UNIVERSITY OF CALIFORNIA, SAN DIEGO

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

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by

Ji-Won Youm

Committee in charge:

Professor Milton H. Saier, Jr., Chair
Professor Russell F. Doolittle
Professor Joshua Fierer

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Abstract, Introduction, Methods, Results, Discussion and overview, Conclusion, and Appendix, all in full, will be submitted for publication of the material as it may appear in Genomics, Genome Biology, or Biochemica acta, 2008, Youm, Ji-Won; Saier, Milton H., Jr, 2008. The thesis author was the primary investigator and author of this paper.

ABSTRACT OF THE THESIS

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

By

Ji-Won Youm

Master of Science in Biology

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Professor Milton H. Saier, Jr., Chair

The co-emergence of multidrug resistant strains and the HIV pandemic has made tuberculosis the 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. As transporters are essential for bacterial growth and persistence, we conducted comparative analyses of transport proteins encoded within the genomes of these organisms. A minimal set of

genes required for intracellular life and extracellular life, and genes that may have been horizontally transferred were identified. Drug efflux systems utilizing primary active transport mechanisms have been preferentially retained in Mle and still others preferentially lost. Transporters involved in adaptation were found in Mtu were mostly lost in Mle. These findings may provide starting points for experimental studies that may elucidate the pathogenesis of these two pathogenic mycobacteria.

INTRODUCTION

One third of the human population is infected with tuberculosis (TB), a deadly infectious disease that claims almost 2 million lives a year ("Tuberculosis Fact Sheet (Revision, March 2004)"). Despite the efficacy of the Bacille Calmette-Guérin vaccine as a means to prevention, TB continues to spread and infect at the staggering rate of one person per second. The development of directly observed short-course chemotherapy (DOTS), a control strategy recommended by the World Health Organization, promises containment. However, tuberculosis mismanagement has led to the increasing prevalence of the multidrug-resistant (MDR) form of TB, which is sensitive only to second-line drugs (Raviglione and Smith). To make matters worse, we now face the challenge of treating extensively drug-resistant (XDR) TB, a form of TB that is not even susceptible to second-line drugs. Even more problematic is that an XDR TB infection in HIV patients is a virtual death sentence (Raviglione and Smith). Therefore, the emergence of the current HIV pandemic may one day lead to a pandemic of untreatable TB. To prevent this grim future from becoming reality, worldwide consortia of physicians and scientists are currently working to shed light onto the molecular pathogenesis of tuberculosis and ultimately eradicate this disease. The causative agent responsible for this deadly disease is *Mycobacterium tuberculosis* (Mtu).

Mtu is an extremely slow growing obligate aerobe. While a fast growing obligate aerobe like the well-studied Gram-positive *Bacillus subtilis* doubles its population every 1.5 hour, Mtu does so every 18-24 hours (Hussain). Its slow growth is generally attributed to the presence of an unusually impermeable cell wall (Jarlier and Nikaido) that is unique to the Mycobacterial genus, which is subdivided into the rapidly-growing (those

that form colonies within 7 days) and slowly-growing species. The cell wall complex contains peptidoglycan, which is more abundant in the slow-growing group, but it is otherwise mainly composed of complex lipids like mycolic acid, cord factor, and wax-D that make up over 60% of the mycobacterial cell envelope. Mycolic acids are strongly hydrophobic molecules that confer upon these mycobacterial organisms the characteristic membrane impermeability and therefore are implicated in having a major role in virulence. Cord factor is toxic to human host cells and inhibits polymorphonuclear neutrophil migration. The mycobacterial lipid layer also confers resistance to osmotic lysis by protecting from the host immune system. It specifically protects against complement attachment as well as lysozyme and oxidating radicals found in phagocytic granules inside macrophages.

To overcome the outermembrane permeability barrier to hydrophilic solutes, it is anticipated that all mycobacteria need β-porins. These proteins form transmembrane pores that usually allow the energy independent passage of solutes across a membrane. The transmembrane portions of these proteins consist exclusively of β-strands, which form a β-barrel. These porin-type proteins are found in the outer membranes of Gram-negative bacteria, mitochondria, plastids and possibly acid-fast Gram-positive bacteria.

Morphology aside, Mtu is a facultative intracellular parasite that can survive outside of its host. On the other hand, *Mycobacterium leprae* (Mle), which evolved relatively recently from the same soil bacteria that Mtu evolved from, is an obligate intracellular parasite. The differences in their lifestyles can be attributed to the differences in their genomes. The genome of *M. tuberculosis* CDC1551 (the clinical strain, as compared to H37Rv, which is the laboratory strain) encodes 4189 proteins. The

Mle genome encodes only 1605 proteins. Previous genomic analyses have revealed that Mle lost many genes through reductive evolution, some of which are undoubtedly required for extracellular life since Mle is an obligate intracellular parasite while Mtu is a facultative intracellular parasite (Lerat and Ochman "Psi-Phi: Exploring the Outer Limits of Bacterial Pseudogenes; Lerat and Ochman "Recognizing the Pseudogenes in Bacterial Genomes"). Various events such as deletions, missense & nonsense mutations, and translocations are thought to have contributed to the inactivation of genes (Gerstein and Zheng). The remnants of the genes resulting from such evolutionary events are called pseudogenes – inactivated genes that no longer produce functional proteins. Pseudogenes are prevalent in Mle but rare in Mtu. More importantly, while Mtu is a facultative aerobe capable of surviving microaerophilic conditions, Mle is an obligate aerobe. Undoubtedly, Mle has lost many genes for anaerobic respiration, but these genes have not yet been identified for several reasons. Infecting mice with Mle does not reproduce the human pathology; Mle can only be grown on mouse footpads and nine-banded armadillos. Taken together with the extremely slow growth of Mle (doubling time of ~14 days), which makes *in vitro* studies virtually impossible, a premium is placed on the genomic approach. An overview of pathogenesis for these mycobacterial pathogens is necessary to lay the contextual framework of our comparative analyses.

The causative agents of tuberculosis and leprosy are, respectively, Mtu and Mle. While both Mtu and Mle are transmitted through the respiratory route, the clinical presentation of tuberculosis and leprosy are markedly different. There are two types of leprosy: the paucibacillary type (tuberculoid) leprosy and the multibacillary (lepromatous) leprosy (Cosma, Sherman and Ramakrishnan). The former type is

characterized by active cell-mediated immunity and granuloma formation. Because of Mle's preferential residency in Schwann cells, Leprosy patients often present with hypopigmented anesthetic patches that result from nerve damage (Ng et al.). The latter type is characterized by the host's inability to control bacterial proliferation due to weak cell-mediated immunity, often resulting in heavily infected and inflamed perineurium, and thickened dermis (Cosma, Sherman and Ramakrishnan). While the incidence of Leprosy is on the decline, Leprosy is a permanently debilitating disease. Although the slow-growing nature of Mle has made it difficult, if not impossible, to study its molecular basis of pathogenesis, the pathogenesis of Mtu has been studied extensively.

Mtu is typically transmitted when aerosol droplets containing Mtu are coughed out by an infected host with active disease and subsequently inhaled by a nearby host. Infection commences when the Mtu bacilli enter the respiratory system and are phagocytosed into phagosomes by alveolar macrophages. Three outcomes arise from a complex interplay between the bacilli and the macrophages: elimination of pathogens (no infection), prolonged dormancy of pathogens (latent infection), and active disease (primary, or reactivation tuberculosis) (Deretic and Fratti). If alveolar macrophages are activated, the bacilli are killed. Macrophages employ several killing mechanisms, including the production of toxic nitrogen and oxygen metabolites (Nathan and Shiloh). Nitric oxide, generated by inducible nitric oxide synthase (iNOS), serves as a potent bacteriocide. More importantly, macrophages acidify the phagosome by fusing it with lysosome, which contains acid hydrolases and the bulk of the proton translocating V-type ATPase to be incorporated into the phagolysosomal membrane (Russell; Haas; Tjelle, Lovdal and Berg). On the other hand, if alveolar macrophages are not activated, the

bacilli replicate intracellularly until bacilliary burden causes host cells to lyse. The bacilli are then released into the surrounding lung tissue and phagocytosed by tissue macrophages. If the tissue macrophages are activated, the bacilli are eliminated. Otherwise, the bacilli replicate until host cell lysis occurs. In an immunocompetent individual, the bacteria are recognized, and this recognition initiates a non-specific, innate immune response in which both the Mtu harboring macrophages and neighboring cells are destroyed. Mtu will be released and subsequently phagocytosed. The adaptive response ensues, and eventually, the bacilli are quarantined in granulomas, which can be caseating or noncaseating. Caseating granulomas, or caseating tubercles, are characterized by a central region of necrosis filled with acellular debris encased by a peripheral cuff of lymphocytes. In noncaseating granulomas, macrophages and lymphocytes replace the necrotic center observed in caseating granulomas. In both types of granuloma, the periphery is composed of epithelioid cells, which are activated macrophages that have fused with one another. This reduces oxygen availability for Mtu residing in granulomas, and this oxygen limitation induces two, metabolically distinct stages of nonreplicating persistence of the tubercle bacilli (Wayne and Hayes "An in Vitro Model for Sequential Study of Shiftdown of Mycobacterium Tuberculosis through Two Stages of Nonreplicating Persistence"). Subsequently, the bacilli are successfully walled off from spreading in the so-called latent infection.

The central paradigm of pathogenesis in latent infection has long been the arrest of Mtu phagosome maturation via phagolysosome biogenesis block (Russell; Deretic and Fratti; Vergne et al.). Mle has also been observed to preclude the fusion of phagosome and lysosome (Frothingham). Typically, an endosomal phosphatidylinositol, a minor

phospholipid component in the eukaryotic membrane, is phosphorylated by phosphatidylinositol 3-kinase (PI3K) in a GTPase (Rab5)-dependent manner to generate phosphatidylinositol 3-phosphate (PIP3) (Vergne et al.). PIP3 binds several proteins required for the fusion of early and late endosomes. Mtu secretes a lipid phosphatase (SapM) that hydrolyzes PIP3, thereby inhibiting phagosome-late endosome fusion. Such fusions are mediated by actin assembly, which is thought to provide tracks along which the lysosome moves toward and fuses with the phagosome (Kjeken et al.). Increased intracellular cAMP in macrophage was found to inhibit actin assembly and phagolysosome fusion, and to stimulate Mtu growth in macrophage (Kalamidas et al.). Furthermore, ω-3 polyunsaturated fatty acids (EPA > DHA) are known to induce small but significant inhibition of actin assembly by Mtu phagosomes, thereby significantly stimulating Mtu growth (Anes et al.). Arachidonic acid, on the other hand, results in significant Mtu killing. Lipoarabinomannan, a cell wall toxin secreted by Mtu, is found to prevent Ca^{2+} signaling required for the formation of PIP3, thereby preventing phagosome maturation (Vergne, Chua and Deretic). Protein kinase G (PknG), a serine-threonine kinase secreted by Mtu, is implicated in having a similar role (Walburger et al.).

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.*, the first to visualize Mtu cellular localization using electron microscopy beyond two days after infection, showed that the Mtu phagosome actually fuses rather rapidly with lysosomes (van der Wel et al.). Furthermore, Mtu secretes virulence factors CFP-10 and ESAT-6 via the RD-1 secretion system (van der Wel et al.; Fortune et al.; MacGurn and Cox) to progressively translocate from phagolysosomes to

the cytosol after two days and ultimately causes apoptosis of its host (van der Wel et al.). It is possible that Mtu halts phagolysosome maturation for a few hours or even days to synthesize the proteins necessary for translocation. How Mtu seizes control over its macrophage host cell for a prolonged period of time and remains in a state of nonreplicating persistence within granulomas without inducing apoptosis remains to be elucidated.

Genomic studies have the potential to provide missing puzzles to the pathogenesis picture. One genome-wide study using transposon mutagenesis revealed a set of genes required for Mtu survival in the macrophage (Rengarajan, Bloom and Rubin). However, this published set of genes does not include genes required for adaptation to stressful environments like those replete with antibiotic drugs, especially because the *de novo* synthesis of multidrug transporters required to pump out such drugs is induced by drugs (Putman, van Veen and Konings). A comprehensive genomic study of transport systems in Mtu and Mle may identify such multidrug efflux systems, secretion systems for the excretion of aforementioned virulence factors, and still others for the uptake of nutrients and extrusion of various end metabolites and toxins.

Transporters are essential for bacterial survival and persistence; Mtu must be able to adapt to the various environmental conditions encountered during pathogenesis. During primary infection, Mtu is phagocytosed by macrophages, and its intracellular residency is diverse: phagosome, phagolysosome or cytosol. In the phagosome or phagolysosome, Mtu must be able to secrete appropriate virulence factors by using secretion systems to hijack the phagosomal machinery, withstand acidic conditions, and extrude various toxins. Mtu must also be able maximize the uptake of nutrients upon

translocating to the cytosolic environment for replicating. Upon release into the extracellular tissues, Mtu would also benefit if it can pump out antibiotic drugs.

