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

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ABSTRACT

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

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1. Introduction

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

Interestingly, Mtu shares evolutionary history with another well-known intracellular parasite, *Mycobacterium leprae* (Mle). Mle is thought to have evolved relatively recently from a common ancestor of both Mle and Mtu [3,4]. However, their lifestyles are dissimilar, as Mtu is a facultative intracellular parasite while Mle is an obligate intracellular parasite. The differences in their lifestyles gave rise to major differences in their genomes. The genome of *M. tuberculosis* CDC1551 (a clinical strain, as compared to H37Rv, which is the laboratory strain) encodes an estimated 4189 proteins while the Mle genome encodes only 1605 proteins. Previous genomic analyses revealed that Mle lost many genes through reductive evolution, some of which are undoubtedly

required for extracellular life [5,6]. Still others remain as recognizable remnants called pseudogenes — inactivated genes that no longer produce functional proteins. Not surprisingly, pseudogenes are prevalent in Mle but relatively rare in Mtu [7,8].

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

Comparative genome analyses of the transport systems in Mtu and Mle has the potential to provide missing puzzles to the pathogenesis picture [15]. Transporters are essential for bacterial survival and persistence, but of these two organisms, only Mtu must be able to adapt to the various environmental conditions encountered before and during pathogenesis. During primary infection, extracellular Mtu is phagocytosed by macrophages, and its intracellular residency is diverse: phagosome, phagolysosome or cytosol. In the phagosome or phagolysosome, Mtu must secrete appropriate virulence factors

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by using secretion systems to hijack the phagosomal machinery, withstand acidic conditions, and extrude various toxins. Mtu must also maximize nutrient uptake upon translocation to the cytosolic environment. Upon release into the extracellular tissues, Mtu may also need to pump out antibiotic drugs.

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

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

2. Methods

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

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

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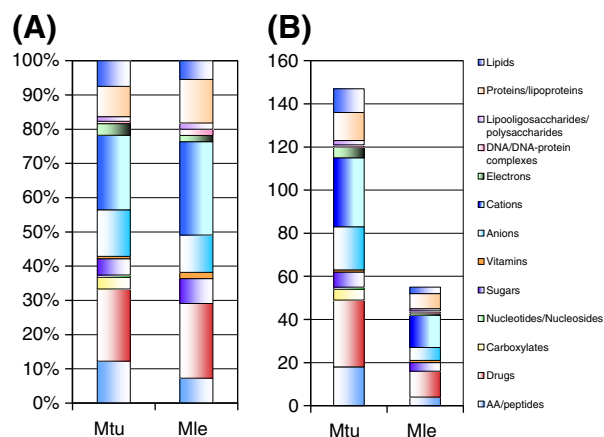

Fig. 1. Relative distribution of predicted substrates of transport systems found in Mtu and Mle according to (A) percent of all transporters identified, and (B) numbers of transport systems. The order of the substrate types, indicated on the far right, is the same for all columns.

pseudogenes, this list of transport proteins was screened for pseudogenes using PSI-FI.

3. Results

3.1. Overview of transporter types

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

Table 1 presents an overall summary of the classes and subclasses of transporters found in Mtu and Mle, according to the TC system. The 285 transport proteins in Mtu comprise 171 transport systems while 132 proteins in Mle make up 59 transport systems. Therefore, 7.2% of Mtu genes and 8.2% of Mle genes encode recognizable transport proteins that correspond to established entries in TCDB, many of which form complete transport systems. These numbers exclude transport proteins most resembling the putative uncharacterized transporters (9.B) because most members of this class of proteins lack functional information. Also excluded are the additional genes encoding potential transporters that give low BLAST scores to TCDB entries. Instead, they are included in Supplemental Tables (see Tables S1 and S2 for Mtu and Mle, respectively). Thus, the total potential percentage of transport protein-encoding genes is likely to be higher than the aforementioned percentages. While 10–15% of the genes in most free-living bacteria encode transport proteins, genomes of intracellular parasites typically encode lower proportions [33]. Not surprisingly, Mtu and Mle encode relatively low proportions of transport proteins. Obligate intracellular bacteria are expected to encode fewer transport proteins than facultative intracellular bacteria, and while this is the case when comparing Mtu with Mle, the Mle genome nevertheless encodes percentagewise more transport proteins than Mtu.

