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

Published: 3 August 2001

*Genome Biology* 2001, 2(8):reviews1023.1–1023.8

The electronic version of this article is the complete one and can be found online at http://genomewww.com/2001/2/8/reviews/1023

© BioMed Central Ltd (Print ISSN 1465-6906; Online ISSN 1465-6914)

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
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.

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].
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) \((-\text{Rha-GlcNAc-P})\) to AG distinguished by furanose sugars \((\text{Gal}^\prime\text{ and Ara})\) [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\(\prime\) units are the mycolic acids (mycolates \(-\text{(Ar}^\prime\text{a)}_{30} (\text{Gal}^\prime)_{30} \text{Rha-GlcNAc-P}-\text{PG}\)), 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](image_url)

**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 MurN Gly, linked by peptide crossbridges) covalently linked to the galactan by a linker unit \((-\text{P-GlcNAc-Rha})\) 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 mycocerosoic 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

| Abbreviation | Definition |
|--------------|------------|
| ACP          | Acyl carrier protein |
| AG           | Arabinogalactan |
| Araf         | Arabinofuranose |
| Galf         | Galactofuranose |
| Glc          | Glucose |
| GlcNAc       | N-acetylgalactosamine |
| 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, polypropenyl 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 (*fbaA*, *fbaB*, and *fbaC*) 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 *cpsi*) [27].

### Polyprenyl phosphates

In all mycobacteria, the polyprenyl-P lipid decaprenyl-P (C₅₀–₇₅P) is central to all aspects of cell-wall biosynthesis as a carrier of the sugar and/or the biosynthetic intermediates. In PG synthesis, a C₅₀–₇₅P-MurNGly-pentapeptide intermediate is formed, to which GlcNAc is added followed by transpeptidation and transglycosylation. LU-arabinogalactan is initiated on C₅₀–₇₅P–P, by successive addition of the GlcNAc, L-Rhamnose, Gal and Araf, before ligation to PG. The sugar donor for arabinan of AG and LAM is C₅₀–₇₅P–Araf while C₅₀–₇₅P-Man is a donor for mannan synthesis of LM and LAM [22,23]. A C₃₅–₇₅P-Man carrier has been proposed as a carrier of mycolic acids [25].
The precursors of all mycobacterial prenyl-Ps, isopentenyl-P-P (IPP) and dimethylallyl-P-P (DMAPP), are generated by the non-mevalonate deoxyxylulose-5-P (DXP) pathway [29]. In *M. tuberculosis*, there are two possible genes for DXP synthesis (*dxx1* and *dxx2*), but *M. leprae* has only *dxx1*. Other putative genes in the DXP → IPP/DMAPP pathway (*dxr, ygbP, ycbB and ygbB*) are present in both genomes. A non-essential IPP isomerase (idli) 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 *gcrCt* is present in *M. leprae*. The *gcrCt* 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 *murL*), 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 arabinosine donor for AG is the novel C95′-P-Araf, which is probably derived by the epimerization of the ribose in 5-phosphoribosyl pyrophosphate followed by transfer to a C95′-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
Putative glycosyltransferases for AG synthesis

| M. leprae | M. tuberculosis Rv | M. bovis | M. avium | C. diphtheriae | Comments |
|-----------|--------------------|---------|----------|---------------|----------|
| **Putative mannosyltransferases for PIM, LM and LAM biosynthesis** | | | | | |
| ML0886 Rv2188c | Y 99/99 | Y 80/85 | Y 50/63 | Homologous to pimB (Rv0557) [39] |
| ML1715 Rv3032 | Y100/100 | Y 87/92 | ? 29/40 | Homologous to pimB |
| ML0452 Rv2610c | Y100/100 | Y 88/91 | Y 48/62 | Homologous to pimB; a candidate mannosyltransferase for PIM, synthesis; part of a cluster of three genes in all these organisms, the other two being phosphatidylinositol synthase gene (pgsA) and a putative acyltransferase [38] |
| ML2583 Rv0225 | Y100/100 | Y 86/94 | Y 50/64 | Homologous to pimB |
| **Putative synthases of polyprenyl-P sugar donors for mannan and arabinin synthesis** | | | | | |
| ML2443 Rv0486 | Y 100/100 | Y 90/95 | Y 51/69 | Homologous to pimB; confers mannosamine resistance in M. smegmatis; 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 wbbI (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 C. diptheriae |
| **Putative glycosyltransferases for PGL synthesis** | | | | | |
| ML2348 Rv1524 | Y 100/100 | Y 65/79 | ? | Homologous to rhamnosyltransferase (rfA) of M. avium [44] and plant and microbial glucosyl or 6-deoxyglucosyl transferases; candidate rhamnosyltransferase for PGL-1 |
| or Rv1526c | Y 100/100 | Y 61/74 | ? | |
| ML0125 Rv2962c | Y 99/99 | ? 27/41 | ? | Clustered with methyltransferases (ML0127/Rv2959c); candidate genes for glycosyltransferases in PGL-1 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 Pseudomonas aeruginosa rhamnosyltransferase |
| **M. tuberculosis glycosyltransferases with no homologs in M. leprae** | | | | | |
| Rv numbers 0539, 0696, 1781c, 1500, 1513, 1514c, 1516c, 1518,1520,1525 | | | | | Possibly involved in synthesis of glycans and glucans; not present in M. leprae |