During latent infection, oxygen is significantly reduced inside granulomas, and Mtu must adapt to microaerophilic conditions by switching from aerobic to mostly anaerobic respiration (uptake of nitrate or another terminal electron acceptor). 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. Specific transporters for the uptake of nutrients and extrusion of toxins are essential for extracellular survival.

Once reactivation occurs in the immunocompromised host, the tubercle may necrotize into vessels and disseminate to the rest of the body or necrotize into bronchioles and transmit this deadly disease. Its varying extracellular conditions throughout the course of pathogenesis will undoubtedly require the ability to adapt to various osmotic pressures as well. It must also be able to withstand abrupt changes in temperature. However, much of the biology behind Mtu's adaptive capabilities, particularly that of transporters, remains to be elucidated.

Identification of transporters may also offer experimental scientists starting points for development of vaccines and drugs. Comparative analyses may reveal which transporters are required for intracellular life as well as extracellular life. We propose that transporters required for adaptive responses will be found in Mtu but not Mle. Moreover, we believe that drug exporting systems will be selectively retained in Mle.

Introduction Acknowledgement

Introduction, in full, will be submitted for publication of the material as it may appear in Genomics, Genome Biology, or Biochemica acta, 2008, Youm, Ji-Won; Saier, Milton H., Jr, 2008. The thesis author was the primary investigator and author of this paper.

METHODS

The proteomes of Mtu and Mle were screened for homologues of all proteins contained in TCDB, a membrane transport protein classification database, initially on 9/11/06 and subsequently on 8/10/08 (http://tcdb.ucsd.edu) (Busch and Saier; Saier, Tran and Barabote). FASTA-formatted protein sequences of the completed genomes for the clinical isolate of Mtu (CDC1551) and the only Mle strain available (TN) were used. The genome sequencing projects for Mtu and Mle are described by Cole et al., 1998 and 2001, respectively (Cole, Brosch et al.; Cole, Eiglmeier et al.). Each putative open-reading frame (ORF) was used as a query in the BLASTP software (Altschul, Gish et al.; Altschul, Madden et al.) to search for homologous proteins in TCDB. The SEG low complexity filter was not used. In addition, each ORF was scanned with HMMTOP (Tusnady and Simon) to predict the number of putative transmembrane segments (TMSs), as reported in Figure 3 and Table 3. WHAT (Zhai and Saier) was used to resolve the differences in the number of TMSs between the Mtu or Mle protein and the TCDB homologue.

These candidate proteins were subsequently examined in greater detail to determine their substrate specificities. On the basis of the number and location of TMSs and sequence similarity, transport proteins were classified into families and subfamilies of homologous transporters according to the classification system presented in TCDB. Operon analyses were performed for all candidate proteins assigned to have transport function, and the subsequent 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 2). Assignment to a family or subfamily within the Transport Classification System usually allows specification of substrate type with high confidence (Busch and Saier; Saier, Tran and Barabote; Saier "A Functional-Phylogenetic Classification System for Transmembrane Solute Transporters"). When an expected transport protein constituent of a multi-component transport system could not be identified with BLASTP, tBLASTn was performed in case of a sequencing error in these genomes.

These transport proteins were then systematically analyzed for unusual properties with an unpublished, in-house software (Youm and Saier, 2007). Unusual properties encompass the result of events such as deletions and fusions (see Table 3) -- that is, the gain of extra domains to the Mtu proteins with respect to the homologous protein in TCDB. Additionally, since the Mle genome is particularly replete with pseudogenes, this list of transport proteins was screened for pseudogenes using PSI-FI.

Methods Acknowledgement

Methods, in full, will be submitted for publication of the material as it may appear in Genomics, Genome Biology, or Biochemica acta, 2008, Youm, Ji-Won; Saier, Milton H., Jr, 2008. The thesis author was the primary investigator and author of this paper.

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 (Busch and Saier; Saier "A Functional-Phylogenetic Classification System for Transmembrane Solute Transporters"). 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 of transporters found in Mtu and Mle. The 300 transport proteins in Mtu make up 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 and form complete transport systems. These numbers exclude transport proteins most resembling the putative uncharacterized transporters (9.B) because this family of proteins lacks functional information. Also excluded are the additional genes encoding potential transporters that do not give good hits in TCDB entries. Instead, they are included in Supplemental Table 1. 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 proportion of transport proteins (Ren and Paulsen). Not surprisingly, Mtu and Mle, also intracellular pathogens, encode relatively

low proportion of transport proteins. However, while obligate intracellular bacteria are expected to encode far fewer transport proteins than facultative intracellular bacteria, the Mle genome encodes percentwise more transport proteins than Mtu.

In most bacteria, approximately 3–8% of all the transport proteins encoded in the genome are channel-type transporters (Ren and Paulsen). 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). Mle has 2 inner-membrane channel proteins (1.5% of the transport proteins) that comprise 2 channels (3.4% of the transport systems), and 1 outer-membrane, porin-type channel-forming proteins (0.8% of the transport proteins), corresponding to 1 system (1.7% of the transport systems). Thus, Mle has somewhat fewer channel-type transporters than Mtu on a percentage basis. These numbers presumably reflect the stable environment of human host cells that have allowed Mle to shed these channels through reductive evolution.

Mtu has substantially more secondary carriers (78 systems, or 53% of transport systems identified) than primary active transporters (43 systems, or 29%). 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 51% for primary active transporters. This observation may reflect the importance of multidrug efflux systems for intracellular survival. Likewise, this observation suggests that many secondary carriers are not

essential for intracellular life. Mtu has 4 transmembrane electron flow carriers but Mle has just 1. 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%) and Mle (7 systems, or 12%). Since these systems have not been characterized, it can be postulated that they exhibit non-essential function that are not commonly found in many bacteria.

Transport substrates

Table 2 presents a breakdown of the transporter systems according to substrate type. 63 (37%) and 21 (36%) of the recognized transporters in Mtu and Mle, respectively, are specific for organic molecules and correspond to a retention rate of 33% (reduction of 67%). 12 Drug transporters were selectively retained in Mle (39%) as were 4 sugar transporters (57%). The only transport system that recognizes 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.

58 (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%. 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 7 (54%) transport systems specific for proteins were retained in Mle, only 3 (27%) transport systems specific for lipids were retained in Mle.

Transporter Membrane Topology

Of the 284 and 135 transport proteins that comprise a total of 148 and 55 transport systems in Mtu and Mle, respectively, 203 and 76 proteins have 2 or more TMSs (Fig. 3). Those with less than 2 TMSs are likely to be secreted proteins such as periplasmic binding proteins, which require an N-terminal leader sequence to exit the cytoplasm via the Sec pathway, although several virulence factors in Mtu do not have any such recognizable leader sequences. No proteins with 3 or fewer TMSs per polypeptide chain that function as carriers have yet been identified (Busch and Saier) and (Saier "Tracing Pathways of Transport Protein Evolution"). Predicted 6- and 12-TMS proteins comprise the majority of TMS-containing transport proteins. Those with 6 TMSs generally function as carriers, and those with 12 TMSs are likely to be ABC transporters.

Channels (TC 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 governs the uptake of ammonia, a primary source of nitrogen used for various biosynthetic needs, but Mle does not. While there are several nitrogen-containing compounds, ammonia is the preferred source of nitrogen as it supports a higher growth rate than any other nitrogen source (Merrick and Edwards). Mle may acquire nitrogen using other nitrogen-containing compounds including amino acids like histidine and arginine, or nucleosides such as cytidine. Mle grows much slower than Mtu (see Introduction), possibly obviating the need for an NH_4^+

channel protein. Alternatively, Mle may acquire nitrogen using lipid soluble ammonia that freely diffuses across the cellular envelope.

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. 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 (Pivetti et al.). In comparison to the MscS-type, the MscL-type requires a greater stimulus, opens to a larger pore, and consequently has larger conductance (Edwards, Booth and Miller). The two MscS-type channels in Mtu most likely provide additional adaptation to various osmotic pressure changes associated with the free lifestyle of Mtu responsible for its pathogenesis. Mle, which resides solely inside the host cell, only requires the MscL-type for intracellular life as it most likely relies on the host cell's homeostatic mechanism for regulating intracellular pressure.

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 (Kehres, Lawyer and Maguire). In many bacteria, and especially in *Salmonella typhimurium*, they provide the primary entry pathway for Mg^{2+} (Snavely et al.). These mycobacterial homologues may, therefore, provide the primary mechanism for divalent cation (Mg^{2+}, Co^{2+}, etc.) uptake.

Outer-membrane porins (TC 1.B)

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. Beta-barrel, outer-membrane porins allow the uptake of nutrients and efflux of waste products across this highly impermeable layer. 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 a member of the OmpA-OmpF Porin family. OmpATb is a low activity channel that is essential for adaptation of Mtu to low pH and survival in mouse macrophage (Niederweis). OmpA-OmpF homologues probably all 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 the phagosome acidification when OmpATb is defective (Schaible et al.).

The beta-barrel porin found in both Mtu and Mle is the 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 (Siroy et al.). The presence of only one beta-barrel porin in Mle may, in part, explain its extremely slow growth.

Secondary carriers (TC 2.A)

The majority of transporters in Mtu and Mle are primary active transporters. Many of these are members 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, six of which most resemble the tetracenomycin:H^+ antiporters (2.A.1.3.12), and Mle, respectively. Two of these putative tetracenomycin exporters have fusions of major 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, reported for Mtu in TCDB (2.A.1.3.32), was found in Mle as well. 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 synthase (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 (Rowe et al.). 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* (Wayne and Hayes "Nitrate Reduction as a Marker for Hypoxic Shiftdown of Mycobacterium Tuberculosis"), but this may be different in vivo. One Mtu NarK2 homologue encoded by the *narK2X* operon is associated with the upregulated nitrate reductase activity in anaerobic environment (Sohaskey and Wayne). Thus, these nitrate uptake porters/nitrite exporters 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. Although it has been reported that siderophore production is lost in Mle (Cole, Brosch et al.; Cole, Eiglmeier et al.), we suggest that his PDTC transporter may, nonetheless, function in iron uptake for Mtu and Mle. Iron is important for enzymatic activities and the electron transport chain and is essential for growth of virtually all aerobic organisms. We suggest that PDTC may be a primary iron uptake permease.

Two of the subfamilies in the Amino Acid-Polyamine-Organocation (APC) superfamily are represented in both organisms. They are predicted to transport asparagine and cationic amino acids. 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) exports 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 the Zinc (Zn^{2+})-Iron (Fe^{2+}) Permease (ZIP) family (> 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 transporters in Mtu. Members of this family are known to catalyze export of lipids in mycobacterial species and antibiotics (e.g. actinorhodin). Mtu encodes 12 such carriers of the HAE2 family, and Mle encodes 5. Two separate operons in both Mtu and Mle encode an ActII3-like protein; ActII3 has been implicated in drug resistance (Tahlan et al.). 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 the vivo growth and persistence of Mtu (Glickman and Jacobs). 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* (Martinez, Torello and Kolter). There exists an extra copy in Mtu (GI: 15841024) but is likely to be a pseudogene that may have arisen by deletion of one of the two MmpL domains. Three and one 2,3-diacyl-α, α'-D-trehalose-2'-sulfate (sulfatide precursor) exporters, MmpL8, are present in both Mtu and Mle.

There are a few additional drug exporting secondary carriers: Mtu and Mle each encode the Mmr multidrug efflux pump (2.A.7.1.2) of the DMT superfamily for which the substrates are tetraphenylphosphonium (TPP), erythromycin, ethidium bromide, acriflavine, safranin O, and pyronin Y when the mmr gene is cloned in *M. smegmatis* (De Rossi et al.). A drug exporter of the MATE family of the MOP superfamily is represented

by Mtu and a putative drug exporter of the MVF family, which is distantly related to the MATE family, is represented by both Mtu and Mle. Proteins in the MVF family have been important virulence factors in *Salmonella typhimurium* when infecting the mouse (Kutsukake et al.), 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^{+} antiporters 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 each organism. While a single arsenite efflux system of the ArsB family was identified in Mle, two orthologs 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 manganese transporters of the Nramp family, previously thought to be present only in Mle (Cole, Eiglmeier et al.). 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 to 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 ArsB 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 bicarbonate transporter may allow uptake of HCO_3^- for CO_2 fixation reactions.