In most bacteria, approximately 3–8% of all the transport proteins encoded in the genome are channel-type transporters [33]. Both Mtu and Mle have a surprisingly small number of channel proteins: 2.6% and 2.3%, respectively. Mtu has 6 inner-membrane channel proteins (1.9% of the transport proteins) that comprise 6 channels (3.5% of the transport systems), and 2 recognized outer-membrane porin-type channel-forming proteins (0.6% of the transport proteins), corresponding to 2 systems (1.2% of the transport systems), but one of

Table 1
Overview of the *M. tuberculosis* and *M. leprae* transporter analyses based on TC class and subclass.

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

[a] Transporter classes 6 and 7 have not been assigned in the TC system and therefore are absent.
[b] Numbers in parentheses represent the number of transport systems. Transport systems are comprised of one or more constituent transport proteins.
[c] Relative reduction in transport systems from Mtu to Mle.
[d] Poorly defined systems here include families in TC subclass 9.A (poorly characterized transporters) but not in TC subclass 9.B (putative transporters); see Tables 3, S1 and S2 for homologues of families within subclass 9.B.

them is in doubt [34]. Mle has 2 inner-membrane channel proteins (1.5% of the transport proteins) that comprise two channels (3.4% of the transport systems) and one recognized outer-membrane porin-type channel-forming protein. These numbers presumably reflect the stable environment of human host cells that have allowed Mle to shed other channels through reductive evolution [15].

Mtu has substantially more secondary carriers (78 systems, or 53% of transport systems identified) than primary active transporters (46 systems, or 31%). However, Mle has roughly the same number of secondary carriers (23 systems, or 42%) as primary active transporters (24, or 44%). This corresponds to a transport system retention rate of 29% for secondary carriers and a staggering 52% for primary active transporters. This observation may reflect the importance of high affinity importers and multidrug efflux systems for intracellular survival. Efflux systems may be more important for intracellular pathogens, which must find ways to cope with toxic drugs that mankind has created as well as excessive cytoplasmic concentrations of natural metabolites and by-products of metabolism. Likewise, this observation suggests that many secondary carriers are not essential for intracellular life. Mtu has four transmembrane electron flow carriers but Mle has just one. This may reflect the massive gene decay by which most of the microaerophilic and anaerobic respiratory chains were lost in Mle. Finally, a much larger proportion of poorly defined systems exists in Mtu (32 systems, or 19%) than in Mle (7 systems, or 12%). It is unclear what role these systems have in the differences between their lifestyles and pathogenesis. However, it can be postulated that they exhibit functions that are commonly found in many bacteria. While systems that have not been characterized necessarily exhibit non-essential functions, most of the well characterized transporters are the ones with more generally important functions, and the ones less well characterized are the ones that have very specialized functions that are likely to be non-essential for ordinary growth [35].

3.2. Transport substrates

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

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

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

3.3. Channels (TC subclass 1.A)

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

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

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

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

Table 2
Overview of transport systems in *M. tuberculosis and M. leprae* based on predicted substrate specificity.

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

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

mycobacterial homologues may, therefore, provide the primary mechanism for divalent cation (Mg^{2+}, Co^{2+}, etc.) uptake.

