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Comparative analyses of transport proteins encoded within the genomes of *Mycobacterium tuberculosis* and *Mycobacterium leprae*

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Abstract

The co-emergence of multidrug resistant pathogenic bacterial strains and the HIV pandemic has made tuberculosis a leading public health threat. The causative agent is *Mycobacterium tuberculosis* (Mtu), a facultative intracellular parasite. *Mycobacterium leprae* (Mle), a related organism that causes leprosy, is an obligate intracellular parasite. Given that different transporters are required for bacterial growth and persistence under a variety of growth conditions, we conducted comparative analyses of transport proteins encoded within the genomes of these two organisms. A minimal set of genes required for intracellular and extracellular life were identified. Drug efflux systems utilizing primary active transport mechanisms have been preferentially retained in Mle and still others preferentially lost. Transporters associated with environmental adaptation found in Mtu were mostly lost in Mle. These findings provide starting points for experimental studies that may elucidate the dependencies of pathogenesis on transport for these two pathogenic mycobacteria. They also lead to suggestions regarding transporters that function in intra- versus extra-cellular growth.

Keywords

Tuberculosis; leprosy; transport proteins; intracellular versus extracellular life; whole genome analyses; comparative genomics

INTRODUCTION

One third of the human population is infected with tuberculosis (TB), a deadly infectious disease that claims almost 2 million lives per year (1). Unfortunately, TB continues to spread at a staggering rate of one person per second despite the invention of vaccines and the development of control strategies. Moreover, TB mismanagement has led to the increasing prevalence of multidrug-resistant (MDR) and even extensively drug-resistant (XDR) forms of TB. This is particularly problematic because an XDR TB infection in HIV patients is a virtual death sentence, and incidence of HIV continues to rise in many countries (2). The causative agent of this deadly disease is *Mycobacterium tuberculosis* (Mtu).

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Interestingly, Mtu shares evolutionary history with another well-known intracellular parasite, *Mycobacterium leprae* (Mle). Mle is thought to have evolved relatively recently from a common ancestor of both Mle and Mtu (3, 4). However, their lifestyles are dissimilar, as Mtu is a facultative intracellular parasite while Mle is an obligate intracellular parasite. The differences in their lifestyles gave rise to major differences in their genomes. The genome of *M. tuberculosis* CDC1551 (a clinical strain, as compared to H37Rv, which is the laboratory strain) encodes an estimated 4189 proteins while the Mle genome encodes only 1605 proteins. Previous genomic analyses revealed that Mle lost many genes through reductive evolution, some of which are undoubtedly required for extracellular life (5, 6). Still others remain as recognizable remnants called pseudogenes – inactivated genes that no longer produce functional proteins. Not surprisingly, pseudogenes are prevalent in Mle but relatively rare in Mtu (7, 8).

The central paradigm of pathogenesis in latent infection has long been the arrest of Mtu phagosome maturation via phagolysosome biogenesis blockage (9-11). However, there is strong evidence for a paradigm shift in the mechanism behind Mtu's ability to evade destruction and remain metabolically quiescent. A relatively recent study by van der Wel *et al.* showed that the Mtu phagosomes actually fuse rapidly with lysosomes (12). Furthermore, Mtu secretes virulence factors CFP-10 and ESAT-6 via the RD-1 secretion system (12-14) to progressively translocate from phagolysosomes to the cytosol after two days and ultimately causes apoptosis of its host (12). It is possible that Mtu halts phagolysosome maturation, allowing it to synthesize the proteins necessary for translocation. How Mtu seizes control over its macrophage host cell for prolonged periods of time and remains in a state of nonreplicating persistence within granulomas without inducing apoptosis remains to be elucidated.

Comparative genome analyses of the transport systems in Mtu and Mle has the potential to provide missing puzzles to the pathogenesis picture (15). Transporters are essential for bacterial survival and persistence, but of these two organisms, only Mtu must be able to adapt to the various environmental conditions encountered before and during pathogenesis. During primary infection, extracellular Mtu is phagocytosed by macrophages, and its intracellular residency is diverse: phagosome, phagolysosome or cytosol. In the phagosome or phagolysosome, Mtu must secrete appropriate virulence factors by using secretion systems to hijack the phagosomal machinery, withstand acidic conditions, and extrude various toxins. Mtu must also maximize nutrient uptake upon translocation to the cytosolic environment. Upon release into the extracellular tissues, Mtu may also need to pump out antibiotic drugs.

During latent infection, oxygen is significantly reduced in granulomas, and Mtu must adapt to microaerophilic conditions by switching from aerobic to mostly anaerobic respiration using nitrate or another terminal electron acceptor (16, 17). Furthermore, it must remain metabolically quiescent or adopt different metabolic states and express lipid transporters to maximize growth in the granuloma environment that is most likely replete with lipid-rich, acellular debris (18). Specific transporters for the uptake of nutrients and extrusion of toxins and pathogenicity-promoting proteins are essential for extracellular survival (19).

Identification of transporters should lead to a greater understanding of mycobacterial diseases, such as tuberculosis and leprosy (20), and offer experimental scientists starting points for development of vaccines and novel drugs or drug combinations (21). Comparative analyses may reveal which transporters are required for intracellular life as well as extracellular life and possibly offer various points to interfere with pathogenesis (22, 23). We propose that transporters required for adaptive responses will be found in Mtu to a much greater degree than in Mle.

METHODS

The proteomes of Mtu and Mle were screened for homologues of all proteins contained in the Transporter Classification Database (TCDB; www.tcdb.org), (24, 25). FASTA-formatted protein sequences of the completed genomes for the clinical isolate of Mtu (CDC1551) and Mle strain TN were used. The genome sequencing projects for Mtu and Mleare described by Cole et al., 1998 and 2001, respectively (3, 26). Each putative open-reading frame (ORF) was used as a query in the BLASTP software (27, 28) to search for homologous proteins in TCDB. The SEG low complexity filter was not used. In addition, each ORF was scanned with HMMTOP (29) to predict the number of putative transmembrane segments (TMSs), as reported in Table 3. WHAT (30) was used to resolve the differences in the numbers of TMSs between Mtu or Mle proteins and the TCDB homologues (31).

Candidate proteins were subsequently examined in greater detail to estimate their substrate specificities. On the basis of the numbers and locations of TMSs and sequence similarity, transport proteins were classified into families and subfamilies of homologous transporters according to the classification system presented in TCDB. Regions of sequence similarity were examined to insure that homology was in the transmembrane region and not in a hydrophilic domain. Proteins encoded within single operons were identified; operon analyses were performed for all candidate proteins assigned to have transport functions. Operon clusters are indicated by grey shading in Table 3. The substrate specificities of particular homologues identified in the sequenced genomes have been predicted based on homology to functionally characterized genes and from their genomic context (see Table 1). Assignment to a family or subfamily within the TC System often allows prediction of substrate type with confidence (24, 25, 32). When an expected transport protein constituent of a multi-component transport system could not be identified with BLASTP, tBLASTn was performed because such expected proteins are sometimes undetectable by BLASTP due to sequencing errors or pseudogene formation.

These transport proteins were then systematically analyzed for unusual properties with in-house software (unpublished material). Unusual properties can result from events such as genetic deletion and fusion (see Table 3), sometimes resulting in the gain or loss of extra domains or the generation of multifunctional proteins. Additionally, since the Mle genome is particularly replete with pseudogenes, this list of transport proteins was screened for pseudogenes using PSI-FI.

RESULTS

Overview of transporter types

According to the Transporter Classification (TC) System, transporters are classified into five well-defined categories (classes 1 to 5) and two poorly defined categories (classes 8 and 9). The well-defined categories are (1) channels, (2) secondary carriers, (3) primary transporters, (4) group translocators, and (5) transmembrane electron flow carriers (24, 32). The less-well-defined proteins include auxiliary transport proteins (class 8) and transporters or putative transporters of unknown mechanism of action or function (class 9).

Table 1 presents an overall summary of the classes and subclasses of transporters found in Mtu and Mle, according to the TC system. The 285 transport proteins in Mtucomprise 171 transport systems while 132 proteins in Mle make up 59 transport systems. Therefore, 7.2% of Mtu genes and 8.2% of Mle genes encode recognizable transport proteins that correspond to established entries in TCDB, many of which form complete transport systems. These numbers exclude transport proteins most resembling the putative uncharacterized transporters (9.B) because most members of this class of proteins lack functional

information. Also excluded are the additional genes encoding potential transporters that give low BLAST scores to TCDB entries. Instead, they are included in Supplemental Tables (see Tables S1 and S2 for Mtu and Mle, respectively). Thus, the total potential percentage of transport protein-encoding genes is likely to be higher than the aforementioned percentages. While 10-15% of the genes in most free-living bacteria encode transport proteins, genomes of intracellular parasites typically encode lower proportions (33). Not surprisingly, Mtu and Mle encode relatively low proportions of transport proteins. Obligate intracellular bacteria are expected to encode fewer transport proteins than facultative intracellular bacteria, and while this is the case when comparing Mtu with Mle, the Mle genome nevertheless encodes percentagewise more transport proteins than Mtu.

In most bacteria, approximately 3–8% of all the transport proteins encoded in the genome are channel-type transporters (33). Both Mtu and Mle have a surprisingly small number of channel proteins: 2.6% and 2.3%, respectively. Mtu has 6 inner-membrane channel proteins (1.9% of the transport proteins) that comprise 6 channels (3.5% of the transport systems), and 2 recognized outer-membrane porin-type channel-forming proteins (0.6% of the transport proteins), corresponding to 2 systems (1.2% of the transport systems) but one of them is in doubt (34). Mle has 2 inner-membrane channel proteins (1.5% of the transport proteins) that comprise two channels (3.4% of the transport systems) and one recognized outer-membrane porin-type channel-forming protein. These numbers presumably reflect the stable environment of human host cells that have allowed Mle to shed other channels through reductive evolution (15).

