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MINIREVIEW

A Balancing Act: Efflux/Influx in Mycobacterial Drug Resistance[▽]

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Since the discovery of the tubercle bacillus by Robert Koch in 1882 (110), a greater understanding of the dynamics and survival mechanisms of this pathogen has led to more questions than answers. Despite stringent control strategies and many advances in our knowledge of the epidemiology of tuberculosis (TB) and the biology of the causative agent *Mycobacterium tuberculosis*, TB still remains one of the most common and deadly infectious diseases worldwide. The emergences of multidrug-resistant TB (MDR-TB) (with resistance to at least the first-line drugs isoniazid [INH] and rifampin [rifampicin] [RIF]) (39) and extensively drug-resistant TB (XDR-TB) (with additional resistance to a fluoroquinolone [FQ] and any one of the injectable drugs kanamycin [KAN], amikacin [AMI], and capreomycin [CAP]) (43, 67) are a major concern in the control of the global TB epidemic.

Drug resistance is not acquired through horizontal gene transfer in *M. tuberculosis*, since this pathogen does not contain plasmids and the transfer of genomic DNA has not been demonstrated (125). Thus, resistance to anti-TB drugs develops by spontaneous mutation and the resulting resistant mutants are selected by subsequent treatment with anti-TB drugs to which the mutants are resistant.

Resistance to various first-line anti-TB drugs, such as INH, RIF, pyrazinamide (PZA), ethambutol (EMB), and classes of second-line drugs (FQs, aminoglycosides, thionamides, peptides, and cycloserines) is attributed to specific mutations in target genes or regulatory domains (10, 11, 28, 69, 107–109) (Table 1). It is thus believed that a specific gene alteration (mutation, insertion, or deletion) will alter the structure of the target protein, thereby influencing the degree of susceptibility to the drug (116). For example, the *katG* gene codes for both catalase and peroxidase enzyme activity, which is essential for the conversion of INH to its active form. Mutations in the *katG* gene lead to a decrease in catalase activity, thereby resulting in less INH being activated and *M. tuberculosis* being resistant to high levels of INH (40). This relationship was confirmed by Ramaswamy et al., who showed that INH-resistant isolates with MICs of >256 μg/ml INH all had low or no catalase activity levels (89). In contrast, mutations in the regulatory or structural regions of the *inhA* gene result in low-level resistance to INH in *M. tuberculosis* (41, 89). Interestingly, mutations within the promoter and the coding region of *inhA* were found to also confer ethionamide resistance (7, 69). This demonstrates that mutations in the same genes or regulatory domain can result in different drug resistance phenotypes.

However, resistance in a proportion of clinical *M. tuberculosis* isolates cannot be explained by classical gene mutations such as those described above. For example, approximately 20 to 30% of clinical INH-resistant *M. tuberculosis* isolates do not have mutations in any of the known genes (Table 1) associated with INH resistance (88, 89). Similarly, approximately 5% of clinical RIF-resistant *M. tuberculosis* isolates do not harbor mutations in the RIF resistance-determining region of the *rpoB* gene (112). Therefore, it is evident that other, more-undefined mechanisms could play a role in drug resistance.

Additional mechanisms that contribute to drug resistance in mycobacteria exist. These mechanisms include the production of drug-modifying and -inactivating enzymes, low cell wall permeability, and efflux-related mechanisms (1, 9, 12, 88, 120, 121). Mycobacteria produce enzymes that degrade or modify certain antibiotics, leading to their inactivation (61, 111). For example, *Mycobacterium smegmatis* is naturally resistant to RIF, although no mutations have been identified in the *rpoB* gene (87). This suggests that an alternative mechanism or mechanisms play a role in conferring resistance to RIF. In 1995, it was reported that *M. smegmatis* DSM43756 inactivates RIF by ribosylation, whereby a ribose ring is covalently linked to the RIF molecule (17, 46). Gene disruption experiments provided evidence that RIF inactivation via ribosylation was the principal contributor of RIF resistance in *M. smegmatis* (87). However, only limited data exist for the production of degrading and drug-modifying enzymes in *M. tuberculosis.* It has previously been reported that bacterial resistance to aminoglycosides can be attributed to enzymatic inactivation of aminoglycosides by phosphotransferases, nucleotidyltransferases, and acetyltransferases (18). Acetyltransferase AAC (2′)-Ic and the phosphotransferase encoded by the Rv3225c gene have been shown to display aminoglycoside-modifying activity (25) that resulted in resistance to aminoglycosides in mycobacteria. Individual mechanisms may not be sufficient to confer clinical resistance but may interact with other resistance mechanisms to cause high-level resistance. This accumulation

