

AsnB Is Involved in Natural Resistance of *Mycobacterium smegmatis* to Multiple Drugs

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Mycobacteria are naturally resistant to most common antibiotics and chemotherapeutic agents. The underlying molecular mechanisms are not fully understood. In this paper, we describe a hypersensitive mutant of *Mycobacterium smegmatis*, MS 2-39, which was isolated by screening for transposon insertion mutants of *M. smegmatis* mc²155 that exhibit increased sensitivity to rifampin, erythromycin, or novobiocin. The mutant MS 2-39 exhibited increased sensitivity to all three of the above mentioned antibiotics as well as fusidic acid, but its sensitivity to other antibiotics, including isoniazid, ethambutol, streptomycin, chloramphenicol, norfloxacin, tetracycline, and β -lactams, remained unchanged. Uptake experiment with hydrophobic agents and cell wall lipid analysis suggest that the mutant cell wall is normal. The transposon insertion was localized within the *asnB* gene, which is predicted to encode a glutamine-dependent asparagine synthetase. Transformation of the mutant with wild-type *asnB* of mc²155 or *asnB* of *Mycobacterium tuberculosis* complemented the drug sensitivity phenotype. These results suggest that AsnB plays a role in the natural resistance of mycobacteria.

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), is responsible for 2 million deaths and 10 million new infections each year. Clinical treatments of TB and other mycobacterial infections are difficult because mycobacteria are naturally resistant to most commonly used antibiotics and chemotherapeutic agents (14, 17). For instance, among the commonly used antibiotics against *M. tuberculosis*, the only ones found to be effective are rifampin and streptomycin, which are routinely used, in combination with isoniazid, pyrazinamide, and ethambutol, for chemotherapy of TB. Infections caused by nontuberculous mycobacteria such as the *Mycobacterium avium* complex (MAC) are increasingly common in immunocompromised individuals, which are often more difficult to treat because these organisms are resistant to standard anti-TB drugs including isoniazid and rifampin (14, 17).

The natural resistance of mycobacteria is caused primarily by the impermeability of the mycobacterial cell wall (6, 14, 17). The cell wall forms an asymmetric lipid bilayer with mycolic acids in the inner leaflet and extractable complex lipids in the outer leaflet (17, 20). Mycolic acids are of extraordinary length and are highly saturated; as such, the mycolic acid-containing layer has extremely low fluidity, which forms a strong permeability barrier and contributes to the broad resistance (17, 18, 20). The natural resistance of mycobacteria also involves active efflux processes mediated by various transport systems. Efflux-mediated resistances have been reported for fluoroquinolones (21, 28), tetracyclines (9, 27), isoniazid, ethambutol (7), pyrazinamide (33), erythromycin, and rifamycins (16). These efflux systems, however, often confer only low levels of resistance.

In an attempt to better understand the mechanisms involved in the natural resistance of mycobacteria to antibiotics, we have screened a transposon insertion library of *Mycobacterium smegmatis* for drug-supersusceptible mutants. Using this strategy, we have previously isolated and characterized a rifampin-hypersensitive mutant, which showed that the presence of a rifampin ADP ribosyltransferase (Arr) in *M. smegmatis* confers resistance to rifampin (1). In the present work, we describe a different drug-susceptible mutant in which inactivation of *asnB*, a gene involved in amino acid metabolism, dramatically sensitizes *M. smegmatis* to multiple antibiotics, including rifampin, erythromycin, novobiocin, and fusidic acid.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. Wild-type (WT) *Mycobacterium smegmatis* strain mc²155 and its *mariner* insertion mutants were grown in Middlebrook 7H9 broth or Middlebrook 7H11 agar (Difco) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC; Difco). In preparation for genomic DNA isolation, mycobacteria were grown in 7H9 broth supplemented with 0.1% Tween 80 and 10% albumin-dextrose-catalase (ADC; Difco). Antibiotics (Sigma) were added at the following concentrations: ampicillin, 50 μ g/ml; kanamycin, 25 μ g/ml for mycobacteria and 50 μ g/ml for *Escherichia coli*; hygromycin, 75 μ g/ml for mycobacteria and 150 μ g/ml for *E. coli*.

