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Genomic evidence for the retention of the essential mycobacterial cell wall in the otherwise defective *Mycobacterium leprae*

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Summary The obligate intracellularism of *Mycobacterium leprae* may be attributable to the effects of mutations in major metabolic areas due to a genome capable of encoding only about 1600 proteins. Yet cell wall biosynthesis capability remains relatively intact and comparisons with the genome of *Mycobacterium tuberculosis* provide insights into the genetic basis of a minimal mycobacterial cell wall.

Abbreviations

ACP:acyl carrier protein; AG: arabinogalactan; Ara*f*: arabinofuranose; C_{50}-P: decaprenyl 5-phosphate; CoA: coenzyme A; DAP: meso-diaminopimelic acid; DMAPP: dimethylallyl diphosphate; DPA: decaprenyl phosphoarabinose; DXP: deoxyxylulose 5-phosphate; FPP: farnesyl diphosphate; Gal*f*: galactofuranose; GAP: glyceraldehyde 3-phosphate; GDP: guanosine 5' diphosphate; GlcNAc: *N*-acetyl glucosamine; GPP: geranyl diphosphate; IPP: isopentenyl diphosphate; LAM: lipoarabinomannan; LM: lipomannan; mAGP: mycolyl-arabinogalactan-peptidoglycan; Mal: malonyl; Man: mannose; Me: methyl; MurNAc: *N*-acetylmuramic acid; MurNGly: *N*-glycolylmuramic acid; P: phosphate; PG: peptidoglycan; PDIM: phthiocerol dimycocerosate; PGL: phenolic glycolipids; PIMs: phosphoinositol mannosides; PAPP: 5' phospho arabinofuranosyl pyrophosphate; PRPP: 5' phospho ribosyl pyrophosphate; Rha: rhamnose; TDM: trehalose dimycolate; TDP: thymidine 5'-diphosphate; TMM: trehalose monomycolate; UDP: uridine 5′-diphosphate

Introduction

In the last decade of biological research dominated by the pursuit of genomic sequence of organisms, the genus *Mycobacterium* has not been left behind. In fact, several species of mycobacteria have been or are currently being sequenced to aid in the prevention and treatment of diseases such as leprosy and tuberculosis in humans and Johne's disease and tuberculosis in cattle.[1–3] The sequences of the virulent *M. tuberculosis* H37Rv, avirulent fast growing *M. smegmatis* and that of the slow-growing *M. leprae* are a valuable data set for comparative studies on physiology and virulence of mycobacteria. Unraveling the genetics of

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essential biosynthetic pathways is the preferred approach for identifying new dug targets and has already yielded several candidates.[4] The sequencing of the *M. leprae* bacterium is especially significant to medicine because it gives the genetic blue print of an organism yet to be cultivated in a laboratory. The sequence has revealed 'a decaying genome' with a dramatic loss of functional capacity by mutations that gave rise to 1116 pseudogenes in every aspect of central and intermediary metabolism resulting in the obligate *in vivo* and intracellular habitat of *M. leprae* [1,5](see also 'The decaying genome of *Mycobacterium leprae*', this issue). The very long doubling time of 11–12 days[6] and the preference for a cooler environment (30°C)[7] may also be due the presence of temperature sensitive mutations in some of the remaining 1600 or so genes. Nevertheless, *M. leprae* can sustain growth and cause disease. The survival of mycobacteria under unfavorable conditions has been attributed to the characteristic permeability barrier of the cell envelope and its role in infection of host macrophages and modulation of immune responses.[8] The properties of the cell envelope are particularly relevant for *M. leprae*, because the organism may depend on the host for several nutrients due to defects in the synthesis of methionine, cysteine, purine rings, and uptake of several amino acids, ions and sugars[1,5] (see also 'The microbial physiologist's guide to the leprosy genome', this issue). In this report we have therefore analyzed the impact of the genome down sizing and decay on the cell wall of *M. leprae*. We have compared the genomes of *M. leprae* and *M. tuberculosis* and assume that genes for the biosynthesis of similar molecules are orthologous (same gene in different genomes) or at least homologous (similar gene) and those genes that are present in *M. tuberculosis*, but absent or pseudogenes in *M. leprae* are probably not involved in these syntheses or are redundant (spare).

We refer the readers to the reviews by Brennan and Nikaido[8], McNeil *et al.*[9], Chatterjee and Khoo[10], Barry *et al.*[11] Baulard *et al.*[12], Belanger and Inamine[13] and Crick *et al.*[14] for details of the structure, function, biosynthesis and distribution among mycobacteria of these envelope components and only provide a brief outline in this review. Instead we have focused primarily on gene assignments for *M. leprae* in relation to those for *M. tuberculosis* based on the completed and annotated genome sequences.