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TABLE 1. Genes associated with resistance to various anti-TB drugs

Drug(s)[a]	Yr of discovery	Drug mode of action	Gene	Target enzyme	Frequency of mutations (%) associated with resistance	References
INH*	1952	Inhibits cell wall synthesis	*katG*	Catalase peroxidase	30–60	52, 55, 88, 90, 101, 128
			InhA	Fatty acid enoyl acyl carrier protein reductase A	70–80	
			ahpC	Alkyl hydroperoxidase reductase	Not known	
			kasA	B-ketoacyl-ACP synthase	Not known	
			ndh	NADH dehydrogenase	9.5	
RIF*	1966	Inhibits RNA synthesis	*rpoB*	B subunit of RNA polymerase	95	88, 90, 108, 128
STR***	1944	Inhibits translation	*rpsL*	Ribosomal protein S12	65–67	77, 88, 128
			rrs	16S rRNA		
			gidB	7-Methylguanosine methyltransferase	33	67
EMB*	1961	Inhibits cell wall synthesis	*embCAB*	Arabinosyl transferase	70–90	48, 88, 128
PZA*	1952	Disrupts plasmamembrane and energy metabolism	*pncA*	Pyrazinamidase	>70	62, 96, 128
			IS*6110* insertion		Not known	
FQ**	1963	Introduces negative supercoils in DNA molecules	*gyrA*	DNA gyrase	42–85	16, 32, 33, 128
			gyrB			
KAN, AMI**	1957	Inhibits translation	*rrs*	16S rRNA	>60	2, 53, 105, 109, 128
CAP, VIO**	1957		*rrs*	16S rRNA	40–100	66, 105
			tlyA	rRNA methyltransferase	80	
ETH**	1956	Disrupts cell wall biosynthesis	*InhA*	Fatty acid enoyl acyl carrier protein reductase A	>60	7, 57, 69, 91, 128
			ethA	Flavin monooxygenase	>60	
			ethR	Transcriptional repressor	Not known	

[a] All drugs were hydrophilic. VIO, viomycin; ETH, ethionamide. *, first-line drug for treatment of TB; **, second-line drug for treatment of MDR-TB; ***, alternative first-line drug for retreatment TB cases. The FQ for treatment of MDR-TB consists of OFL-CIP-moxifloxacin.

of resistance mechanisms may contribute to the variation in level of resistance that is seen in *M. tuberculosis* isolates that have identical mutations in resistance-causing genes (45).

In order to design new anti-TB drugs and to develop novel diagnostics, it is essential to gain an in-depth insight into the mechanisms, other than the classical mutations in known target genes, that confer resistance. This is of particular importance since pathogenic mycobacteria, such as *M. tuberculosis*, are becoming increasingly resistant to many of the first- and second line anti-TB drugs. This review gives a broad perspective on the regulation of intracellular anti-TB drug concentration by efflux-related mechanisms in mycobacteria.

MECHANISMS OF THE MYCOBACTERIAL INTRINSIC RESISTOME

The intrinsic resistome is an evolutionary ancient phenotype and can be defined as the intrinsic resistance of any bacterial species that has not been acquired as a result of exposure to antibiotics (27). Intrinsic resistance is usually the result of the reduced permeability of the bacterial envelope and the activity of multidrug efflux pumps (73). This suggests that the main physiological role of the components of intrinsic resistance involves the prevention of influx of toxic components by restricting the permeability of the cell or the active export of toxic compounds or their metabolites out of the cell.

In mycobacteria, the influx of toxic compounds is significantly restricted by the complex cell wall and lipid bilayer. This waxy, lipid-rich cell wall, comprising three covalently linked molecules, i.e., peptidoglycan, arabinogalactan, and mycolic acid, presents a significant barrier to the influx of antibiotics (9). The reduction in membrane permeability leads to a decrease in the influx of the drug, thus leading to a decrease in intracellular drug accumulation (71, 79, 116). However, the first porin (MspA) was identified in mycobacteria, and it allows hydrophilic compounds to enter the cell (72). MspA was also shown to increase susceptibility to β-lactams in *Mycobacterium bovis* BCG and *M. tuberculosis* (63). Thus, hydrophilic compounds diffuse across the mycolic acid layer via porins.