3.4. Outer-membrane porins (TC subclass 1.B)

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

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

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

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

3.5. Secondary carriers (TC subclass 2.A)

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

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

Mtu encodes three nitrate/nitrite antiporters for nitrite extrusion (NarK2) and one nitrate/H^+ symporter for nitrate uptake (NarK1) of the NNP family (2.A.1.8). Mle only encodes one NarK2. Nitrate, a vital source of assimilable nitrogen, is reduced to nitrite under hypoxia and serves as a terminal electron acceptor for anaerobic respiration [49]. Nitrite is subsequently excreted by transporters of the NNP family or further reduced by two forms of nitrite reductases. Both nitrite reductases, present in Mtu, are absent in Mle. Lowering intracellular nitrite by reduction does not occur *in vitro* [50], but this may be different *in vivo*. One Mtu NarK2 homologue, encoded by the *narK2X* operon, is associated with the upregulated nitrate reductase activity that functions under anaerobic conditions [51]. Thus, these observations may, in part, help to explain why Mtu is able to adapt to various extracellular environments with low concentrations of oxygen while Mle cannot.

Perhaps more intriguing is the presence of an iron siderophore transporter, the iron (Fe^{3+}) · pyridine-2,6-bis(thiocarboxylic acid (PDTC)) uptake transporter, encoded by both Mtu and Mle. The ability to acquire iron is thought to be severely limited in Mle because it has reportedly lost the mbt operon, which encodes the non-ribosomal peptide synthase required for production of siderophores like

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 Family name TC#	Transport System TC#	No. of comp.[a]	Acc. # in TCDB[b]	Size (aa)	TC TMS[c]	Comments	GI # (CDC1551)	GI # (H37Rv)	Size (aa)	TMS	% Identity Mtu/TC	Comments (Mtu vs. TC)	GI #	Size (aa)	TMS	% Identity Mle/TC	% Identity Mtu/Mle	No. of transport systems Mtu[e]	Mle[f]
1.A alpha-Type Channel-forming Proteins and Peptides																			
1.A.1 (VIC) Superfamily	1.A.1.13.2	1	O27564	336	2		15842786	15610336	355	2	26	Mtu-N: [1,2,3]	-	-	-	-	-	1	0
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		-	-	-	-	-		
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	Rv1698 (H37Rv)	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		

Transport Classification (TC)								M. tuberculosis (Mtu)						M. leprae (Mle)				Mtu/Mle		
Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # in TCDB[b]	Size (aa)	TC TMS[c]	Comments	GI # (CDC1551)	GI # (H37Rv)	Size (aa)	TMS	% Identity Mtu/TC	Comments (Mtu vs. TC)	GI #	Size (aa)	TMS	% Identity Mle/TC	% Identity Mtu/Mle	No. of transport systems Mtu[e]	Mle[f]
	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		-	-	-	-	-		
	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	-	-	-	-	-		
								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		-	-	-	-	-		

Table 3 (continued)

Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # in TCDB[b]	Size (aa)	TC TMS[c]	Comments	GI # (CDC1551)	GI # (H37Rv)	Size (aa)	TMS	% Identity Mtu/TC	Comments (Mtu vs. TC)	GI #	Size (aa)	TMS	% Identity Mle/TC	% Identity Mtu/Mle	Mtu[e]	Mle[f]
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	8 1	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 15842223	15609821 57117014	429 428	11 10	22 22	Mle ortholog (15827501) hits equally well to both.	15827501 15827501	429 429	10 10	23 23	74 74	2	1
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	4 2		-	-	-	-	-	1	0
2.A.53	Sulfate Permease (SulP) Family	2.A.53.4.1 2.A.53.5.1 2.A.53.8.1	1 1 1	P72770 Q7U617 Q8F8H7	566 556 750	13 12 10		15841203 15841167 15842863	15608877 15608845 15610409	560 486 764	11 12 10	33 34 29	different # of TMS but good e-value	- - -	- - -	- - -	- - -	- - -	3	0
2.A.55	Metal Ion (Mn2+-iron) Transporter (Nramp) Family	2.A.55.3.1	1	P77145	412	11		1584034 7 1584034 7	57116797 57116797	429 429	11 11	43 43		15828133 15828438	426 618	11 12	42 25	74 25	1	2
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
2.A.64	Twin Arginine Targeting (Tat) Family	2.A.64.1.1	4	O69415 P69423 P69428	171 258 89	1 6 1	B C A	15840668 15841584 161350070	15608364 15609230 15609231	131 284 83	1 6 1	3 3 3 1 3 0	Mtu-C: [1,2,3] Mtu-N: [1]	15827529 15827693 161723267	120 286 88	1 6 1	36 33 40	73 86 69	1	1
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 15839879	15609123 15607629	199 201	6 6	3 1 3 2		- -	- -	- -	- -	- -	2	0
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		-	-	-	-	-		