Morphology of *M. leprae* and the ultrastructure of the cell wall

M. leprae is a strongly acid fast staining rod 1–8 μm long and 0.3 μm in diameter and thus does not differ remarkably from *M. tuberculosis*. Studies of the ultrastructure of *M. leprae*, in sections and as whole bacteria from man, mouse and armadillo, have been extensive[15], but have not shown any gross unique features compared to other mycobacteria. However, Draper[16] has described three ultrastructural features of the cell walls that may be characteristic of *M. leprae*: aberrant morphology, wall bands and paracrystalline bodies. He had observed departure from the classical cylindrical shape of a bacillus in suspensions prepared from armadillos, in that cells typically have a tapered or double-tapered shape with hemispherical ends, which he attributed to a defect in the normal process of cell wall construction. Wall bands first observed by Nishiura *et al.* are described as 'circumferential ridges on the outer surface of the cell', very numerous and positioned at random along the length of the cell.[17] These may be scars left when the cell wall separated during the division process and their randomness may reflect a defect in the cell wall construction. The paracrystalline, quasi-crystalline bodies seen in sectioned *M. leprae*[18] probably correspond to the capsular matrices and foamy structures responsible for binding hundreds of bacilli into

'clumps' or globi and into smaller clumps where the individual cells occur in parallel arrays, the noted 'bundles of cigars'.

Biochemical structure and composition

Current knowledge on the biosynthesis and genetics of several components of the mycobacterial cell wall that are described below has evolved primarily from studies with *M. smegmatis, M. tuberculosis*, *M. bovis BCG* and *M. avium* using a combination of methods including chemical and structural analysis, metabolic labeling, cell free assay systems, isolation and characterization of naturally occurring variants or mutants and, more recently, genetic manipulation such as mutagenesis of the genome and recombinant gene expression. However, such opportunities are limiting for *M. leprae* research due to the inability to cultivate the organism *in vitro* and the lack of proven genetic tools. Despite these limitations, sufficient information was gathered on the chemical and structural composition using small amounts of cells obtained from animal or human sources to conclude that the basic architecture of the cell wall is the covalently linked peptidoglycan-arabinogalactan-mycolic acids complex (mAGP) seen amongst all mycobacteria and the related corynebacteria and nocardia, except for few modifications.[19,20]

The peptidoglycan (PG) of *M. leprae* is characteristic of the chemotype IV group that includes mycobacteria, corynebacteria and nocardia because they contain *meso*-diaminopimelic acid (DAP), in the peptide chains.[8] The muramic acids of the sugar backbone are modified with *N*-glycolyl rather than *N*-acetyl groups in mycobacteria and nocardia.[8] A feature unique to *M. leprae* is the substitution of L-ala with glycine in the peptide of peptidoglycan.[21] The effect of this change on the physical properties of the peptidoglycan is not known.

Arabinogalactan (AG) is a polymer of furanose sugars of galactose and arabinose, not found in humans.[22] Typically, a homogalactan (~30 units in *M. tuberculosis*) composed of alternating 5 and 6 linked βD-Gal$_f$ residues is linked to the peptidoglycan via a disaccharide bridge (-L-Rha-D-GlcNAc-P-) called the linker unit (LU).[23] Three branches of 5-linked arabinan are attached near the reducing end of the galactan. The arabinan is composed of 5-linked Ara$_f$, which further branch (3- and 5-linked Ara$_f$ -α). The non reducing ends are composed of the hexaarabinofuranosyl motif [βD-Ara$_f$ -(1→2)-α-D-Ara$_f$]$_2$-3,5-α-D-Ara$_f$-(1→5)-α-D-Ara$_f$ (Ara$_6$).[24] All these major motifs are identical in *M. tuberculosis* and *M. leprae*, with the exception that *M. leprae* has 40–50% fewer galactan residues.[20] Two-thirds of the terminal arabinoses of the arabinan chains are esterified with mycolic acids in *M. tuberculosis*.[25] The extent in *M. leprae* has not been determined.

The α-alkyl branch of the α-alkyl, β-hydroxy fatty acids called mycolic acids which range from C_{14} to C_{26}, is C_{20} in *M. leprae*.[11] The β-hydroxy (meromycolate) chain is often modified with double bonds (cis and trans), cyclopropane, methyl, epoxy, keto, and methoxy groups that render flexibility (fluidity) to the wall. *M. leprae* does not have methoxymycolates[26] due to the lack of a functional *mmaA3* gene, as demonstrated in *Mycobacterium bovis* BCG (Pasteur).[27] It appears that ketomycolates have a more specific role for growth in macrophages in *M. tuberculosis* as their abundance increases 5-fold *in vivo*, and under low oxygen tensions *in vitro* while the methoxymycolates decrease 2-fold. The absence of ketomycolates reduces ability to survive in macrophage like cell lines.[28] Therefore, the lack of methoxymycolates in *M. leprae* may not impair viability in macrophages.