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 family (Tat) 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 distinct operon. It has been shown that TatE is rare, and most organisms have either the TatA and TatC, or TatABC (Yen et al.). 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 (Wayne and Hayes "Nitrate Reduction as a Marker for Hypoxic Shiftdown of Mycobacterium Tuberculosis"). A single member from each of the following families was identified in Mtu: OPT family, LysE family, and ThrE family, but none of these putative peptide

uptake and amino acid export permeases were found in Mle, consistent with the previous study of the LysE carrier protein (Vrljic et al.). This suggests that toxic levels of intracellular amino acids, generated by peptide hydrolysis, is not problematic in Mle although it is in Mtu. This may be due to the exclusive presence of the OPT homologue in Mtu.

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 uptake systems and 13 potential 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 may 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. The 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 their constituent components are encoded in the same operon. All seven systems are encoded by distinct operons. The Mtu operon encoding transporters most resembling the *E. coli* homologues of 3.A.1.1.3 is absent in Mle. Each of the two operons in Mtu and Mle encode all four components of the maltose-type system (3.A.1.1.7), including the cytoplasmic component that is missing in *Thermococcus litorali* and *Pyrococcus furiosus*.

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 (1 M and 1 C) most resemble the fructose/mannose/ribose porter (3.A.1.2.7), the other two (1 M and 1 R) most resemble the ribose and autoinducer 2 porter (3.A.1.2.1). As the receptor specificity determines the function of the transport system, this particular operon in Mle probably encodes four proteins that function together as a ribose transporter instead of as a fructose/mannose/ribose porter. This arrangement of having four components comprise a system resembles that of a minority of CUT2 transporters. Others have a single membrane constituent and thus have only three constituents, one C, one M, and one R. The *E. coli* ribose system has the equivalent, four gene products, RsbABCD, where A is the cytoplasmic ATPase, B is the periplasmic receptor, and C and D are the channel-forming membrane proteins.

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 domain of proteins in the PAS family, which have been found to bind ligands and act as sensors for light and oxygen in signal transduction. Aas 143-294 show high sequence similarity to the GGDEF domain, which suggests that this protein probably has a 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 uptake of ribose or autoinducer-2, interspecies communication molecule produced by G+ and G-, is unclear. Whether or not this may

explain some differences in pathogenesis between Mtu and Mle remains to be investigated (See Discussion).

There are two complete oligopeptide uptake systems (3.A.1.5) in Mtu and one in Mle. TC 3.A.1.5.2, which belongs to the PepT family, is known to be a five-component transport system in *Bacillus subtilis*. That is, the transport system requires five different proteins (2 M's, 1 R, 2 C's) encoded by five different genes. Generally, the genes encoding the proteins that work in concert towards a specific goal such as a transport mechanism are present within the same operon. However, only one C constituent is encoded within the operon coding for the constituents of this system. Several members of the PepT family possess only one cytoplasmic subunit, so there is no need to propose the existence of a second ABC protein.

Four of the five proteins encoded within this operon in Mtu correspond to 2 M's, 1 R, and 1 C of 3.A.1.5.2. The fifth protein, while expected to be the second cytoplasmic component, does not encode for a protein that is homologous to any constituent in TCDB. It belongs to the Filamentation in response to cAMP (Fic) family. Therefore, this protein and the peptide transporter might function together to regulate cell division. In G+ bacteria, many pheromones are peptides.

A glutathione transport system (3.A.1.5.11) was identified in both Mtu and Mle. The high sequence similarity between the two organisms indicates that this transport system, which has been selectively retained during the reductive evolutionary processes of Mle, may be important for intracellular life. Indeed, glutathione is an important antioxidant against free radicals (Struzynska, Chalimoniuk and Sulkowski) that are toxic

to these 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 family was identified in Mtu but not Mle. The four components of this system are encoded by the *cysAWTsubI* 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 (resembling IrtAB of Mtu; 3.A.1.21.2; 1 Mtu and 0 Mle), (6) thiamine (ThiW of *M. tuberculosis*; 3.A.1.26.4; 1 Mtu and 0 Mle), and (7) peroxysomal long chain fatty acid (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. ABC efflux systems 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, viral agents, cancer agents, long-chain fatty acids, peptides, phospholipid, 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 aas and 349 aas) that are homologous to the entirety of the MacB homolog (~660 aas) when put together. They may function together as a single heterodimeric system. Members of the 3.A.1 family display such domain splittings and still retain function (Linton and Higgins; Higgins). This suggests a transport mechanism that may not require a MacA or TolC homologue, both of which are required in *E. coli* (Kobayashi, Nishino and Yamaguchi).

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 the gamma pyrophosphate bonds in ATP

using the proton electrochemical gradient (the pmf) as the driving force. Surprisingly, in both Mtu and Mle, delta subunits are found fused to the C-termini of a b subunit, 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 may have led to such fused proteins. It clearly shows that delta and b must function together (e.g., as part of the rotor (Kinosita et al.)). 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^{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 those in Mu. Two of these are probably specific for Cu^{2+} (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 has been identified 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 gene duplication event. ArsB proteins all possess twelve transmembrane spanners and may also have arisen by a tandem gene 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 (the ArsB family; 2.A.45). 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, 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 shows sequence similarity to the SecD domain of the Conserved Domain Database (Wheeler et al.). Furthermore, 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 (Sharp and Pogliano).

Primary active transporters – cation-translocation electron transfer complexes

Many bacteria possess H^+-translocating NADH dehydrogenase complexes of 14 dissimilar subunits (TC 3.D.1) (Hirst; Sapra, Bagramyan and Adams; Steuber et al.). Mtu has these 14 proteins encoded within one operon. The majority of these proteins are most similar to the *Thermus thermophilus* homologues, but the minority of most resemble homologues of other organism. 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 more similar in sequence to the *Paracoccus denitrificans* homologue of subunit, which also has 14 TMSs. The NADH dehydrogenase complex is not present in Mle. Mutations to this complex have been implicated in Mtu strains resistant to isoniazid (Lee, Teo and Wong), which is a first-line medication used to treat tuberculosis.

Both Mtu and Mle encode the H^+-translocating NAD(P) transhydrogenase complex of two dissimilar subunits that are 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, as is the case in *Bdellovibrio bacteriovorus* (Barabote et al.), 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 PSI-FI. Nonetheless, these enzyme complexes may be capable of coupling proton export to electron flow (Flock, Reimann and Adelroth).

Group translocators – The 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 believed to be coupled to transport. A role in group translocation is not fully accepted, and many acyl-CoA ligases clearly do not function directly in transport. Indeed, the FAT family (4.C.1) includes hundreds of sequenced homologues that include fatty acyl CoA ligases (fatty acyl CoA synthases), carnitine CoA ligases, and putative fatty acid transporters (Hirsch, Stahl and Lodish). Animals yeast and bacteria have numerous paralogues that may exhibit 2-4 TMSs and maybe up to 500-600 residues long (Black and DiRusso). 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 was identified with confidence as a lipid transporter. Many of these candidate lipid transporters (not shown in Table 3) 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. 4, the nitrate reductase system has 3 components; in the order in which it is transcribed in the operon, we have the 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 (Sohaskey and Wayne). This entire operon is absent in Mle, consistent with it being an obligate aerobe.

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. 4). 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 (Fenhalls et al.). However, induction alone during hypoxia (Sohaskey and Wayne; Sherman et al.) does not implicate NarX with a functional role in nitrate reduction. *narX* mutants display wild-type nitrate reductase levels, suggesting that NarX is not essential for the nitrate reductase activity of Mtu (Sohaskey and Wayne). The absence of NarX in Mle suggests that NarX may be a pseudogene on its way out.

Two additional members of the PMO family were identified in Mtu but not Mle: the biotin d-sulfoxide reductase (BisC) of 5.A.3.4.3 and the thiosulfate reductase precursor protein (PhsC) of 5.A.3.5.1 (PhsABC). 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*. Furthermore, these findings suggest that, in addition to oxygen, nitrate, thiosulfate, and biotin d-sulfoxide may also serve as final electron acceptor of the electron transport cycle.

An Mtu protein annotated as a putative formate dehydrogenase (not shown in Table 3) most resembling the FdnG of the anaerobic, respiratory, membrane-bound formate dehydrogenase, FdnGHI, was also identified. The Mtu protein (779 aas) aligns to aas 101-159 of the *E. coli* homolog but not the N-terminal segment, which form the two transmembrane segments. Formate dehydrogenase homologs in *Mycobacterium marinum, Mycobacterium sp. MCS, Mycobacterium avium* 104, and many other

mycobacterial species also lack the characteristic two-TMS domain. The identity value is low (23%). Homology could not be established to an experimentally established formate dehydrogenase in *Mycobacterium vaccae* (GI: 15982577). Although another protein encoded within this putative formate dehydrogenase operon in Mtu is annotated as a formate dehydrogenase accessory protein, Mtu proteins homologous to either FdnH or FdnI were not identified. FdnI, the cytochrome b556 (fdn) subunit of this complex, is not encoded by other mycobacterial species. Thus, function could not be assigned with confidence.

Auxiliary transport protein

Mtu and Mle homologues of the *Saccharomyces cerevisiae* GET3 (Arr4p) regulator of chloride transport (8.A.26.1.1) were identified. GET3 (Arr4) is an ATPase homologous to the ArsA protein of bacteria (TC #3.A.4) such as *E. coli* (TC #3.A.4.1.1). It is the ATPase of the GET heterotrimeric complex that mediates ATP-dependent retrieval of endoplasmic reticular proteins from the Golgi apparatus. It may also be involved in low-level resistance to oxyanions such as arsenite, and in heat tolerances (Shen et al.). GET3 (Arr4) inhibits Cl^- transport via Gelf1p (TC #1.A.11.1.1) (Metz et al.).

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) (Burian et al.), and (3) a Co^{2+} transporters of the HlyC/CorC (HCC) family of Putative Transporters (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* (Gibson et al.). 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 (Wards, de Lisle and Collins; Hsu et al.; Stanley et al.; Guinn et al.). ESAT6, CFP-10, and several components of the ESX-1 system are encoded by the RD1 gene cluster (Andersen et al.; Berthet et al.), 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 (Mahairas et al.; Philipp et al.; Brosch, Philipp et al.; Brosch, Gordon et al.; Gordon et al.; Behr et al.; Zumarraga et al.). 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 cluster (Brosch, Gordon et al.; Behr et al.).

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 adenosine triphosphatase (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 (Renshaw, Panagiotidou et al.; Renshaw, Lightbody et al.), 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 (Stanley et al.). A second, non-RD1 gene cluster, the Rv3614c-Rv3616c locus, is also required for ESAT-6 secretion (MacGurn et al.). 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 in RD1. The secretion of ESAT-6 and CFP-10 is critical for *M. tuberculosis* 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 for 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 number of genes and run the gamut from 7 to 18. These clusters probably arose from gene duplication (Tekaia et al.). Surprisingly, these systems do not complement one another, although ESX-3 and ESX-5 appear to be essential (Abdallah et al.). 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 FtsK/SpoIIE-like ATPase. It is thought that Rv3871 interacts with CFP-10 and delivers the heterodimer complex in an ATP-dependent manner to Rv3870, thereby delivering these virulence factors to the secretion

machinery. The molecular mechanism by which ESX secretes virulence factors remains to be elucidated.

Only ESX-1, ESX-3, 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 (Gey Van Pittius et al.). Two components of ESX-3 are not found in Mle, and many components of ESX-3 are essential for growth (Lamichhane et al.). This suggests that some ESX-3 components may not be essential.

(Putative) transporters of unknown function or mechanism

In the 9B series of putative permeases, we find two Mtu and two Mle homologues of bacterial murein precursor exporters of the MPE family (TC 9.B.30), which are found in many, if not all, bacteria. These porters probably serve the function of exporting precursors essential for bacterial cell wall synthesis (Boyle et al.; Gerard, Vernet and Zapun). 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) (Alix and Blanc-Potard). The results are consistent with a Mg^{2+} uniport or a Mg^{2+}: H^{+} antiport mechanism, but a transport function for MgtC has not yet been established. MgtC is

required for intramacrophage survival. Also identified in Mtu but 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).

Results Acknowledgement

Results, in full, will be submitted for publication of the material as it may appear in Genomics, Genome Biology, or Biochemica acta, 2008, Youm, Ji-Won; Saier, Milton H., Jr, 2008. The thesis author was the primary investigator and author of this paper.

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 encountered during pathogenesis. We have analyzed the transporters in these organisms to determine what systems might confer upon *M. tuberculosis* 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 likely are implicated in having 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.

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 reduced by the NADH dehydrogenase complexes of 14 dissimilar subunits in ETC. Consistent with the previous reports, this complex was identified in Mtu but not Mle (Cole, Eiglmeier et al.). 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^+ (Marques et al.). However, the presence of all subunits of the H^+-translocating F-type ATPase in Mle, which is also present in Mtu, suggests that respiration is active. The presence of the majority of proton pumping cytochrome oxidase complex subunits (3.D.4) in both Mtu

and Mle provides further support to this notion. Interestingly, the subunits are not encoded by the same operons, as seen in *Bdellovirbio bacteriovorus*.