Generation and screening of *M. smegmatis* ϕ MycoMar insertion library. The *mariner*-based transposon system ϕ MycoMarT7 was utilized to generate a transposon insertion mutant library of *M. smegmatis* mc²155 as described previously (1, 2). Kanamycin-resistant (i.e., transposon-containing) colonies were patched onto Middlebrook 7H11 agar to obtain a library of 7,680 clones (i.e., 80 plates \times 96 colonies per plate). To screen this library, clones were replica plated onto Middlebrook 7H11 agar supplemented with either rifampin, erythromycin, or novobiocin, each at one-third the MIC for the WT *M. smegmatis* mc²155. Clones that failed to grow on the drug-containing plates were deemed hypersensitive. A broth dilution method was used to determine the MIC and confirm the drug hypersensitivity of these clones (19).

Localization of the ϕ MycoMar insertion. The method used to localize and identify the disrupted gene has been described previously (1, 2). Briefly, total chromosomal DNA of transposon insertion mutant was cleaved with BamHI. Digested DNA was self-ligated with T4 DNA ligase and transformed into competent *E. coli* DH5 α λ pir116 cells. Plasmid DNA was isolated from Km^r *E. coli* transformants. MycoMar-specific primers were used to determine the DNA sequence of the MycoMar/chromosomal junction. These DNA sequences were

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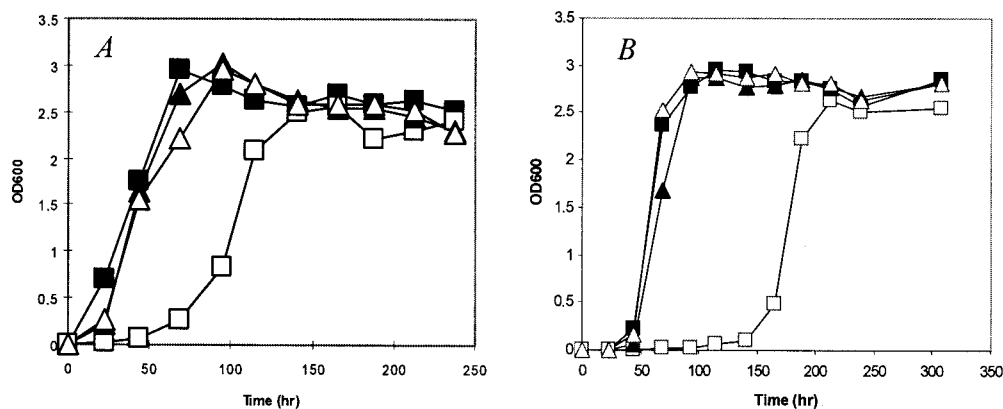


FIG. 1. Growth of mutant MS 2-39. Strains of *mc*²155 (■), MS 2-39 (□), MS 2-39/pASNB_MS (▲), and MS 2-39/pASNB_MTB (△) were grown in 7H9 liquid medium at 37°C (A) or 25°C (B). MS 2-39 exhibited a growth delay at both temperatures, which was complemented by plasmid pASNB_MS or pASNB_MTB. The initial inoculate was 10⁶ cells/ml. OD600, optical density at 600 nm.

compared to the GenBank database and the *M. smegmatis mc*²155 genome database at the Institute for Genomic Research (<http://www.tigr.com/>) using the BLASTN algorithm. Nucleotide sequences were also analyzed with NTI Suite software (Informax).

Cloning of *asnB* gene from *M. smegmatis* and *M. tuberculosis*. The *asnB* gene of *M. smegmatis* WT strain *mc*²155 was amplified by PCR from its genomic DNA using the forward primer 5'-TGCAGCCGCTCAGCAGTAGC-3' and reverse primer 5'-CCGAGACCCACAAGGAGGAT-3'. The *asnB* gene of *M. tuberculosis* H37Rv was amplified by PCR using the forward primer 5'-TACTTGTCC TTGCCCTCTGG-3' and reverse primer 5'-TCCTAAGGGTGTGCGATTGG-3'. An ordered BAC library of *M. tuberculosis* H37Rv genome (obtained from S. Cole, Institut Pasteur, Paris, France) was used as the DNA template for cloning. Vector pDrive (QIAGEN) was used for cloning of PCR products. To construct pASNB plasmids, a 2.4-kb EcoRI fragment containing *asnB* of *M. smegmatis* or a 3.2-kb StuI fragment containing *asnB* of *M. tuberculosis* was cloned into the *E. coli*-*Mycobacterium* shuttle vector pNBV1 (11), which contains a hygromycin resistance cassette. The resulting plasmids were named pASNB_MS and pASNB_MTB, respectively. Plasmids pASNB and pNBV1 were transformed into mycobacterial cells by electroporation according to standard protocol. Transformants were selected on Middlebrook 7H11 agar containing hygromycin (75 µg/ml). Drug sensitivities of the transformants were determined by a broth dilution method in Middlebrook 7H9 supplemented with OADC (19).

