

Minireview

The genome of *Mycobacterium leprae*: a minimal mycobacterial gene set

Varalakshmi D Vissa and Patrick J Brennan

Address: Department of Microbiology, Colorado State University, Fort Collins, CO 80523, USA.

Correspondence: Varalakshmi D Vissa. E-mail: Varalakshmi.Vissa@colostate.edu

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Abstract

Comparison of the recently sequenced genome of the leprosy-causing pathogen *Mycobacterium leprae* with other mycobacterial genomes reveals a drastic gene reduction and decay in *M. leprae* affecting many metabolic areas, exemplified by the retention of a minimal set of genes required for cell-wall biosynthesis.

Mycobacterium leprae, 'Hansen's Bacillus', was the first human pathogenic bacterium to be identified, predating the discovery of the tubercle bacillus (*Mycobacterium tuberculosis*) by a decade. The genomes of both have now been decoded [1-4]. The genomes of other mycobacteria are also being sequenced, including those that cause opportunistic infections in people with AIDS (*Mycobacterium avium*) [5], bovine tuberculosis (*Mycobacterium bovis*) [6], and Johne's disease of cattle (*Mycobacterium avium* subsp. *paratuberculosis*) [7]. The sequencing of *Mycobacterium smegmatis* [5], the laboratory model strain used for studying mycobacterial physiology and genetics, and of the phylogenetically related *Corynebacterium glutamicum* [5] and *Corynebacterium diphtheriae* [8], are also under way. Although clinical aspects of the virulent mycobacterial strains vary, they are all intracellular pathogens that are transmitted by the respiratory route and occupy macrophages as their preferred niche [9]. A number of antibodies crossreact amongst these bacterial species, indicating similarities in protein composition, and the basic cell-wall architecture is the same [10]. Thus, comparative genomics is a useful tool for identifying common and divergent pathways.

Cole *et al.* [1,3] have found that, compared to the *M. tuberculosis* H37Rv genome of 4,411,529 base-pairs (bp), which can potentially encode 3,924 genes [3], the *M. leprae* genome of 3,268,203 bp encodes only 1,604 proteins and

contains 1,116 pseudogenes [1]. They have annotated and classified all these genes into various functional categories. Figure 1 depicts this drastic gene reduction and decay in *M. leprae* compared to *M. tuberculosis*, which affects nearly every aspect of metabolism.

Despite numerous experiments that demonstrated metabolic activity by labeling macromolecules such as phenolic glycolipid (PGL)-I, proteins, nucleic acids and lipids with radioactive precursors in bacteriological media or in macrophages infected with host-derived *M. leprae*, multiplication of *M. leprae* cells has not been achieved. The only sources of *M. leprae* are tissues from infected humans, armadillos or mouse footpads [11]. The failure to grow *M. leprae* cells *in vitro* may result from the combined effects of gene reduction and mutations in several metabolic areas (Figure 1b). Mutations are found in genes involved in regulation (encoding repressors, activators, two-component systems, serine-threonine kinases and phosphatases), detoxification (genes encoding peroxidases), DNA repair (the *mutT*, *dnaQ*, *alkA*, *dinX*, and *dinP* genes) and transport or efflux of metabolites such as amino acids (arginine, ornithine, D-alanine, D-serine and glycine), peptides, cations (magnesium, nickel, mercury, ammonium, ferrous and ferric ions and potassium), and anions (arsenate, sulfate and phosphate). In general, pseudogenes are found more frequently in degradative, rather than synthetic, pathways. Genes for the synthesis