Transport Classification (TC)								M. tuberculosis (Mtu)						M. leprae (Mle)				Mtu/Mle		
Family Family name TC#		Transport System TC#	No. of comp.a	Acc. # in TCDBb	Size (aa)	TC TMSc	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 Mtue	Mlef
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]h	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		
		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		–	–	–	–	–		

Table 3 (*continued*)

Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # in TCDB[b]	Size (aa)	TC TMS[c]	Comments	GI # (CDC1551)	GI # (H37Rv)	Size (aa)	TMS	% Identity Mtu/TC	Comments (Mtu vs. TC)	GI #	Size (aa)	TMS	% Identity Mle/TC	% Identity Mtu/Mle	Mtu[e]	Mle[f]
	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]	–	–	–	–	–		
	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] lolC-lolE	15840412	15608127	855	10	23	Mtu-C: FtsX(66381), SalY(30922)[1,2,3,4]	–	–	–	–	–		
				P75958	413	4	[M] lolE	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: 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

Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # in TCDB[b]	Size (aa)	TC TMS[c]	Comments	GI # (CDC1551)	GI # (H37Rv)	Size (aa)	TMS	% Identity Mtu/TC	Comments (Mtu vs. TC)	GI #	Size (aa)	TMS	% Identity Mle/TC	% Identity Mtu/Mle	No. of transport systems Mtu[e]	Mle[f]
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	Mtu-N: [1,2,3]	15828075	766	8	35	77		
								15840395	15608109	792	8	46		-	-	-	-	-		
								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		
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		
				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

(continued on next page)

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. # in TCDBb	Size (aa)	TC TMSc	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 Mtue	Mlef
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		
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	Mtu-C: [1,2,3,4]	-	-	-	-	-		
				P19318	514	0	Beta	15840605	15608302	558	0	56		-	-	-	-	-		
				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

Family TC#	Family name	Transport System TC#	No. of comp.[a]	Acc. # in TCDB[b]	Size (aa)	TC TMS[c]	Comments	GI # (CDC1551)	GI # (H37Rv)	Size (aa)	TMS	% Identity Mtu/TC	Comments (Mtu vs. TC)	GI #	Size (aa)	TMS	% Identity Mle/TC	% Identity Mtu/Mle	Mtu[e]	Mle[f]
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	-	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	24	84		
				O53935	932	1		15839670	15607425	1330	1	32	"Rv1783-Rv1784"	15828366	1329	1	31	84		
				O53943	300	1		15839675	15607430	295	1	25		15828362	289	1	25	75		
				O53944	503	12		15839676	15607431	472	12	25		15828361	485	11	25	76		
				O53945	585	3		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		
				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		-	-	-	-	-		

Table 3 (*continued*)

Transport Classification (TC)							M. tuberculosis (Mtu)						M. leprae (Mle)				Mtu/Mle		
Family Family name TC#	Transport System TC#	No. of comp.[a]	Acc. # in TCDB[b]	Size (aa)	TC TMS[c]	Comments	GI # (CDC1551)	GI # (H37Rv)	Size (aa)	TMS	% Identity Mtu/TC	Comments (Mtu vs. TC)	GI #	Size (aa)	TMS	% Identity Mle/TC	% Identity Mtu/Mle	No. of transport systems	
																		Mtu[e]	Mle[f]
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 of limited value.