Uptake assay. Radiolabeled chemicals were obtained from NEN. Uptake of [¹⁴C]erythromycin (specific activity, 55.1 mCi/mmol) and [¹⁴C]chenodeoxycholate (specific activity, 48.6 mCi/mmol) by mycobacterial cells was measured as described previously (18, 19). Briefly, [¹⁴C]erythromycin or [¹⁴C]chenodeoxycholate was added to 1-ml cell suspensions of *mc*²155 or MS 2-39 in 0.1 M K-phosphate buffer (pH 7.0) at time zero to a final concentration of 10 µM. At various time points thereafter (0, 3, 5, 10, 15, 20, 25, 30 min), 50-µl portions of the suspension were removed, filtered, and washed. The radioactivity retained on the filter was determined by scintillation counting.

Thin-layer chromatography analysis of cell wall mycolic acids. Mycolic acids were extracted and analyzed by thin-layer chromatography as previously described (31).

RESULTS

Isolation and characterization of a hypersensitive mutant MS 2-39. To identify mechanisms that may contribute to the intrinsic drug resistance of mycobacteria, we generated a transposon insertion mutant library of *M. smegmatis mc*²155 (~8,000 clones) and screened for mutants that exhibit increased sensitivity to antibiotics. This was achieved by replica plating of each clone onto 7H11 agar plates containing kanamycin (25 µg/ml) and supplemented with either rifampin, erythromycin, or novobiocin, each at one-third the MIC for the WT *M. smegmatis mc*²155. These plates were incubated at 37°C

for 7 days, by which time all colonies on the drug-free (i.e., master) plates fully grew. Colonies on the drug-containing plates were visually inspected to identify mutants that did not grow in the presence of sublethal concentrations of drugs. One mutant, MS 2-39, was unable to grow on plates containing either rifampin, erythromycin, or novobiocin, suggesting that this mutant was hypersensitive to all three drugs.

The MS 2-39 mutant was patch purified on 7H11 agar plates, and single colonies were inoculated in 7H9 liquid medium containing kanamycin. The mutant exhibited a growth delay; when grown in 7H9 broth at 37°C, there was an approximately 75-h lagging period at the early stage of growth compared to the WT strain *mc*²155 (Fig. 1A). However, once it started to grow, the mutant had a similar growth rate as the WT strain and reached the same cell density at stationary phase. Microscopic examination of mutant cells revealed typical rod shape of normal size (not shown).

To determine the influence of temperature on bacterial growth, MS 2-39 and *mc*²155 were cultured at 25 and 45°C in 7H9 broth. At 25°C, the mutant exhibited a ~100-h delay at the early growth phase (Fig. 1B). At 45°C, there was no visible growth of the mutant after 10 days, while the WT strain reached stationary phase after only 48 h (data not shown). These results suggest that growth of MS 2-39 is affected at all temperatures but the effect is most pronounced at 45°C.

To confirm the hypersensitivity of the mutant, MICs of MS 2-39 were determined in 7H9 liquid medium at 37°C using the broth dilution method (19). MICs for the WT strain were determined after 4-day incubation, whereas MICs for the mutant were determined after 7-day incubation because the mutant exhibits a 3-day growth delay at 37°C. Prolonged growth (e.g., 10 days) of the mutant did not alter the MIC reading. The result confirmed that MS 2-39 is hypersensitive to erythromycin, rifampin, novobiocin, and fusidic acid (Table 1). MICs of the mutant are between 16- and 64-fold lower than the WT strain for these antibiotics (Table 1). Sensitivity of MS 2-39 to other antibiotics, including isoniazid, ethambutol, streptomycin, chloramphenicol, norfloxacin, nalidixic acid, tetracycline, ampicillin, nafcillin, and cephaloridine, remained unchanged (data not shown).