Mtu has substantially more secondary carriers (78 systems, or 53% of transport systems identified) than primary active transporters (46 systems, or 31%). However, Mle has roughly the same number of secondary carriers (23 systems, or 42%) as primary active transporters (24, or 44%). This corresponds to a transport system retention rate of 29% for secondary carriers and a staggering 52% for primary active transporters. This observation may reflect the importance of high affinity importers and multidrug efflux systems for intracellular survival. Efflux systems may be more important for intracellular pathogens, which must find ways to cope with toxic drugs that mankind has created as well as excessive cytoplasmic concentrations of natural metabolites and by-products of metabolism. Likewise, this observation suggests that many secondary carriers are not essential for intracellular life. Mtu has four transmembrane electron flow carriers but Mle has just one. This may reflect the massive gene decay by which most of the microaerophilic and anaerobic respiratory chains were lost in Mle. Finally, a much larger proportion of poorly defined systems exist in Mtu(32 systems, or 19%) than in Mle (7 systems, or 12%). It is unclear what role these systems have in the differences between their lifestyles and pathogenesis. However, it can be postulated that they exhibit functions that are commonly found in many bacteria. While systems that have not been characterized necessarily exhibit non-essential functions, most of the well characterized transporters are the ones with more generally important functions, and the ones less well characterized are the ones that have very specialized functions that are likely to be non-essential for ordinary growth.

Transport substrates

Table 2 presents a breakdown of the transporter systems according to substrate type. Sixty-three (37%) and 21 (36%) of the recognized transporters in Mtu and Mle, respectively, are specific for organic molecules and correspond to a retention rate in Mle of 33% (reduction of 67%). Twelve drug transporters were selectively retained in Mle (39%), as were four sugar transporters (57%). A single transport system that is known to recognize vitamins was retained in Mle. While 14 of the 18 amino acid/peptide transport systems in Mtu were selectively lost in Mle (68%), those for carboxylates (5) and nucleotides/nucleosides (1) were completely lost in Mle.

Fifty eight (34%) and 22 (38%) of the recognized transporters in Mtu and Mle, respectively, are specific for inorganic molecules and correspond to a retention rate 38% for Mle. Of these, 15 cation-transporting systems were selectively retained (45%) while only 1 of 5 electron-transporting systems was retained in Mle (20%).

Transporters for macromolecules display the highest retention rate (44%). Surprisingly, while seven (54%) transport systems specific for proteins were retained in Mle, only 3 (27%) transport systems specific for lipids were retained in Mle. Transporters for lipids in Mycobacterial organisms, whose uniquely lipid-rich outer membrane has been implicated in intracellular survival and pathogenicity, would be expected to have been preserved, not lost.

Channels (TC subclass 1.A)

As noted above, Mtu and Mle have small numbers of channel types. As shown in Table 3, Mtu encodes a putative calcium-gated potassium channel of the VIC family (TC 1.A.1) that is absent in Mle. K^+ channels maintain ionic and pressure homeostasis.

Mtu encodes an Amt channel (1.A.11.1) that allows the uptake of ammonia, but Mle does not. Ammonia generally enters cells via the NH_3/NH_4^+ transporters. Ammonia uptake may be possible by free diffusion but only at high concentrations. Thus, ammonia is unlikely to be the primary source of nitrogen in Mle. Mle may acquire nitrogen by uptake of organic compounds instead.

Mtu possesses three putative mechanosensitive channels, one of the MscL-type (1.A.22) and two of the MscS-type (1.A.23). One of the MscS-types (1.A.23.4.1) in Mtu has a cAMP-binding regulatory domain fused to the C-terminus of the MscS homologue, suggesting that it is gated by cAMP (37). Mle possesses only the MscL-type with 70% sequence identity to the Mtu ortholog. These channels open during hypoosmotic stress, and the resultant efflux of solutes provides the cell with pressure relief (38). In comparison to the MscS-type, the MscL-type requires a greater stimulus, opens to a larger pore, and consequently has larger conductance (39). The two MscS-type channels in Mtu most likely provide additional adaptation to various osmotic pressure changes associated with the extracellular lifestyle of Mtu. Mle, which resides solely inside the host cell, only retains the MscL-type as it relies on the host cell's homeostatic mechanism for regulating intracellular pressure (4).

Finally, Mtu and Mle have a single, putative divalent metal ion channel of the MIT or CorA family (1.A.35). CorA family members can be specific for a single divalent cation or can allow entry of several (40). In many bacteria, and especially in *Salmonella typhimurium*, they provide the primary entry pathway for Mg^{2+} (41). These mycobacterial homologues may, therefore, provide the primary mechanism for divalent cation (Mg^{2+}, Co^{2+}, etc.) uptake.

Outer-membrane porins (TC subclass 1.B)

Mtu is a slow growing obligate aerobe. While *Bacillus subtilis* doubles its population every 1.5 hour, Mtu does so every 18-24 hours (7). Its slow growth is generally attributed to the presence of an unusually impermeable cell wall that is unique to Actinobacteria (42). Mycobacteria contain an outer membrane composed of mycolic acids and a large variety of other lipids. Its protective function is an essential virulence factor for both Mtu and Mle.

While the mycobacterial lipid layer offers extensive protection against various environmental insults, Mtu must be able to acquire hydrophilic nutrients. To overcome this outer membrane permeability barrier to hydrophilic solutes, it is anticipated that all mycobacteria need outer membrane porins. These proteins form transmembrane pores that usually allow the energy independent passage of solutes across a membrane.

Two recognizable beta-barrel porins were identified in Mtu, but only one of these is retained in Mle. The beta-barrel porin found in Mtu but not Mle, OmpATb (1.B.6.1.3), is reported to be a member of the OmpA-OmpF Porin family. OmpATb has been reported to be a low activity channel that is essential for adaptation of Mtu to low pH and survival in mouse macrophage (43). OmpA-OmpF homologues form structures consisting of eight transmembrane, all next neighbor, antiparallel, amphipathic β-strands. They form small β-barrels with short turns at the periplasmic barrel ends, and long flexible loops at the external ends. OmpATb may be important in the Mtu pathogenesis as activated macrophages are able to override Mtu's arrest of phagosome acidification when OmpATb is defective (44, 45). However, a recent publication has questioned the conclusion that OmpATb is a porin (34).

The beta-barrel porin found in both Mtu and Mle is an acid-fast, bacterial, outer-membrane porin (1.B.50.1.1) of the AFB-OMP family. This transport protein has single homologs in Corynebacteria and is the first characterized member of a new class of channel proteins found exclusively in mycolic acid-containing outer membranes of acid fast bacteria (46). However, the presence of only one β-barrel porin in Mle cannot alone explain its extremely slow growth. The number of β-barrel proteins cannot necessarily be correlated with growth rate. More likely, most of the porins in Mycobacteria have not yet been identified. Of note, Mah et al. identified as many as 629 outer-membrane proteins in Mtu and 242 in Mle (47). Song et al. identified as many as 144 outer-membrane proteins (48). However, these are predictions based on methodology with higher false-positive rate, and not all outer-membrane proteins are porins.

Secondary carriers (TC subclass 2.A)

The majority of transporters in Mtu are secondary active transporters. Many of these are members of the MFS (TC# 2.A.1). These include three sugar porters of the SP family (2.A.1.1, one) and the ACS family (2.A.1.14, two), all of which are only present in Mtu. Sugar efflux systems (2.A.1.20) were not found in either of these mycobacterial species. MFS transporters also found only in Mtu are four carboxylate transporters of the MHS family (2.A.1.6, three) and SHS family (2.A.1.12, one) as well as a nucleoside transporter of the AzgA family (2.A.1.40).

Many more MFS transporters are involved in drug efflux. Two drug exporters of the DHA1 family (2.A.1.2) are found in Mtu but not Mle. Twelve and one drug export systems of the DHA2 family (2.A.1.3) were identified in Mtu and Mle respectively. Six of the twelve in Mtu most resemble the tetracenomycin:H^+ antiporters (2.A.1.3.12). Two of these putative tetracenomycin exporters have fusions of large domains to the C-termini of the MFS homologues. Both have a cAMP-binding regulatory domain (CAP_ED) followed by a phosphodiesterase domain (RssA), with sequences of about 200 amino acyl residues of unknown function separating these two domains. These particular fusions are undocumented in the literature for any Mtu strain or for other mycobacterial species. A two-component transport system of the DHA2 family, requiring a lipoprotein in addition to the MFS carrier (2.A.1.3.32), was found in Mle as well as Mtu. Two drug exporters of the DHA3 family, most resembling TC entry 2.A.1.21, are present in Mtu but not Mle.

Mtu encodes three nitrate/nitrite antiporters for nitrite extrusion (NarK2) and one nitrate/H^+ symporter for nitrate uptake (NarK1) of the NNP family (2.A.1.8). Mle only encodes one NarK2. Nitrate, a vital source of assimilable nitrogen, is reduced to nitrite under hypoxia and serves as a terminal electron acceptor for anaerobic respiration (49). Nitrite is subsequently excreted by transporters of the NNP family or further reduced by two forms of nitrite reductases. Both nitrite reductases, present in Mtu, are absent in Mle. Lowering intracellular nitrite by reduction does not occur *in vitro* (50), but this may be different *in vivo*. One Mtu NarK2 homologue, encoded by the *narK2X* operon, is associated with the upregulated

nitrate reductase activity that functions under anaerobic conditions (51). Thus, these observations may, in part, help to explain why Mtu is able to adapt to various extracellular environments with low concentrations of oxygen while Mle cannot.

Perhaps more intriguing is the presence of an iron siderophore transporter, the iron (Fe^{3+})·pyridine-2,6-bis(thiocarboxylic acid (PDTC)) uptake transporter, encoded by both Mtu and Mle. The ability to acquire iron is thought to be severely limited in Mle because it has reportedly lost the mbt operon, which encodes for the non-ribosomal peptide synthase required for production of siderophores like mycobactin/exochelin (3). However, this does not necessarily preclude usage of other siderophores. Presence of genes encoding MoeZ (GI: 15827360) in Mle, a protein thought to be essential for biosynthesis of PDTC (52), provides additional evidence that PDTC may function as an important siderophore in Mle. Furthermore, the presence of iron-PDTC transporter, an MFS carrier, and the absence of IrtAB, an ABC primary active transporter (53), in Mle are all consistent with previous findings that iron uptake is pmf-driven rather than ATP-dependent (54, 55). Therefore, we suggest that this PDTC transporter functions in iron uptake for both Mtu and Mle.