[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.*

[h]Abbreviations for constituents of ABC transporters: C, the cytoplasmic ATPase; M, the integral membrane constituent(s); R, the extra-cytoplasmic receptor.

[i]Mtu-N:[] (or Mtu-C:[]) indicates the presence of any N-terminal (or C-terminal) sequences of the query protein (Mtu) outside the BLAST alignment window that is > 30 AAs long. [1] = found in other *M. tuberculosis* strains, [2] = other mycobacterial species, [3] = other Corynebacteriaceae suborders, [4] = other actinobacterial phyla. For example, Mtu-C: UgpA (31368) [1,2,3,4] indicates that AAs of the query protein flanking the C-terminal end of this alignment window was found to contain the UgpA domain, which was found not only in other Mtu species but other Actinobacteria as well.

*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 a related function. Missing YajC (increases secretion rate by ~10× esp at low temp, but not essential).

mycobactin/exochelin [3]. However, this does not necessarily preclude usage of other siderophores. The presence of genes encoding MoeZ (GI: 15827360) in Mle, a protein thought to be essential for biosynthesis of PDTC [52], provides additional evidence that PDTC may function as an important siderophore in Mle. Furthermore, the presence of an iron-PDTC transporter, an MFS carrier, and the absence of IrtAB, an ABC primary active transporter [53], in Mle are all consistent with previous findings that iron uptake is pmf-driven rather than ATP-dependent [54,55]. Therefore, we suggest that this PDTC transporter functions in iron uptake for both Mtu and Mle.

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

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

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

A few additional drug exporting secondary carriers were identified. Mle encodes the Mtu Mmr multidrug efflux pump orthologue of the characterized Mtu protein (2.A.7.1.2) of the DMT superfamily for which the identified substrates are tetraphenylphosphonium (TPP), erythromycin, ethidium bromide, acriflavine, safranin O, and pyronin Y [59]. A drug exporter of the MATE family within the MOP superfamily is present in Mtu, and a putative carbohydrate or drug exporter of the MVF family is present by both Mtu and Mle. Proteins in the MVF family have been shown to be important virulence factors in *S. typhimurium* when infecting the mouse [60], but otherwise, little is known about these putative exporters. Interestingly, both Mtu and Mle homologues have a serine/threonine protein kinase domain fused to the C-termini of these homologues although such domains are lacking in the *S. typhimurium* transporter.

One putative $Ca^{2+}:H^+$ antiporter is present in each organism, and two and one putative phosphate uptake permeases of the Pit family were found in Mtu and Mle, respectively. A single, probable monovalent-cation exchanger of the CPA1 family was identified in Mtu but not Mle, and a single monovalent-cation exchanger of the CPA2 family was identified in both organisms. While a single arsenite efflux system of the ArsB family was identified in Mle, two paralogs were surprisingly found in the same operon of Mtu. One ammonium transporter of the AMT family, one Ni^{2+}–Co^{2+} transporter of the NiCoT family, and three similar sulfate permeases of the SulP family

were identified in Mtu, but all of these transporters were lacking in Mle. More intriguing are the putative manganese transporters of the Nramp family, previously thought to be present only in Mle [3]. Two Mle manganese transporter homologues correspond to an Mtu ortholog; one of the Mle homologues displays significant sequence divergence and a fusion of 170 aas at the C-terminus that is observed in other mycobacterial organisms like *Mycobacterium ulcerans*, *M. marinum*, *M. avium*, and *M. abscessus* but not *M. tuberculosis* (both CDC1551 and H37Rv strains). However, the other Mle homologue has very high sequence similarity to the Mtu ortholog and thus, a functional manganese transporter probably exists in both organisms. An arsenite/antimonite exporter of the ACR3 family was found only in Mtu. Interestingly, a protein-tyrosine phosphatase domain (Wzb) is fused to the C-terminus of this homologue. Moreover, a putative Na^+-dependent bicarbonate importer of the SBT family was found only in Mtu. These cation and anion facilitators probably function primarily in the maintenance of ionic homeostasis, but they may also play secondary roles in adaptation to various types of stress.