TABLE 1. MIC of various antibiotics for *M. smegmatis asnB* mutant strain MS 2-39

Strain	MIC ($\mu\text{g/ml}$)			
	Rifampicin	Erythromycin	Novobiocin	Fusidic acid
mc ² 155	64	32	128	128
MS 2-39	2	0.5	4	8
MS 2-39/pASNB_MS	64	32	128	128
MS 2-39/pASNB_MTB	64	32	128	128

Mutant MS 2-39 is defective in AsnB. The transposon insertion in MS 2-39 was localized as described in Materials and Methods. Upon comparison, our DNA sequencing data matched the *M. smegmatis* mc²155 genome data available at The Institute for Genomic Research (www.tigr.org). The transposon had inserted at a TA dinucleotide, 305 bp downstream from the GTG start codon of an open reading frame, which is predicted to encode a 658-amino acid polypeptide (Fig. 2A). A homology search using the BLAST algorithm revealed a significant sequence identity with predicted asparagine synthetase B (AsnB) of various organisms including *Mycobacterium avium* subsp. *paratuberculosis* (82% amino acid identity), *M. tuberculosis* (80%), *M. leprae* (77%), *Rhodococcus erythropolis* (72%), *Nocardia farcinica* (71%), *Corynebacterium glutamicum* (63%), and *Bacillus licheniformis* (52%). Based on the sequence homology, we named the gene inactivated in mutant MS 2-39 *asnB*.

AsnB catalyzes the transfer of the γ amino residue of glutamine to the carboxyl residue of aspartate (Fig. 2B) and is a member of the *purF* family of glutamine-dependent amidotransferases, which includes glucosamine-6-phosphate synthase (GlmS) and glutamine phosphoribosylpyrophosphate amidotransferase (32). Like other AsnB homologs, the deduced sequence of *M. smegmatis* AsnB is composed of a glutaminase domain (residues 2 to 247) and a synthetase domain (residues 270 to 577). The N-terminal glutaminase domain hydrolyzes glutamine to glutamate and ammonia. It contains a conserved N-terminal second cysteine residue, which is characteristic of all *purF* enzymes and is essential for glutamine-dependent amidotransferase activity (5, 30). Family members that contain the C-terminal synthetase domain catalyze the

conversion of aspartate to asparagine. The three-dimensional architecture of the N-terminal domain of AsnB is similar to that observed for glutamine phosphoribosylpyrophosphate amidotransferase while the molecular motif of the C-terminal domain is reminiscent to that observed for GMP synthetase (15).

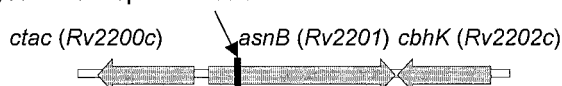
Complementation of mutant MS 2-39. To confirm that the hypersensitive phenotype of MS 2-39 was due to disruption of the *asnB* gene and not a polar effect caused by the transposon insertion, we cloned the *asnB* gene from the parental *M. smegmatis* strain mc²155 and the *asnB* ortholog of *M. tuberculosis* into a shuttle vector pNBV1 (11). The resulting plasmids, pASNB_MS and pASNB_MTB, respectively, were transformed into MS 2-39. Recombinant strains of MS 2-39 containing either pASNB_MS or pASNB_MTB exhibited MICs and growth curves that were indistinguishable from the WT strain (Table 1 and Fig. 1A and B), suggesting that the *asnB* gene of *M. smegmatis* or *M. tuberculosis* complemented the mutant phenotypes.

Uptake of erythromycin and chenodeoxycholate. To determine whether the enhanced drug sensitivity of MS 2-39 is caused by increased cell wall permeability, uptakes of [¹⁴C]erythromycin and [¹⁴C]chenodeoxycholate by mutant cells were performed and compared with WT cells. Erythromycin was one of the four drugs that showed enhanced activity against mutant MS 2-39. In addition, since all four drugs that showed increased activities against the mutant are hydrophobic molecules, we also measured the uptake of chenodeoxycholate, which is a lipophilic agent that has been widely used for assessing cell wall permeability to hydrophobic agents (18, 19). There is no difference in the rate and levels of uptakes of erythromycin or chenodeoxycholate between mutant and WT cells (data not shown), suggesting that the permeability of the mutant cell wall was not affected. Thin-layer chromatography analysis of mycolic acids, a major cell wall component that plays a critical role on cell wall permeability (18, 19), did not reveal any difference between the WT and the mutant (data not shown).