DNA sequencing has predicted that the genome of *M. tuberculosis* strain H37Rv encodes multiple putative efflux proteins, of which the majority have not yet been characterized (1,

116). These efflux pump mechanisms probably have a preexisting physiological role, protecting the bacillus against low intracellular levels of toxic molecules. In addition, they maintain cellular homeostasis and physiological balance through transport of the toxins or metabolites to the extracellular environment. Recent evidence suggests that mycobacteria extrude many drugs (61, 111, 115) via active efflux systems (64, 79, 94). However, the efflux of a broad range of structurally unrelated toxic compounds can be considered an "accidental and opportunistic" side effect of the transport of unidentified physiological substrates in bacterial and mycobacterial species (12, 13, 86, 130). Some efflux pumps are specific for certain antibiotics, while others extrude structurally and functionally unrelated compounds, as is the case for multidrug resistance efflux pumps (54, 61, 64). Experimental procedures for the identification of these pumps are limited to laboratory-induced mutants overexpressing efflux pumps (14). Very few studies have been done on clinical isolates. Therefore, the specific conditions required for the induction of these pumps are not known yet, although it is well recognized that the expression of efflux pump genes is tightly regulated (73, 86, 92). This ensures that efflux pump genes are available when required by the cell to perform their physiological function.

Multidrug resistance efflux pumps. Multidrug resistance efflux pumps by definition reduce the intracellular concentrations of more than one antibiotic to subinhibitory levels (61, 64) and thereby are thought to promote the emergence of resistance to multiple drugs. Genes encoding multidrug resistance pumps are constitutively expressed in wild-type cells (74) and thereby confer a low level of resistance. Current evidence suggests that *M. tuberculosis* contains more than one efflux pump capable of extruding the different antibiotics, e.g., the Tap efflux pump extrudes both aminoglycosides and tetracyclines (TETs) (1). Multidrug resistance efflux pumps and transporters can be overexpressed due to mutations in regulatory regions (73). Alternatively, antibiotics can induce the expression of a multidrug resistance efflux pump by interacting with regulatory systems (92), e.g., TET-specific pumps possess regulatory controls that sense the presence of TET and thereby act as an inducer (35, 36). Both of the above-mentioned mechanisms lead to an increase in the level of drug resistance. Thus, inactivation or silencing of the efflux pumps (including multidrug resistance efflux pumps) could be a possible mechanism for controlling drug resistance. This would render the bacterium more susceptible, allowing lower doses of drugs to be used in the treatment of TB.

Multidrug resistance transporters are grouped into the following two major classes, based on their bioenergetic and structural profiles.

(i) Secondary multidrug transporters (influx/efflux pumps). Secondary multidrug transporters utilize the transmembrane electrochemical gradient of protons or sodium ions to extrude the drugs from the cell (19). They are divided into four families: the major facilitator superfamily (MFS), the small multidrug resistance family (SMR), the resistance nodulation cell division family (RND), and the multidrug and toxic compound extrusion family (1, 54, 64). Most RND proteins are either multidrug or multication transporters (74) and are mostly found in gram-negative bacteria. The SMR proteins bind cationic, lipophilic antibiotics and transport them across the membrane in exchange for protons. In *M. tuberculosis*, Mmr is an example of an SMR protein that confers resistance to acriflavine, ethidium bromide, and erythromycin (6). The expression of MFS and RND superfamily transporters is known to be subject to regulatory controls (79, 86, 114). In some instances, the transported substrates act as the inducer, while in others, the transported substrates act as the repressor by binding to the specific regulatory domains, thereby modulating gene expression. An example is the *M. tuberculosis tap* gene designated Rv1258c, which is a proton-dependent TET efflux pump. In the presence of a compound that inhibits the activity of this pump, the level of resistance to TET was decreased (1). The expression of this transporter is dependent on the expression of the MDR effector gene *whiB7* (70).