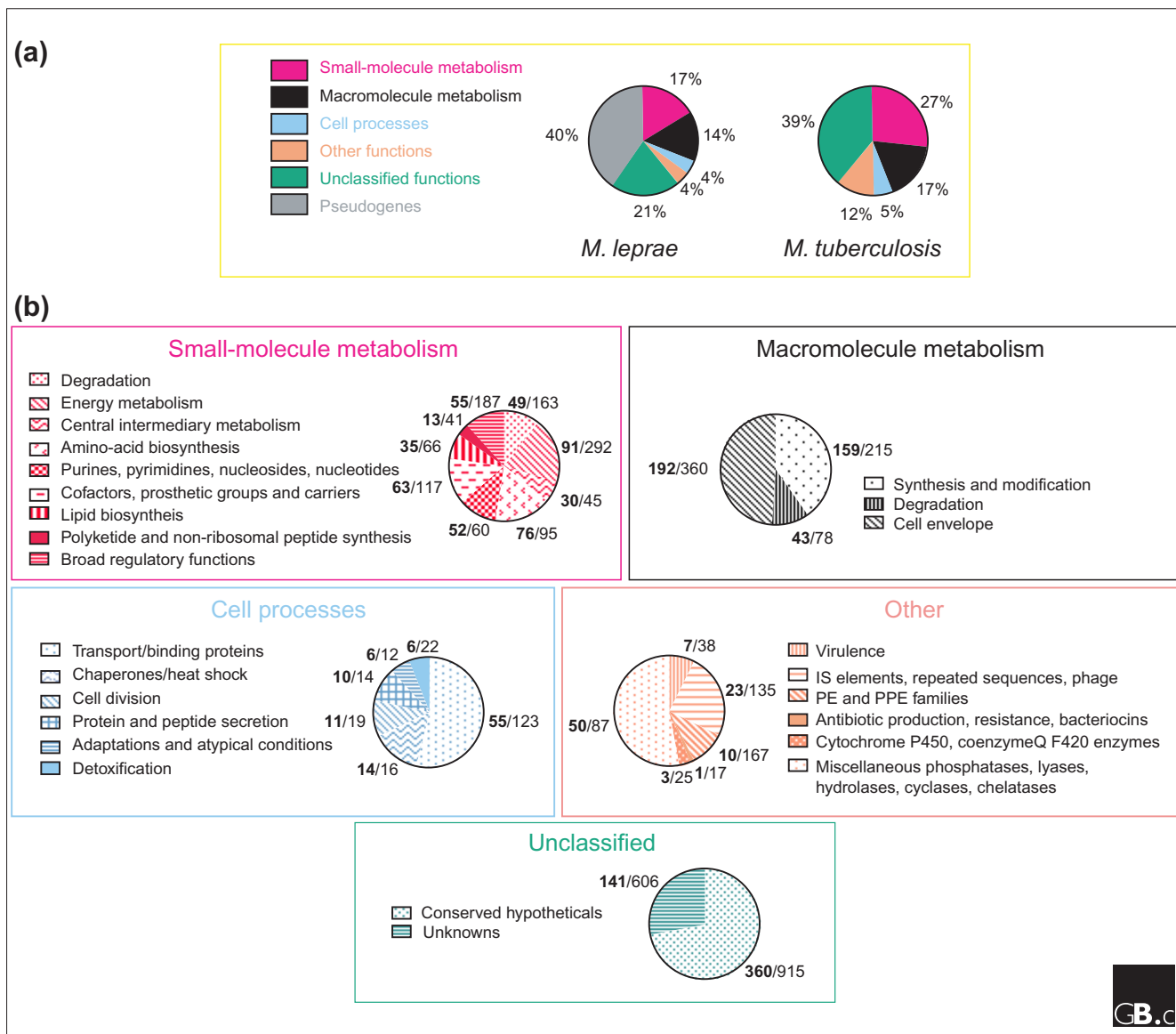


Figure 1

The extent of gene reduction and decay in the genome of *M. leprae*. **(a)** The percentage of the total potential open reading frames assigned to major cellular functions are shown. **(b)** Each category has been sub-classified and the number of putative functional genes in *M. leprae* (after eliminating the pseudogenes) for each subclass are indicated by bold numbers, followed by the corresponding number in *M. tuberculosis*. The data were obtained from the databases of the *M. leprae* and *M. tuberculosis* genome projects [2,4] as annotated by Cole *et al.* [1,3].

of most small molecules, such as amino acids, purines, pyrimidines and fatty acids, and for the synthesis of macromolecules such as ribosomes, aminoacyl tRNAs, RNA and proteins, are reasonably intact.

In terms of gene reduction, there are fewer genes in almost every category, but notably affected are insertion sequences (IS) and the acidic, glycine-rich families of proteins that have proline-glutamic acid (PE) or proline-proline-glutamic acid (PPE) motifs at the amino terminus; these proteins may confer antigenic variation. Repressors, activators, oxidoreductases

and oxygenases are also affected. Thus, while preserving genes required for its transmission, establishment and survival in the host, *M. leprae* has discarded genes that can be compensated for by a host-dependent parasitic lifestyle. Analysis of the *M. leprae* genome therefore provides a useful paradigm for all mycobacteria, because of its smaller genome size, obligate intracellularism, and limited complement of genes. The availability of several completely or partially sequenced mycobacterial genomes allows us to dissect the genetics of conserved and dissimilar pathways, such as those for cell-wall biosynthesis.