Mutant MS 2-39 is not an asparagine auxotroph. Some bacteria such as *E. coli* and *Klebsiella aerogenes* contain two types of asparagine synthetase (13, 25), AsnB and a mechanically distinct asparagine synthetase, AsnA, which utilizes ammonia as an amide donor. AsnB is the only asparagine synthetase found in mycobacterial genomes. To determine whether the *asnB* mutant is auxotrophic for asparagine, we compared the growth of the mutant in 7H9 supplemented with or without 10 mM asparagine. The result showed that supplementation of asparagine did not facilitate the growth of mutant (Fig. 3A). We also examined the effect of other amino acids including glutamine, glutamate, and aspartate, since the intracellular pools of these amino acids could be affected by an AsnB-mediated reaction (Fig. 2B). Like asparagine, supplementation of glutamine or glutamate had no effect on the growth of mutant MS 2-39 (Fig. 3A). Intriguingly, supplementation of aspartate inhibited the growth of the mutant; there was no viable growth at 37°C after 230 h (Fig. 3B). Supplementation of the same concentration of aspartate to 7H9 caused only a slight growth delay (~20 h) of the WT strain (Fig. 3B). The mechanism of growth inhibition of aspartate is unknown.

A

ϕMycoMar transposon insertion



B



FIG. 2. (A) The *asnB* region of *M. smegmatis* mc²155. The *asnB* gene and flanking genes are depicted. Block arrows represent open reading frames. Arrow indicates insertion of the transposon to generate the hypersensitive mutant MS 2-39. The genome of *M. tuberculosis* has the same genetic organization and the corresponding genes are shown (*Rv2200c*, *Rv2201*, *Rv2202c*). (B) Reaction catalyzed by glutamine-dependent asparagine synthetase, AsnB.

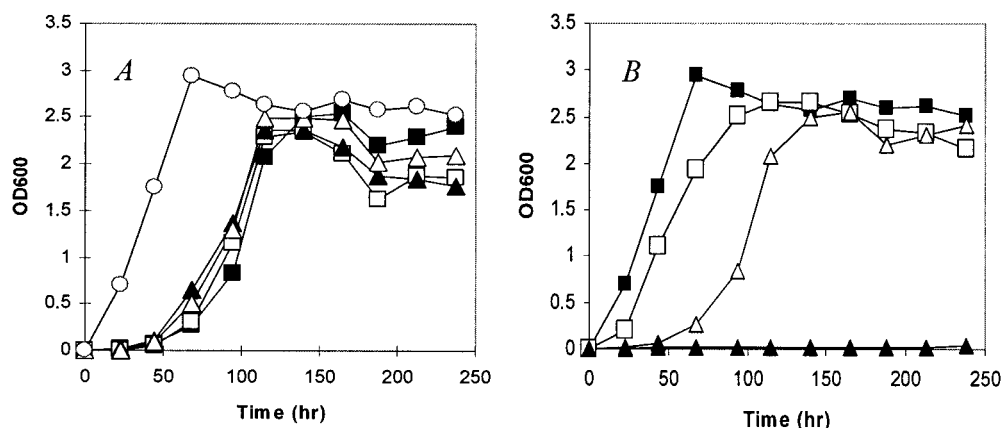


FIG. 3. Growth of mutant MS 2-39 in 7H9 supplemented with amino acids. (A) MS 2-39 was inoculated (10^6 cells/ml) in 7H9 supplemented with either asparagine (\square), glutamine (\triangle), or glutamate (\blacktriangle), each at 10 mM and incubated at 37°C . Growth of MS 2-39 (\blacksquare) and mc^2155 (\circ) in 7H9 was shown for comparison. Supplementation of asparagine, glutamine, or glutamate did not facilitate the growth of MS 2-39, which showed a growth delay at the early stage. OD600, optical density at 600 nm. (B) Supplementation of aspartate (10 mM) to 7H9 inhibited the growth of MS 2-39 (\blacktriangle). Only a slight delay of growth was observed for WT strain mc^2155 (\square).