In addition to the mycolates esterified to AG, mycolic acids are also present in the extractable lipids as esters of trehalose: 6-*O*-mycolyl and 6, 6'-O-dimycolyl trehalose (TMM and TDM respectively). Small amounts of TMM but not TDM were identified in *M. leprae*.[29]

The wall of *M. leprae* is also endowed with an unusually abundant proportion of the extractable intercalated lipoglycans, phosphatidylinositol mannosides (PIMs), lipomannans (LM) and lipoarabinomannans (LAM) characterized in several mycobacteria which may be anchored in the plasma membrane via the acyl chains (tuberculostearic and palmitic acid) of phosphatidyl inositol (PI).[30] LM and LAM are made up of linear α-1→6 linked mannan chain originating from PIM_2 in which each mannose is further branched with α-1→2 D-mannose.[31] LAM is a heterogeneous macromolecule arising from LM that contains arabinan branches similar in composition and structure to that of AG that may be terminated with 'caps' of variable numbers of mannose or inositol residues.[10,32] The LAM of *M. leprae* has fewer Ara_6 termini, and a lesser degree of mannose capping than that of *M. tuberculosis*.

However, there are other solvent extractable components[8] such as glycopeptidolipids (GPLs) typical of *M. avium* spp that define serovar specificity and colony morphology; trehalose based lipids such as acylated trehaloses (containing straight chain, mycerocerosic acids, mycolipanolic and mycolipenic fatty acids) and sulpholipids (trehalose 2' sulphate acylated with phthioceranic and hydroxyphthioceranic acids) present in strains of *M. tuberculosis*; and lipooligosaccharides (LOSs) isolated from several species are absent in *M. leprae*. Instead, the dominant lipid is the phenolic glycolipid PGL-1 [33], a glycosylated derivative of the phenolphthiocerol dimycocerosate. The trisaccharide-of PGL-1 of *M. leprae* is β-D-3, 6, di-*O*-methyl Glu (1→4)-α-2,3-di-*O*-methyl-L-Rha-(1→2)-α-3-*O*-methyl-L-Rha and is highly antigenic. Synthetic glycoconjugates containing this trisaccharide are sensitive tools for serodiagnostics of leprosy.[34] Recently the trisaccharide was shown to be involved in the specific interaction of *M. leprae* with the laminin of Schwann cells.[35] This discovery is an important step towards the identification of a mechanism for entry of *M. leprae* into nerve cells that can initiate the subsequent nerve damage that is the hallmark of leprosy. *M. tuberculosis* H37Rv contains only phthiocerol dimycocerosates.[8]

Biosynthesis and genetics of cell wall (envelope) in *M. leprae*: insights from the genome sequence

The biosynthetic pathway for the individual components and their assembly to form the mAGP complex, as deciphered from the approaches mentioned before, is depicted in Figure 1. In the recent review by Crick *et al.*[14], only the 13 *M. tuberculosis* genes for AGP synthesis that have been functionally characterized have been highlighted. Eleven of these were identified by the 'cloning by homology' approach. In Table 1, we show the homologs for these genes in *M. leprae*. We predict that these genes will be functional in *M. leprae* without the need for their functional characterization as the homology is high, and genetic context is similar. Furthermore, we have included putative genes for reactions not yet characterized in mycobacteria, by finding homologs for known genes in other organisms using the BLAST algorithm.[36] The genes for the mycolic acid and phenolphthiocerol dimycocerosate synthesis are also included. In the following section, some of the biosynthetic pathways are described in brief. The common names of the genes involved are indicated in italic font in parenthesis.