Retention of the essence of mycobacterial cell walls in *M. leprae*

Extensive studies of the ultrastructure of the cell wall of *M. leprae*, both embedded in sections and as whole bacteria isolated from infected tissue in man, mouse, and armadillo, have shown properties common to all mycobacteria: beyond the plasma membrane is a rigid, moderately dense layer composed of an innermost electron-dense layer (probably consisting of peptidoglycan, PG, and arabinogalactan, AG), an intermediate electron-transparent zone (the mycolate layer), and an outermost electron-dense layer (probably composed of assorted lipoglycans, free polysaccharides, glycolipids, and phospholipids) [12,13] (see Figure 2).

The underlying framework or 'core' of all mycobacterial cell walls consists of PG, which is covalently attached through a linker unit (LU) (-Rha-GlcNAc-P-) to AG distinguished by furanose sugars (Gal_f and Ara_f) [10,14]; the abbreviations we use in the glycoconjugate and sugar names in this review are defined in Box 1. Attached to the terminal Ara_f units are the mycolic acids (mycolates - (Ara_f)_{~30}-(Gal_f)_{~30}-Rha-GlcNAc-P-PG), the lipophilicity of which provides the dominant physiological features of all mycobacteria [15]. Lipoarabinomannan (LAM), lipomannan (LM), the phosphatidylinositol-mannosides (PIMs), cord factor (trehalose dimycolate), sulfolipids, and proteins are associated with this framework in a physical arrangement that is poorly understood [10] (Figure 2).