DISCUSSION

In this study, we have identified a novel mechanism of natural resistance of mycobacteria. Wild-type *M. smegmatis* is naturally resistant to rifampin, erythromycin, novobiocin, and fusidic acid. We demonstrated that inactivation of the *asnB* gene in *M. smegmatis* confers hypersensitivity to these drugs, indicating that *asnB* is involved in the natural resistance of *M. smegmatis* to multiple drugs. To the best of our knowledge, the involvement of an amino acid metabolic enzyme in antibiotic resistance has not been described previously in mycobacteria.

The mutant MS 2-39 was isolated by screening the transposon insertion library for mutants that exhibited increased sensitivity to rifampin, erythromycin, or novobiocin. This mutant turned out to be hypersensitive to all three antibiotics examined, as well as fusidic acid. The transposon insertion was localized within the *asnB* gene, which is predicted to encode a glutamine-dependent asparagine synthetase. This was somewhat unexpected. However, *asnB* gene from the WT strain of *M. smegmatis* and *asnB* ortholog of *M. tuberculosis* fully complemented the mutant hypersensitive phenotype, confirming that inactivation of *asnB* is responsible for the enhanced drug sensitivity of the mutant.

Two families of asparagine synthetase have been reported. One is the AsnA family, whose members are found in prokaryotes such as *E. coli* and *K. aerogenes* (13, 25). AsnA is able to use only ammonia as the amino group donor. The other is the AsnB family, whose members are found in both prokaryotes and eukaryotes (12, 29, 30). Members of AsnB are able to use both glutamine and ammonia as the nitrogen donor, but glutamine is preferred. Structure and enzymatic mechanism have been studied extensively for AsnB of *E. coli* (5, 15). On the basis of sequence analyses and structural studies, AsnB has been shown to belong to a larger family of enzymes referred to as the *purF* family of glutamine amidotransferases. In all these enzymes, glutamine serves as the preferred source of nitrogen whereby an acceptor molecule is subsequently aminated. A cysteine residue at the N terminus is absolutely re-

quired for enzyme activity in the amidotransfer reaction (5, 30).

AsnB of mycobacteria shows a low level of sequence identity to AsnB of *E. coli* (29% amino acid identity), but it shares significant sequence identity with predicted AsnB of gram-positive bacteria including *Rhodococcus*, *Nocardia*, *Corynebacterium*, and *Bacillus*. AsnB of gram-positive bacteria has not been studied extensively. Nevertheless, an *asnB* mutant of *Corynebacterium glutamicum* was isolated as a lysozyme-sensitive mutant, hence its name *ltsA* (10). Intriguingly, wild-type *asnB* (or *ltsA*) gene of *C. glutamicum* did not complement asparagine auxotroph of the *E. coli* *asnA* *asnB* double mutant. Based on these observations, it was suggested that AsnB/LtsA of corynebacteria is not an asparagine synthetase; instead, it may catalyze the amidotransfer reaction from glutamine onto some unknown cell surface component(s) (10). AsnB of *M. smegmatis* shares 63% amino acid identity with AsnB/LtsA of *C. glutamicum*, suggesting that they may perform a similar function. Like the *asnB* mutant of *C. glutamicum*, which is temperature sensitive for growth at 37°C (10), the growth of the *M. smegmatis* *asnB* mutant was arrested at a high temperature (45°C). However, unlike the *asnB* mutant of *C. glutamicum*, which grows normally at a permissive temperature (30°C) (10), the growth of the *M. smegmatis* *asnB* mutant was affected at all temperatures; there was a growth delay at both 25 and 37°C , but once the growth commenced, the actual growth rates were normal. In addition, the *asnB* mutant of *M. smegmatis* did not exhibit detectable change on cell size, which was observed for the *asnB* mutant of *C. glutamicum* (10), and sensitivity to lysozyme remained unchanged compared to the WT *M. smegmatis* strain (data not shown).