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.

Iron-sulfur centers are essential to life as they 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, with the exception of a protein homologous to the receptor component of the sulfate porter, suggesting that the primary means to acquiring sulfur in Mle may be through sulfur-containing organic compounds like cysteine. Although the ABC-type uptake system for cysteine is present in Mtu but not Mle, additional transporters of cysteine or other organic compounds may 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 such iron-siderophore complexes facilitate the uptake of ions that are otherwise very insoluble. One such transporter is the iron (Fe^{3+}) · pyridine-2,6-bis(thiocarboxylic acid (PDTC)) uptake transporter, encoded by both Mtu and Mle. A transporter of the Zinc-Permease (ZIP) family was found in Mtu but not Mle. The Fe^{3+}-carboxymycobactin transporter was also found in Mtu but not Mle. Taken together, the absence of these two transporters in Mle suggests that PDTC may be the primary means of iron uptake in Mtu and Mle.

When oxygen is not readily available to Mtu, nitrate can be used as the final electron acceptor of ETC in place of oxygen and undergo anaerobic respiration. Nitrate is reduced by the nitrate reductase, and iron-sulfur centers are found in the NarG and NarH subunits of the nitrate reductase complex. Additionally, NarG requires the molybdopterin cofactor, which contains molybdenum. Molybdate uptake in Mtu is achieved by a transport system that most resemble 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.

There may be additional compounds that serve as the final electron acceptor of the electron transport chain. 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.

Evasion of host immunity, persistence, and proliferation

Several types of specialized secretion systems like ESX1-5 are dedicated for the secretion of virulence factors and play important roles in many stages of pathogenesis of both Mtu and Mle. ESX-3 is regulated by iron and zinc availability (Maciag et al.; Rodriguez and Smith). Zinc may be acquired by Mtu with the zinc-iron permease of the ZIP family. Mle does not appear to have an ortholog of this permease and how zinc is obtained by Mle is unclear, but decreased zinc concentration in the disease-affected skin of leprosy patients (Jain et al.) may suggest the presence of a zinc transporter in Mle.

ESAT-6 and CFP-10, and possibly other virulence factors, are implicated in arresting the maturation of phagosomes in macrophages. The fusion of phagosomes to

lysosomes is blocked, and so, the acidification of these pathogens' environment is averted. However, these macrophages may overcome this arrest upon activation by cytokines like interferon-gamma. Phagosomal compartment are acidified. OmpATb (1.B.6.1.3) is essential for Mtu's adaptation to low pH and survival in macrophage (Niederweis). 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.

Several killing mechanisms such as the generation of free radicals may also be employed (see Introduction) by macrophages. 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 against free radicals (Struzynska, Chalimoniuk and Sulkowski). 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 Mtu and Mle survival 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) (Glickman and Jacobs), 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 (James et al.).

Under conditions of low oxygen, Mtu relies heavily upon nitrate respiration using the *narKX2* operon in addition to the *narGHIJ* operon. Intriguingly, two domains are fused to a permease with specificity for ribose and autoinducer-2 in Mle: 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 aerobic 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. Autoinducers are interspecies communication molecule produced by both G+ and G- bacteria and are sugar derivatives that bind borate with extremely high affinity. The particular autoinducer-2 imported by this permease may be an aerotactic pheromone that amplifies this signal transduction to move toward areas more abundant in oxygen or away from areas low in oxygen.

The granuloma, particularly the necrotic center, is replete with lipids and proteins from dead macrophages, lymphocytes and mycobacteria. The glyoxylate shunt is the choice biochemical pathway in the persistent mycobacteria. As such, Mtu most likely uses a variety of lipid transporters to import a diverse set of fatty acids. Acyl-CoA synthetases are implicated as possible lipid transporters. There are as many as 38 acyl-CoA synthetases encode by Mtu, but only one acyl-CoA synthetase, the one that is listed in TCDB, was assigned with confidence to be a lipid transporter (see Discussion).

Latent infection may become active tuberculosis when granulomas caseate and liquefy. Mtu and Mle undergo tremendous proliferation upon "reactivation." To proliferate, mycobacteria must undergo cell division. Surprisingly, a protein belonging to the Filamentation in response to cAMP (Fic) family was identified in Mtu and encoded within the operon that encodes for the oligopeptide uptake system (3.A.1.5.2) of the PepT family. In G+ bacteria, many pheromones are peptides. This cell-to-cell communication may allow rapid proliferation that is observed in Mtu. Therefore, this protein and the peptide transport might function together to regulate cell division. Additionally, the presence of septal DNA translocators (3.A.12) in both Mtu and Mle suggest that septum formation precedes the transfer of DNA. 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 the next set of host cells. Cell-to-cell spread may be achieved by the TmtpC proteins (2.A.6.5.3), which are implicated in sliding motility. Surprisingly, seven paralogues were discovered in Mtu, one of which is likely to be a pseudogene (GI: 15841024). The first ~600 aas of TmtpC have been effectively deleted upstream of the ~370 aas of this putative pseudogene in Mtu, as confirmed by tBLASTn. 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, especially in Mtu, allow for adaptation to varying conditions of osmolarity and pH (particularly in the extracellular

environment). 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 (Pivetti et al.). As such, Mle possesses only one of the MscL-type for intracellular life presumably because it probably relies on the host cell's homeostatic mechanisms to regulate intracellular pressure. These two MscS type channels likely provide additional adaptation to various osmotic pressure changes associated with the free lifestyle of Mtu responsible for its pathogenesis. Perhaps not surprisingly, one of the MscS types in Mtu is gated by cAMP for finer control. Other transporters involved in intracellular pressure homeostasis include: a K^+ channels, a BetS of 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 (as discussed earlier).

Antimicrobial drugs

Many drug efflux systems utilizing secondary active transport as carriers 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). SULFATIDE EXPORTERS? Of these, only 1 of DHA2, 1 of DMT, and 1 of MVF are found in Mle, suggesting that secondary active transport may not the primary means of drug efflux for intracellular life. Primary active pumps were 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 DrugRA are found in Mle. This suggests that

the primary active drug efflux systems are preferentially retained for intracellular survival.

Other toxins and virulence factors

Mtu and Mle are resistant to a panoply of toxic metals and organic compounds. Mtu and Mle both have ArsB permeases that may confer upon these organisms resistance to arsenite, which can react with free thiols of proteins, particularly those that are involved in the citric acid cycle. Mtu also possesses ArsA, the cytoplasmic ATPase that energizes efflux of arsenite against concentration gradients. Mle may have lost ArsA through reductive evolution perhaps because it is less likely that Mle would encounter an environment so rich in arsenite as to require a pump to transport out arsenite against an electrochemical gradient. Mle host cells would not be expected to have such high levels of arsenite.

Mtu and Mle may also be resistant to acetate as they both express the acetate exporter (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.

Nutrient uptake

Many transporters in Mtu and Mle are involved in the uptake of nutrients. Mtu probably acquires nitrogen as ammonium either through the Amt channel transporter (1.A.11) or the Amt carrier transporter (2.A.49). Both transporters are absent in Mle, suggesting that ammonium may not be the primary source of nitrogen. Instead, Mle may acquire nitrogen as ammonia, which can freely diffuse across the cell envelope, or

acquire nitrogen from the uptake of organic compounds. This may also explain Mle's extremely slow growth.

Mtu and Mle possess many carriers and primary active uptake systems dedicated for acquiring carbohydrates. Three sugar porters (1 of the SP family and 2 of the ACS family) and four carboxylate symporters (many carboxylates are metabolic intermediates) are the carrier type that utilizes secondary active transport. None of these secondary active transport utilizing carrier types are found in Mle. Instead, relatively more primary active uptake systems for sugars are retained in Mle. Four, maltose type, uptake systems (CUT1) are encode by Mtu and three of these are retained in Mle. Moreover, a complete ribose type uptake system (CUT2) is found in Mle, but only an incomplete system is found in Mtu.

Likewise, Mtu and Mle also encode for many transporters of both the carrier type and primary active type that are dedicated 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, ThrE, and two of LysE are only found in Mtu. This suggests that peptide hydrolysis may be toxic to Mtu but not to Mle. Uptake systems include that of the PepT family, two in Mtu and one in Mle. This may be involved in inducing cell division as discussed earlier.

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 synthase 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 the N-termini: a cAMP binding domain followed by a phosphodiesterase domain, suggesting that this transporter may also be involved in regulating enzymes that break down cyclic nucleotide phosphodiesterase. Drug transporters of the MVF family (2.A.66.4) in Mtu and Mle have the serine/threonine protease domains at the N-termini. We believe that this transporter has a novel regulatory function in Mtu and Mle, but little is known about the MVF family.

Putative transporters with little confidence

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 this esterification (Saier and Kollman; Dirusso and Black). Faergeman et al. have presented evidence that fatty acyl-CoA synthetase function as components of fatty acid uniport systems in yeast by linking import and activation of exogenous fatty acids (Faergeman et al.). Further, Zou et al. isolated FAT1 mutants of S. cerevisiae that are deficient for either transport or acyl-CoA synthetase activity (Zou et al.). 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.

These set of proteins were difficult to assign to transport families for a variety of reasons. Many of the acyl-coA synthetases were difficult to assign. Although there functional information for the Proposed Fatty Acid Transporter (FAT) family (4.C.1) is sparse, acyl-coA synthetases with 2-4 TMSs are likely to be transporters and those with

no TMS are not likely to be. While a particular ABC permease can be correctly predicted to have a certain number of TMSs by TMHMM and WHAT programs, such consensus was not achieved amongst the these programs for these putative FAT family proteins in this genome. The peroxysomal fatty acyl-CoA synthase (ligase) of *Homo sapiens* is predicted to have 3 TMSs by TMHMM and 4 TMSs by WHAT. The *M. tuberculosis* homologue is predicted to have 0 TMS by TMHMM and 4 TMSs by WHAT albeit with less pronounced hydropathy scores. Based on our analyses using the WHAT program, as many as 38 acyl-coA synthetases may be involved in fatty acid transport. Given the different lengths of fatty acids that may exist in the extracellular environment, particularly in granulomas, the presence of many different types of fatty acid transporters is feasible. Orphan ABC transporters that most resemble constituents of incomplete multi-component systems could not be assigned to particular families with confidence.

Future direction

As more genomes are sequenced, the transport database will undoubtedly expand, and this may allow the identification of transporters or the assignment of putative transporters with greater confidence. Alternatively, a putative transporter may not be assigned as a transporter. Our findings suggest many areas that can serve as the starting point for experimental analyses to investigate aspects of pathogenesis that remains to be elucidated. Furthermore, there is potential for drug development. Isoniazid enters the Mtu cell and becomes active only when an Mtu enzyme acts on it. Similarly, fatty acids could be attached to particular drugs to mediate uptake by Mtu. This might hinder Mtu's ability to survive during latency.

Discussion and overview Acknowledgement

Discussion and overview, in full, will be submitted for publication of the material as it may appear in Genomics, Genome Biology, or Biochemica acta, 2008, Youm, Ji-Won; Saier, Milton H., Jr, 2008. The thesis author was the primary investigator and author of this paper.

CONCLUSION

147 and 55 complete transport systems were identified in Mtu and Mle, respectively. Transport proteins may be selectively retained in reductive evolutionary processes. Drug exporting transport systems display the highest retention rate. P-P-bond hydrolysis-driven subclass of transporters display the highest retention rate (52%), as many of these are drug exporting transport systems. Many Mtu and Mle proteins display intra-operon gene fusions, possibly as a result of evolutionary pressure to make genomes more compact. Terminal fusions of regulatory domains to proteins in Mtu and Mle is frequent, reinforcing the notion that evolution tends toward complexity. Many transport protein that may allow Mtu to persist in granulomas during latent infection, especially those involved in anaerobic respiration or fatty acid transport, were identified.

Conclusion Acknowledgement

Conclusion, in full, will be submitted for publication of the material as it may appear in Genomics, Genome Biology, or Biochemica acta, 2008, Youm, Ji-Won; Saier, Milton H., Jr, 2008. The thesis author was the primary investigator and author of this paper.