Two of the families in the Amino Acid-Polyamine-Organocation (APC) superfamily are represented in both organisms. They are predicted to transport asparagine and cationic amino acids, respectively. Interestingly, two putative asparagine transporters are encoded within the same operon of Mle.

The CDF family of heavy-metal divalent-cation transporters is also represented, with one member in each of Mtu and Mle. However, they do not appear to be orthologous; instead, they probably have different substrate specificities. The Mtu efflux permease resembling (2.A.4.1.1) may export Cd^{2+}, Zn^{2+}, Co^{2+} and also binds Cu^{2+} and Ni^{2+}. The Mle efflux permease resembling (2.A.4.1.2) may export only Zn^{2+} and Co^{2+}. Another transporter encoded by Mtu is distantly related to members of the Zinc (Zn^{2+})-Iron (Fe^{2+}) Permease (ZIP) family. Homology was established since the GAP program gave a comparison score of > 9 S.D..

All four transport systems of the HAE2 family (2.A.6.5) listed in TCDB, a subfamily of the RND superfamily, are identical or similar to corresponding known transporters in Mtu. Members of this family are known to catalyze export of lipids in mycobacterial species as well as antibiotics (e.g., actinorhodin). Mtu encodes 12 carriers of the HAE2 family, and Mle encodes 5. Two separate operons in both Mtu and Mle encode an ActII3-like drug resistance protein (56). Mtu and Mle also encode the MmpL7 protein (2.A.6.5.2) that catalyzes the export of an outer membrane lipid, phthiocerol dimycocerosate (PDIM), a lipid shown to be required for *in vivo* growth and persistence of Mtu (57). The Mtu genome encodes six putative glycopeptidolipid exporters resembling TmtpC (2.A.6.5.3), which has been implicated in sliding motility in *M. smegmatis* and *M. avium* (58). There exists an extra copy in Mtu (gi: 15841024) that may be a pseudogene. Three and one 2,3-diacyl-α, α'-D-trehalose-2'-sulfate (sulfatide precursor) exporters resembling the Mtu MmpL8 protein are present in Mtu and Mle, respectively.

A few additional drug exporting secondary carriers were identified. Mle encodes the Mtu Mmr multidrug efflux pump orthologue of the characterized Mtu protein (2.A.7.1.2) of the DMT superfamily for which the identified substrates are tetraphenylphosphonium (TPP), erythromycin, ethidium bromide, acriflavine, safranin O, and pyronin Y (59). A drug exporter of the MATE family within the MOP superfamily is present in Mtu, and a putative carbohydrate or drug exporter of the MVF family is present by both Mtu and Mle. Proteins in the MVF family have been shown to be important virulence factors in *Salmonella typhimurium* when infecting the mouse (60), but otherwise, little is known about these

putative exporters. Interestingly, both Mtu and Mle homologues have a serine/threonine protein kinase domain fused to the C-termini of these homologues although such domains are lacking in the *S. typhimurium* transporter.

One putative Ca^{2+}:H^{+} antiporter is present in each organism, and two and one putative phosphate uptake permease of the Pit family were found in Mtu and Mle, respectively. A single, probable monovalent-cation exchanger of the CPA1 family was identified in Mtu but not Mle, and a single monovalent-cation exchanger of the CPA2 family was identified in both organisms. While a single arsenite efflux system of the ArsB family was identified in Mle, two paralogs were surprisingly found in the same operon of Mtu. One ammonium transporter of the AMT family, one Ni^{2+}-Co^{2+} transporter of the NiCoT family, and three similar sulfate permeases of the SulP family were identified in Mtu, but all of these transporters were lacking in Mle. More intriguing are the putative manganese transporters of the Nramp family, previously thought to be present only in Mle (3). Two Mle manganese transporter homologues correspond to an Mtu ortholog; one of the Mle homologues displays significant sequence divergence and a fusion of 170 aas at the C-terminus that is observed in other mycobacterial organisms like *M. ulcerans, M. marinum, M. avium, M. abscessus* but not *M. tuberculosis* (both CDC1551 and H37Rv strains). However, the other Mle homologue has very high sequence similarity to the Mtu ortholog and thus, a functional manganese transporter probably exists in both organisms. An arsenite/antimonite exporter of the ACR3 family was found only in Mtu. Interestingly, a protein-tyrosine phosphatase domain (Wzb) is fused to the C-terminus of this homologue. Moreover, a putative Na^{+}-dependent bicarbonate importer of the SBT family was found only in Mtu. These cation and anion facilitators probably function primarily in the maintenance of ionic homeostasis, but they may also play a secondary role in adaptation to various types of stress.

The remaining carriers may transport proteins, amino acids, and carboxylates. A single member of the Oxa1 family (TC 2.A.9) was found in both Mtu and Mle. Bacterial Oxa1 family members facilitate insertion of proteins into the cytoplasm membrane. Present only in Mtu, is a glycine-betaine/proline-betaine:Na^{+} symporter, BetS, of the BCCT family, which may facilitate osmotic stress adapation, and a dicarboxylate transporter of the DAACS family. A TatABC translocase of the twin arginine targeting (Tat) family was identified in both Mtu and Mle. TatA and TatC are encoded within the same operon in both Mtu and Mle, but TatB is present within a distinct operon. It has been shown that TatE proteins are rare, and most organisms have either TatA and TatC, or TatABC (61). The *E. coli* system translocates several redox enzymes to the *E. coli* periplasm including nitrate reductase (NapA) and trimethylamine N-oxide reductase (TorA), but non-redox enzymes can also be exported. Indeed, nitrate reductase activity increases during the anaerobic non-replicating persistence stage (50). A single member from each of the following families was identified in Mtu: the OPT family, the LysE family, and the ThrE family, but none of these putative peptide uptake and amino acid export permeases was found in Mle, consistent with a previous study of the LysE carrier protein (62). This suggests that toxic levels of intracellular amino acids, generated by peptide hydrolysis, are not problematic in Mle although they may be in Mtu. This may be due to the exclusive presence of an Oligopeptide Transporter (OPT) Family member in Mtu (63).

Primary active transporters – ABC superfamily (TC # 3.A.1)

The ABC superfamily of ATP-driven transporters is the largest transporter superfamily represented in the Mtu and Mle genomes. 14 potential ABC uptake systems and 13 potential ABC efflux systems were identified in Mtu, and all of these systems appear to be complete, having all of the expected constituents. Nine potential uptake systems and six potential efflux systems were identified in Mle. This suggests that transporters of the ABC superfamily have been preferentially retained in Mle. Four and three maltose-type systems

of the CUT1 family (TC 3.A.1.1) were identified in Mtu and Mle, respectively. Uptake systems such as these typically have 2 transmembrane permease proteins (M), 1 receptor protein with sugar-specificity (R), and 1 cytoplasmic component (C) that binds and hydrolyzes ATP to provide energy for sugar uptake. All seven of these CUT1 systems in Mtu and Mle are complete, and the constituent components of such a system are encoded within a single operon. The system present in Mtu but lacking in Mle is a transporter most resembling the *E. coli* system (3.A.1.1.3). Each of two operons in Mtu and Mle encode all four components of a related maltose-type system (3.A.1.1.7).

A complete, ribose-type system of the CUT2 family (3.A.1.2), composed of four constituents (1 R, 2 M's, 1 C) encoded within the same operon, was identified in Mle. While two constituents most resemble the fructose/mannose/ribose porter (3.A.1.2.7), the other two most resemble the ribose and autoinducer 2 porter (3.A.1.2.1). As the receptor specificity generally determines the substrate of a transport system, this particular operon in Mle probably encodes four proteins that function together as a ribose transporter. This arrangement of having four components comprising a transport system resembles that of a minority of CUT2 transporters, which usually have just one M subunit.

Interestingly, the Mle specific permease (gi: 15827122) displays a particular fusion that is not observed in any other organism. Aas 20-127 of this Mle protein show homology to the conserved protein domains of the PAS family, which have been found to bind ligands and act as sensors for light and oxygen in signal transduction (64, 65). Aas 143-294 show high sequence similarity to the GGDEF domain, which suggests that this protein has diguanylate cyclase activity. Taken together, the presence of these additional domains suggests that this novel transport system may also initiate signal transduction pathways, possibly depending on the availability of oxygen. How this regulatory activity functions with the ABC uptake of a sugar or an autoinducer-2 interspecies communication molecule will prove to be an interesting area of study. Whether or not this accounts for differences in pathogenesis between Mtu and Mle remains to be investigated (See Discussion).

Two complete ABC oligopeptide uptake systems (3.A.1.5) in Mtu and one in Mle belong to the PepT family. These systems are usually five-component transport systems requiring all five different proteins (2 M's, 1 R, 2 C's) for activity. Only one C constituent is encoded within the operon coding for the other constituents of this system, as is true for a few other members of the PepT family. Four of the five proteins encoded within this operon in Mtu correspond to the 2 M's, 1 R, and 1 C of 3.A.1.5.2. The fifth protein encoded in this operon is not homologous to any constituent in TCDB, and instead belongs to the Filamentation in response to cAMP (Fic) family. Therefore, this protein and the peptide transporter may function together to regulate cell division. In Gram positive bacteria, many pheromones are peptides, so we predict that this transporter is involved in intracellular signaling.

A glutathione transport system (3.A.1.5.11) was identified in both Mtu and Mle. The high sequence similarity between these homologues in the two organisms indicates that they are orthologous and may be important for intracellular life. Indeed, glutathione is an important antioxidant against free radicals (66) that are toxic to mycobacterial cells, and the two enzymes required for glutathione biosynthesis are absent in both Mtu and Mle.