(ii) ABC-type multidrug transporters. ATP-binding cassette (ABC)-type multidrug transporters utilize the free energy of ATP hydrolysis to pump drugs out of the cell. The ABC transporters occupy about 2.5% of the total genome content of *M. tuberculosis* (12) and can be classified as importers or exporters on the basis of the direction of translocation of their substrate (50, 51). As their names suggest, importers are involved in the uptake of extracellular molecules while exporters export substrates from the cytoplasm to the external environment. The ABC importers are composed of four domains: two membrane-spanning domains associated with two cytoplasmic nucleotide-binding domains (NBDs) (50). Sequence conservation is marked in five of the NBD motifs. Two of the five motifs (namely, the WALKER A and WALKER B motifs) are present in a vast majority of ATP-binding proteins. The remaining motifs characteristically consist of histidine and glutamine loops that are unique to ABC transporters (42, 118). Evidence suggests that phosphorylation of the NBD of the ABC transporter Rv1747 by the serine/threonine kinase PknF is a possible mechanism for regulation of this transporter in *M. tuberculosis* (68).

EVIDENCE OF EFFLUX MECHANISMS LEADING TO MYCOBACTERIAL DRUG RESISTANCE

(i) INH. There is substantial evidence for efflux-related mechanisms in *M. smegmatis*, which is 300-fold more resistant to INH than *M. tuberculosis*. The overexpression of the *M. tuberculosis mmpL7* gene in *M. smegmatis* confers high-level INH resistance. With the addition of the efflux pump inhibitors reserpine, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), *ortho*-vanadate, reserpine, and verapamil in *M. smegmatis* (13), the level of INH resistance decreases. These results suggest that *mmpL7* is involved in the energy-dependent efflux of INH (79). Earlier reports demonstrate that efflux systems could be induced by prolonged exposure of *M. tuberculosis* to INH, resulting in an increased resistance phenotype (117). More evidence for efflux-related INH resistance comes from analysis of the *iniA* gene in *M. tuberculosis*. It was shown, by gene knockout experiments, that the *iniA* gene is essential for the activity of an efflux pump that confers resistance to INH and EMB (14). This suggests that *iniA* confers resistance to multiple drugs that might lead to the development of MDR- or XDR-TB. *M. tuberculosis* strains lacking the *iniA* gene showed increased susceptibility to INH. The *iniA* deletion also results in an accumulation of intracellular ethidium bromide, thereby suggesting that *iniA* plays a role in efflux (14). This was

supported by the observation that on the addition of reserpine, the resistances to both INH and ethidiumbromide were reversed (14). Recent investigation of gene expression differences by quantitative reverse transcription-PCR with clinical isolates of *M. tuberculosis* showed that various MFS efflux pump genes (*efpA*, *pstB*, Rv1258c, and Rv1410c) and ABC transporters (Rv1819c and Rv2136c) were overexpressed in the presence of INH (38, 47, 119). Collectively, these results suggest that INH resistance in *M. tuberculosis* may be attributed to mutations in known genes and could also be influenced or modulated by efflux-related mechanisms.

(ii) RIF. Although mutations in the *rpoB* gene in RIF-resistant *Mycobacterium avium* and *M. tuberculosis* isolates have been described, certain clinical isolates of *M. avium* and *Mycobacterium intracellulare* demonstrate a significant level of innate resistance to RIF in the absence of *rpoB* mutations, possibly as a result of the permeability barrier (37, 75, 85). Low-level RIF resistance in wild-type *M. smegmatis*, *Mycobacterium aurum*, and *M. tuberculosis* has been shown to be due to an efflux mechanism that extrudes the drug (83). Even though 95% of RIF-resistant clinical *M. tuberculosis* isolates are attributed to mutations in the RIF resistance-determining region, there is evidence to suggest that efflux-related RIF resistance mechanisms may play a role in *M. tuberculosis*, as RIF has been shown to upregulate the expression of the *tap*-like pump Rv1258c and other putative efflux pumps (Rv1410c, Rv1819c, and Rv2136c) in a clinical isolate of *M. tuberculosis* (38, 47, 99). In addition, the ABC transporter *pstB* has been shown to be overexpressed in the presence of RIF (38, 47, 81, 95).