APPENDIX

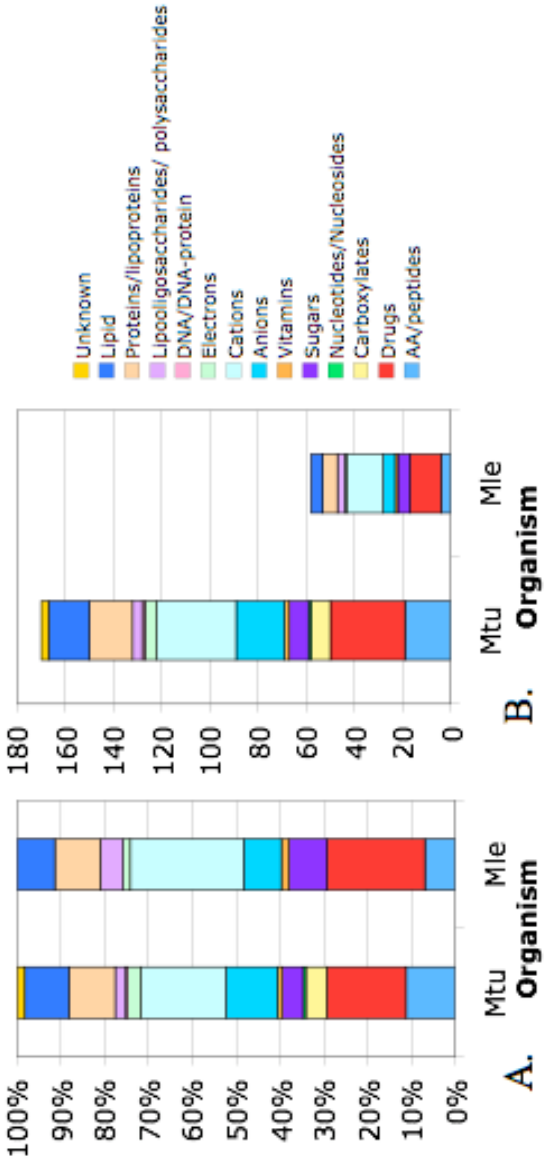


Figure 1: Distribution of transporters in *Mtu* and *Mle*

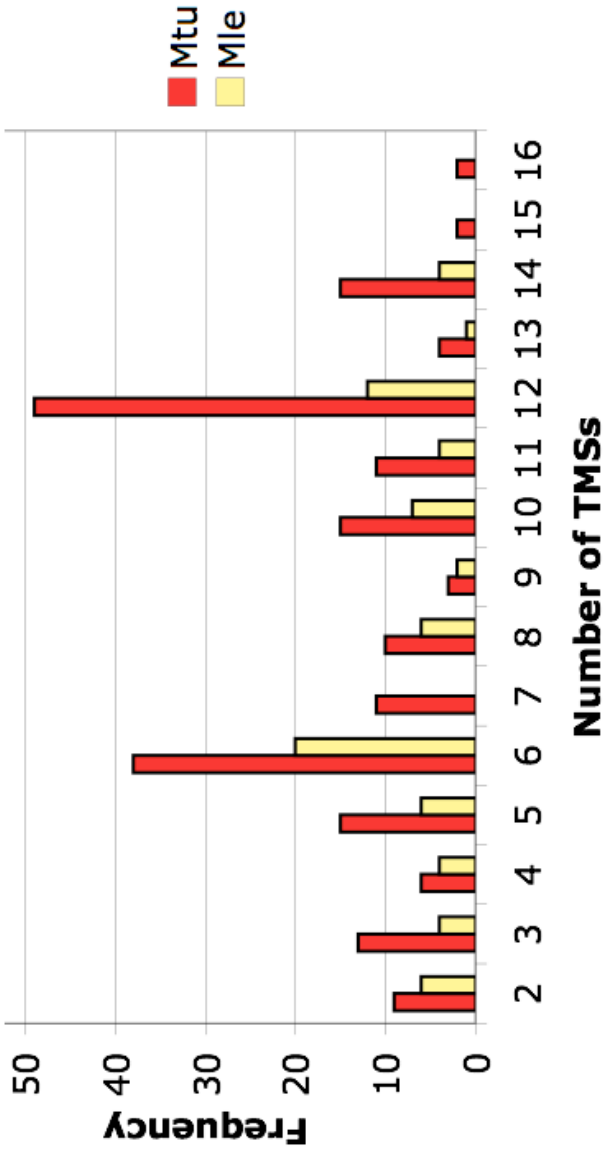
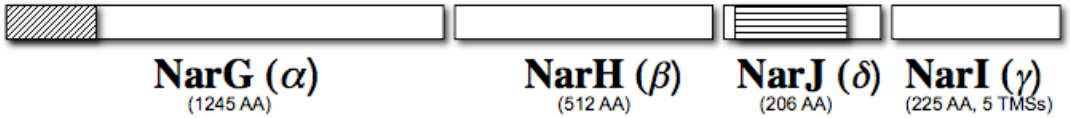


Figure 2: Membrane topology of transporters in Mtu and Mle

Figure 3: Nitrate reductase(s) in Mtu

A) The *narGHJI* operon



B) The *narK2X* operon

Table 1: Overview of the *M. tuberculosis and M. leprae* transporter analyses

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	133 (43)	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	7 (4)	1 (1)	25%	5.A	Transmembrane two-electron transfer carriers	7 (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 system	52 (12)	32 (3)	25%	9.A	Recognized transporters of unknown biochemical mechanism	53 (12)	32 (3)	25%
	Total	**284 (148)**	**135 (55)**	**37%**			**284 (148)**	**135 (55)**	**37%**

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

[b] Numbers in parentheses represent the number of transport systems. Transport systems are comprised of constitudent transport proteins.

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

Table 2: Overview of Transport Systems by Substrate Specificity in *M. tuberculosis and M. leprae*

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	58	34%	22	38%	38%	Anions	20	12%	6	10%	30%
						Cations	33	19%	15	26%	45%
						Electrons	5	3%	1	2%	20%
Macromolecule	27	16%	12	21%	44%	DNA/DNA-protein	1	1%	1	2%	100%
						Lipooligosaccharides/polysaccharides	2	1%	1	2%	50%
						Proteins/lipoproteins	13	8%	7	12%	54%
						Lipid	11	6%	3	5%	27%
Total	148	100%	55	100%	37%		148	100%	55	100%	37%

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

Table 3: TC classification and functional predictions of putative transport proteins from *M. tuberculosis* and *M. leprae*

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle	
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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.A.11	Ammonia Channel Transporter (Amt) Family	1.A.11.1.3	1	Q79VF1	438	10		15842463	15610057	477	10	52	Mtu-C: [1,2]	-	-	-	-	-	1 0
1.A.22	Large Conductance Mechanosensitive Ion Channel (MscL) Family	1.A.22.1.2	1	O53898	151	2		15840410	15608125	151	2	100		15826991	154	2	71	70	1 1
1.A.23	Small Conductance Mechanosensitive Ion Channel (MscS) Family	1.A.23.3.1	1	O34897	267	2		15842675	15610241	308	2	30		-	-	-	-	-	2 0
		1.A.23.4.1	1	Q58543	361	6		15841954	15609571	481	6	24	Mtu-C: Crp(31008), CAP_ED(28920)[1,2,3,4]	-	-	-	-	-	
1.A.35	CorA Metal Ion Transporter (MIT) Family	1.A.35.3.1	1	Q58439	317	3		15840684	15608379	366	3	19	Mtu-N: [1,2,3,4]	15827535	369	3	19	79	1 1
1.B Outer Membrane Porins (beta-structure)																			
1.B.6	OmpA-OmpF Porin (OOP) Family	1.B.6.1.3	1	P65593	326	1		15840318	15608039	326	1	100		-	-	-	-	-	1 0
1.B.50	Acid-fast Bacterial, Outer Membrane Porin (AFB-OMP)	1.B.50.1.1	1	P64883	314	1		15841155	15608836	314	1	100		15827709	317	1	-	56	1 1
2.A Carrier-type Facilitators																			
2.A.1	Major Facilitator Superfamily (MFS)																		
	SP	2.A.1.1.1	1	P37021	464	12		15842926	15610467	500	12	30	Mtu-N: [1,2]	-	-	-	-	-	1 0
	DHA1 (12-Spanner)	2.A.1.2.7	1	Q9S3J9	395	12		15840256	15607982	442	12	21	Mtu-N: [1] Mtu-C: [1]	-	-	-	-	-	2 0
								15839570	15607332	413	12	32		-	-	-	-	-	
	DHA2 (14-Spanner)	2.A.1.3.5	1	Q54806	501	14		15841836	15609470	537	14	32	Mtu-C: [1,2]	-	-	-	-	-	12 2
		2.A.1.3.11	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	
		2.A.1.3.12	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		-	-	-	-	-	
		2.A.1.3.25	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.A.1.3.32	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	
	MHS	2.A.1.6.3	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]	-	-	-	-	-	
		2.A.1.6.6	1	P76350	438	12		15840644	15608340	425	12	36		-	-	-	-	-	

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle		
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
	NNP	2.A.1.8.1	1	P10903	463	12	Nark2	15841832	15609466	515	12	35	Mtu-C: [1]; NarK2	15827374	517	12	35	69	4	1
		2.A.1.8.11	1	Q93PW1	905	24	NarK1-NarK2	15841201	15608875	395	12	31	NarK1	-	-	-	-	-		
							NarK1-NarK2	15839643	15607402	469	12	37	NarK2	-	-	-	-	-		
							NarK1-NarK2	15839648	15607408	463	12	41	NarK2	-	-	-	-	-		
	SHS	2.A.1.12.2	1	P36035	616	12		15841373	15609039	408	12	29		-	-	-	-	-	1	0
	ACS	2.A.1.14.1	1	P42237	455	12		15842550	15610131	445	12	21	Mtu-C: [1,2]	-	-	-	-	-	2	0
		2.A.1.14.3	1	P70786	449	12		15841127	15608810	443	12	38		-	-	-	-	-		
	DHA3	2.A.1.21.1	1	P95827	405	12		15839414	15607179	433	12	22	Mtu-N: [1,2,3,4]	-	-	-	-	-	2	0
		2.A.1.21.4	1	O32859	409	12		15840704	15608398	419	12	69		-	-	-	-	-		
	AzgA	2.A.1.40.2	1	O34987	440	12		15840752	15608442	405	12	27		-	-	-	-	-	1	0
	PDTC-T	2.A.1.55.1	1	Q2M5P1	426	12		15842036	15609645	448	12	49		15827137	454	12	26	75	1	1
2.A.3	Amino Acid-Polyamine-Organocation (APC) Family																			
	AAT	2.A.3.1.5	1	P46349	469	12		15839916	57116749	434	12	52		-	-	-	-	-	4	2
		2.A.3.1.7	1	P39312	470	12		15841162	15608842	556	12	59	Mtu-C: [1,2,3]	-	-	-	-	-		
		2.A.3.1.8	1	P40812	497	12		15841619	57116950	462	12	61		15827675	505	12	59	83		
								15839732	57116723	487	12	58		15827674	498	12	59	83		
	CAT	2.A.3.3.1	1	Q09143	622	14		15841825	15609457	471	14	34	Mtu-C: [1,2,3,4]	-	-	-	-	-	2	0
		2.A.3.3.3	1	Q96241	533	14		15842842	15610389	495	14	35	Mtu-C: [1,2,3,4]	-	-	-	-	-		
	EAT	2.A.3.5.1	1	Q53148	488	12		15842228	15609827	657	12	20	Mtu-N: [1,2,3,4] Mtu-C: [1,2,3,4]	-	-	-	-	-	1	0
	ABT	2.A.3.6.1	1	O28661	736	12		15841455	15609116	481	12	25		15828069	572	12	24	77	2	1
								15841481	15609136	440	12	29		-	-	-	-	-		
2.A.4	Cation Diffusion Facilitator (CDF) Family	2.A.4.1.1	1	P13512	316	6		-	-	-	-	-		15827060	268	6	37	-	1	1
		2.A.4.1.2	1	O85143	326	6		15841512	15609162	332	6	26		-	-	-	-	-		
2.A.5	The Zinc (Zn2+)-Iron (Fe2+) Permease (ZIP) Family	2.A.5.5.1	1	P0A8H3	257	8		15839705	57116721	258	8	NA	Mtu-C: COG0428(30777)[1,2,3,4]; distantly related (GAP post-optimization SD = 9.28)	-	-	-	-	-	1	0
2.A.6	Resistance-Nodulation-Cell Division (RND) Superfamily																			
	HAE2	2.A.6.5.1	1	Q53902	711	12		15839582	15607343	966	11	28	Mtu-N: COG2409(32544)[1,2,3] Mtu-C: [1,2,3,4]	15828413	1013	11	26	72	12	5
								15839586	15607347	944	12	22	Mtu-N: COG2409(32544)[1,2,3] Mtu-C: [1,2]	15828415	955	12	23	75		
		2.A.6.5.2	1	P96289	920	12		15842488	15610079	920	12	100		15826968	902	12	69	68		
		2.A.6.5.3	1	Q9KH53	994	12		15841024	15608695	397	7	68	pseudogene; putative deletion [1] ("deleted sequence" implicated in sliding motility); putatively nonfunctional	-	-	-	-	-		