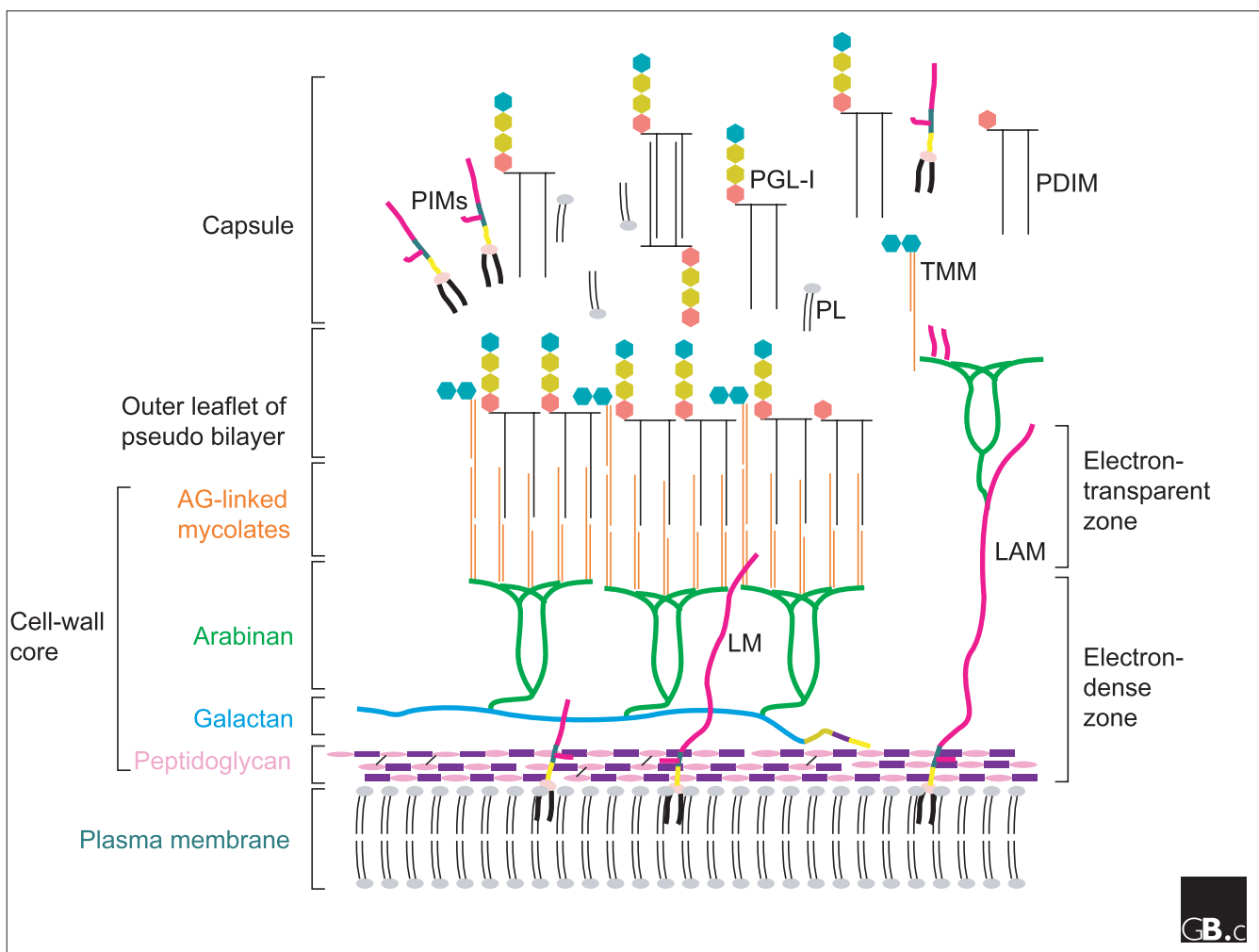


Figure 2

A schematic model of the cell envelope of *M. leprae*. The plasma membrane is covered by a cell-wall core made of peptidoglycan (chains of alternating GlcNAc and MurNGly, linked by peptide crossbridges) covalently linked to the galactan by a linker unit (-P-GlcNAc-Rha-) of arabinogalactan. Three branched chains of arabinan are in turn linked to the galactan. The peptidoglycan-arabinogalactan layer forms the electron-dense zone. Mycolic acids are linked to the termini of the arabinan chains to form the inner leaflet of a pseudo lipid bilayer. An outer leaflet is formed by the mycolic acids of TMM and mycoceroic acids of PDIMs and PGLs as indicated. The pseudo-bilayer forms the electron-transparent zone. A capsule presumably composed largely of PGLs and other molecules such as PDIMs, PIMs and phospholipids surrounds the bacterium. Lipoglycans such as PIMs, LM and LAM, known to be anchored in the plasma membrane, are also found in the capsular layer as shown. Abbreviations are as used in the text and Box 1.

Box 1**A list of abbreviations used in the glycoconjugate and sugar names**

ACP	Acyl carrier protein
AG	Arabinogalactan
Araf	Arabinofuranose
Galf	Galactofuranose
Glc	Glucose
GlcNAc	N-acetylglucosamine
LAM	Lipoarabinomannan
LM	Lipomannan
Me	Methyl
MurNGly	N-glycolylmuramic acid
P	Phosphate
PDIM	Phthiocerol dimycocerosate
PG	Peptidoglycan
PGL	Phenolic glycolipids
PIMs	Phosphatidylinositol mannosides
Rha	Rhamnose
TDP	Thymidine 5'-diphosphate
TMM	Trehalose monomycolate
UDP	Uridine 5'-diphosphate

The limited chemical analysis conducted on the *M. leprae* cell wall to date suggests that it conforms to this pattern, but with modifications [16]. Small amounts of trehalose monomycolate (TMM) are present, but there is no cord factor [17], and, apparently, *M. leprae* contains the full complement of PIMs but is devoid of the trehalose-based mycolipenic-acid-containing sulfolipids characteristic of virulent strains of *M. tuberculosis*. The application of freeze-etching techniques to *M. leprae* in phagolysosomes isolated from infected human, mouse, and armadillo cells showed large quantities of 'peribacillary substances', which appeared as 'spherical droplets', a feature unique to *M. leprae*-infected cells [18]. This material proved to be made up of the *M. leprae*-specific phenolic glycolipids (PGL-I, PGL-II and PGL-III) and the related phthiocerol dimycocerosate (PDIM) [19]. PGL-I consists of the basic phenol-PDIM with the *M. leprae*-specific trisaccharide (3,6-di-O-Me-Glc)-(2,3-di-O-Me-Rha)-(3-O-Me-Rha) in glycosidic link to the phenol component. Lepromatous leprosy is characterized by high titers of antibodies to the trisaccharide unit of PGL-I, and a synthetic derivative has proved useful for serodiagnosis of this condition [20]. Recently, the trisaccharide - notably the terminal 3,6-di-O-Me-Glc unit - was shown to be the *M. leprae*-specific ligand in the characteristic interaction of *M. leprae* and Schwann cells, the glial cells of the peripheral nervous system, which are invaded by *M. leprae* *in vivo* [21]. This discovery is important as it identified an *M. leprae* virulence factor that is involved in causing the characteristic nerve damage observed in some leprosy patients. The glycosyltransferases for the synthesis of PGL-I are therefore good candidate drug targets.