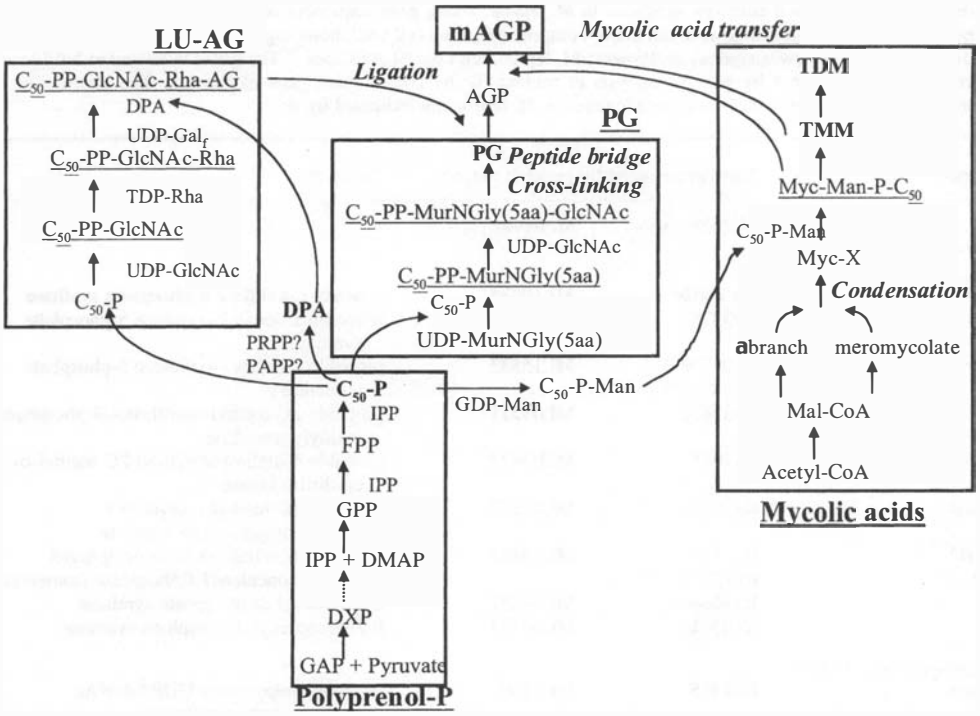

Figure 1. Biosynthesis of the mycolic acid-arabinogalactan-peptidoglycan complex (mAGP) of the cell wall of mycobacteria. The pathways for the synthesis of the individual components and the stages at which they are assembled to form the mAGP complex are shown schematically. The genes involved in these reactions and in the synthesis of the sugar donors are listed in Table 1. Polyprenyl-phosphate linked biosynthetic intermediates have been underlined. 5aa represents the pentapeptide linked to MurNGly in PG synthesis. The Myc-X represents mycolic acid esterified to an unknown carrier. Myc-Man-P-C_{50} is a polyprenyl-phosphate linked mycolate, shown to be involved in transport of mycolic acids through the membrane for deposition on AG (Besra *et al*, *Proc Natl Acad Sci USA*, 1994, **91:** 12735–12739).

PEPTIDOGLYCAN

The basic peptidoglycan biosynthetic machinery is similar to *E. coli* and appears to be remarkably well preserved in *M. tuberculosis*[13,14] and *M. leprae.* The main events are the synthesis of the unusual sugar-nucleotide UDP-MurNAc from UDP-GlcNAc (*murA, murB*), the sequential addition of five amino acids to the MurNAc (*murC, murD, murE* and *murF*), transfer of the sugar-pentapeptide to a polyprenyl-phosphate carrier (*murX*), followed by attachment of GlcNAC from UDP-GlcNAc (*murG*). Glycan chains are formed by transglycosylation reactions of the disaccharide—pentapeptide chains (releasing the lipid carrier from the incoming unit), followed by cross-linking of such chains via DAP-DAP and DAP-D-ala bridges in the final stage of peptidoglycan synthesis. The enzymes for transglycosylation and transpeptidation are members of the penicillin-binding protein (PBP) family. Two *M. leprae* genes (*ponA* and *pon1*) have been cloned and expressed but their role in peptidoglycan synthesis has not been reported.[37,38] The *M. leprae* genome also contains several pseudogenes with homology to PBPs. In mycobacteria, amidation of the carboxyl side chains of glutamate and DAP and the oxidation of the *N*-acetyl group of MurNAc to a glycolyl moiety occur at an undetermined stage in this pathway.

Table 1. Genetics of cell envelope synthesis in *M. leprae*. Using gene sequences of the *M. tuberculosis* or *E. coli* genes involved in the biosynthesis of the major components of the cell wall, homologs were identified in the genome of *M. leprae* (http://www. sanger.ac.uk/Projects/M_leprae) with the BLAST tool.[36] The genes indicated in **bold** have been characterized either by genetic analysis of mutants or by recombinant gene expression. The absence of a homolog is indicated by a dash (–). Pseudogenes in *M. leprae* are indicated by ψ