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle		
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
								15841224	15607591	945	12	31		-	-	-	-	-		
								15841845	15609476	962	12	55		-	-	-	-	-		
								15839785	15607543	958	12	54		-	-	-	-	-		
								15839838	15607591	967	12	63		15828278	959	12	62	78		
								15839900	15607648	968	12	57		-	-	-	-	-		
								15840079	15607816	964	12	60		-	-	-	-	-		
		2.A.6.5.4	1	O07800	1089	12		15840626	15608323	1002	12	58		15827629	983	12	56	70		
								15840989	15608660	1146	12	50	Mtu-C: [1,2]	-	-	-	-	-		
								15843447	15610959	1089	12	100		-	-	-	-	-		
2.A.7	Drug/Metabolite Transporter (DMT) Superfamily	2.A.7.1.2	1	P95094	107	4		15842634	57117052	107	4	100		15827941	107	4	79	78	1	1
2.A.9	Cytochrome Oxidase Biogenesis (Oxa1) Family	2.A.9.2.1	1	O04665	462	6		15843555	15611057	366	5	28	Mtu-N: [1,2,3]	15828468	380	5	30	77	1	1
2.A.15	Betaine/Carnitine/Choline Transporter (BCCT) Family	2.A.15.3.2	1	Q8VTN3	706	12		15840338	15608057	593	12	48	Mtu-C: [1,2,4]	-	-	-	-	-	1	0
2.A.19	Ca2+:Cation Antiporter (CaCA) Family	2.A.19.1.1	1	P31801	366	10		15841060	15608745	360	10	35		15827650	364	10	36	77	1	1
2.A.20	Inorganic Phosphate Transporter (PIT) Family	2.A.20.1.2	1	P43676	499	10		15839941	15607685	417	10	33	Mtu-C: PitA(30654), PitA(30654)[1,2,3,4]	15828208	414	9	30	81	2	1
		2.A.20.2.4	1	Q38954	587	12		15841772	15609418	552	12	43	Mtu-N: [1,2,3,4] Mtu-C: PHO4(65198), PitA(30654)[1,2,3,4]	-	-	-	-	-		
2.A.23	Dicarboxylate/Amino Acid:Cation (Na+ or H+) Symporter (DAACS) Family	2.A.23.1.3	1	Q01857	444	12		15841966	15609580	491	12	45	Mtu-C: [1]	-	-	-	-	-	1	0
2.A.36	Monovalent Cation:Proton Antiporter-1 (CPA1) Family	2.A.36.3.1	1	P32703	549	12		15841778	15609424	542	12	28		-	-	-	-	-	1	0
2.A.37	Monovalent Cation:Proton Antiporter-2 (CPA2) Family	2.A.37.5.1	1	O50576	390	13		15842825	57117079	385	13	28		15827342	385	13	27	81	1	1
2.A.45	Arsenite-Antimonite (ArsB) Efflux Family	2.A.45.1.1	1	P30329	429	11		15842222	15609821	429	11	22	Mle ortholog (15827501) hits equally well to both.	15827501	429	10	23	74	2	1
								15842223	57117014	428	10	22		15827501	429	10	23	74		
2.A.49	Ammonium Transporter (Amt) Family	2.A.49.14.1	1	Q57753	395	10		15839524	15607285	492	10	27	Mtu-N: [1,2] Mtu-C: [1,2]	-	-	-	-	-	1	0
2.A.52	Ni2+-Co2+ Transporter (NiCoT) Family	2.A.52.1.1	1	P23516	351	8		15842397	15609993	372	8	42		-	-	-	-	-	1	0
2.A.53	Sulfate Permease (SulP) Family	2.A.53.4.1	1	P72770	566	13		15841203	15608877	560	11	33	different # of TMS but good e-value	-	-	-	-	-	3	0
		2.A.53.5.1	1	Q7U617	556	12		15841167	15608845	486	12	34		-	-	-	-	-		
		2.A.53.8.1	1	Q8F8H7	750	10		15842863	15610409	764	10	29		-	-	-	-	-		
2.A.55	Metal Ion (Mn2+-iron) Transporter (Nramp) Family	2.A.55.3.1	1	P77145	412	11		15840347	57116797	429	11	43		15828133	426	11	42	74	1	2
								15840347	57116797	429	11	43		15828438	618	12	25	25		
2.A.59	Arsenical Resistance-3 (ACR3) Family	2.A.59.1.2	1	P45946	346	10		15842183	15609780	498	10	50	Mtu-C: Wzb(30743)[1,2,3,4]	-	-	-	-	-	1	0

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle	
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
2.A.64	Twin Arginine Targeting (Tat) Family	2.A.64.1.1	4	O69415	171	1	B	15840668	15608364	131	1	33	Mtu-C: [1,2,3]	15827529	120	1	36	73	1 1
				P69423	258	6	C	15841584	15609230	284	6	31		15827693	286	6	33	86	
				P69428	89	1	A	161350070	15609231	83	1	30	Mtu-N: [1]	161723267	88	1	40	69	
2.A.66	Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily																		
	MATE	2.A.66.1.4	1	P28303	459	13		15842377	15609973	436	13	29		-	-	-	-	-	1 0
	MVF	2.A.66.4.1	1	P37169	524	14		15843543	15611046	1184	14	27	Mtu-C: [1,2,3,4]	15828460	1206	14	27	75	1 1
2.A.67	Oligopeptide Transporter (OPT) Family	2.A.67.4.1	1	P44016	633	15	Mtu(18)	15841909	15609532	667	15	42	Mtu-N: [1,2,3,4] Mtu-C: [1,4] Mtu(18)	-	-	-	-	-	1 0
2.A.75	L-Lysine Exporter (LysE) Family	2.A.75.1.1	1	P94633	236	6		15841464	15609123	199	6	31		-	-	-	-	-	2 0
								15839879	15607629	201	6	32		-	-	-	-	-	
2.A.79	Threonine/Serine Exporter (ThrE) Family	2.A.79.1.1	1	Q93PN2	489	10		15843358	15610873	529	10	38	Mtu-N: [1,2] Mtu-C: [1,2]	-	-	-	-	-	1 0
2.A.83	Na+-dependent Bicarbonate Transporter (SBT) Family	2.A.83.1.1	1	P73953	374	10		15841900	15609524	430	10	24		-	-	-	-	-	1 0
3.A. P-P-Bond Hydrolysis-driven Transporters																			
3.A.1	ATP-binding Cassette (ABC) Superfamily																		
	CUT1	3.A.1.1.3	4	P10907	356	1	[C]	15842373	15609969	360	1	45		-	-	-	-	-	4 3
				P10904	438	1	[R]	15842374	15609970	350	1	33	Mtu-N: [1,2,3,4] Mtu-C: [1,2,3,4]	-	-	-	-	-	
				P10906	281	6	[M]	15842375	15609971	275	6	33		-	-	-	-	-	
				P10905	295	6	[M]	15842376	15609972	303	6	29		-	-	-	-	-	
		3.A.1.1.7	4	O51923	450	1	[R]	15840680	15608375	468	1	29	Mtu-N: [1,2,3]	15827531	468	1	27	77	
				O51924	300	8	[M]	15840681	15608376	307	8	38		15827532	304	8	39	81	
				O51925	276	6	[M]	15840682	15608377	274	6	40		15827533	287	6	37	89	
				?	330	1	[C]	15840683	15608378	393	1	?	Mtu-C: MalK(33631), PotA(33633)[1,2,3,4]	15827534	392	1	?	79	
				O51924	300	8	[M]	15841821	15609453	290	8	35		15827944	328	8	34	83	
				Q9R9Q5	278	6	[M]	15841822	57116968	274	6	30		15827945	274	6	27	85	
				O51923	450	1	[R]	15841823	15609455	426	1	24	Mtu-N: [1,2] Mtu-C: [1,2]	15827946	446	1	22	75	
				?	330	1	[C]	15842226	15609825	317	1	?	Mtu-N: [1,3,4]	15827943	71	1	?	23	

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle		
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
		3.A.1.1.20	4	AAO21860	368	0	[C]	15841526	15609175	357	0	46		15827749	356	0	44	79		
				AAO21858	285	6	[M]	15841527	15609176	280	6	33		15827750	283	6	32	79		
				AAO21857	285	6	[M]	15841528	15609177	300	6	21	Mtu-C: UgpA(31368)[1,2,3,4]	15827751	319	6	30	81		
				AAO21856	431	1	[R]	15841529	15609178	439	1	20	Mtu-N: [1,2] Mtu-C: [1,2,3]	15827752	445	1	20	77		
	CUT2	3.A.1.2.1	4	P0AGI1	321	10	[M]	-	-	-	-	-	not found in other Mtu or myco	15827122	602	10	39	-	0	1
				P02925	296	1	[R]	-	-	-	-	-		15827123	345	1	29	-		
		3.A.1.2.7	3	Q9F9B1	360	10	[M]	15840917	15608595	261	6	22		15827235	265	6	?	83		
				Q9F9B0	260	0	[C]	15840918	15608596	313	0	31	Mtu-C: [1,2,3,4]	15827236	315	0	35	87		
	PepT	3.A.1.5.1	6	P06202	542	1	[R]	15842124	15609722	557	1	58		15827166	555	1	23	78	2	1
		3.A.1.5.2	4	P26905	335	0	[C]	15843277	15610799	548	0	44	Mtu-C: COG1123(31320)[1,2,3,4]	-	-	-	-	-		
				P26904	320	6	[M]	15843278	15610800	266	6	40		-	-	-	-	-		
				P26903	308	6	[M]	15843279	15610801	308	6	34		-	-	-	-	-		
				P26906	543	1	[R]	15843280	15610802	541	1	22	Mtu-N: []	-	-	-	-	-		
		3.A.1.5.11	4	P75797	512	1	[R]	15840727	15608420	591	1	21	Mtu-N: [1,2,3,4]	15827554	557	1	21	80		
				P75796	629	0	[C]	15840728	15608421	612	0	44		15827555	609	0	42	84		
				P75799	303	6	[M]	15840729	15608422	291	6	34		15827556	299	6	31	82		
				P75798	306	6	[M]	15840730	15608423	325	6	33		15827557	325	6	32	83		
	SulT	3.A.1.6.3	4	P71747	353	1	[C]	15841913	57116983	353	1	100		-	-	-	-	-	1	0
				P71746	272	6	[M]	15841914	15609535	272	6	100		-	-	-	-	-		
				P71745	283	6	[M]	15841915	15609536	283	6	100		-	-	-	-	-		
				P71744	356	1	[R]	15841916	15609537	356	1	100		15827252	348	1	77	76		
	PhoT	3.A.1.7.1	4	P06128	346	1	[R]	15840351	57116798	389	1	30	Mtu-N: [1,2]	15828132	369	1	31	77	2	1
				P07653	319	6	[M]	15840352	15608069	324	6	35		15828131	319	6	34	82		
				P07654	296	6	[M]	15840353	57116799	304	6	36		15828130	304	6	35	80		
				P07655	257	0	[C]	15840233	15607960	258	0	54		15828171	258	0	53	91		
				P06128	346	1	[R]	15840355	57116800	370	1	32	Mtu-N: PstS(30575)[1,2,3]	15828172	429	4	28	31		
				P07655	257	0	[C]	15840356	15608073	276	0	44		-	-	-	-	-		
				P06128	346	1	[R]	15840357	57116801	374	1	33	Mtu-N: [1,2]	-	-	-	-	-		
				P07653	319	6	[M]	15840358	57116802	338	6	37		-	-	-	-	-		
				P07654	296	6	[M]	15840359	15608076	301	6	30		-	-	-	-	-		
	MolT	3.A.1.8.1	3	P37329	257	1		15841325	15608994	261	1	28		-	-	-	-	-	1	0
				P09834	229	5		15841326	15608995	245	5	40		-	-	-	-	-		
				P09833	352	1		15841327	15608996	369	1	33	Mtu-C: CysA(31315)[1,2,3,4]	-	-	-	-	-		
	QAT	3.A.1.12.3	4	Q45461	217	5	[M]	15843376	15610892	239	5	32		-	-	-	-	-	1	0
				P39775	226	5	[M]	15843377	15610893	229	5	29		-	-	-	-	-		
				Q45460	381	0	[C]	15843378	15610894	367	0	42	Mtu-C: [1,2,3]	-	-	-	-	-		
				Q45462	306	1	[R]	15843379	15610895	343	1	21	Mtu-N: [1]	-	-	-	-	-		