A complete sulfate porter (3.A.1.6.3) of the Sulfate Uptake Transporter (SUT) family was identified in Mtu but not Mle. The four components of this system are encoded by the *cysAWTIsubI* operon in Mtu. The absence of a complete system in Mle, as well as our inability to identify any type of sulfate transporter in Mle, suggests that this organism might utilize organic sulfur compounds as preferential sulfur sources.

Complete ABC uptake transporters specific for (1) phosphate (resembling PstABC/PstS of *E. coli*; TC 3.A.1.7.1; 2 in Mtu and 1 in Mle), (2) molybdate (resembling ModABC of *E. coli*; 3.A.1.8.1; 1 Mtu and 0 Mle), (3) choline (resembling OpuBA, BB, BC, BD of *B. subtilis*; 3.A.1.12.3; 1 Mtu and 0 Mle), (4) iron/zinc/copper (resembling MtsABC of *Streptococcus pyogenes*; 3.A.1.15.6; 0 Mtu and 1 Mle), (5) Fe^{3+}-carboxymycobactin (IrtAB of Mtu; 3.A.1.21.2; 1 Mtu and 0 Mle), (6) thiamine (ThiW of Mtu; 3.A.1.26.4; 1 Mtu and 0 Mle), and (7) long chain fatty acids (resembling PMP70 of *Homo sapiens*; 3.A.1.203.1; 1 Mtu and 1 Mle) were found. Interestingly, a complete iron/zinc/copper uptake system (3.A.15.6) is found in Mle but not Mtu, although a receptor was identified in Mtu.

Many ABC efflux systems were found in Mtu and Mle and they most resemble: (1) the lipopolysaccharide exporter (RfbAB of *Klebsiella pneumoniae*; 3.A.1.103.1; 1 Mtu and 1 Mle), (2) that for daunorubicin and doxorubicin (resembling DrrAB of *Streptomyces peucetius*; 3.A.1.105.1; 1 Mtu and 1 Mle), (3) one exporting oleandomycin (resembling OleC4-OleC5 of *Streptomyces antibioticus*; 3.A.1.105.2; 1 Mtu and 1 Mle), (4) the oleandomycin ATPase (OleB of *Streptomyces antibioticus*; 3.A.1.120.3; 1 Mtu and 1 Mle), (5) the acetate exporter (AatA of *Acetobacter aceti* (BAE71146); 3.A.1.120.5; 2 Mtu and 1 Mle), and (6) a lipid/MDR porter (LmrA of *Lactococcus lactis*; 3.A.1.117.1; 1 Mtu and 1 Mle). The membrane constituent is unknown for the oleandomycin ATPase. ABC efflux systems in Mtu but not Mle most resemble those in other organisms. They may be specific for: (1) lipooligosaccharides (resembling NodIJ of *Rhizobium galegae*; 3.A.1.102.1), (2) macrolides (resembling MacAB of *E. coli*; 3.A.1.122.1; 1 Mtu), (3) lipoproteins (resembling LolCDE of *E. coli*; 3.A.1.125.1), (4) cysteine (resembling CydDC of *E. coli*; 3.A.1.129.1), (5) organic cations and amphiphilic compounds of unrelated structure like antibiotics, anti-viral agents, anti-cancer agents, long-chain fatty acids, peptides, phospholipids, and more (resembling MDR1 of *Homo sapiens*; 3.A.1.201.1), (6) miloxantrone, daunorubicin, doxorubicin, rhodamine, reduced folates, mono-, di- and tri-glutamate derivatives of folic acid and methotrexate (resembling BCRP of *Homo sapiens* (AAC97367); 3.A.1.204.3). Interestingly, two adjacent genes encode two proteins (300 and 349 aas) that together are homologous to the entirety of the MacB homolog (~660 aas). They may function together as a single heterodimeric system. Members of the 3.A.1 family display such domain splittings and still retain function (67, 68). This suggests a transport mechanism that may not require a MacA or TolC homologue, both of which are required in *E. coli* (69).

Primary active transporters – other cation-transporting ATPases

Both Mtu and Mle encode one complete H^+-translocating F-type ATPase (TC 3.A.2). This enzyme can reversibly synthesize ATP using the proton electrochemical gradient (the pmf) as the driving force. Surprisingly, in both Mtu and Mle, ∂-subunits are found fused to the C-termini of the b subunits, and in contrast to all known F-type ATPases, there are 3 b subunits, all encoded within the same operon. Both features are unique and undocumented in the literature. It is possible that the evolutionary pressure to make the genome more compact and the transcription/translation of genes more efficient has led to such fused proteins. It clearly shows that subunits ∂ and b must function together (e.g., as part of the rotor (70)). However, as noted above, protein fusions appear to be far more common in Mycobacteria than in most other prokaryotes.

Equally striking is that Mtu has twelve P-type ATPases (3.A.3) while Mle has just four. In Mtu, two are likely to be specific for Ca^{2+} (efflux), three for Cu^+ or Cu^{2+} (uptake or efflux), four for Zn^{2+}, Cd^{2+}, Pb^{2+}(efflux), and one for K^+(uptake). The four P-type ATPases in Mle are orthologous to some of those in Mtu. Two of these are probably specific for copper (uptake or efflux) and one for Zn^{2+}, Cd^{2+}, Pb^{2+} (efflux). Two P-type ATPases in Mtu are of the functionally uncharacterized P-type ATPase family (FUPA24); only one ortholog is present in Mle.

Primary active transporters – anion-transporting ATPases

The arsenite resistance (Ars) efflux pumps of bacteria consist either of two proteins (ArsB, the integral membrane constituent with twelve transmembrane spanners, and ArsA, the ATP-hydrolyzing, transport energizing subunit, as for the chromosomally-encoded *E. coli* system), or of one protein (the ArsB integral membrane protein of the plasmid-encoded *Staphylococcus* system). ArsA proteins have two ATP binding domains and probably arose by a tandem intragenic duplication event. ArsB proteins all possess twelve transmembrane spanners and may also have arisen by a tandem intragenic duplication event. Structurally, the Ars pumps superficially resemble ABC-type efflux pumps, but there is no significant sequence similarity between the Ars and ABC pumps. When only ArsB is present, as in the Arsenite-Antimonite (ArsB) Efflux family (2.A.45), the system operates by a pmf-dependent mechanism, and consequently belongs in TC subclass 2.A. When ArsA is also present, ATP hydrolysis drives efflux, and consequently the system belongs in TC subclass 3.A. These pumps actively expel both arsenite and antimonite. In Mtu, two proteins most resembling the cytoplasmic ArsA are encoded in the same operon as ArsB. Two Mle orthologs of ArsA are also encoded in the same operon, but an ArsB homolog is lacking in this operon. However, these Mle cytoplasmic constituents probably function together with the aforementioned ArsB protein encoded elsewhere in the genome.

Primary active transporters – ATP-dependent protein secretion systems

As reported previously, Mtu has an essentially complete (10 of 11 components) Sec system (TC 3.A.5), including SecYEG, SecA-1 and SecA-2, SecDF, FtsY, FtsE, Ffh, and the 4.5S RNA. Surprisingly, it has two SecAs but no YajC. Moreover, broken parts of the N-terminal half of the SecD homolog in Mtu show sequence similarity to the SecD domain in the Conserved Domain Database (71). Further, one of the proteins encoded within this operon shows sequence similarity to the receptor component of an ABC peptide transporter, 3.A. 1.5.1. All four of these variations seen in Mtu hold true for Mle, except that Mle has only one SecA. Both Mtu and Mle possess a single member of the septal DNA translocator family (3.A.12), essential for DNA translocation after septum formation in many bacteria. This suggests that septum formation can precede DNA segregation in these organisms (72).

Primary active transporters – cation-translocation electron transfer complexes

Many bacteria possess H^+-translocating NADH dehydrogenase complexes of 14 dissimilar subunits (TC 3.D.1) (73-75). Mtu has these 14 proteins encoded within one operon. The majority of these proteins are most similar to the *Thermus thermophilus* homologues, but a minority most resemble homologues of other organisms. While the *Thermus thermophilus* homologue of subunit M has 11 TMSs, the Mtu homologue has 14 TMSs because an extra ~85 aas at the N-terminus contain 3 TMSs. The subunit M of Mtu is similar to the *Paracoccus denitrificans* homologue, which also has 14 TMSs. The NADH dehydrogenase complex is not present in Mle. Mutations to this complex in Mtu have been implicated in resistance to isoniazid (76), a first-line medication used to treat TB.

Both Mtu and Mle encode the H^+-translocating NAD(P) transhydrogenase complex of two dissimilar subunits most similar to 3.D.2.2.1 of *Rhodospirillum rubrum*. However, an Mtu homologue of the alpha-2 subunit of *Rhodospirillum rubrum* could not be identified, even with tBLASTn. Proteins with demonstrable homology to this alpha-2 subunit were not found in any other actinomycetes.

Both Mtu and Mle also encode an essentially complete proton pumping cytochrome oxidase complex (3.D.4) with five of the six expected proteins (Cox1-3, CoxX, and CtaA). Cox4 was not identified in either Mtu or Mle. Although the cytochrome oxidase complex subunits are all encoded in a single operon in *Bdellovibrio bacteriovorus* (77), each subunit identified

in Mtu and Mle is encoded within a different operon (e.g., five subunits within five, entirely different operons). Interestingly, the CoxX homologue in Mle was predicted to be a pseudogene by the PSI-FI program. Nonetheless, these enzyme complexes may be capable of coupling proton export to electron flow (78).

Group translocators –Acyl-CoA Ligase-coupled Transporters

The putative acyl-CoA ligase-coupled transporters (4.C.1, 2 and 3) use the energy of ATP to thioesterify fatty acids and other acids such as carnitine in a process thought to be coupled to transport. A role in group translocation is not fully accepted, and many acyl-CoA ligases clearly do not function in this capacity. Indeed, the FAT family (4.C.1) includes fatty acyl-CoA ligases (fatty acyl-CoA synthetases), carnitine CoA ligases, and putative fatty acid transporters (79). Animals, yeast and bacteria have numerous paralogues that may exhibit 2-4 TMSs and may be up to 500-600 residues long (80). The proteins with 2-4 TMSs may be transporters, but those with none are not likely to be. Of the 38 putative fatty acyl-CoA synthetases in Mtu, only one has been identified as a potential lipid transporter based on previous finding (79). Many of these candidate lipid transporters (see Supplemental Table S3) display transmembrane segments and may serve as transporters. These putative lipid transporters may be essential for adaptation to the lipid-rich, oxygen-poor granulomas during latent infection.