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), mycolyltransferase (fbpA) and galactan genes (gltT 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 \( \text{Rv3799c-Rv3809c} \) (including homologs for *fbpA*, *glt* and *gltT*).

**Mannans**

The *psaA* gene (previously called *pis*) for the synthesis of the PI core of PIMs, LM and LAM was identified in an operon consisting of an acetyltransferase 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 PIM, is made by an unknown manniosyltransferase, 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 mannos donor for the synthesis of the bulk of the mannan of LM and LAM is \( \text{C}_{100}-\text{P Man} \) [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 phthiocol, 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 phthiocol 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 manniosyltransferases for LM and LAM biosynthesis, rhansmol and glycosyltransferases for PGL-I and polypreinyl-P-glycosyltransferases (for \( \text{C}_{100}-\text{P-Araf} \)). 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 *gltT*), 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 yield benefits to medicine, but we can be hopeful that they are guiding us in the right direction.

**Acknowledgements**

Research conducted in the authors’ laboratory was supported by NIH, NIAID, DMD Contract NO1 AI-55262 from the National Institute of Allergy and Infectious Diseases, NIH, and by the Heiser Program for Research in Leprosy and Tuberculosis, New York City, USA.

**References**

1. Cole ST, Egielmeier K, Parkhill J, James KD, Thomson NR, Wheeler PR, Honore N, Garnier T, Churcher C, Harris D, et al.: *Massive gene decay in the leprosy bacillus*. Nature 2001, 409:1007-1011.

2. The *Mycobacterium leprae* genome project [http://www.sanger.ac.uk/Projects/M_leprae/]

3. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Egelmeier K, Gas S, Barry CE 3rd, et al.: *Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence*. Nature 1998, 393:537-544.

4. The *Mycobacterium tuberculosis* genome project [http://www.sanger.ac.uk/Projects/M_tuberculosis/]

5. The Institute for Genomic Research [http://www.tigr.org]

6. The *Mycobacterium bovis* genome project [http://www.sanger.ac.uk/Projects/M_bovis/]

7. The *Mycobacterium paratuberculosis* genome project [http://www.cbc.umn.edu/ResearchProjects/AGAC/Mtbp/MtbpHome.html]

8. The *Corynebacterium diphtheriae* genome project [http://www.sanger.ac.uk/Projects/C_diphtheriae/]

9. Russell DG: *Mycobacterium and the seduction of the macrophage*. In Mycobacteria. Molecular Biology and Virulence. Edited by Ratledge, C, Dale, J: Oxford: Blackwell Science. 1999: 371-388.

10. Bellisle JT, Brennan FP: *Mycobacteria*. In Encyclopedia of Microbiology. San Diego: Academic Press, 2000: 312-327.

11. Hastings RC, Gillis TP, Krahenbuhl JL, Frenzblau SG. *Leprosy*. Clin Microbiol Rev 1988, 1:330-348.

12. Hirata T: *Electron microscopic observations of cell wall and cytoplasmic membrane in murine human leprosy bacilli*. Int J Lepr 1985, 53:433-440.

13. Draper P: *The bacteriology of Mycobacterium leprae*. Tuberce 1983, 64:43-56.

14. McNeil M, Daffe M, Brennan FP: *Evidence for the nature of the link between the arabinogalactan and peptidoglycan of mycobacterial cell walls*. J Biol Chem 1990, 265:18200-18206.

15. McNeil M, Daffe M, Brennan FP: *Location of the mycolyl ester substituents in the cell walls of mycobacteria*. J Biol Chem 1991, 266:13217-13223.

16. Daffe M, McNeil M, Brennan FP: *Major structural features of the cell wall arabinogalactans of Mycobacterium, Rhodococcus, and Nocardia spp*. Carbaryl Res 1993, 249:383-398.

17. Dharwal KR, Yang YM, Fales HM, Goren MB: *Detection of trehalose monomycolate in Mycobacterium leprae* grown in armadillo tissues. J Gen Microbiol 1987, 133:201-209.