Name	Number assigned by genome project		Function
	M. tuberculosis	*M. leprae*	
Polyprenyl-P synthesis			
C_{50}-P synthesis			
dxs-I	**Rv 2682c**	ML10388	1-deoxy-D-xylulose 5-phosphate synthase
dxs-II	Rv3379c	–	probable 1-deoxy-D-xylulose 5-phosphate synthase
dxr	Rv2870c	ML15833	probable 1-deoxy-D-xylulose 5-phosphate reductase
ygbP	Rv3582c	ML03211	probable 2C-methyl-D-erithritol-4-phosphate cytidylyltransferase
ychB	Rv1011	ML02422	probable 4-diphosphocytidyl-2C-methyl-D-erythritol kinase
ygbB	Rv 3581c	ML03222	probable 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
lytB2	Rv1110	ML19388	gene function has not been deciphered
idi	Rv1745c	–	probable isopentenyl diphosphate isomerase
	Rv1086	ML24677	E,Z farnesyl disphosphate synthase
	Rv2361c	ML06344	E,Z decaprenyl diphosphate synthase
Peptidoglycan synthesis			
murA	**Rv1315**	ML1150	phosphoenolpyruvate:UDP-GlcNAc enolypyruvate transferase
murB	Rv0482	ML2447	UDP-*N*-acetylenolpyruvoylglucosamine reductase
murC	**Rv2152c**	**ML0915**	UDP-MurNAC: L-alanine ligase
murD	**Rv2155c**	ML0912	UDP-MurNAc-L-ala: D-glutamate ligase
murE	Rv2158c	ML0909	UDP-*N*-acetylmuramyl-tripeptide synthetase
murF	Rv2157c	ML0910	UDP-MurNAc-pentapeptide synthetase
murX	Rv2156c	ML0911	UDP-acetylmuramyl-tripeptide synthetase
murG	Rv2153	ML0915	pentapeptide pyrophosphoryl-decaprenol *N*-GlcNAc transferase
ponA	Rv0050	**ML2688**	PBP; transpeptidase or transglycosylase
ponA′	Rv3682	**ML2308**	PBP; transpeptidase or transglycosylase
Linker unit-arabinogalactan synthesis			
dTDP-rhamnose synthesis			
rmlA	**Rv0334**	ML2503	D-glucose 1-phosphate thymidylyl transferase
rmlB	**Rv3464**	ML1964	dTDP-D-glucose-4,6 dehydratase
rmlC	**Rv3465**	ML1965	dTDP-4-dehydrorhamnose 3,5-epimerase
rmlD	**Rv3266c**	ML0751	dTDP-4-dehydro-rhamnose reductase
UDP-galactofuranose synthesis			
galE	**Rv3634c**	ML0204	UDP-glucose-4-epimerase
glf	**Rv3809c**	ML0092	UDP-galactopyranose mutase
Lipid linked linker unit-arabinogalactan polymerization			
rfe	Rv1302	ML1137	probable UDP-GlcNAc: C_{50}-P GlcNAc transferase
wbbl	Rv3265c	ML0752	probable dTDP-rhamnose: C_{50}-PP-GlcNAc rhamnose transferase
glfT	**Rv3808c**	ML0093	UDP-galactofuranose transferase
embC	Rv3793	ML0106	arabinofuranose transferase? (arabinan synthesis)
embA	Rv3794	ML0105	
embB	Rv3795	ML0104	

Table 1. Continued

Name	Number assigned by genome project		Function
Mycolic acid synthesis and deposition			
α-branch synthesis			
fas	Rv2524c	ML1191	fatty acid synthase (FASI)
Meromycolic acid synthesis			
accD6	Rv2247	ML1657	acetyl-CoA carboxylase (malonyl-CoA synthase)
acpM	**Rv2244**	ML1654	acyl carrier protein
fabD	Rv2243	ML1653	malonyl-CoA-[ACP]-transacylase (malonyl-ACP) synthase
fabH	**Rv0533c**	–	β-ketoacyl-ACP synthase III
kasA	**Rv2245**	ML1655	β-ketoacyl-ACP synthase
kasB	**Rv2246**	ML1656	"
mabA	Rv1483	ML1807	3-ketoacyl-ACP reductase
inhA	**Rv1484**	ML1806	enoyl-ACP reductase
Meromycolic acid modification			
cmaA1	**Rv3392c**	ML0404	cyclopropane mycolic acid synthase (distal)
cmaA2	**Rv0503c**	ML2426	cyclopropane mycolic acid synthase (proximal)
mmaA1	**Rv0645c**	ML1900	trans cyclopropane mycolic acid synthase (oxygenated mycolates)
mmaA2	**Rv0644c**	ML1901ψ	cyclopropane mycolic acid synthase (oxygenated mycolates)
mmaA3	**Rv0643c**	ML1902ψ	methoxymycolate synthase
mmaA4	**Rv0642c**	ML1903	hydroxymycolate synthase
umaA1	Rv0469	ML2460ψ	probable mycolic acid methyltransferase
umaA2 (pcaA)	**Rv0470c**	ML2459	cyclopropane mycolic acid synthase (proximal, α-mycolates)
desA1	Rv0824c	ML2185	probable acyl-ACP desaturase
desA2	Rv1094	ML1952	"
desA3	Rv3229c	ML0789ψ	"
Deposition of mycolic acids			
fbpA	**Rv3804c**	ML0097	mycolyltransferase (TMM, TDM and mAGP synthesis?)
fbpB	**Rv1886c**	ML2028	"
fbpC2	**Rv0129c**	ML2655	"
PGL-1 synthesis			
Mycocerosoic acid synthesis			
mas	**Rv2940c**	ML0139	mycocerosoic acid synthase
fadD28	**Rv2941**	ML0138	probable acyl-CoA synthase
mmpL7	Rv2942	ML0137	mycobacterium membrane protein (transport of PDIM)
Phthiocerol synthesis			
fadD26	**Rv2930**	ML2358	probable acyl-CoA synthase
ppsA	**Rv2931**	ML2357	(phenol) phthiocerol synthase
ppsB	**Rv2932**	ML2356	"
ppsC	**Rv2933**	ML2355	"
ppsD	**Rv2934**	ML2354	"
ppsE	**Rv2935**	ML2353	"
drrA	**Rv2936**	ML2352	?
drrB	**Rv2937**	ML2351	?
drrC	**Rv2938**	ML2350	transport of PDIM
papA5	**Rv2939**	ML2349	polyketide associated protein (transport of PDIM)
Glycosylation of PDIM (trisaccharide synthesis)			
	Rv1524	ML2348	probable TDP-Rhamnose:phenol PDIM rhamnose transferase
	Rv1526c	ML2348?	
	Rv2962c	ML0125	probable UDP-glucose: phenol PDIM-rhamnose glucose transferase

