin as the parent strain. GPLs maybe do not play an expectantly essential role in the integration of permeability barrier of the mycobacteria cell walls.

There are some relationship between GPL and mycobacteriophage. It was reported that nsGPL was the receptor of mycobacteriophage D4 and I3, and the methylated Rha in GPLs was the active sit, while the mutant absent of GPLs remained sensitive to D29 and Bxz1 [Dhariwal et al., 1986; Chen et al., 2009]. The GPL-deficient mutant of *M. smegmatis* was sensitive to mycobacteriophage D29 and TM4, indicating GPL is the selective receptor for the particular mycobacteriophage.

IMPACT ON SLIDING MOTILITY AND BIOFILM FORMATION

Mycobacteria surface motility exemplified by the transparent halo can be an indication of the feature of their cell wall [Recht et al., 2000]. Both fast-growing saprophytic *M. smegmatis* and slow-growing conditions pathogenic *M. avium* can slide [Martínez et al., 1999; Carter, 2003]. In the natural environment, mycobacteria can effectively colonize and spread by sliding motility or biofilm formation. In the water distribution systems, *M. avium* in the biofilms represents a source of infection of NTM [Freeman et al., 2006]. In animals, the formation of biofilms or sliding favors the epithelial cell invasion and drug-tolerance of mycobacteria [Ojha et al., 2008].

NTM GPLs are believed to contribute to biofilm formation or sliding motility. In *M. smegmatis*, the formation of biofilm is affected significantly by the deletion [Recht et al., 2000] or change [Recht and Kolter, 2001] of GPLs. This is true for *M. avium* [Martínez et al., 1999] and *M. abscessus* [Howard et al., 2006] as well, but the core GPL is dispensable for *M. avium* biofilm formation, at least on the Permanox and silanized glass surface

[Freeman et al., 2006]. The precise mechanism of the effect of GPLs on mycobacterium biofilms formation or sliding motility remains unknown. The electrostatic interaction between the fatty acyl tails of GPLs and the hydrophilic agar surfaces represents one explanation [Recht et al., 2000; Recht and Kolter, 2001]. Currently, no experimental data supporting this interpretation can be found.

ROLE IN THE MYCOBACTERIUM PATHOGENESIS

The location and immunogenecity of *M. avium* GPLs imply that they might play a role in the pathogenesis. The macrophages infected with GPL-deficient *M. avium* 2151 strain produces more cytokines and inflammatory chemokines like tumor necrosis factor-α (TNF-α) interleukin-6 (IL-6), interleukin-12p40 (IL-12p40), and RANTES [Bhatnagar and Schorey, 2006]. It was found that ssGPLs as one of toll-like receptor 2 (TLR2) agonists could promote macrophages activation in myeloid differentiation primary-response protein88 (MyD88)-dependent manner, which stimulated MAPKs p38, Jun N-terminal kinase (JNK), and NF-κB to facilitate cytokines secretion [Sweet, 2006]. ssGPLs differs in their ability to activate host immune systems with serotype 1 and 2 stimulating the activation of MAPK and NF-κB as well as the release of TNF-α in MyD88 and TLR2-dependent manner, while serotype 4 fails to function in the same manner [Sweet, 2006]. It was also reported in the early study that serovar 8-specific GPLs but not serovar 4 and serovar 20 had the capability to induce secretion of prostaglandin E2 (PGE2) in human peripheral blood mononuclear cells (PBM) [Barrow et al., 1995]. In macrophage infections, GPLs can interact with the host membranes and promote mycobacterium survival in the host cells by interfering with membrane-mediated functions [Bhatnagar and Schorey, 2007]. Acetylation plays a key role in the recognition for GPLs by the host immune systems. Only when C4 of 6dTal is acetylated, the serovar 2-specific GPL can induce MyD88- and TLR2-dependent signaling response [Sweet et al., 2008]. The acetylation occurring in the serovar 13-specific GPL is also required for its cognition by TLR2 [Naka et al., 2011]. It is generally believed that nsGPLs cannot stimulate the immune response of the hosts, however it was reported that IgG responses to nsGPLs with acetylated dTal indeed occurred in the guinea pigs with MAC infection. In addition, the pool of anti-ssGPL antibodies was different from that of anti-nsGPL antibodies due to the different recognition fragments (oligosaccharide and acetylation respectively) [Matsunaga et al., 2008]. The acetylation at C4 of dTal and the methylation at C3 of Rha are indispensable for nsGPLs to activate macrophages in MyD88- and TLR2-dependent manner [Sweet et al., 2008]. nsGPLs as well as the mannosylated arabinan can delay phagosome maturation, which depends on the expression of the mannose receptor (MR) [Sweet et al., 2010]. The surface-exposed nsGPLs of *M. smegmatis* can inhibit the phagocytosis of many mycobacteria including *M. smegmatis*, *M. kansasii*, *M. avium*, and *M. tuberculosis*, by human macrophages [Villeneuve, 2003]. The internalization of GPL-deficient *M. smegmatis* by macrophages is also more rapid than that of the parent strain [Etienne et al., 2002].