Transmembrane electron transport systems

Cytochrome c is a major component of the respiratory electron transport chain. An Mtu protein and its Mle ortholog most resembling the cytochrome c-type biogenesis protein (CddA) of the Disulfide Bond Oxidoreductase D (DsbD) family (5.A.1) were identified. Additionally, a putative mercuric ion reductase (MerA) was found in Mtu but not Mle.

Nitrate reduction allows for growth under anaerobic conditions. The *narGHJI* operon of Mtu encodes protein subunits that most resemble those of the anaerobic, respiratory, membrane-bound nitrate reductase (5.A.3.1.2) of the Prokaryotic Molybdopterin-containing Oxidoreductase (PMO) family. As shown in Fig. 2, the nitrate reductase system has 3 components. They are transcribed in the order: alpha chain (NarG, 1245 aas, the hydrophilic component that reduces NO_3^- to NO_2^-), the beta chain (NarH, 512 aas, the hydrophilic component that has 4 iron-sulfur centers), and the gamma chain (NarI, 225 aas, the 5 TMS hydrophobic component that anchors the alpha and beta chains to the membrane). Assembly of this system is aided by a chaperon protein, the delta chain (NarJ, 206 aas, located in between the two genes encoding the beta and gamma chains). The nitrate reductase activity in Mtu is associated with this operon (51). This entire operon is absent in Mle, consistent with it being an obligate aerobe.

There are other known electron acceptors including fumarate and formate. Fumarate reductase was found in Mtu (26) (Cole et al., 1998), but an incomplete system of it (FrdA and FrdB only) was found in Mle. Formate dehydrogenase (with the exception of a likely pseudogene of FdnG in Mtu) was not found in either Mtu or Mle.

The *narK2X* operon of Mtu encodes NarK2, the aforementioned nitrite exporter, and NarX, which is annotated as a nitrate reductase because of its homology to the various subunits of the aforementioned nitrate reductase (see Fig. 2). Two deletions from the duplication of *narGHJI* may have given rise to NarX. *narX* expression is localized to the lymphocyte cuff and transition zone but not in the necrotic zone, as evidenced by an *in situ* detection of Mtu transcripts in human lung granulomas (81). However, induction alone during hypoxia (51, 82) does not establish a functional role in nitrate reduction (8). *narX* mutants display wild-

type nitrate reductase levels in Mtu (51), and the absence of NarX in Mle suggests that the Mtu NarX may be a pseudogene.

Two additional members of the PMO family were identified in Mtu but not Mle: the biotin d-sulfoxide reductase (BisC; 5.A.3.4.3) and the thiosulfate reductase precursor protein (PhsC; 5.A.3.5.1) of the PhsABC complex. While the thiosulfate reductase electron transport protein, PhsB, is encoded in the same operon as PhsC in *S. typhimurium*, none of the genes from the operon in which the Mtu PhsC homolog is encoded encode proteins homologous to PhsB. However, the beta subunit of nitrate reductase, NarH, shows significant sequence similarity to PhsB, suggesting that PhsC might function together with subunits of the nitrate reductase encoded by *narGHJI*. Oxygen, nitrate, thiosulfate, and biotin d-sulfoxide may serve as final electron acceptors in Mtu. An Mtu protein annotated as a putative formate dehydrogenase (see Supplemental Table S1), with no such Mle orthologue, may be a pseudogene lacking functionality.

Recognized transporters of unknown biochemical mechanism

In the 9A category of incompletely characterized transporters, we find many transporters encoded by Mtu but not Mle that most resemble: (1) a Mg^{2+}, Co^{2+} transporter of the MgtE family (9.A.19; 1 in Mtu), (2) a tellurium ion resistance efflux permease of the TerC family (9.A.30; 1 Mtu) (83), and (3) a Co^{2+} transporters of the HlyC/CorC (HCC) family (9.A.40; 2 Mtu). A CorC homologue is probably not present in Mle because the CorC homologue of *Bacillus subtilis*, YrkA, is believed to function as an auxiliary protein to the CorA Co^{2+}/Mg^{2+} channel of *S. typhimurium* (84). CorA, found in both Mtu and Mle, is a member of the Metal Ion Transporter (MIT) family of α-type channels (TC #1.A.35).

A specialized secretion system in mycobacteria, the ESX-1 system (9.A.25), is required for the secretion of virulence factors like ESAT-6 and CFP-10, which are small proteins of the Esx family and which lack the traditionally recognizable Sec-signal sequence (85-88). ESAT6, CFP-10, and several components of the ESX-1 system are encoded by the RD1 gene cluster (89, 90), which is one of the five regions of difference (RD) that were identified by comparing *M. tuberculosis* H37Rv, *M. tuberculosis* H37Ra, *M. bovis* and the attenuated *M. bovis* BCG (91-97) strains. The RD1 operon has been deleted from the *M. bovis* strain to give rise to the attenuated BCG strain, presumably from serial passage for the development of the BCG vaccine. Moreover, virulence is restored when the attenuated *M. bovis* BCG strain is complemented with the RD1 gene cluster (94, 96).

Although the exact number of components to the ESX-1 system is still debated, a set of RD1 proteins with domains of known function has been implicated as essential to virulence. This system includes a multitransmembrane protein, Rv3877 (Snm4), and two putative SpoIIIE/FtsK ATPase family members, Rv3870 (Snm 1) and Rv3871 (Snm2). These three proteins are required for secretion of ESAT-6 and CFP-10. ESAT-6 (product of the *esxA* gene) and CFP-10 (product of the *esxB* gene) interact to form a 1:1 dimer (98, 99), and the stability of these proteins is interdependent *in vivo*. CFP-10, but not ESAT-6, interacts with the C-terminal domain of Rv3871, a cytosolic component of the ESX-1 system (87). A second, non-RD1 gene cluster, the Rv3614c-Rv3616c locus, is also required for ESAT-6 secretion (100). They are homologous to Rv3864-Rv3867 from RD1. All known components of this system, as listed in TCDB, were identified in Mtu (the CDC1551 strain) and Mle. In Mle, no recognizable homologue of Rv3872 was identified. The secretion of ESAT-6 and CFP-10 is critical for Mtu virulence, but the molecular mechanisms of ESX-1 substrate selection and secretion are unclear.

Additional homologous pairs of ESAT-6/CFP-10 exist in Mtu, but only four of these pairs, each of which is encoded by tandem genes, are surrounded by genes encoding the

components of ESX-1 as listed in Table 3. These four gene clusters are: ESX-2, ESX-3, ESX-4, and ESX-5 (9.A.40). These clusters vary in the numbers of genes present from 7 to 18. These clusters probably arose by gene duplication (101). Surprisingly, these systems do not complement one another, although ESX-3 and ESX-5 appear to be essential (102). Rv3870-Rv3871 homologues in each of the ESX2-5 clusters in Mtu are found fused to one another, supporting the current notion of Rv3870 and Rv3871 functioning together as an FtsK/SpoIIE-like ATPase. It is thought that Rv3871 interacts with CFP-10 and delivers the heterodimeric complex in an ATP-dependent manner to Rv3870, thereby delivering these virulence factors to the secretory machinery. The molecular mechanism by which ESX secretes virulence factors remains to be elucidated.

Only ESX-1, ESX-3, and ESX-5 were identified in Mle. While the majority of ESX-2 components were lost in Mle, ESX-4 was lost in its entirety. This is surprising because ESX-4 is thought to be the most archaic of the ESX clusters (103). Two components of ESX-3 are not found in Mle although several components of ESX-3 are thought to be essential for growth of Mtu (104).

(Putative) transporters of unknown function or mechanism

In the 9B series of putative permeases, we find two Mtu and two Mle homologues of bacterial murine precursor exporters of the MPE family (TC 9.B.30, recently changed to 2.A.103), which are found in many, if not all, bacteria. These porters probably serve the function of exporting precursors essential for bacterial cell wall synthesis (105, 106). A putative Mg^{2+} transporter-C of the MgtC family (9.B.20) was identified in Mtu but not Mle. MgtC was thought to be an auxiliary protein for the MgtB protein, which is known to be a Mg^{2+} transporting P-type ATPase (3.A.3). However, this MgtC homologue in Mtu is found in a region of the genome that does not encode a comparable MgtB homologue. Moreover, loss of MgtC, due to an *mgtC* knock-out mutation, prevents growth of the bacteria at low Mg^{2+} concentrations (10-50 μM) under low pH conditions (pH 6.2—6.8). Growth was restored at higher concentrations of Mg^{2+} (100 μM) (107). The results are consistent with a Mg^{2+} uniport mechanism, but a transport function for MgtC has not yet been established. MgtC is required for intramacrophage survival. Also identified in Mtu but not Mle are proteins homologous to transporters of: the PTT family (9.B.22), the PF27 family (9.B.26), the YdjX-Z family (9.B.27), the Hly III family (9.B.30), the ExeAB family (9.B.42), the YnfA family (9.B.45), and the CstA family (9.B.59).

DISCUSSION AND OVERVIEW

While *M. leprae* (Mle) is an obligate intracellular pathogen, *M. tuberculosis* (Mtu) is a facultative intracellular pathogen that adapts to its changing environment prior to and during its various stages of pathogenesis. We have analyzed the transporters in these organisms partly to determine what systems might confer upon Mtu its ability to survive in various extracellular environments. We also wanted to determine what systems might be essential for intracellular survival, as those retained in Mle are implicated in such roles. We identified several putative transport proteins in addition to those reported in the original genome annotation efforts for both Mtu and Mle. Our most interesting and provocative findings will be summarized here with emphasis on the potential physiological and pathological importance of some of our observations. Actual physiologic conditions faced by Mtu and Mle are largely unknown and require much more investigation (108). Transcriptome data are scattered throughout the literature and infrequently confirmed (109). Nonetheless, transcriptome data for a variety of different conditions are abundant (110-112). Much of it can be found on the Tuberculosis Database (113), which contains microarray data for specific conditions and URLs to publications, if any, that generated the data.