18. Fujikawa Y: *Electron microscopic findings of the peripheral nerve lesions of nude mouse inoculated with *M. leprae* perineural lesions*. Int J Lepr 1988, 56:501.

19. Hunter SW, Brennan FP: *A novel phenolic glycolipid from Mycobacterium leprae* possibly involved in immunogenecity and pathogenicity. J Bacteriol 1981, 147:728-735.
20. Brennan PJ, Chatterjee D, Fujiwara T, Cho S-N: Leprosy-specific neoglycoconjugates: synthesis and application to serodiagnosis of leprosy. Methods Enzymol 1994, 242:27-37.

21. Ramakrishnan AK: Molecular basis for the peripheral nerve predilection of Mycobacterium leprae. Curr Opin Microbiol 2001, 4:21-27.

22. Baulard AR, Besra GS, Brennan PJ: The cell-wall core of Mycobacterium: structure, biogenesis and genetics. In Mycobacteria. Molecular Biology and Virulence. Edited by Ralridge C, Dale J. Oxford: Blackwell Science; 1999: 240-259.

23. Belanger AE, Inamine JM: Genetics of cell wall biosynthesis. In Molecular Genetics of Mycobacteria. Edited by Hatfull GF, Jacobs WR Jr. Washington, DC: ASM Press; 2000: 191-202.

24. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997, 25:3389-3402.

25. Barry CE 3rd, Lee RE, Mdluli K, Sampson AE, Schroeder BG, Slaby RA, Yuan Y: Mycolic acids: structure, biosynthesis and physiological functions. Prog Lipid Res 1998, 37:143-179.

26. Crema KH, Kremer L, Besra GS, Rock CO: Identification and substrate specificity of beta-ketoacyl (acyl carrier protein) synthase III (mtFabH) from Mycobacterium tuberculosis. J Biol Chem 2000, 275:28201-28207.

27. Puech V, Bayan N, Salim K, Leblon G, Daffe M: Characterization of the in vivo acceptors of the mycoloyl residues transferred by the corynebacterial PS1 and the related mycobacterial antigens B5. Mol Microbiol 2000, 35:1026-1041.

28. Mikusova K, Mikus M, Besra GS, Hancock I, Brennan PJ: Biosynthesis of the linkage region of the mycobacterial cell wall. J Biol Chem 1996, 271:7820-7828.

29. Crick DC, Brennan PJ: Antituberculosis drug research. Curr Opin Anti-Infect Invest Drugs 2000, 2:154-163.

30. Schulbach MC, Brennan PJ, Crick DC: Identification of a short (C15) chain Z-isoprenyl diphosphate synthase and a homologous long (C50) chain Z-isoprenyl diphosphate synthase in Mycobacterium tuberculosis. J Biol Chem 2000, 275:22876-22881.

31. Malapantha S, Crick DC, Brennan PJ: Comparison of the UDP-N-acetylmuramato-L-alanine ligase enzymes from Mycobacterium tuberculosis and Mycobacterium leprae. J Bacteriol 2000, 182:6827-6830.

32. Ma Y, Stern RJ, Scherman MS, Vissa VD, Yan W, Jones VC, Zhang F, Franzblau SG, Lewis WH, McNeil MR: Drug targeting Mycobacterium tuberculosis cell wall synthesis: genetics of dTDP-rhamnose synthetic enzymes and development of a microtitre plate-based screen for inhibitors of conversion of dTDP-glucose to dTDP-rhamnose. Antimicrob Agents Chemother 2001, 45:1407-1416.

33. Weston A, Stern RJ, Lee RE, Nassau PM, Monsey D, Martin SL, Scherman MS, Besra GS, Duncan K, McNeil MR: Biosynthetic origin of mycobacterial cell wall galactofuranosyl residues. Tubercle Lung Dis 1997, 78:123-131.

34. Scherman MS, Kalbe-Bournonville L, Bush D, Xiy D, McNeil M: Polypropylphosphate-pentoses in mycobacteria are synthesized from 5-phosphoribose pyrophosphate. J Biol Chem 1996, 271:29652-29658.

35. Mikusova K, Yagi T, Stern R, McNeil MR, Besra GS, Crick DC, Brennan PJ: Biosynthesis of the galactan component of the mycobacterial cell wall. J Biol Chem 2000, 275:33890-33897.

36. Besra GS, Morehouse CB, Rittner CM, Waechter CJ, Brennan PJ: Biosynthesis of mycobacterial lipoarabinomannan. J Biol Chem 1997, 272:18460-18466.