(iii) PZA. PZA resistance in *M. tuberculosis* is primarily attributed to a wide spectrum of mutations in the *pncA* gene, which encodes the enzyme pyrazinamidase (PZase) (49, 62, 88). PZase activates PZA by converting it into active pyrazinoic acid (POA) (126). About 70% of PZA-resistant clinical isolates can be attributed to mutations in the *pncA* gene (96, 97, 103). The *pncA* gene can also be inactivated by the insertion of IS*6110*, thereby conferring the resistance phenotype (58). However, studies by Zhang et al. confirmed that efflux pumps also play a role in mycobacterial resistance to PZA (104, 130). Notably, in nontuberculous *M. avium* and *M. smegmatis* isolates, innate resistance to PZA is not due to a defective *pncA* gene but is due to a highly active efflux mechanism that extrudes the active POA from the cell as soon as PZA is converted by PZase (104). The unique susceptibility of *M. tuberculosis* to PZA is due to efficient PZase activity at acidic pH as well as to a defective POA efflux mechanism (130). The definitive components of the POA efflux mechanism remain to be described, although accumulation of radioactive POA in *M. tuberculosis* and its extrusion by nontuberculous mycobacteria have been demonstrated (104). The efflux pump inhibitor reserpine has been shown to be an effective inhibitor of the POA efflux pump, increasing the susceptibility of *M. tuberculosis* to PZA threefold (129).

(iv) FQs and cationic dyes. The FQs target and inactivate DNA gyrase and type II DNA topoisomerase (32, 90), which are encoded by *gyrA* and *gyrB*, respectively (90). Missense mutations within the quinolone resistance-determining-region have been identified and are associated with FQ resistance (32). However, not all FQ resistance in clinical *M. tuberculosis* isolates can be explained by mutations in the *gyrA* and *gyrB* genes.

An FQ efflux pump of the MFS superfamily, *lfrA*, was the first efflux pump to be described for *M. smegmatis* (60). This efflux pump exhibits broad substrate specificity to more hydrophilic FQs. When expressed on a plasmid, *lfrA* mediates low-level resistance to FQs and other toxic compounds, such as ethidium bromide. The disruption of the *lfrA* gene in *M. smegmatis* causes increased sensitivity to ethidium bromide (60) and a minor decrease in the level of ciprofloxacin (CIP) resistance (106). Recently, an automated method that allows for detection and quantification of ethidium bromide transport across *M. avium* and *M. smegmatis* cell walls was developed. This study shows that the intrinsic resistance of *M. avium* and *M. smegmatis* is affected by thioridazine or chlorpromazine (93).

Recently, it has been shown that FQ-resistant *M. tuberculosis* strains with mutations in the *gyrA* and *gyrB* genes were influenced by the efflux pump inhibitors MC207.110 and reserpine. The levels of resistance for ofloxacin (OFL) and CIP were reduced between two- and sixfold (26). The disruption of the Pst phosphate-specific protein of *M. smegmatis* was also correlated with a decrease in CIP efflux. This resulted in an increased susceptibility to CIP, suggesting an involvement in the efflux of this antimicrobial agent (84).

The *M. tuberculosis* Rv2686c-Rv2687c-Rv2688c operon encodes an ABC transporter that confers resistance to CIP. Pasca et al. showed that the addition of the efflux pump inhibitors reserpine, verapamil, and CCCP to CIP-resistant *M. tuberculosis* increased the intracellular accumulation of CIP. It was also observed that *M. smegmatis* cells containing the Rv2686c-Rv2687c-Rv2688c operon actively pumped out CIP by using ATP hydrolysis as an energy source (78) (Table 2).

(v) Aminoglycosides and TETs. Streptomycin (STR), KAN, and AMI are some of the known aminoglycosides used for the treatment of MDR-TB. STR targets the 30S subunit of the ribosome by binding to the 16s RNA and S12 ribosomal proteins in *M. tuberculosis* specifically. Mutations in the *rrs* gene (encoding 16s RNA) and the *rpsL* gene (encoding the S12 protein) have been shown to confer resistance to this aminoglycoside (44, 98, 113). Approximately 65 to 67% of STR-resistant clinical isolates harbor mutations in one of these two genes; however, some resistant isolates lack identifiable gene mutations. Recently, mutations in a highly conserved gene, *gidB*, which functions as an rRNA methyltransferase, were identified. It was observed that mutations in *gidB* result in low-level STR resistance and contribute approximately 33% of STR resistance in *M. tuberculosis* (77). It has been suggested that the blocking of STR uptake as a result of membrane impermeability (9) could explain resistance in these cases; however, efflux-related mechanisms leading to aminoglycoside resistance have recently been observed (12, 102).