The electron transport chain

The electron transport chain (ETC) is essential for both Mtu, a facultative aerobe, and Mle, an obligate aerobe. Typically, catabolic processes generate NADH, which is generated by the NADH dehydrogenase complexes of 14 dissimilar subunits in bacteria. Consistent with previous reports, this complex was identified in Mtu but not Mle (3). A proteomic study of Mle did not identify this NADH dehydrogenase; instead, alcohol dehydrogenase and lactate dehydrogenase were identified, suggesting an alternative pathway to regenerate NAD^+ (114). The H^+-translocating F-type ATPases in Mle and Mtu provide a means to interconvert chemiosmotic and chemical energy. The presence of the majority of proton pumping cytochrome oxidase complex subunits (3.D.4) in both Mtu and Mle provides a direct means to generate a pmf. Interestingly, the subunits of a single complex are not encoded within one operon, as seen in many other bacteria.

Iron-sulfur centers are present in several complexes of the ETC. In Mtu, sulfur is probably acquired by one of three sulfate permeases (2.A.53) or by the complete ABC-type sulfate porter (3.A.1.6.3). These transporters are not encoded by Mle suggesting that the primary means to acquire sulfur in Mle may be through the uptake of sulfur-containing organic compounds. Although the ABC-type uptake system for cysteine is present in Mtu but not Mle, additional transporters of other sulfur-containing organic compounds exist. Fe^{3+} ions have a very low solubility at low pH, but siderophores can chelate these ions to increase solubility. Transporters with specificity for iron-siderophore complexes such as the iron (Fe^{3+}) · pyridine-2,6-bis(thiocarboxylic acid (PDTC)) uptake transporter are encoded by both Mtu and Mle. In general, iron uptake can be mediated by facilitated diffusion with secondary carriers or by active transport. A transporter of the Zinc-Permease (ZIP) family and the Fe^{3+}-carboxymycobactin transporter were found in Mtu but not Mle. Taken together, the absence of these two transporters in Mle and the observation that iron uptake may be mediated by facilitated diffusion in Mle suggests that PDTC may be the primary means of iron uptake in Mle.

When oxygen is not readily available to Mtu, nitrate can be used as the terminal electron acceptor. Nitrate is reduced by the nitrate reductase. NarG requires the molybdenum-containing molybdopterin cofactor. Molybdate uptake in Mtu is achieved by a transport system that resembles ModABC of *E. coli* (3.A.1.8.1). Consistent with the observation that Mle is an obligate aerobe, neither the nitrate reductase complex nor the molybdate transporter is found in Mle.

Additional compounds can serve as the terminal electron acceptor in Mtu. The presence of biotin d-sulfoxide reductase (BisC of 5.A.3.4.3) and the thiosulfate reductase (PhsC of 5.A. 3.5.1) suggests that biotin-d-sulfoxide and thiosulfate may be utilized in lieu of oxygen and nitrate. Fumarate likely also serves as an electron acceptor in Mtu (26) but not in Mle, as only an incomplete system was found.

Evasion of host immunity, persistence, and proliferation

Several types of specialized secretion systems are dedicated to the secretion of virulence factors and play important roles in many stages of both Mtu and Mle pathogenesis. There are several clusters of ESX named ESX-2-5. ESX-3 is regulated by iron and zinc availability (115, 116). Zinc may be acquired by Mtu with the zinc-iron permease of the ZIP family. Mle does not have an ortholog of this permease, and how it obtains zinc is unclear.

ESAT-6 and CFP-10, and possibly other virulence factors, are implicated in arresting maturation of phagosomes in macrophages. The fusion of phagosomes to lysosomes is blocked, preventing acidification of their environment. However, macrophages may overcome this arrest upon activation by cytokines like interferon-gamma. OmpATb (1.B.

6.1.3) is essential for adaptation to low pH and survival in macrophage (43). Surprisingly, Mle does not possess an OmpATb ortholog. This may explain why the human host is more successful in killing Mle and containing them in granulomas. However, the role of OmpATb as a porin (34, 45) has been seriously questioned, leading to more questions about its physiological function.

Several killing mechanisms such as the generation of free radicals may also be employed by macrophages (117). However, Mtu may be able to thwart this attack by importing glutathione using the glutathione transport system (3.A.1.5.11) because glutathione is an important antioxidant protecting against free radicals (66). This glutathione transport system appears to have been selectively retained in Mle during the reductive evolutionary processes. Taken together with the absence of the two enzymes required for glutathione biosynthesis, this glutathione transport system may be essential for the survival of Mtu and Mle in macrophages.

During latency, various transporters are required for nonreplicating persistence in the peculiar extracellular environment of granulomas. Persistence and *in vivo* growth may require phthiocerol dimycocerosate (PDIM) (57), which is exported by a member of the HAE2 family (2.A.6.5). As the granulomas become increasingly microaerophilic toward the necrotic center, Mtu is able to sense changes in oxygen and switch to anaerobic respiration. DosT and DevS are important protein kinases that confer upon Mtu this switching mechanism, and proper transport of Mg^{2+} with any one of the magnesium transporters identified here may be important for such protein kinase activities. Perhaps this may explain why MgtC is essential for intramacrophage survival. Lower oxygen is observed in the activated phagocytes as compared to that in unstimulated phagocytes (118).

Under conditions of low oxygen, Mtu relies upon nitrate respiration. The *narGHJI* operon of Mtu encodes protein subunits that assemble into an active nitrate reductase complex, but Mle lacks such proteins. Intriguingly, two domains are fused to a permease with specificity for ribose, or more likely autoinducer-2, in Mle. This protein contains an oxygen-sensing domain and a diguanylate cyclase domain. Because Mle is an obligate aerobe that lacks the ability to switch between different types of respiration like Mtu, this oxygen-sensing domain and the GGDEF domain may function together to initiate a signal transduction cascade and induce expression of proteins for movement toward areas of higher oxygen concentration. Autoinducer-2 is an interspecies communication molecule produced by both Gram-positive and Gram-negative bacteria and is a sugar derivative that contains borate. Autoinducer-2 may be an aerotactic pheromone.

Latent mycobacterial infections may reactivate when granulomas caseate and liquefy. Mtu and Mle undergo tremendous proliferation upon reactivation. Surprisingly, a protein belonging to the Filamentation in response to cAMP (Fic) family is encoded within the operon that encodes the oligopeptide uptake system (3.A.1.5.2) in Mtu. In Gram-positive bacteria, peptide pheromones provide cell-to-cell communication that could regulate cell proliferation. Therefore, peptide transport might regulate cell division. The presence of septal DNA translocators (3.A.12) in both Mtu and Mle suggests that septum formation precedes the full transfer of the chromosome. Lipopolysaccharide exporters (3.A.1.103.1) identified in both Mtu and Mle are important for building the cell envelope.

Upon proliferation, mycobacteria may proceed to adjacent host cells. Cell-to-cell spreading is dependent on the TmtpC proteins (2.A.6.5.3), implicated in sliding motility. Seven paralogues were discovered in Mtu, one of which (gi: 15841024) is likely to be a pseudogene. The first ~600 aas of TmtpC have become deleted. Only two homologues were

identified in Mle. These two homologues may be the minimal set of TmtpC proteins required for obligate intracellular life.

Adaptation to various extracellular environments

Many types of stress-response transporters allow for adaptation to varying conditions of osmolarity and pH. Mtu possesses three putative mechanosensitive channels, one of the MscL-type (1.A.22) and two of the MscS-type (1.A.23). These channels open during hypoosmotic stress and provide the cell with pressure relief (38). Mle possesses only one of the MscL-type presumably because it relies, in part, on the host cell's homeostatic mechanisms to regulate intracellular pressure. The two MscS type channels in Mtu likely provide additional adaptation to various osmotic pressure changes associated with the lifestyle of Mtu. One of the MscS types in Mtu is gated by cAMP for fine control. Although cyclic nucleotides are known to be synthesized in Mtu and play important roles in pathogenesis, their mechanisms of action and physiological control consequences are poorly understood (119). Our finding gives a clue to a possible mechanism of its action (120). Other transporters potentially involved include: a K^+ channel, a BetS osmolyte porter of the BCCT family, and a member of the SBT family. Not surprisingly, these three transporters were identified in Mtu but not Mle. Furthermore, OmpATb is required for survival in low pH conditions (121).

Antimicrobial drugs

Many secondary active transport drug efflux systems were identified in Mtu and Mle. Mtu possesses varying numbers of members in the following families: 2 of DHA1 (2.A.1.2), 12 of DHA2 (2.A.1.3), 2 of DHA3 (2.A.1.21), 2 of HAE2 (2.A.6.5), 1 of DMT (2.A.7.1), 1 of MATE (2.A.66.1), and 1 of MVF (2.A.66.4). Of these, only 1 of DHA2, 1 of DMT, and 1 of MVF are found in Mle, suggesting that secondary active transport may not be the primary means of drug efflux for intracellular life. Primary active pumps identified in Mtu are: 2 of DrugE1 (3.A.1.105), 3 of Drug RA1 (3.A.1.120), 1 of MacB (3.A.1.122), and 1 of MDR (3.A.1.201). Of these, 2 of DrugE1 and 3 of DrugRA1 are found in Mle. This shows that certain primary active drug efflux systems were preferentially retained for intracellular survival. These observations suggest the need for high affinity, low efficiency drug exporters, again reflective of a strictly intracellular life style. The secondary exporters may be more appropriate for extracellular life where much higher concentration of toxic substances may be encountered (122).