Comparative genomics of cell-envelope synthesis

Understanding of the biosynthesis of mycobacterial cell walls is still evolving, and our knowledge to date is confined to understanding individual components of the cell wall separately; the pathways and regulation of final assembly are not understood. The genetics of some of the pathways that have been elucidated in different mycobacterial species, such as *M. tuberculosis*, *M. smegmatis*, *M. avium* or *M. bovis*, have been compiled in reviews on the mycobacterial cell wall, and putative genes of *M. tuberculosis* have also been predicted on the basis of homology to genes in other bacteria [22,23]. Here, we update these analyses for various wall components - mycolic acids, polyprenyl phosphates, peptidoglycan, linker-unit arabinoglycan, mannans and PGL-I - by including and comparing the findings for the condensed genome of *M. leprae*.

Mycolic acids

The major aspects of acyl-chain elongation leading to the synthesis of mycolic acids in *M. tuberculosis* have been well-defined and are catalyzed by the two fatty-acid synthases FASI and FASII [25]. The *M. leprae* genome contains the full complement of the genes encoding FASII enzymes (*fabD*, *acpM*, *kasA*, *kasB* and *accD6*). In *M. tuberculosis*, it has been proposed that the disassociated FASII is primed by lauroyl-CoA generated by FASI, a reaction that is catalyzed by the β -ketoacyl-ACP synthase FabH [26]. We find that there is no apparent FabH homolog in *M. leprae*, however, pointing to an alternative linking reaction. The lack of methoxymycolates in *M. leprae*, which was demonstrated previously by chemical analysis, may be explained by the fact that the gene for the responsible methoxymycolic acid synthase (*mmaA3*) is in fact a pseudogene. The mechanism of condensation of the α chain (from FASI) and the monomycolate chain (from FASII) to form the mature mycolic acid is not yet understood in any mycobacterium. The three mycolyltransferase genes (*fbpA*, *fbpB*, and *fbpC*) in *M. tuberculosis* that have been implicated in the synthesis of cord factor and also in the transfer of mycolates, to AG, are conserved in *M. leprae* and incidentally also, at least to some extent, in *C. diphtheriae* and *C. glutamicum* (as *cps1*) [27].

Polyprenyl phosphates

In all mycobacteria, the polyprenyl-P lipid decaprenyl-P (C_{50} -P) is central to all aspects of cell-wall biosynthesis as a carrier of the sugar and/or the biosynthetic intermediates. In PG synthesis, a C_{50} -P-P-MurNGly-pentapeptide intermediate is formed, to which GlcNAc is added followed by transpeptidation and transglycosylation. LU-arabinogalactan is initiated on C_{50} -P [28], by successive addition of the GlcNAc, L-Rhamnose, Galf and Araf, before ligation to PG. The sugar donor for arabinan of AG and LAM is C_{50} -P-Araf while C_{50} -P-Man is a donor for mannan synthesis of LM and LAM [22,23]. A C_{35} -P-Man carrier has been proposed as a carrier of mycolic acids [25].