Table 1. Continued

Name	Number assigned by genome project		Function
	Rv2958c	ML0128	"
	Rv2959c	ML0127	probable methyltransferase
	Rv2952	ML0130	probable methyltransferase
PIMs, LM and LAM synthesis			
	Rv0486	ML2443	probable GDP-mannose: polyprenyl-P mannosyl transferase
	Rv2051c	ML1440	"
pgsA	**Rv2612c**	ML0454	CDP-diacylglycerol: inositol phosphitidyl transferase
	Rv2611c	ML0452	probable phosphitidylinositol: GDP-mannose mannose transferase
	Rv2610c	ML0453	probable diacylphosphitidylinositol mannose: palmitoyl-CoA acyltransferase
pimB	**Rv0557**	ML2272ψ	triacylphosphitidylinositol mannose: GDP-mannose mannosyl transferase
	Rv2188c	ML0886	probable mannose transferase (mannan synthesis?)
	Rv3032	ML1715	"
	Rv0225	ML2583	"

Since the *M. leprae* peptidoglycan has glycine rather than L-alanine in the peptide cross-links, it was thought there might be a genetic basis for this substitution. Mahapatra *et al.*[39] analysed the genome but could not find a second ligase gene. In fact they demonstrated by *in vitro* studies with the recombinant MurC enzyme, that it can use L-alanine or glycine substrate with comparable affinities. Therefore the presence of glycine in *M. leprae* is perhaps an *in vivo* phenomenon driven by the ambient amino acid milieu.

ARABINOGALACTAN

The synthesis of AG requires the sugar donors UDP-GlcNAc,[40] TDP-rhamnose (*rmlA, rmlB, rmlC* and *rmlD*)[40,41], UDP-galactofuranose UDP-gal$_f$ (*galE* and *glf*)[42] and decaprenyl-phospho-arabinose DPA.[43] Successive addition of GlcNAc (*rfe*), rhamnose (*wbbl*), galacto-furanose and arabinofuranose on a prenyl-phosphate lipid carrier[44] occurs before the entire LU-AG is transferred (ligated) to approximately 1 in 10 MurNAc units of peptidoglycan.

The synthesis of DPA is interesting and is proposed to originate from the pentose phosphate pathway as phosphoribose pyrophosphate (PRPP).[45] Epimerization of the ribose to arabinose may occur before or after transfer to a decaprenyl-phosphate carrier. Regarding the galactosyltransferases, Mikusova *et al.*[44] showed that the gene Rv3808c (*glfT*) of *M. tuberculosis,* is a galactosyltransferase in AG synthesis. There is an ortholog in *M. leprae*. Furthermore, Kremer *et al.*[46] suggest that it encodes a bi-functional transferase for the alternating 5 and 6 linked galactose residues of the galactan by use of synthetic acceptors. It has not been demonstrated if GlfT can also catalyse the addition of the first galactose unit to the rhamnose sugar of the linker unit and the second galactose of the galactan. GlfT contains the sugar nucleotide binding motif hhhhDxDxh where 'h' represents an amino acid with hydrophobic nature.