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle	
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
	MZT	3.A.1.15.6	3	Q9RNI8	284	8		-	-	-	-	-		15827091	286	8	29	-	0 1
				Q9RNI9	241	0		-	-	-	-	-		15827092	275	0	26	-	
				P0A4G4	310	1	[R]	15841548	15609196	511	1	43		15827093	302	1	22	64	
	SIUT	3.A.1.21.2	2	P63391	859	6		15840803	15608488	859	6	100		-	-	-	-	-	1 0
				P63393	579	6		15840804	15608489	579	6	100		-	-	-	-	-	
	ThiW	3.A.1.26.4	1	NP_336866.1	697	5	[M-C-C]	15841829	15609463	697	5	100		15827376	724	5	26	77	1 1
	LOSE	3.A.1.102.1	2	P50333	262	6		15841143	15608824	226	5	24	Mtu-N: [1,2,3,4]	-	-	-	-	-	1 0
				P50332	347	0		15841144	15608825	255	0	36		-	-	-	-	-	
	LPSE	3.A.1.103.1	2	Q48476	246	0		15843404	15610917	273	0	32		15826947	272	0	34	91	1 1
				Q48475	259	6		15843406	15610919	280	6	23	Mtu-N: [1,2,3]	15826945	276	6	26	84	
	DrugE1	3.A.1.105.1	2	P32010	330	0		15842482	15610073	331	0	46		15828268	331	0	48	85	2 2
				P32011	283	6		15842483	15610074	289	6	28	Mtu-N: [1] Mtu-C: [1,2]	15828267	288	6	26	63	
		3.A.1.105.2	2	Q53717	273	6		15842484	15610075	276	6	26	Mtu-N: [1]	15828266	276	6	23	78	
	Drug RA1	3.A.1.120.3	1	Q53712	569	0		15840933	15608611	542	0	36	Mtu-C: [1,2,3,4]	15827973	545	0	34	88	3 2
		3.A.1.120.5	1	Q2PGB8	591	0		15841123	15608806	591	0	31	Mtu-C: [1,2,3,4]	-	-	-	-	-	
								15842003	15609614	558	0	33		15827638	556	0	32	92	
	MacB	3.A.1.122.1	1	P75831	648	4	[M-C]	15842101	15609700	349	4	23	[M], Mtu-N: [1,3,4]	-	-	-	-	-	1 0
								15842102	15609701	330	0	40	[C], Mtu-C: CAP_ED(28920), cNMP_binding(63916)[1,2,3,4]	-	-	-	-	-	
								15839452	15607215	330	1	38	Mtu-C: CAP_ED(28920), Crp(31008)[1,2,3,4]	-	-	-	-	-	
	LPT	3.A.1.125.1	3	P75957	233	1	[C]	15840411	15608126	248	1	39		-	-	-	-	-	1 0
				P75956	399	4	tc:[M] Mtu:[M-M] IolC-IolE	15840412	15608127	855	10	23	Mtu-C: FtsX(66381), SalY(30922)[1,2,3,4]	-	-	-	-	-	
				P75958	413	4	[M] IolE	15839451	15607214	349	4	21		-	-	-	-	-	
	CydDC-E	3.A.1.129.1	2	P23886	573	6	[M-C]	15841075	15608758	576	6	29		-	-	-	-	-	1 0
				P29018	588	6	[M-C]	15841076	15608759	527	6	33	Mtu-N: [1,2]	-	-	-	-	-	
	DrugE2	3.A.1.135.1	2	Q9CIP5	664	6		15840719	15608412	631	5	38	Mtu-N: [1,2,3,4]	15827547	629	5	36	75	1 1
				Q9CIP6	573	5		15840720	15608413	582	5	34	Mtu-N: [1,2,3,4]	15827548	584	5	34	78	
	MDR	3.A.1.201.1	1	P08183	1280	12		15839573	15607335	1241	12	29	Mtu-N: MdlB(31327), SunT(32455)[1,2,3]	-	-	-	-	-	1 0
	P-FAT	3.A.1.203.1	1	P28288	659	6		15841289	15608956	639	6	27	Mtu-N: COG4178(33918)[1,2,3,4]	15828127	638	6	25	74	1 1
	EPP	3.A.1.204.3	1	Q9UNQ0	655	6		15841211	15608885	863	6	25	Mtu-N: COG1716(31902), COG1716(31902)[1,2,3,4] Mtu-C: [1,2,3]	-	-	-	-	-	1 0

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle	
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
3.A.2	H+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily	3.A.2.1.2	8	P21903	289	6	a	15840755	15608444	250	6	33		15827570	251	6	30	84	1 1
				P21905	89	2	c	15840756	15608445	81	2	31		15827571	81	2	28	92	
				P21904	168	1	b	15840757	15608446	171	1	23	Mtu-N: [1,2]	15827572	170	1	21	79	
				P29708	174	0	F1 (b+delta)*	15840758	15608447	446	0	22	Mtu-N: AtpF(31055), ATP-synt_B(64302)[1,2,3,4]	15827573	446	1	20	77	
				P29706	471	0	alpha	15840759	15608448	549	0	58	Mtu-C: [1,2,3,4]	15827574	558	0	58	90	
				P29710	281	0	gamma	15840760	15608449	305	0	37		15827575	298	0	36	83	
				P29707	467	0	beta	15840761	15608450	486	0	62		15827576	485	0	62	92	
				P29709	136	0	epsilon	15840762	15608451	121	0	32		15827577	121	0	34	87	
				P21904	168	1	b	15842473	15610064	247	1	30	Mtu-N: [1,2,3,4] Mtu-C: DivIVA(33399)[1,2,3,4]	15827879	247	0	28	90	
3.A.3	P-type ATPase (P-ATPase) Superfamily	3.A.3.2.4	1	P37367	905	10		15840327	15608048	797	10	26	Mtu-C: [1,2,3]	-	-	-	-	-	12 4
								15841479	15609134	901	10	46		-	-	-	-	-	
		3.A.3.5.1	1	P32113	727	8		15839473	15607234	761	8	37		15828075	766	8	35	77	
								15840395	15608109	792	8	46	Mtu-N: [1,2,3]	-	-	-	-	-	
								15839483	15607245	752	8	37		15828087	750	8	38	77	
		3.A.3.6.1	1	P20021	727	9		15840929	15608607	657	7	32	Mtu-N: [1,2]	-	-	-	-	-	
								15841474	15609129	771	6	39	Mtu-N: [1,2,3,4]	-	-	-	-	-	
								15842860	15610406	718	8	34	Mtu-N: [1,2,3,4]	15827315	725	8	35	85	
								15843364	15610879	660	6	35	Mtu-N: [1]	-	-	-	-	-	
		3.A.3.7.1	3	P03959	557	11	A	15840459	15608169	571	11	50		-	-	-	-	-	
				P03960	682	7	B	15840460	15608170	709	7	63		-	-	-	-	-	
				P03961	190	1	C	15840461	15608171	189	1	46		-	-	-	-	-	
		3.A.3.24.1	1	Q7U2U7	1625	2		15839812	15607566	1539	1	27		-	-	-	-	-	
								15839487	15607249	1625	2	31		15828441	1609	2	30	81	
3.A.4	Arsenite-Antimonite (ArsAB) Efflux Family	3.A.4.1.1	2	P37310	429	13	[M]	15843191	15610714	413	13	31		-	-	-	-	-	1 1
				P08690	583	0	[C]	15843297	15610815	340	0	27	Mtu-C: [1,2,3]	15828237	341	0	25	84	
								15843298	15610816	386	0	31	Mtu-C: ArsA(30352)[1,2,3,4]	15828238	381	0	24	88	

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle	
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
3.A.5	Type II (General) Secretory Pathway (IISP) Family[x]	3.A.5.1.1	11	P10408	901	0	SecA2 (ATPase)	15840015	15607753	855	0	36	Mtu-N: [1,2] Mtu-C: [1,2,3,4]	-	-	-	-	-	1 1
				P16920	127	3	SecE	15840041	57116765	80	1	37		15828021	146	1	35	75	
				P03844	443	10	secY (translocating channel) was reassigned	15840139	15607872	441	10	42		15827980	438	10	42	91	
				P33582	110	3	SecG*	15840898	57116863	77	3	27		15827226	77	2	29	96	
				P10408	901	0	SecA-1 (ATPase)	15841291	15608958	808	0	35	Mtu-N: [1,2] Mtu-C: [1,2,3,4]	15828125	778	0	35	87	
				P19674_b	323	6	SecF	15842125	15609723	442	6	27	Mtu-N: [1,2,3,4] Mtu-C: [1,2]	15827165	471	6	24	71	
		Mle has been replcaed		P19673_b	615	6	SecD	15842126	15609724	554	5	27	Mtu-N: SecD(30690)[1,2,3,4] Mtu-C: [1,2,3]	15827164	597	5	26	80	
				P07019	453	0	Ffh (SRP)	15842459	15610053	525	0	51	Mtu-C: [1,2,3]	15827854	521	0	50	88	
				P10121	497	0	FtsY (cell division protein)	15842465	15610058	422	1	40	Mtu-N: [1,2,3,4]	15827857	430	1	40	81	
				P0A9R7	222	1	FtsE (cell division ATP-binding protein)	15842673	15610239	229	1	49		15827279	229	1	49	91	
3.A.12	Septal DNA Translocator (S-DNA-T) Family	3.A.12.1.1	1	P21458	787	4		15842288	15609885	968	4	51	Mtu-N: FtsK(31860)[1,2,3,4]	15827463	886	4	38	77	1 1
3.D. Oxidoreduction-driven Active Transporters																			
3.D.1	Proton-translocating NADH Dehydrogenase (NDH) Family	3.D.1.3.1	14	Q56217	119	3	alpha	15842721	15610281	128	3	34		-	-	-	-	-	1 0
				Q56218	181	2	beta	15842722	15610282	184	2	64		-	-	-	-	-	
				Q56219	207	0	C	15842723	15610283	210	0	36	Mtu-N: [1,2,3,4]	-	-	-	-	-	
				Q56220	409	1	delta	15842724	15610284	398	1	47		-	-	-	-	-	
				Q56221	181	0	E Mtu(1)	15842725	15610285	252	1	41	Mtu-N: [1,2,3,4] Mtu-C: [1,2,3]	-	-	-	-	-	
				Q56222	438	0	F	15842726	15610286	445	0	53		-	-	-	-	-	
				Q56223	783	1	gamma	15842727	15610287	791	1	32	Mtu-C: MopB_CT_NDH-1_NuoG2-N7(30320), NuoG(31237)[1,2,3,4]	-	-	-	-	-	
				Q60019	365	9	H	15842728	15610288	410	9	46	Mtu-C: [1,2,3,4]	-	-	-	-	-	
				Q56224	182	0	I Mtu(1)	15842729	15610289	211	1	52	Mtu-N: [1,2]	-	-	-	-	-	
				Q56225	176	5	J	15842730	15610290	262	5	33	Mtu-C: [1,2,3,4]	-	-	-	-	-	
				Q56226	95	3	kappa	15842731	15610291	99	3	54		-	-	-	-	-	
				Q56227	606	16	L	15842732	15610292	633	16	43		-	-	-	-	-	
				Q56228	469	11	M Mtu(14)*	15842733	15610293	554	14	35	Mtu-N: NuoM(31212)[1,2,3,4]	-	-	-	-	-	
				Q56229	431	13	N	15842734	15610294	531	13	38	Mtu-N: [1,2,3,4]	-	-	-	-	-	
3.D.2	Proton-translocating Transhydrogenase (PTH) Family	3.D.2.2.1	3	P0C188	464	10	beta	15839537	15607299	475	10	46		15828423	472	10	47	87	1 1
				P0C186	384	0	alpha-1	15842318	15609917	371	0	32	Mtu-C: Ald(31030)[1,2,3,4]	15827805	371	0	30	85	