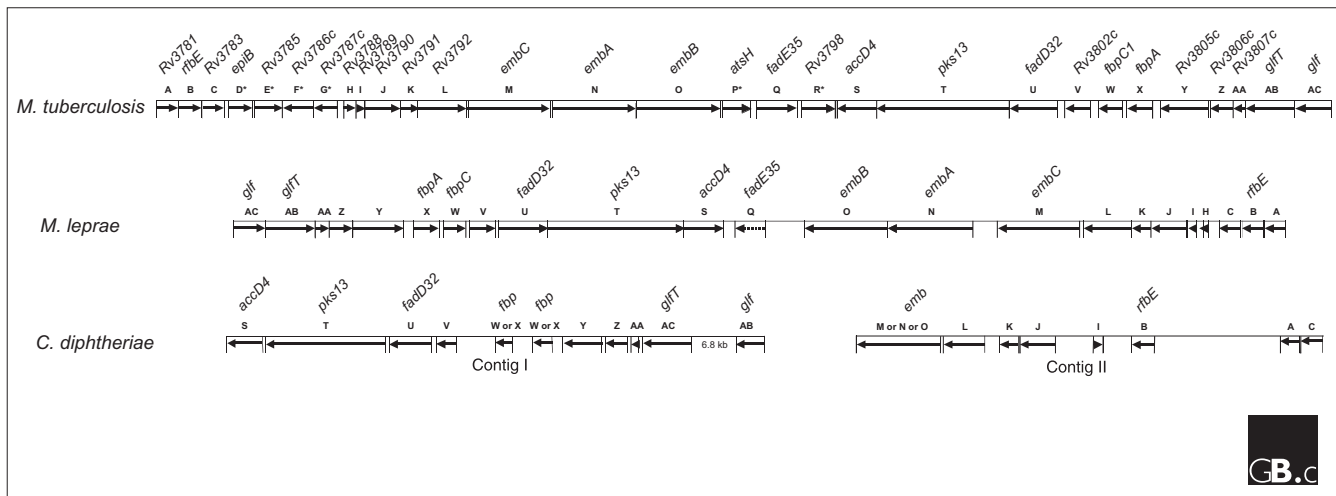


Figure 3

Genetic organization of the putative AG biosynthetic cluster in *M. tuberculosis* [23] and identification of a similar cluster in *M. leprae* and *C. diphtheriae*. The *M. tuberculosis* genes are represented by a letter (A-AC), along with an Rv number or a gene name as annotated in the Sanger Centre *M. tuberculosis* database [4]. Genes D, E, F, G, P and R (asterisked) are absent from both *M. leprae* and *C. diphtheriae*. In *C. diphtheriae*, homologs of genes S-AC and A-M were found on two different contigs (represented here as I and II). *M. leprae* *fadE35* (Q; dotted arrow) is a pseudogene.

The precursors of all mycobacterial polyprenyl-Ps, isopentenyl-P-P (IPP) and dimethylallyl-P-P (DMAPP), are generated by the non-mevalonate deoxyulose-5-P (DXP) pathway [29]. In *M. tuberculosis*, there are two possible genes for DXP synthesis (*dxs1* and *dxs2*), but *M. leprae* has only *dxs1*. Other putative genes in the DXP → IPP/DMAPP pathway (*dxr*, *ygbP*, *ygbB* and *ygbB*) are present in both genomes. A non-essential IPP isomerase (*idi*) is present in *Escherichia coli* for the interconversion of IPP and DMAPP, and a homolog was found in *M. tuberculosis* but not in *M. leprae*. The two isoprenyl-PP synthase genes (Rv1086 and Rv2361c) in *M. tuberculosis* shown to catalyze the synthesis of decaprenyl phosphate [30] have homologs in *M. leprae*. Of five other putative isoprenyl diphosphate synthase genes involved in making other isoprenoid molecules in *M. tuberculosis*, only *grcC1* is present in *M. leprae*. The *grcC1* gene is clustered with genes in the menaquinone pathway in both species and may be involved in the prenylation of menaquinone. *M. tuberculosis* also has genes for sterol synthesis that are absent from *M. leprae*.

Peptidoglycan

The entire *mur* operon of *E. coli* and associated genes involved in PG synthesis have previously been shown to be replicated in *M. tuberculosis* and *M. leprae* (*ftsZ*, *ftsQ*, *murC*, *murG*, *ftsW*, *murD*, *mraY*, *murF*, *murE*, and *ftsI*) [31]. The genes for synthesis of D-alanine and D-glutamic acid from their L-isomers (*alr* and *murI*), and for making D-alanine-D-alanine (*ddlA*) are found in *M. leprae*. Homologs of the *murA* and *murB* are also present, but no good candidate genes encoding key enzymes in meso-diaminopimelic acid synthesis (*dapC* and *dapD*) have been

found in *M. tuberculosis* or *M. leprae*. A hydroxylase for the formation of UDP-MurNGly from UDP-MurNAc has not been identified, and, despite the presence of glycine rather than L-alanine in the peptide crosslinks, *M. leprae* appears to use the conserved ligase MurC for the addition of glycine or L-alanine to UDP-MurNGly rather than having specialized ligases for the two amino acids [31]. Of the putative *M. tuberculosis* genes for transpeptidation and/or transglycosylation, two are found in *M. leprae* (*ponA* and *ponA'*) and three are pseudogenes.

Linker unit arabinogalactan

Genes required for the synthesis of the sugar donor TDP-rhamnose (*rmlA*, *rmlB*, *rmlC* and *rmlD*) for the linker unit, and for the synthesis of UDP-Galf (*galE* and *glf*) for galactan have been cloned and characterized in *M. tuberculosis* and are present in *M. leprae* [32,33]. The arabinose donor for AG is the novel C₅₀-P-Araf, which is probably derived by the epimerization of the ribose in 5-phosphoribose pyrophosphate followed by transfer to a C₅₀-P [34]. Rv3808c (*glfT*) of *M. tuberculosis* encodes a bifunctional galactosyl transferase responsible for adding both the 5- and 6-linked Galf sugars during galactan polymerization [35,36]. There is an ortholog of Rv3808c in a similar genetic context in *M. leprae* (as described below).