With regard to arabinosyltransferases, the work of Belanger[47] suggests that the *embA* or *embB* genes of *M. avium* encode putative transferases for AG. A third gene, *embC,* also exists in all mycobacteria sequenced thus far and Escuyer *et al.*[48] have shown that knocking out the *embA or embB* genes of *M. smegmatis* causes changes in the arabinan content and structure of AG. These Emb proteins are very homologous to each other; they are large and hydrophobic, and span membranes. The genes are well conserved amongst many mycobacteria and are intact in *M. leprae* in a gene cluster very similar to that seen in *M. tuberculosis.* However, the proteins they encode have no significant homology to any other proteins in the database and no known domains or motifs. It is not clear if the Emb proteins are involved in the actual glycosyltransferase catalysis or in the assembly of the arabinan.

PRENYL-PHOSPHATE CARRIERS

The biosynthesis of many of the key cell wall polymers requires prenyl-phosphate carriers as sugar donors (DPM and DPA) and for carriers of the intermediates of cell wall polysaccharide synthesis (AG and PG).[14] The synthesis begins with the formation of deoxyxylulose phosphate from pyruvate and glyceraldehydes-3P (*dxs-I*), which is converted to the 5-carbon isoprene compounds isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) by the non-mevalonate pathway, also called the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway.[49] In *M. tuberculosis* there are two possible genes for this function (*dxs-I* and *dxs-II*). DXS-I has been shown to be functional by expression in *E. coli.*[50] Studies on DXS-II, are in progress. However, *dxs-II* may be redundant in *M. tuberculosis*, since the *M. leprae* genome has only one gene that is homologous to *dxs-I*. The complete pathway for the synthesis of IPP and DMAPP is not known. However, for all genes identified in *E. coli*[51,52], homologous genes have been found in *M. tuberculosis* and *M. leprae* for this essential pathway. A non-essential IPP isomerase (*idi*) for the interconversion of IPP and DMAPP is present in *E. coli*[53] and *M. tuberculosis* but not in *M. leprae*. The gene responsible for condensation of IPP with DMAP, to form geranyl diphosphate (GPP, C_{10}-PP) has not been identified in any organism. The addition of IPP to GPP results in farnesyl diphosphate (FPP, C_{15}-PP), which is subsequently elongated by seven cycles of polymerization to form decaprenyl diphosphate in a specific stereochemistry in *M. tuberculosis*[54] and also probably in *M. leprae* because homologous polymerase genes exist. In terms of synthesis of other isoprenoids, there are no homologs in *M. leprae* for four other prenyldiphosphate synthase genes found in the *M. tuberculosis* genome, except for the *grcC1* gene, which may be involved in the transfer of a prenyl moiety in the menaquinone pathway. There are also no homologs in *M. leprae* for the *M. tuberculosis* squalene synthase, monoxygenase and cyclase genes probably involved in steroid synthesis.

Biosynthesis of PI, PIMs, LM and LAM

The biosynthesis of phosphatidylinositol mannosides is initiated on the precursor PI using the gene product of *pgsA*[55], followed by mannosylation using GDP-mannose as the sugar donor.[56] Genes for a mannosyltransferase and an acyltransferase are linked to *pgsA* and may be required for the synthesis of PIM_1 and the acylation of mannose to form triacyl PIM_1, respectively. The gene product of Rv0557 (*pimB*) of *M. tuberculosis* has been identified as the second mannosyltransferase.[57] The rPimB was shown to convert labeled

tri-acylated PIM_1 to PIM_2 in the presence of GDP-mannose. PimB belongs to the family of glycosyltransferases that have a conserved C terminal motif EX_7E.[58] This gene is a pseudogene in *M. leprae* and we predict that one of the other genes sharing a homologous active site is used (Table 1). Subsequent mannosylation with undefined enzymes generates LM.

Of the many putative glycosyltransferases in the *M. tuberculosis* genome, several (Rv0539, Rv0696, Rv1781c, Rv1500, Rv1513, Rv1514c, Rv1516c, Rv1518, Rv1520 and Rv1525) do not have orthologs in *M. leprae*. We propose that these enzymes are not involved in the biosynthesis of mannan, arabinan and galactan, since there are no significant structural differences between these molecules in *M. tuberculosis* and *M. leprae*.

Mycolic acids, TMM and TDM and deposition on AG

The synthesis of mycolates occurs in several stages: synthesis of the α-alkyl chain and the primer for the meromycolic acid by the multifunctional fatty acyl synthase FASI enzyme (*fas*)[59]; linking by the β-keto acyl synthase III (*fabH*)[60] and elongation of the primer[11] by the disassociated fatty acyl synthase complex FASII (*accD6, fabD, kasA, kasB, acpM, mabA, inhA*);[61,62] modification of the meromycolic acid (introduction of double bonds, cyclopropane rings, keto, methyl and methoxy groups), probably in parallel with elongation;[63] and finally condensation of the α-alkyl chain and meromycolate. The condensation step and the carrier on which this reaction occurs are not known. The mycolates are then transferred to AG and trehalose to form mAGP and TMM/TDM respectively and may occur using the mycolyltransferases encoded by the members of the antigen 85 complex (*fbpA, fbpB* and *fbpC2*)[64,65]. All of these steps have been characterized in *M. tuberculosis* and homologs for the genes are present in *M. leprae*, with the exception of the gene encoding the linking enzyme FabH. The mechanism in *M. leprae* is not clear.