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle		
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
3.D.4	Proton-translocating Cytochrome Oxidase (COX) Superfamily	3.D.4.4.1	6	P24009	305	9	CoxX	15840911	15608589	308	9	31	15827232g	15840911[g]	321	9	32	82	1	0
				P24010	622	14	Cox1	15842607	15610180	573	14	12	Mtu-C: [1,2,3,4]	15827927	574	14	48	94		
				P24011	356	3	Cox2	15841691	15609337	363	3	23	Mtu-N: [1,2]	15827398	353	3	24	85		
				P24012	207	5	Cox3	15841684	15609330	203	5	33		15827405	202	5	35	90		
				P12946	306	8	CtaA (biogenesis protein)	15840916		312	8	22		15827233	311	8	24	83		
4.C. The Acyl CoA Ligase-Coupled Transporters																				
4.C.1	Proposed Fatty Acid Group Translocation (FAT) Family	4.C.1.1.3	1	O05307	597	4		15840650	15608346	597	4	100		-	-	-	-	-	1	0
5.A. Transmembrane Electron Transfer Carriers																				
5.A.1	Disulfide Bond Oxidoreductase D (DsbD) Family	5.A.1.2.1	1	P45706	235	6		15839921	57116750	259	6	30		15828297	262	6	28	78	2	1
		5.A.1.4.1	1	P30344	319	7		15842419	57117033	287	7	33		-	-	-	-	-		
5.A.3	Prokaryotic Molybdopterin-containing Oxidoreductase (PMO) Family	5.A.3.1.1	3	P09152	1246	0	NarX	15841200	15608874	652	5	60	Mtu-C: Nitrate_red_gam(66360), NarI(32364)[1,2,3,4]	-	-	-	-	-	2	0
		5.A.3.1.2	3	P19319	1245	0	Alpha	15840604	15608301	1232	0	47		-	-	-	-	-		
				P19318	514	0	Beta	15840605	15608302	558	0	56	Mtu-C: [1,2,3,4]	-	-	-	-	-		
				P19316	226	5	Gamma	15840607	15608304	241	5	31		-	-	-	-	-		
		5.A.3.4.3	1	P20099	777	1	Alpha	15840899	15608580	766	1	39		-	-	-	-	-		
		5.A.3.5.1	3	P37600	758	1		15839577	15607338	749	1	22		-	-	-	-	-		
8.A. Auxiliary Transport Proteins																				
8.A.26	Golgi to ER Trafficking Protein (GET3) Family	8.A.26.1.1	1	Q12154	354	0		15841674	15609321	380	0	25	Mtu-C: [1,2,3,4]	15827412	415	0	25	79	1	1
9.A. Transporters of Unknown Classification																				
9.A.19	Mg2+ Transporter-E (MgtE) Family	9.A.19.2.1	1	Q52398	356	6		15839748	15607503	460	6	47	Mtu-N: MgtE(32420)[1,2,3,4]	-	-	-	-	-	1	0
9.A.25	Protein Secretion System (RD1) Family	9.A.25.1.1	11	Q79F93	99	0		15839671	57116715	102	0	32		15828365	102	0	31	73	1	1
				Q79F93	99	0		15840844	57116857	102	0	30		15827194	102	0	?	72		
				P0A564	94	0		15843521	Rv3890c	95	0	23	ESAT-6-like (ESX-2)							
				P0A564	94	0		15843522	Rv3891c	107	0	32	ESAT-6-like (ESX-2)							
				P0A564	94	0		15839674	Rv0288	96	0	29	ESAT-6-like (ESX-3)							
				P0A564	94	0		15842582	15610156	96	0	27	ESAT-6-like (ESX-3)	15828363	96	0	35	66		
				P0A564	94	0		15843039	15610580	100	0	24	ESAT-6-like (ESX-4)	15827096	104	0	27	71		
				P0A564	94	0		15843040	Rv3445c	135	0	34	ESAT-6-like (ESX-4)							
				P0A564	94	0		?	Rv1792	98	0	?	ESAT-6-like (ESX-5); pseudogene							
				P0A564	94	0		15841262	Rv1793	94	0	21	ESAT-6-like (ESX-5)							
				O06269	184	0	Rv3614c; Snm10	15843224	Rv3614c	184	0	100	espA operon	15827126	216	0	71	71		
				P65087	103	0	Rv3615c; Snm9	15843225	Rv3615c	103	0	100		15827125	106	0	56	56		
				?	?	?	Rv3616c; EspA	15843226	Rv3616c	392	2?	99		15827124	389	5?	62	62		
				Q79F93	99	1		15843367	57117152	123	1	51		-	-	-	-	-		
				Q79F93	99	1		-	-	-	-	-		15827127	100	0	31	-		

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle	
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
				Q79F93	99	1		15843367	57117152	123	1	51		-	-	-	-	-	
				Q79F93	99	1		-	-	-	-	-		15827127	100	0	31	-	
				O69735	747	3	Rv3870	15843500	15611006	747	3	100	FtsK-like ATPase (RD1)	15826903	744	3	85	85	
				O69736	591	2 or 3	Rv3871	15843502	15611007	591	2 or 3	100		15826902	597	2 or 3	81	80	
				Q79F93	99	1	Rv3872	15843503	57117163	112	1	100		-	-	-	-	-	
				Q79F92	368	1	Rv3873	15843504	57117164	371	1	100		15826901	302	1	45	44	
				P0A566	99	0	Rv3874; CFP-10	15843505	15611010	100	0	100		15826900	100	0	39	40	
				P0A564	94	0	Rv3875; ESAT-6	15843506	57117165	95	0	100		15826899	95	0	36	36	
				O69740	666	0	Rv3876	15843507	15611012	666	0	100		15826898	586	0	49	48	
				O69741	511	11	Rv3877	15843508	15611013	479	11	100		15826897	512	11	74	74	
				P0A566				15843537	Rv3905c	103	0	23	CFP-10-like	-	-	-	-	-	
9.A.30	Tellurium Ion Resistance (TerC) Family	9.A.30.1.1	1	Q7B1W8	346	9		15842261	15609860	397	9	29	Mtu-C: [1,2,3]	-	-	-	-	-	1 0
9.A.40	HlyC/CorC (HCC) Family	9.A.40.2.1	1	P54428	434	4		15841309	15608978	345	1	27		-	-	-	-	-	2 0
								15841310	15608979	455	3	28		-	-	-	-	-	
9.A.42	Mycobacterial PPE41 Protein Secretion System (MPSS) Family	9.A.42.1.1	8	O53933	506	1		15839669	15607424	538	1	39	ESX-3	15828367	552	1	37	70	4 2
				O53934	435	1		15839670	15607425	1330	1	30	"Rv1783-Rv1784"	15828366	1329	1	?	84	
				O53935	932	1		15839670	15607425	1330	1	32	"Rv1783-Rv1784"	15828366	1329	1	?	84	
				O53943	300	1		15839675	15607430	295	1	25		15828362	289	1	25	75	
				O53944	503	12		15839676	15607431	472	12	25		-	-	-	-	-	
				O53945	585	1		15839677	15607432	414	1	43		15828360	475	1	44	72	
				O53946	406	1		-	-	-	-	-		-	-	-	-	-	
				P63744	610	3		15839668	15607423	613	3	26		15828368	640	0	32	85	
				O53933	506	1	Rv1782	15841251	15608920	506	1	100	ESX-5	15827812	506	1	-	82	
				O53934	435	1	Rv1783	15841252	15608922	1391	1	100	Rv1783-Rv1784	15827811	1325	1	-	87	
				O53935	932	1	Rv1784	15841252	15608922	1391	1	100	Rv1783-Rv1784	15827811	1325	1	-	87	
				O53943	300	1	Rv1794	15841263	15608931	300	1	100		15827810	300	0	-	88	
				O53944	503	12	Rv1795	15841264	15608932	503	12	100		15827809	503	11	-	76	
				O53945	585	1	MycP5	15841265	15608933	585	1	100		15827808	567	1	-	73	
				O53946	406	1	Rv1797	15841266	15608934	406	1	100		15827807	401	2	-	67	
				P63744	610	3	Rv1798	15841267	15608935	610	3	100		15827806	610	1	-	89	
				O53933	506	1		15843045	15610586	482	1	34	ESX-4	-	-	-	-	-	
				O53934	435	1		15843042	15610583	1200	1	35	"Rv1783-Rv1784"	-	-	-	-	-	
				O53935	932	1		15843042	15610583	1200	1	35	"Rv1783-Rv1784"	-	-	-	-	-	
				O53943	300	1		-	-	-	-	-		-	-	-	-	-	
				O53944	503	12		15843043	15610584	467	12	24		-	-	-	-	-	
				O53945	585	1		15843044	15610585	455	1	44		-	-	-	-	-	
				O53946	406	1		-	-	-	-	-		-	-	-	-	-	
				P63744	610	3		-	-	-	-	-		-	-	-	-	-	
				O53933	506	1		15843499	15611005	480	1	34	ESX-1	15826904	481	1	33	75	
				O53934	435	1		-	-	-	-	-		-	-	-	-	-	
				O53935	932	1		-	-	-	-	-		-	-	-	-	-	
				O53943	300	1		-	-	-	-	-		-	-	-	-	-	
				O53944	503	12		-	-	-	-	-		-	-	-	-	-	
				O53945	585	1		15843510	15611015	723	1	29		-	-	-	-	-	
				O53946	406	1		-	-	-	-	-		-	-	-	-	-	
				P63744	610	3		15843498	15611004	573	3	31		15826905	573	1	32	89	

Table 3: Continued

Transport Classification (TC)								*M. tuberculosis* (Mtu)						*M. leprae* (Mle)				Mtu/Mle		
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # TC[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]
				O53933	506	1		15843499	15611005	480	1	34	ESX-1	15826904	481	1	33	75		
				O53934	435	1		-	-	-	-	-		-	-	-	-	-		
				O53935	932	1		-	-	-	-	-		-	-	-	-	-		
				O53943	300	1		-	-	-	-	-		-	-	-	-	-		
				O53944	503	12		-	-	-	-	-		-	-	-	-	-		
				O53945	585	1		15843510	15611015	723	1	29		-	-	-	-	-		
				O53946	406	1		-	-	-	-	-		-	-	-	-	-		
				P63744	610	3		15843498	15611004	573	3	31		15826905	573	1	32	89		
				O53933	506	1		15843526	15611031	495	1	41	ESX-2	-	-	-	-	-		
				O53934	435	1		15843525	15611030	1396	1	30	"Rv1783-Rv1784"	-	-	-	-	-		
				O53935	932	1		15843525	15611030	1396	1	30	"Rv1783-Rv1784"	-	-	-	-	-		
				O53943	300	1		15843520	15611025	276	1	24		-	-	-	-	-		
				O53944	503	12		15843518	15611023	469	12	24		-	-	-	-	-		
				O53945	585	1		15843515	15611019	411	1	45		15826894	446	4	37	79		
				O53946	406	1		15843514	15611018	462	1	26		15826895	467	3	25	72		
				P63744	610	3		15843516	15611020	624	3	30		-	-	-	-	-		
9.B. Putative Uncharacterized Transporters																				
9.B.3	Putative Bacterial Murein Precursor Exporter (MPE) Family	9.B.3.1.3	1	P07373	366	10		15839391	15607159	469	12	32	Mtu-N: [1,2,3,4]	15826882	465	12	32	82	2	2
						10		15841646	15609291	524	10	37	Mtu-N: [1,2,3] Mtu-C: [1,2,3,4]	15827433	534	10	37	75		
9.B.20	Putative Mg2+ Transporter-C (MgtC) Family	9.B.20.1.2	1	O07221	234	4		15841281	15608948	234	4	100		-	-	-	-	-	1	0
9.B.22	Putative Thiamin Transporter (PTT) Family	9.B.22.1.1	2	P77406	353	8		15840540	15608241	385	8	25	Mtu-C: [1,2,4]	-	-	-	-	-	1	0
				P37622	349	8		15839585	15607346	365	8	26		-	-	-	-	-		
9.B.26	PF27 (PF27) Family	9.B.26.1.1	1	P52876	206	6		15843479	15610984	302	6	31	Mtu-C: [1,2,3,4]	-	-	-	-	-	1	0
9.B.27	YdjX-Z (YdjX-Z) Family	9.B.27.1.2	1	P76221	235	5		15840954	15608629	252	5	32	Mtu-N: [1,2] Mtu-C: [1,2]	-	-	-	-	-	1	0
9.B.30	Hly III (Hly III) Family	9.B.30.1.1	1	P54176	219	7		15840522	15608225	242	7	29	Mtu-N: [1,2] Mtu-C: [1,2,3,4]	-	-	-	-	-	1	0
9.B.42	ExeAB (ExeAB) Secretin Assembly/Export Complex	9.B.42.1.1	1	P45754	547	2		15842349	15609950	270	0	33		-	-	-	-	-	1	0
9.B.45	YnfA (YnfA) Family	9.B.45.1.1	1	P76169	108	4		15842180	15609776	110	4	37		-	-	-	-	-	1	0
9.B.59	Putative Peptide Transporter Carbon Starvation CstA (CstA) Family	9.B.59.1.1	1	P15078	701	18		15842631	15610200	758	16	51	Mtu-N: [1,2]	-	-	-	-	-	1	0
	Total																		147	55

[a] Number of components per transport system as annotated by TCDB.

[b] Accession number for a given component of a transport system.

[c] The numbers of putative α-helical transmembrane segments (TMSs) were calculated using the TMHMM program. Unfortunately, in the case of outer-membrane porins (TC 1.B), the numbers do not reflect the number of β strands and therefore are

[d] An asterisk (*) indicates requirement for survival in macrophage [15].

[e] Number of transport systems in *M. tuberculosis* per family.

[f] Number of transport systems in *M. leprae* per family.

[g] A putative pseudogene in *M. leprae*.

* See Table 2

[x] The 4.5S rRNA component of 3.A.5.1.1 was not included here. Also, 15842124 is in the same operon as 15842125 and 15842126 but does not appear to have related function. Missing YajC (increases secretion rate by ~10x esp at low temp, but

** Different shades of grey indicate operon clustering.

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