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

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

The ABC superfamily of ATP-driven transporters is the largest transporter superfamily represented in the Mtu and Mle genomes. 14 potential ABC uptake systems and 13 potential ABC efflux systems were identified in Mtu, and all of these systems appear to be complete, having all of the expected constituents. Nine potential uptake systems and six potential efflux systems were identified in Mle. This suggests that transporters of the ABC superfamily have been preferentially retained in Mle. Four and three maltose-type systems of the CUT1 family (TC 3.A.1.1) were identified in Mtu and Mle, respectively. Uptake systems such as these typically have 2 transmembrane permease proteins (M), 1 receptor protein with sugar-specificity (R), and 1 cytoplasmic component (C) that binds and hydrolyzes ATP to provide energy for sugar uptake. All seven of these CUT1 systems in Mtu and Mle are complete, and the constituent components of such a system are encoded within a single operon. The system present in Mtu but lacking in Mle is a transporter most resembling the *E. coli* system (3.A.1.1.3). Each of two operons in Mtu and Mle encode all four components of a related maltose-type system (3.A.1.1.7).

A complete, ribose-type system of the CUT2 family (3.A.1.2), composed of four constituents (1 R, 2 M's, 1 C) encoded within the same

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

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

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

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

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

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

Many ABC efflux systems were found in Mtu and Mle and they most resemble: (1) the lipopolysaccharide exporter (RfbAB of *Klebsiella pneumoniae*; 3.A.1.103.1; 1 Mtu and 1 Mle), (2) that for

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

3.7. Primary active transporters — other cation-transporting ATPases

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

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

3.8. Primary active transporters — anion-transporting ATPases

The arsenite resistance (Ars) efflux pumps of bacteria consist either of two proteins (ArsB, the integral membrane constituent with twelve transmembrane spanners, and ArsA, the ATP-hydrolyzing, transport energizing subunit, as for the chromosomally-encoded *E. coli* system), or of one protein (the ArsB integral membrane protein of the plasmid-encoded *Staphylococcus* system). ArsA proteins have two ATP binding domains and probably arose by a tandem intragenic duplication event. ArsB proteins all possess twelve transmembrane spanners and may also have arisen by a tandem intragenic duplication event. Structurally, the Ars pumps superficially resemble ABC-type efflux pumps, but there is no significant sequence similarity

between the Ars and ABC pumps. When only ArsB is present, as in the Arsenite–Antimonite (ArsB) Efflux family (2.A.45), the system operates by a pmf-dependent mechanism, and consequently belongs in TC subclass 2.A. When ArsA is also present, ATP hydrolysis drives efflux, and consequently the system belongs in TC subclass 3.A. These pumps actively expel both arsenite and antimonite. In Mtu, two proteins most resembling the cytoplasmic ArsA are encoded in the same operon as ArsB. Two Mle orthologs of ArsA are also encoded in the same operon, but an ArsB homolog is lacking in this operon. However, these Mle cytoplasmic constituents probably function together with the aforementioned ArsB protein encoded elsewhere in the genome.

3.9. Primary active transporters — ATP-dependent protein secretion systems

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

3.10. Primary active transporters — cation-translocation electron transfer complexes

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

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

Both Mtu and Mle also encode an essentially complete proton pumping cytochrome oxidase complex (3.D.4) with five of the six expected proteins (Cox1-3, CoxX, and CtaA). Cox4 was not identified in either Mtu or Mle. Although the cytochrome oxidase complex subunits are all encoded in a single operon in *Bdellovibrio bacteriovorus* [77], each subunit identified in Mtu and Mle is encoded within a different operon (e.g., five subunits within five, entirely different operons). Interestingly, the CoxX homologue in Mle was predicted to be a pseudogene by the PSI-FI program. Nonetheless, these enzyme complexes may be capable of coupling proton export to electron flow [78].