Phenolic glycolipids PGLs (glycosylphenolpthiocerol dimycocerosates)

The phthiocerol moiety is synthesized using a set of multifunctional enzymes (*ppsA, ppsB, ppsC, ppsD and ppsE*) that contain one or more of the acyltransferase, ketoacyl synthase, keto reductase, dehydratase, enoyl reductase and acyl carrier modules for the polymerization of malonyl-CoA and methylmalonyl-CoA units on a C_{22}-CoA fatty acid precursor.[66] Mycocerosic acid syntheisis occurs by the elongation of fatty acyl-CoA primers with methylmalonyl-CoA[67] (*mas),* followed by the transfer to the phthiocerol using a specific acyl-CoA synthase (*fadD28*). Two membrane associated proteins MmpL7 and DrrC have been shown to be responsible for the transport of the PDIM. In *M. tuberculosis*, genes for all these functions are clustered on the genome and mutations in these genes result in disruption of PDIM synthesis and loss of virulence.[68] In *M. leprae*, the *ppsA-E* genes are intact but have been separated from the *mas*, *fadD28* and *mmpL7* genes. For the addition of the first rhamnose in the trisaccharide of PGL-1 in *M. leprae*, we have analyzed the genome for genes homologous to the rhamnosyltransferases such as *rtfA* of *M. avium* (for addition of L-rhamnose to the 6-deoxy talose in GPL synthesis[69]), and the *wbbL* gene of *M. tuberculosis* (involved in linker unit synthesis). Based on homology searches with *rtfA*, we have

identified the gene ML2348 in *M. leprae* as a candidate. Co-incidentally, ML2348 is located where the phthiocerol gene cluster of *pps/drr/papA5* has separated from the *mas/fadD28/mmpL7* in *M. leprae* and may indicate its role in PGL-1. ML2348 is also homologous to genes used for the synthesis of glycosylated steroids in plants, and for antibiotics such as balhimycin and tylosin (tylN, 6-deoxyallosyltransferase) in microbes.[70] All these homologs use sterol/phenol like acceptors. Furthermore, *rtfA and tylN* encode glycosyltransferases for sugars other than glucose (particularly 6-deoxy hexoses). Combining these pieces of information, we propose that ML2348 is a good candidate for the enzyme that transfers the first rhamnose. We postulate that the genes ML0125 and ML0128 are glycosyltransferases and ML0127 and ML0130 are methylases for the synthesis of the second and third sugars of PGL-1. These genes are located close to the mycocerosoic acid gene cluster *mas/fadD28/mmpL7* genes. Genes that are highly homologous to ML2348 are also present in *M. tuberculosis* (RV1524 and 1526c). However, these are clustered with another *pks* system (pks5). There are also homologs in *M. tuberculosis* for the candidate glycosyltransferases (Rv2958c and Rv2962c) and methyltransferases (RV 2952 and Rv2959c) in a cluster similar to that in *M. leprae*, but may have no function since only phthiocerols and not the phenolpthiocerols are found in *M. tuberculosis*.[8]

Concluding remarks

In this review, we have identified putative genes of *M. leprae* for some biosynthetic pathways by homology searches with known genes of other organisms. We are comfortable with the premise that the genome of *M. leprae* approaches a minimal and perhaps also an 'essential' gene set for all basic structural and biological properties shared by virulent and avirulent mycobacteria, particularly for cell wall core synthesis. Genes for the synthesis of precursor molecules that cannot be obtained from the host environment such as UDP-Gal_f and TDP-Rha for AG, PI for PIMs, LM and LAM and DXP for polyprenyl phosphates have been retained in *M. leprae*.

In terms of the practical approaches to verifying and harnessing this genetic information, recombinant proteins can be purified and used in suitable assays. In the future, it may even be possible to set up *de novo* synthesis of complex macromolecules in crude extracts of *M. leprae*, since viable and high titre *M. leprae* are now available from nude mouse foot pads.[71] In addition, *M. leprae* from this source have been kept viable for up to 6 weeks in broth culture at 30°C. Since the number of functional ORFs is smaller than *M. tuberculosis*, use of microarrays and comparative proteomics, is a reasonable approach to identify genes that are preferentially regulated under defined and modified test conditions *in vitro*. We believe the *M. leprae* sequence information will be valuable towards efforts for elimination of leprosy.

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