The *tap*-like Rv1258c gene, from the MFS superfamily, has been identified to confer low-level resistance to aminoglycosides and TET when expressed in *M. smegmatis* (1). Rv1258c also showed increased expression in the presence of INH and RIF in an MDR-TB isolate (47). Another example of efflux-related drug resistance to aminoglycosides and TET includes the characterization of the P55 protein isolated from *M. bovis*, which was shown to be related to aminoglycoside and TET efflux pumps in mycobacteria (100). Addition of CCCP, vera-

TABLE 2. Reported putative efflux pump genes and transporters that play a role in drug resistance in *M. tuberculosis*

Gene	Possible drug(s) extruded	Transporter	Function	Protein product	Reference(s)
pstB	INH, RIF, EMB, CIP	ABC	Active import of inorganic phosphate and export of drugs	Phosphate-transport ATP-binding protein	5, 8, 15, 95
Rv2686c	CIP	ABC	Active transport of drugs	Integral membrane ABC transporter	8, 15, 78
Rv2687c	CIP	ABC	Export of highly hydrophobic drugs	Antibiotic transport integral protein	8, 15, 78
Rv2688c	CIP	ABC	Export of toxic compounds	Antibiotic-transport ATP-binding protein	8, 15, 78
Rv1747	INH	ABC	Transport of drug across the membrane.	Conserved transmembrane ATP-binding protein	8, 15
drrA	TET, STR, EMB	ABC	Export of antibiotic in the cell wall.	ATP-binding protein DrrA	8, 12, 15
drrB	TET, STR, EMB	ABC	Export of antibiotic in the cell wall.	ATP-binding protein DrrB	8, 12, 15
drrC	TET, STR, EMB	ABC	Export of antibiotic in the cell wall.	ATP-binding protein DrrC	8, 12, 15
Rv1348	Multiple drugs	ABC	Active export/translocation of drugs across the membrane.	Probable drug transport-associated transmembrane ATP-binding protein	15
Rv1456c	Undetermined	ABC	Active export of antibiotic across the membrane	Integral membrane protein	15
Rv1463	Undetermined	ABC	Active transport and energy coupling across the membrane.	Probable conserved ATP-binding protein	15
Rv1258c	INH, RIF, EMB, OFL	MFS	Export of drugs	Conserved membrane protein	15, 20, 47, 99
Rv2994	Undetermined	MFS	Efflux of drugs	Conserved membrane protein	15, 20, 59
Rv1877	TET, KAN, erythromycin	MFS	Efflux of drugs	Conserved membrane protein	15, 20, 59
Rv1634	Undetermined	MFS	Efflux of sugars and drugs	Drug efflux membrane protein	15, 19, 20, 59
efpA	Possibly INH	MFS	Export of drugs	Integral membrane efflux protein	15, 24, 59
Rv2333c	TET	MFS	Efflux of drugs	Conserved integral membrane transport protein	15, 20
Rv2459c	Drugs	MFS	Transport of substrates	Conserved integral membrane transport protein	15, 20
Rv3239c	Sugar or drugs	MFS	Could be involved in efflux	Conserved transmembrane transport protein	15, 20
Rv3728	Sugar or drugs	MFS	Involved in efflux	Conserved two-domain membrane protein	15, 20
mmpL7	INH	RND	Export of antibiotic	Transmembrane transport protein	15, 23, 79, 82
emrB	Undetermined	SMR	Export of multiple drugs	Integral membrane efflux protein	15, 21
mmr	Erythromycin	SMR	Export of multiple drugs	Integral membrane efflux protein	6, 15
whiB7	RIF	Regulatory protein	Transcriptional regulation	Transcriptional regulatory protein and effector gene	99
Rv2989	Undetermined	Transcriptional regulator	Transcriptional mechanism	Transcriptional regulatory protein	15
iniA	INH, EMB	Membrane protein	Drug transport	INH-inductible protein IniA	3, 14, 15
iniB	INH	Membrane protein	Drug transport	INH-inductible protein IniB	3, 14, 15
iniC	INH	Membrane protein	Transcriptional mechanism	INH-inductible protein IniC	3, 14, 15
Rv1002c	Undetermined	Membrane protein	Unknown function	Integral membrane protein	15
Rv3806c	Undetermined	Membrane protein	Unknown Function	Integral membrane protein	15
Rv3679	Undetermined	ATPase	Extrusion of anions	Probable anion transporter	15