The *embA* and *embB* genes of *M. avium* that confer resistance to ethambutol in *M. smegmatis* have been implicated as arabinosyl transferases; and there is an additional *embC* gene in *M. tuberculosis* [37]. These homologous genes are conserved among many mycobacteria and are intact in *M. leprae* within a gene cluster proposed to be involved in

Table 1**Putative glycosyltransferases for cell-wall synthesis**

<i>M. leprae</i>	<i>M. tuberculosis</i> Rv	<i>M. bovis</i>	<i>M. avium</i>	<i>C. diphtheriae</i>	Comments
Putative mannosyltransferases for PIM, LM and LAM biosynthesis					
ML0886	Rv2188c	Y 99/99	Y 80/85	Y 50/63	Homologous to <i>pimB</i> (Rv0557) [39]
ML1715	Rv3032	Y 100/100	Y 87/92	? 29/40	Homologous to <i>pimB</i>
ML0452	Rv2610c	Y 100/100	Y 88/91	Y 48/62	Homologous to <i>pimB</i> ; a candidate mannosyltransferase for PIM ₁ synthesis; part of a cluster of three genes in all these organisms, the other two being phosphatidylinositol synthase gene (<i>pgsA</i>) and a putative acyltransferase [38]
ML2583	Rv0225	Y 100/100	Y 86/94	Y 50/64	Homologous to <i>pimB</i>
Putative synthases of polyprenyl-P sugar donors for mannan and arabinan synthesis					
ML2443	Rv0486	Y 100/100	Y 90/95	Y 51/69	Homologous to <i>pimB</i> ; confers mannosamine resistance in <i>M. smegmatis</i> ; probably involved in LM and LAM biosynthesis [39]
ML1440	Rv2051c	Y 99/99	Y 69/76	? 39/56	Probable polyprenyl-P mannosyltransferase
ML0207	Rv3631	Y 100/100	Y 84/91	? 39/54	
Putative glycosyltransferases for AG synthesis					
ML0752	Rv3265c	Y 98/98	Y 83/88	Y 51/66	Probably <i>wbbI</i> (rhamnosyltransferase for linker-unit synthesis)
ML0113	Rv3782	Y 100/100	Y 87/91	Y 62/73	Putative ligase of lipid-linked AG to PG; part of the putative AG-biosynthetic gene cluster [23]; also found in a similar cluster in <i>C. diphtheriae</i>
Putative glycosyltransferases for PGL synthesis					
ML2348	Rv1524 or Rv1526c	Y 100/100 Y 100/100	Y 65/79 Y 61/74	? ?	Homologous to rhamnosyltransferase (<i>rtfA</i>) of <i>M. avium</i> [44] and plant and microbial glucosyl or 6-deoxyglucosyl transferases; candidate rhamnosyltransferase for PGL-I
ML0125	Rv2962c	Y 99/99	? 27/41	?	Clustered with methyltransferases (<i>ML0127/Rv2959c</i>); candidate genes for glycosyltransferases in PGL-I synthesis
ML0128	Rv2958c	Y 99/99	C-terminal	?	See comments for ML0125
Putative mannosyltransferase for glycoproteins (O-linked)					
ML0192	Rv1002c	Y 99/99	Y 85/91	Y 42/59	Some homology to protein mannosyltransferases in yeast
Unassigned glycosyltransferases					
ML1064	Rv1208	Y 100/100	Y 81/87	Y 49/59	
ML0985	Rv2739c	Y 100/100	Y 85/90	?	Similar to <i>Pseudomonas aeruginosa</i> rhamnosyltransferase
<i>M. tuberculosis</i> glycosyltransferases with no homologs in <i>M. leprae</i>					
Rv numbers 0539, 0696, 1781c, 1500, 1513, 1514c, 1516c, 1518, 1520, 1525					Possibly involved in synthesis of glycans and glucans; not present in <i>M. leprae</i>