3.11. Group translocators — Acyl-CoA ligase-coupled transporters

The putative acyl-CoA ligase-coupled transporters (4.C.1, 2 and 3) use the energy of ATP to thioesterify fatty acids and other acids such

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

3.12. Transmembrane electron transport systems

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

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

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

Fig. 2. Nitrate reductase genes in Mtu. The Mtu genome includes two operons that respectively encode a nitrate reductase and a partial nitrate reductase. The latter probably includes nitrate-reductase pseudogenes resulting from two genomic deletions, the first being large, and the second being small. Both operons are absent in Mle. (A) The *narGHJI* operon encodes the three nitrate reductase subunits, NarG (alpha), NarH (beta), and NarI (gamma). NarJ, the delta subunit, is a chaperone protein required for the assembly of this nitrate reductase complex. (B) The narK2X operon encodes two proteins: NarK2, a nitrite exporter, and NarX, a truncated nitrate reductase. The N-terminus of NarX is homologous to the first 256 aas of NarG. The next 160 aas of NarX are homologous to aas 5–164 of NarJ. The C-terminus of NarX is homologous to all 241 aas of NarI. narK2 and narI (γ) may be functional.

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

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

3.13. Recognized transporters of unknown biochemical mechanism

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

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

Although the exact number of components to the ESX-1 system is still debated, a set of RD1 proteins with domains of known function has been implicated as essential to virulence. This system includes a multitransmembrane protein, Rv3877 (Snm4), and two putative SpoIIIE/FtsK ATPase family members, Rv3870 (Snm 1) and Rv3871 (Snm2). These three proteins are required for secretion of ESAT-6 and CFP-10. ESAT-6 (product of the *esxA* gene) and CFP-10 (product of the *esxB* gene) interact to form a 1:1 dimer [98,99], and the stability of these proteins is interdependent *in vivo*. CFP-10, but not ESAT-6, interacts with the C-terminal domain of Rv3871, a cytosolic component of the ESX-1 system [87]. A second, non-RD1 gene cluster, the Rv3614c-Rv3616c locus, is also required for ESAT-6 secretion [100]. They are homologous to Rv3864-Rv3867 from RD1. All known

components of this system, as listed in TCDB, were identified in Mtu (the CDC1551 strain) and Mle. In Mle, no recognizable homologue of Rv3872 was identified. The secretion of ESAT-6 and CFP-10 is critical for Mtu virulence, but the molecular mechanisms of ESX-1 substrate selection and secretion are unclear.

Additional homologous pairs of ESAT-6/CFP-10 exist in Mtu, but only four of these pairs, each of which is encoded by tandem genes, are surrounded by genes encoding the components of ESX-1 as listed in Table 3. These four gene clusters are: ESX-2, ESX-3, ESX-4, and ESX-5 (9.A.40). These clusters vary in the numbers of genes present from 7 to 18. These clusters probably arose by gene duplication [101]. Surprisingly, these systems do not complement one another, although ESX-3 and ESX-5 appear to be essential [102]. Rv3870–Rv3871 homologues in each of the ESX2-5 clusters in Mtu are found fused to one another, supporting the current notion of Rv3870 and Rv3871 functioning together as an FtsK/SpoIIE-like ATPase. It is thought that Rv3871 interacts with CFP-10 and delivers the heterodimeric complex in an ATP-dependent manner to Rv3870, thereby delivering these virulence factors to the secretory machinery. The molecular mechanism by which ESX secretes virulence factors remains to be elucidated.