AsnB is the only putative asparagine synthetase found in mycobacterial genomes, and yet the *M. smegmatis* *asnB* mutant does not seem to be an asparagine auxotroph. Although the *asnB* mutant exhibits a delayed growth at 25 and 37°C in 7H9 medium, supplementation of asparagine (10 mM) does not facilitate the mutant growth. The apparent paradox could be

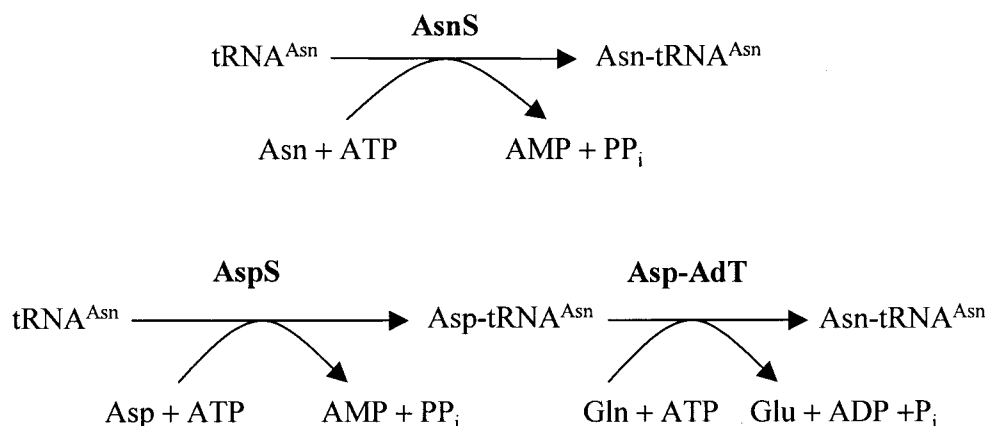


FIG. 4. Pathways of Asn-tRNA^{Asn} synthesis. The top reaction is the direct pathway, which synthesizes Asn-tRNA^{Asn} with asparagine either imported or made from aspartate by the AsnA or AsnB enzymes. The bottom reaction is the transamidation pathway, which consists of a nondiscriminating AspS capable of generating the misacylated Asp-tRNA^{Asn}, and a heterotrimeric Asp-tRNA^{Asn} amidotransferase (Asp-AdT, encoded by the *gatCAB* genes). The genome of *M. tuberculosis* does not contain *asnS*, encoding asparaginyl-tRNA synthetase, but does contain the following genes for the transamidation pathway: *aspS* (*Rv2572c*), *gatCAB* (*Rv3012c*, *Rv3011c*, *Rv3009c*).

explained by the presence of a tRNA-dependent transamidation mechanism (*gatCAB*) for the conversion of aspartate to asparagine in mycobacteria (Fig. 4) (8, 23). Such a mechanism has been shown to be an essential route to asparagine formation in organisms such as *Thermus thermophilus* and *Deinococcus radiodurans*, which lack both *asnA* and *asnB* (23, 26).

The mechanism by which AsnB confers antibiotic resistance in mycobacteria is currently unknown. AsnB could catalyze the synthesis of a cell wall component(s) other than asparagine, as suggested for AsnB of *C. glutamicum* (10). As such, inactivation of *asnB* disrupts the cell wall structure and confers hypersensitivity to antibiotics. On the other hand, the hypersensitivity of the *M. smegmatis* *asnB* mutant is restricted only to several hydrophobic drugs, and sensitivity of the mutant to a panel of other antibiotics including isoniazid, ethambutol, streptomycin, chloramphenicol, norfloxacin, tetracycline, and β -lactams was not affected. This is different from typical cell wall-deficient mutants, which exhibit increased sensitivity to a wide range of antibiotics, for example, a mutant of *Pseudomonas aeruginosa* in which GlnS (also a member of the *purF* family of glutamine amidotransferases) was affected (24). In addition, uptake experiments with erythromycin and chenodeoxycholate, as well as analysis of the mycolic acid composition of the mutant cell wall, did not reveal any difference between the WT and the mutant strains.

To our knowledge, there is no published literature on the involvement of *ansB* in antibiotic resistance. Interestingly, there appears to be an inverse relationship between the susceptibility of leukemia cells to cancer drug therapy and their capacity for intracellular asparagine biosynthesis (3, 4, 22). As a consequence, L-asparaginase, which catalyzes the hydrolysis of asparagine, is widely used in chemotherapeutic protocols for treating acute lymphoblastic leukemia.

In summary, our results indicate that the *asnB* is involved in the multidrug resistance of mycobacteria. This finding may provide a strategy for designing new drugs or novel combinations of drugs for the treatment of mycobacterial infections including TB.

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