pamil, and reserpine to the cells expressing P55 decreased the levels of resistance to both STR and TET. Therefore, it has been suggested that P55 is sensitive to the efflux pump inhibitors and that this protein uses energy from the proton gradient to drive transport of drugs (100). Other examples of aminoglycoside- and TET-related efflux include the expression of the ABC transporter genes *drrAB* in *M. smegmatis*, which confer resistance to a broad range of clinically relevant antibiotics, including TET, STR, and EMB. The addition of reserpine and verapamil to these cells reversed the resistance phenotype (12). Recently, it has been shown that the transcriptional regulator *whiB7* of *M. tuberculosis* is upregulated in the presence of STR and KAN, although its target gene has not been determined (30). The phosphate transport ATP-binding protein PstB was also shown to extrude STR in *M. smegmatis* (38).

For TET specifically, an energy-dependent efflux pump, Tet(V), was identified in *M. smegmatis*. The *tet*(*V*) gene encodes an efflux protein which uses proton motive force to extrude TET from *M. smegmatis*. The Tet(V) protein is not homologous to any known specific TET efflux pump and remains restricted to the *M. smegmatis* and *Mycobacterium fortuitum* species (22, 59).

Reports of efflux-related drug resistance are not limited to mycobacteria. Studies of other bacteria also provide evidence that proteins involved in efflux can contribute to drug resistance as well as resistance to other toxic compounds and heavy metals (31, 56, 74, 76, 122–124).

EFFLUX PUMP GENES NOT IMPLICATED IN DRUG RESISTANCE

Antibiotic resistance characteristics have facilitated the identification of many efflux proteins. However, *efpA* was discovered fortuitously during the screening of genes for novel *M. tuberculosis* membrane proteins (24). EfpA is predicted to be highly related to members of the QacA transporter family, which is also known as the drug resistance transporter family. Thus far, *efpA* has not been implicated in drug resistance, although all other members of the QacA transporter family mediate resistance to antibiotics (29, 34, 80). The investigation of intrinsic resistance in *M. smegmatis*

showed that *efpA* was expressed at detectable levels. Deletion of the *efpA* gene resulted in increased susceptibility to FQs, acriflavine, and ethidiumbromide. However, in contrast, deletion of *efpA* also resulted in decreased susceptibility to rifamycins, INH, and chloramphenicol (59). It was shown that the exposure of resistant *M. tuberculosis* to INH resulted in induced expression of *efpA.* However, the researchers suggested that this induced expression could be used to mediate metabolic processes that are linked to the toxic consequences of INH (119). EfpA mutations were also detected in drug-susceptible and drug-resistant *M. tuberculosis* isolates examined (24, 127). Therefore, on the basis of these contradictory studies, it remains questionable whether the *efpA* gene plays a role in drug resistance.

CONCLUDING REMARKS

Dogma informs us that mutations in drug target genes are the principal cause of drug resistance in *M. tuberculosis*. Thus, the identification of these mutations has been proposed as a means to genetically identify drug resistance and to thereby improve the time to diagnosis and prevent the transmission of drug resistance. However, this review highlights the observation that *M. tuberculosis* may have adopted certain intrinsic mechanisms, normally used to remove toxic compounds from the cell, to evade the killing and toxic effect of anti-TB drugs. These intrinsic mechanisms include regulation of the intracellular concentration of the anti-TB drug by efflux pumps. This suggests that drug resistance may be more complex than the simple relationship between the presence and absence of mutations in drug target genes. From the data presented, we propose that drug resistance can be attributed to mutations in drug target genes and/or upregulation of efflux mechanisms. Although the clinical relevance of the upregulation of efflux activity remains to be determined, we postulate that such mechanisms may influence the level of resistance as well as conferring cross-resistance to different anti-TB drugs. The observation that certain compounds (such as thioridazine and SILA 421) were able to restore susceptibility by inhibiting efflux activity suggests that such compounds may have an important role in the future treatment of MDR- and XDR-TB (4, 65). Therefore, it is imperative to broaden our knowledge of the mechanisms of efflux and their role in drug resistance. This will enable the identification of new drug targets and the development of new drugs to counteract the natural mechanisms of defense against toxic compounds.

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