The impact of GPLs on the virulence is often reflected indirectly by the virulences of the strains with different colony morphologies. In MAC, the relation between the virulence and the colony morphology is complex. *M. avium* Rg strain is more virulent than SmT and SmO variants in chickens and mice [Schaefer et al., 1970]. Compared with SmT strain, *M. avium* 101 RgT variant leads to 6–8 times fatality rate in the mice-infection model [Kansal et al., 1998]. It seems plausible that GPL-deficient Rg strain is more virulent than GPL-expressing SmT strain, but there were some contradictory reports, for example, *M. avium* 104 Rg strain with the lower virulence in mice significantly induced high level of TNF-α production [Krzywinska et al., 2005]. In *M. abscessus*, the reports about the relation between the colony morphology and the virulence are consistent. It was observed that *M. abscessus* Rg strain could persist and propagate in the lungs of the infected murine while the smooth variant was cleared soon [Byrd and Lyons, 1999]. Catherinot also found that the GPL-deficient Rg strain led to the higher death rate in the infected mice by intravenous injection [Catherinot et al., 2007]. The absence of GPLs because of the disruption of *mmpL4b* facilitates the *M. abscessus* mutant to replicate in the macrophages and activate TLR2 [Nessar et al., 2011]. Clinical data and epidemiological data also showed that the *M. abscessus* Rg strain was more virulent [Sanguinetti et al., 2001; Jonsson et al., 2007; Catherinot et al., 2009].

The variation among the studies may result from the differences in the strains used and the underlying causes of the morphological changes [Schorey and Sweet, 2008]. For example, the mechanism of conversion between the *M. avium* strains with colony morphologies is different from that of *M. abscessus* strains. In *M. avium*, the irreversible conversion from the smooth strain to the rough variant is due to the deletion of some genes involved in GPL biosynthesis, while the conversion between *M. abscessus* different strains is bidirectional, indicating they adopt a reversible mechanism rather than lost in GPL synthesis genes. In fact, *M. abscessus* Rg strain can produce few GPLs instead of the complete deficiency of GPLs [Howard et al., 2006]. The virulence of *M. abscessus* is not directly reflected with GPLs for it only expresses nsGPLs without strong immunogenicity. These surface-exposed GPLs will mask underlying cell wall lipids involved in stimulating the host immune responses. When GPLs lose or reduce, the underlying bioactive lipids are exposed to interact with the host immune systems, for example, the underlying mannose-containing lipids can activate TLR2 to promote IL-8 and HβD2 expression [Rhoades et al., 2009; Fessler et al., 2011; Nessar et al., 2011; Roux et al., 2011].

Some facets of the GPLs have been established, such as the structure, biosynthesis, and function. However, many outstanding questions remain, such as the regulation of the GPLs biosynthesis, the mechanisms underlying the effect of GPLs on the sliding motility and biofilm formation, the roles of GPLs in the permeability barrier of mycobacteria cell walls and in the interaction between mycobacteria and the hosts. Given the important contributions of GPLs to the physiology and pathogenesis of mycobacteria, the genes participating in the GPLs biosynthesis or/ and the regulation might be potential drug target candidates for the drugs against mycobacterium infection, particularly the NTM-caused infection in the immuno-compromised or aged population.

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