These findings beg the question as to what extent MDR transporters are responsible for drug resistance in Mtu (i.e., against isoniazid (INH), rifampin, pyrazinamide, and ethambutol) and Mle. This is an important question that unfortunately cannot be answered by an *in silico* approach. However, there is evidence to support the hypothesis that MDR transporters play an important role and is summarized in a review article by Louw et al. (123). There was a microarray analysis of efflux pump genes in MDR-TB strains induced by common anti-TB drugs (124). More specifically, overexpression of the Mtu *mmpL7* gene, encoding a hypothetical RND transporter, in *M. smegmatis* has been shown to confer high-level INH resistance. Also, INH resistance decreased with the addition of various efflux pump inhibitors in *M. smegmatis* (125). Gene KO experiments have shown that the *iniA* gene in Mtu is essential for activity of an MDR-efflux pump that confers resistance to INH and EMB (126).

There is also evidence for an important role of MDR transporters for resistance to rifampicin and pyrazinamide (PZA) as well. In the case of resistance to PZA, 70% of PZA-resistant clinical strains can be attributed to mutations in the *pncA* gene, which encodes an enzyme

that converts PZA into an active form; nonetheless, the role of efflux pumps as primary mechanism of resistance to PZA has been documented.

In summary, the primary mechanism of resistance appears to be different for each of the aforementioned drugs, and how much of this resistance we can attribute to MDR transporters is unclear.

Toxins and virulence factors

Mtu and Mle are resistant to a panoply of toxic metals and organic compounds. They both have ArsB/ArsA permeases that may confer resistance to arsenite, which can react with free thiols of proteins, particularly those that are involved in the citric acid cycle. Whether the ArsA energizers function with the ArsB transporters has yet to be determined.

Mtu and Mle may also be resistant to high concentrations of acetate as they both express acetate exporters (3.A.1.120.5). While Mtu expresses two paralogs, Mle expresses just one acetate porter, suggesting that only one is sufficient to maintain acetate resistance. Acetate would be expected to be present in the host cell cytoplasm as a result of normal metabolism.

Nutrient uptake

Many transporters in Mtu and Mle are involved in nutrient uptake. Mtu probably acquires nitrogen as ammonium through the Amt channel (1.A.11), but Mle lacks such a protein. Ammonia is the preferred source of nitrogen as it supports a higher growth rate than any other nitrogen source (36). Ammonia may be acquired by free diffusion but only at high concentrations. How much nitrogen is available inside the host cell varies with conditions but ammonia generally enters cells via NH_3/NH_4^+ transporters. Therefore, ammonia may not be the primary source of nitrogen in Mle; instead, Mle may acquire nitrogen using other nitrogen-containing compounds including amino acids like histidine and arginine, or nucleosides such as cytidine. This may help explain why Mle grows much slower than Mtu (generation time of 14 days).

Mtu and Mle possess many carriers and primary active uptake systems dedicated to the acquisition of carbohydrates. Three sugar porters (1 of the SP family and 2 of the ACS family) and four carboxylate symporters utilize secondary active transport in Mtu. None of these transporters is found in Mle. Instead, primary active uptake systems for sugars are retained. This can be explained since cytoplasmic sugar concentrations are likely to be low due to rapid metabolism. Secondary active transport usually allows lower affinity uptake for more rapid growth, conditions that would be favored for an organism living in a continuously changing extracellular environment (122).

Four, maltose-type uptake systems (CUT1) are encoded by Mtu, and three of these are retained in Mle. Moreover, a complete ribose or autoinducer type uptake system (CUT2) is found in Mle although only an incomplete system is found in Mtu. Perhaps this system plays a role in communication inside the host cell.

Mtu and Mle encode many primary and secondary active transporters for the uptake of amino acids. Carriers include two of the APC family and are found in both Mtu and Mle. However, one of OPT and ThrE, and two of LysE are only found in Mtu. This suggests that amino acid accumulation during peptide hydrolysis may be problematic in Mtu but not Mle. Uptake systems include ABC-type PepT family members, two in Mtu and one in Mle, possibly involved in regulating cell division as discussed earlier. The presence of secondary amino acid transporters in Mle may reflect higher concentrations of these nutrients compared to those of free sugars.

It is clear that Mtu scavenges nutrients from host cells as does Mle. The use of host triacylglycerol by Mtu is yet another example (127). Some inorganic ions such as iron (128, 129) and phosphate (130) are acquired from the host. They are undoubtedly required for pathogen survival, while others may be toxic when present in excessive amounts (131, 132). However, there is also evidence for de novo synthesis of various cofactor like NAD (133). Mtu therefore likely relies on external sources when available and makes what is not available. Very little is known about how many compounds limit or promote growth. Further studies will be required to provide this knowledge.

Fusion of genes and extra domains

Supporting the notion that evolution tends toward complexity is the prevalence of gene fusions and fusions of extra domains to transport proteins in Mtu and Mle. In the F-type ATP synthases of both Mtu and Mle, the delta subunits were found fused to the b subunits. Two transporters of the DHA2 family (2.A.1.3) have the following extra domains at their N-termini: a cAMP binding domain followed by a phosphodiesterase domain, suggesting that this transporter may also be regulated by cyclic nucleotides or be involved in regulating enzymes that break down cyclic nucleotide phosphodiesters. Drug transporters of the MVF family (2.A.66.4) in Mtu and Mle have threonine protease domains at their N-termini. We suggest that these transporters have a novel regulatory function in Mtu and Mle, but little is known about the functions of MVF family members.

It is thought that the fatty acyl-CoA synthetase transport proteins (FATP) catalyze and energize transport using a carrier or channel mechanism, trapping the fatty acids in the cell cytoplasm as a result of covalent modification by thioesterification (134, 135). Faergeman et al. have presented evidence that fatty acyl-CoA synthetases function as components of fatty acid uniport systems in yeast by linking import and activation of exogenous fatty acids (136). Further, Zou et al. isolated FAT1 mutants of *S. cerevisiae* that are deficient for either transport or acyl-CoA synthetase activity (137). Loss of acyl-CoA synthetase activity in yeast or animal cells results in greatly reduced fatty acid uptake activity, suggesting that uptake and CoA esterification are linked (Stuhlsatz-Krouper et al., 1998, 1999). If transport is coupled to thioesterification, these systems provide a novel mechanism of group translocation.

Of the 38 putative fatty acyl-CoA synthetases in Mtu, evidence for only one that may function as a lipid transporter has been provided (79). Although functional information for the proposed Fatty Acid Transporter (FAT) family (4.C.1) is sparse, acyl-CoA synthetases with 2-4 TMSs are more likely to be transporters than those with no TMSs.

CONCLUSION

147 and 55 complete transport systems were identified in Mtu and Mle, respectively. Some types of transport proteins have been selectively retained in the reductive evolutionary process that Mle has undergone. For example, P-P-bond hydrolysis-driven transporters display the highest retention rate (52%) when Mle is compared with Mtu, and among these are drug exporters. Many Mtu and Mle proteins display intra-operon gene fusions, possibly as a result of evolutionary pressure to make genomes more compact, or to ensure proper protein-protein interactions. Terminal fusions of regulatory domains to transport proteins in Mtu and Mle are also abundant. Many transport proteins that may allow Mtu to persist in granulomas during latent infection, especially those involved in anaerobic respiration or fatty acid transport, were identified.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Starting point for experimental studies in dependencies for mycobacteria
- Suggestions regarding transporters that function in intra- v. extracellular growth
- Co-emergence of multidrug resistant bacterial strains as a public health threat

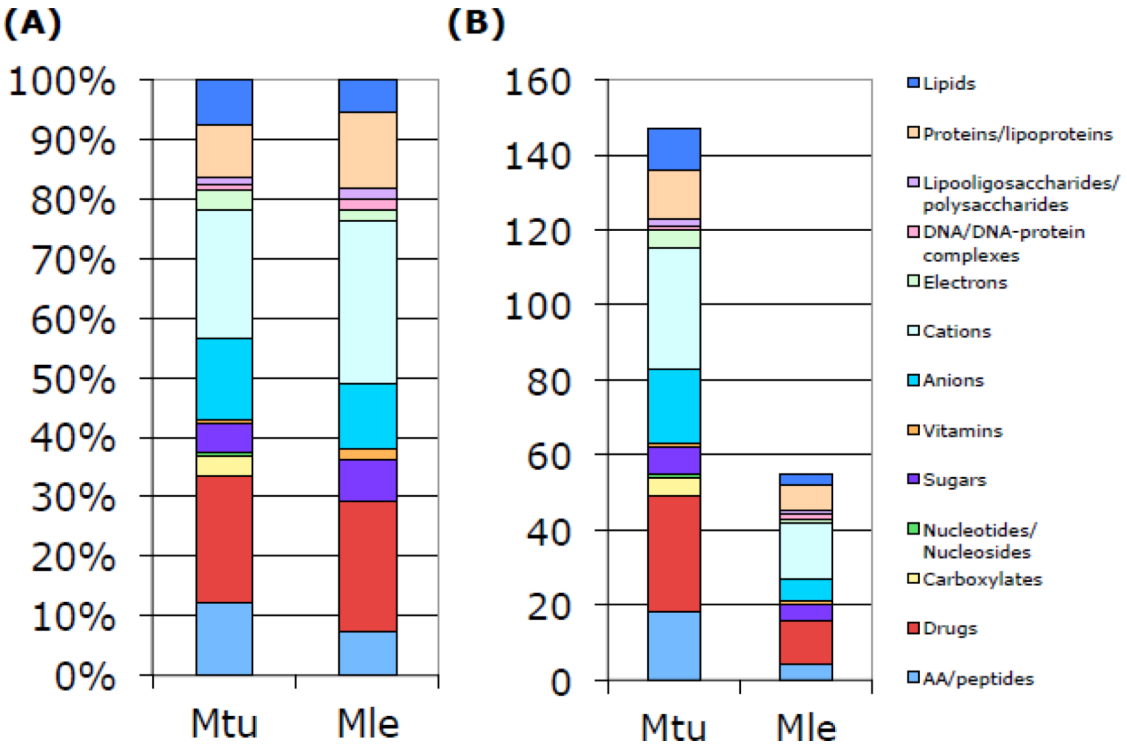

Figure 1.
Relative distribution of predicted substrates of transport systems found in Mtu and Mle according to (A) percent of all transporters identified, and (B) numbers of transport systems.