Genes were identified by finding homologs for known glycosyltransferases in *M. tuberculosis* and *M. leprae* genomes. The Rv and ML numbers are as listed in the Sanger Centre databases [2,4]. Entries for the unfinished genomes of *M. bovis*, *M. avium* and *C. diphtheriae* are represented by 'Y' for yes, followed by % identity / % similarity at the amino-acid level, if homologous regions could be found, and '?' if no homologs were found at this stage of the sequencing. Funding sources for unfinished genome sequencing are: Beowulf Genomics (*C. diphtheriae*), MAFF and Beowulf Genomics (*M. bovis*) and NIAID (*M. avium*).

several aspects of AG synthesis [23]. This putative AG cluster of *M. tuberculosis* (Rv3781-Rv3809c) includes genes homologous to O-antigen export proteins (Rv3781, Rv3783), unknown glycosyltransferases (Rv3782, Rv3789), mycolyl-transferase (*fbpA*) and galactan genes (*glfT* and *glf*)

(Figure 3). Except for three genes of unknown function, this cluster is present in *M. leprae*. Interestingly, in the unfinished genome of *C. diphtheriae*, this cluster was also found to a large extent, but it appears to be split between two contigs: one contains portions of Rv3781-Rv3793 (which

includes the O-antigen export proteins and has only one *emb* gene); the other contains all the 11 genes Rv3799c-Rv3809c (including homologs for *fbpA*, *glf* and *glfT*).

Mannans

The *pgsA* gene (previously called *pis*) for the synthesis of the PI core of PIMs, LM and LAM was identified in an operon consisting of an acyltransferase and mannosyltransferase in *M. tuberculosis* and *M. smegmatis* and was shown to be essential in the latter [38]. *M. leprae* has a similar operon. In *M. tuberculosis*, it has been shown that after PIM₁ is made by an unknown mannosyltransferase, the gene *pimB*, which encodes the second mannosyltransferase, is responsible for synthesis of PIM₂, the precursor of LM and LAM [39]. Peculiarly, *pimB* is a pseudogene in *M. leprae*. The mannose donor for the synthesis of the bulk of the mannan of LM and LAM is C₅₀-P Man [40] and the mannosyl-transferase gene responsible for its synthesis has been identified in both *M. tuberculosis* (Rv2051c) and *M. leprae*.

PGL-I

In *M. tuberculosis* and *M. bovis BCG*, a cluster of genes for the synthesis of phthiocerol, mycocerosic acids, their ligation and transport to the cell wall have been characterized (*fadD26*, *ppsA-E*, *drrA-C*, *papA5*, *mas*, *fadD28* and *mmpL7*) [41,42]. Interestingly, in *M. leprae*, the genes for phthiocerol synthesis are intact but have been separated from those for mycocerosic-acid synthesis. We have identified putative genes responsible for the synthesis of the three sugars in PGL-I (for details see Table 1). The associated methyltransferase genes are analogous to those associated with glycopeptidolipid synthesis in *M. avium* [43,44].

As described above, we know little about the glycosyltransferases involved in the synthesis of the mycobacterial cell wall such as mannosyltransferases for LM and LAM biosynthesis, rhamnosyl and glycosyltransferases for PGL-I and polyprenyl-P-glycosyltransferases (for C₅₀-P-Araf). By combining information from annotations in the genome databases of *M. tuberculosis* and *M. leprae* [2,4] with the results of BLAST and RPS-BLAST searches [24] and with what is known about some glycosyltransferases (such as *pimB* and *glfT*), we have compiled a list of glycosyltransferases from the genomes of *M. leprae* and *M. tuberculosis* and tentatively assigned certain functions to them (see Table 1). Also included in the searches were the unfinished genomes of *M. avium*, *C. diphtheriae* and *M. bovis*. Such comparative genome analysis should also be helpful in identifying genes for species-specific pathways such as the pathway for sulfolipid found in virulent strains of *M. tuberculosis*.

Analysis of the genes involved in similar pathways across all mycobacterial genomes and *Corynebacterium* will facilitate a complete understanding of the physiology of *Mycobacterium*, *Corynebacterium* and *Nocardia*, including knowledge about their cell walls, the most characteristic and yet

most obscure features of these pathogens. This will allow identification of novel drug targets, formulation of vaccines, and development of new diagnostics. The sequencing of a *Rhodococcus* genome will be a welcome addition. In the case of *M. leprae*, recombinant-protein expression and proteomics will further our understanding, because, as of today, there are no genetic tools for manipulating this pathogen. It will be some time before the insights from comparative genomics of mycobacteria yields benefits to medicine, but we can be hopeful that they are guiding us in the right direction.

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