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

3.14. (Putative) transporters of unknown function or mechanism

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

4. Discussion and overview

While *M. leprae* (Mle) is an obligate intracellular pathogen, *M. tuberculosis* (Mtu) is a facultative intracellular pathogen that adapts to its changing environment prior to and during its various stages of pathogenesis. We have analyzed the transporters in these organisms partly to determine what systems might confer upon Mtu its ability to survive in various extracellular environments. We also wanted to determine what systems might be essential for intracellular survival, as those retained in Mle are implicated in such roles. We identified several putative transport proteins in addition to those reported in the original genome annotation efforts for both Mtu and Mle. Our most interesting and provocative findings will be summarized here with emphasis on the potential physiological and pathological

importance of some of our observations. Actual physiologic conditions faced by Mtu and Mle are largely unknown and require much more investigation [108]. Transcriptome data are scattered throughout the literature and infrequently confirmed [109]. Nonetheless, transcriptome data for a variety of different conditions are abundant [110–112]. Much of it can be found on the Tuberculosis Database [113], which contains microarray data for specific conditions and URLs to publications, if any, that generated the data.

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

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

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

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

4.2. Evasion of host immunity, persistence, and proliferation

Several types of specialized secretion systems are dedicated to the secretion of virulence factors and play important roles in many stages of both Mtu and Mle pathogenesis. There are several clusters of ESX named ESX-2-5. ESX-3 is regulated by iron and zinc availability [115,116]. Zinc may be acquired by Mtu with the zinc–iron permease

of the ZIP family. Mle does not have an ortholog of this permease, and how it obtains zinc is unclear.

ESAT-6 and CFP-10, and possibly other virulence factors, are implicated in arresting maturation of phagosomes in macrophages. The fusion of phagosomes to lysosomes is blocked, preventing acidification of their environment. However, macrophages may overcome this arrest upon activation by cytokines like interferon-gamma. OmpATb (1.B.6.1.3) is essential for adaptation to low pH and survival in macrophage [43]. Surprisingly, Mle does not possess an OmpATb ortholog. This may explain why the human host is more successful in killing Mle and containing them in granulomas. However, the role of OmpATb as a porin [34,45] has been seriously questioned, leading to more questions about its physiological function.

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

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

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

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

Upon proliferation, mycobacteria may proceed to adjacent host cells. Cell-to-cell spreading is dependent on the TmtpC proteins

(2.A.6.5.3), implicated in sliding motility. Seven paralogues were discovered in Mtu, one of which (gi: 15841024) is likely to be a pseudogene. The first ~600 aas of TmtpC have become deleted. Only two homologues were identified in Mle. These two homologues may be the minimal set of TmtpC proteins required for obligate intracellular life.

4.3. Adaptation to various extracellular environments

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

4.4. Antimicrobial drugs

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

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

There is also evidence for an important role of MDR transporters for resistance to rifampicin and pyrazinamide (PZA) as well. In the case of resistance to PZA, 70% of PZA-resistant clinical strains can be attributed to mutations in the *pncA* gene, which encodes an enzyme that converts PZA into an active form; nonetheless, the role of efflux pumps as primary mechanism of resistance to PZA has been documented.

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

4.5. Toxins and virulence factors

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

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

4.6. Nutrient uptake

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

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

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

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

It is clear that Mtu scavenges nutrients from host cells as does Mle. The use of host triacylglycerol by Mtu is yet another example [127]. Some inorganic ions such as iron [128,129] and phosphate [130] are

acquired from the host. They are undoubtedly required for pathogen survival, while others may be toxic when present in excessive amounts [131,132].

However, there is also evidence for *de novo* synthesis of various cofactor like NAD [133]. Mtu therefore likely relies on external sources when available and makes what is not available. Very little is known about how many compounds limit or promote growth. Further studies will be required to provide this knowledge.

4.7. Fusion of genes and extra domains

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

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

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

5. Conclusion

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

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