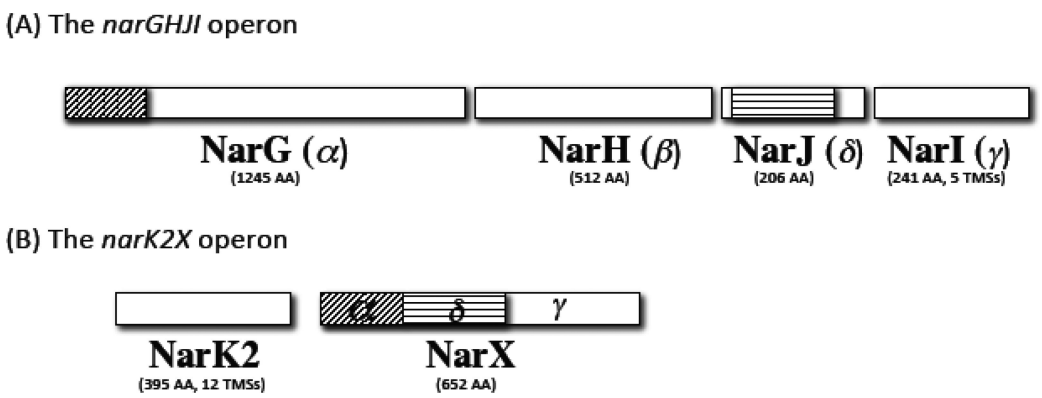

Figure 2.

Nitrate reductase genes in Mtu. The Mtu genome includes two operons that respectively encode a nitrate reductase and a partial nitrate reductase. The latter probably includes nitrate-reductase pseudogenes resulting from two genomic deletions, the first being large, the second being small. Both operons are absent in Mle. (A) The *narGHJI* operon encodes the three nitrate reductase subunits, NarG (alpha), NarH (beta), and NarI (gamma). NarJ, the delta subunit, is a chaperone protein required for the assembly of this nitrate reductase complex. (B) The narK2X operon encodes two proteins: NarK2, a nitrite exporter, and NarX, a truncated nitrate reductase. The N-terminus of NarX is homologous to the first 256 aas of NarG. The next 160 aas of NarX are homologous to aas 5-164 of NarJ. The C-terminus of NarX is homologous to all 241 aas of NarI. narK2 and narI (γ) may be functional.

Table 1

Overview of the *M. tuberculosis and M. leprae* transporter analyses based on TC class and subclass

TC class[a]	Class description	No. of transport proteins[b]		Mle/Mtu[c]	TC subclass	Subclass description	No. of transport proteins		Mle/Mtu
		Mtu	Mle				Mtu	Mle	
1	Channels	8 (8)	3 (3)	38%	1.A	α-Type channels	6 (6)	2 (2)	33%
					1.B	β-Barrel porins	2 (2)	1 (1)	50%
2	Secondary carriers	82 (78)	26 (23)	29%	2.A	Porters (uniporters, symporters, antiporters)	82 (78)	27 (23)	29%
3	Primary active transporters	132 (46)	72 (24)	52%	3.A	P–P-bond-hydrolysis-driven transporters	111 (43)	66 (23)	53%
					3.D	Oxidoreduction-driven transporters	21 (3)	6 (1)	33%
4	Group Translocaters	1 (1)	0 (0)	0%	4.C	Acyl-CoA ligase-coupled transporters	1 (1)	0 (0)	0%
5	Transmembrane electron carriers	8 (4)	1 (1)	25%	5.A	Transmembrane two-electron transfer carriers	8 (4)	1 (1)	25%
8	Auxiliary transport proteins	1 (1)	1 (1)	100%	8.A	Auxiliary transport proteins	1 (1)	1 (1)	100%
9	Poorly defined systems[d]	53 (9)	33 (3)	33%	9.A	Recognized transporters of unknown biochemical mechanism	53 (9)	33 (3)	33%
	Total	**285(147)**	**136 (55)**	**37%**			**285 (147)**	**136 (55)**	**37%**

[a] Transporter classes 6 and 7 have not been assigned in the TC system and therefore are absent.

[b] Numbers in parentheses represent the number of transport systems. Transport systems are comprised ofone or more constituent transport proteins.

[c] Relative reduction in transport systems from Mtu to Mle.

[d] Poorly defined systems here includes families in TC subclass 9.A (poorly characterized transporters) but not in TC subclass 9.B (putative transporters); see Tables 3, S1 and S2 for homologues of families within subclass 9.B.

Table 2

Overview of transport systems in *M. tuberculosis and M. leprae* based on predicted substrate specificity

Substrate Category	Mtu		Mle		Mle/Mtu[a]	Substrate Subcategory	Mtu		Mle		Mle/Mtu
Organic	63	37%	21	36%	33%	AA/peptides	18	11%	4	7%	22%
						Drugs	31	18%	12	21%	39%
						Carboxylates	5	3%	0	0%	0%
						Nucleotides/Nucleosides	1	1%	0	0%	0%
						Sugars	7	4%	4	7%	57%
						Vitamins	1	1%	1	2%	100%
Inorganic	57	34%	22	38%	39%	Anions	20	12%	6	10%	30%
						Cations	32	19%	15	26%	47%
						Electrons	5	3%	1	2%	20%
Macromolecule	27	16%	12	21%	44%	DNA/DNA-protein complexes	1	1%	1	2%	100%
						Lipooligosaccharides/polysaccharides	2	1%	1	2%	50%
						Proteins/lipoproteins	13	8%	7	12%	54%
						Lipids	11	6%	3	5%	27%
Total	147	100%	55	100%	37%		147	100%	55	100%	37%

[a]Relative reduction in transport systems from Mtu to Mle

Table 3

s of putative transport proteins from *M. tuberculosis* and *M. leprae*

Transport Classification (TC)						*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle		
tem TC#	No. of comp.[a]	Acc. # in TCDB[b]	Size (aa)	TC TMS[c]	Comments	GI # (CDC1551)	GI # (H37Rv)	Size (aa)	TMS	% Identity Mtu/TC	Comments (Mtu vs. TC)	GI #	Size (aa)	TMS	% Identity Mle/TC	% Identity Mtu/Mle	No. of transport systems	
																	Mtu[e]	Mle[f]
ides																		
	1	O27564	336	2		15842786	15610336	355	2	26	Mtu-N: [1,2,3]	-	-	-	-	-	1	0
	1	Q79VF1	438	10		15842463	15610057	477	10	52	Mtu-C: [1,2]	-	-	-	-	-	1	0
	1	O53898	151	2		15840410	15608125	151	2	100		15826991	154	2	71	70	1	1
	1	O34897	267	2		15842675	15610241	308	2	30		-	-	-	-	-	2	0
	1	Q58543	361	6		15841954	15609571	481	6	24	Mtu-C: Crp(31008), CAP_ED(28920)[1,2,3,4]	-	-	-	-	-		
	1	Q58439	317	3		15840684	15608379	366	3	19	Mtu-N: [1,2,3,4]	15827535	369	3	19	79	1	1
	1	P65593	326	1		15840318	15608039	326	1	100		-	-	-	-	-	1	0
	1	P64883	314	1		15841155	15608836	314	1	100	Rv1698 (H37Rv)	15827709	317	1	-	56	1	1

Transport Classification (TC)						*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle		
tem TC#	No. of comp.[a]	Acc. # in TCDB[b]	Size (aa)	TC TMS[c]	Comments	GI # (CDC1551)	GI # (H37Rv)	Size (aa)	TMS	% Identity Mtu/TC	Comments (Mtu vs. TC)	GI #	Size (aa)	TMS	% Identity Mle/TC	% Identity Mtu/Mle	No. of transport systems	
																	Mtu[e]	Mle[f]
	1	P37021	464	12		15842926	15610467	500	12	30	Mtu-N: [1,2]	-	-	-	-	-	1	0
	1	Q9S3J9	395	12		15840256	15607982	442	12	21	Mtu-N: [1] Mtu-C: [1]	-	-	-	-	-	2	0
						15839570	15607332	413	12	32		-	-	-	-	-		
	1	Q54806	501	14		15841836	15609470	537	14	32	Mtu-C: [1,2]	-	-	-	-	-	12	2
	1	P42670	503	14		15841089	15608772	471	14	28	Mtu-C: [1,2]	-	-	-	-	-		
						15842387	15609983	530	14	34	Mtu-N: [1,2]	15827823	534	14	34	86		
	1	P39886	538	14		15840696	15608390	579	14	32	Mtu-C: [1,2]	-	-	-	-	-		
						15841347	15609014	687	14	30	Mtu-N: [] Mtu-C: [1,2,3,4]	-	-	-	-	-		
						15841983	15609596	523	14	31		-	-	-	-	-		
						15842828	15610375	1065	14	32	Mtu-N: [1,2] Mtu-C: RssA(31938), CAP_ED(28920)[1,2,3,4]	-	-	-	-	-		
						15843349	15610864	1071	14	34	Mtu-N: [1,2] Mtu-C: RssA(31938), CAP_ED(28920)[1,2,4]	-	-	-	-	-		
						15840198	15607923	545	14	26		-	-	-	-	-		
	1	Q9HS33	420	12		15841758	15609402	409	12	20	Mtu-N: [1,2,3]	-	-	-	-	-		
						15841980	15609593	418	12	24	Mtu-N: [1,2,4]	-	-	-	-	-		
	2	P71678	518	14		15840868	15608548	518	14	28		15827207	509	14	27	82		
		P0A5I8	236	1		15840869	15608549	236	1	100		15827208	238	1	100	68		
	1	Q52000	429	12		15843086	15610612	449	12	40		-	-	-	-	-	3	0
						15840290	15608016	559	12	28	Mtu-N: [1,2,3,4] Mtu-C: [1,2,3,4]	-	-	-	-	-		
	1	P76350	438	12		15840644	15608340	425	12	36		-	-	-	-	-		

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