The mabA gene from the inhA operon of Mycobacterium tuberculosis encodes a 3-ketoacyl reductase that fails to confer isoniazid resistance

Asesh Banerjee,† Michele Sugantino,‡ James C. Sacchettini‡+ and William R. Jacobs, Jr†

A target of the anti-tuberculosis drugs isoniazid (INH) and ethionamide (ETH) has been shown to be an enoyl reductase, encoded by the inhA gene. The mabA (mycolic acid biosynthesis A) gene is located immediately upstream of inhA in Mycobacterium tuberculosis, Mycobacterium bovis and Mycobacterium smegmatis. The MabA protein from M. tuberculosis was expressed in Escherichia coli and shown to have 3-ketoacyl reductase activity, consistent with a role in mycolic acid biosynthesis. In M. smegmatis, inhA and mabA are independently transcribed, but in M. tuberculosis and M. bovis BCG, mabA and inhA constitute a single operon. Several INH-ETH-resistant M. tuberculosis clinical isolates contain point mutations in the ribosome-binding site of mabA in the mabA-inhA operon. However, genetic dissection of this operon reveals that the INH-ETH-resistance phenotype is encoded only by inhA, and not by mabA.

Keywords: Mycobacterium tuberculosis, isoniazid, ethionamide, drug resistance, inhA gene

INTRODUCTION

Tuberculosis is one of the world’s most deadly infectious diseases (World Health Organization, 1997). The recent emergence of multidrug-resistant strains of Mycobacterium tuberculosis poses a serious threat to tuberculosis-control programs worldwide (Snider et al., 1994). Understanding the mechanisms of resistance to the available antimycobacterial drugs and discovery of new drug targets are necessary for the treatment of tuberculosis.

The gene (inhA) encoding a target for isoniazid (INH) and its analogue, ethionamide (ETH), was cloned in 1994 (Banerjee et al., 1994). Mutations in inhA were shown to confer resistance to both INH and ETH in drug-resistant M. tuberculosis clinical isolates (Heym et al., 1994; Kapur et al., 1996; Musser et al., 1996; Ristow et al., 1995; Rouse et al., 1995; Telenti et al., 1997; Victor et al., 1997). KatG- (Zhang et al., 1992) activated INH is proposed to inhibit the inhA-encoded enoyl reductase (ER) enzyme, which is involved in the biosynthesis of mycolic acids (long-chain fatty acids of mycobacteria) (Johnsson et al., 1995; Wheeler & Anderson, 1996; Quemard et al., 1995; Dessen et al., 1995; Rozwarski et al., 1998).

An ORF has been found immediately upstream of inhA in all the mycobacteria tested thus far (Banerjee et al., 1994). This ORF, a strong homologue of many 3-ketoacyl reductases (KARs), was previously designated orfI and later renamed mabA (Musser et al., 1996), due to its probable involvement in mycolic acid biosynthesis (Kikuchi et al., 1989; Quemard et al., 1995). mabA from Mycobacterium smegmatis, which is transcribed separately from inhA, has been shown not to mediate INH-ETH resistance. Despite the fact that, unlike in M. smegmatis, mabA constitutes an operon with inhA in...
M. tuberculosis, it was not initially tested whether MabA from M. tuberculosis plays a role in mediating drug resistance. Based on the high (82% identity) homology between these two MabAs, it was assumed that M. tuberculosis MabA is not involved in INH resistance. However, later on, INH-resistant clinical isolates of M. tuberculosis were frequently reported to have mutations in the ribosome-binding site (RBS) of mabA (Kapur et al., 1996; Musser et al., 1996; Rouse et al., 1995; Telenti et al., 1997; Victor et al., 1997). This observation supported the speculation that, in M. tuberculosis, MabA could be an additional target for INH and ETH, and overexpression of MabA could mediate INH resistance by virtue of increased drug titration (Musser, 1995). Hence, we decided to characterize the mabA-encoded activity and examined, by a genetic approach, whether it can be a target for INH and ETH in M. tuberculosis.

METHODS

Strains, plasmids and media. Strains and plasmids used in this study are shown in Table 1. All the antibiotics used were purchased from Sigma if not mentioned otherwise. Luria broth (Difco) and Luria agar containing 50 µg ampicillin ml⁻¹ were used for the growth of Escherichia coli strains (Sambrook et al., 1989). Mycobacteria were grown in liquid culture using M-OADC-TW broth (Jacobs et al., 1991). Middlebrook 7H10 agar (supplemented with 10% OADC and 0.5% glycerol) was used for growth on solid media, colony titrations and plating transformations of mycobacteria. Selections for mycobacteria were performed using this solid medium containing kanamycin (10–25 µg ml⁻¹), INH (0.2, 1, 10, 20 and 50 µg ml⁻¹) and ETH (10, 30, 50, 100 and 200 µg ml⁻¹). Cycloheximide was added to liquid and solid media at a final concentration of 50 µg ml⁻¹ to inhibit growth of moulds.

DNA manipulation, cloning and sequencing. The original M. smegmatis mabA-inhA cosmids were isolated from a M. smegmatis genomic library by their ability to confer INH resistance in the M. smegmatis host (Banerjee et al., 1994). Analogous M. tuberculosis and Mycobacterium bovis BCG cosmids were obtained by screening genomic libraries of these two organisms using colony blotting. The plasmids used in this study (Table 1) are subclones of these parent cosmids. For subcloning, cosmid DNAs were digested with the restriction enzymes (New England Biolabs) indicated in Table 1, and the desired DNA fragments were isolated by using the SEAKEM (FMC) agarose gel following the manufacturer's instructions.

Table 1. Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype/phenotype/description</th>
<th>Reference/source</th>
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<tr>
<td><strong>Strains</strong></td>
<td></td>
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<tr>
<td>E. coli DH5α</td>
<td>F- endA1 hisD17 supE44 thi-1 recA1 gyrA96 relAα (argF– lacZYA) U169</td>
<td>Bethesda Research Laboratories</td>
</tr>
<tr>
<td>E. coli Epicurian Coli SCS1</td>
<td>recA1 endA1 gyrA96 thi-1 hisD17(rK⁺ mK⁻) supE44 relA</td>
<td>Stratagene</td>
</tr>
<tr>
<td>E. coli BL21(DE3) pLysS</td>
<td>A lysogenic strain containing the gene for T7 RNA polymerase under the control of a lacUV promoter; F- ompT hisD17B(rK⁺ mK⁻) gal dcm (DE3) pLysS (Cm8')</td>
<td>Novagen</td>
</tr>
<tr>
<td>M. smegmatis mc6 ATCC607</td>
<td>Wild-type strain from which the M. smegmatis mabA and inhA genes were cloned</td>
<td>W. Jones, Centers for Disease Control, Atlanta, GA, USA</td>
</tr>
<tr>
<td>M. smegmatis mc6 ATCC607</td>
<td>Electroporation-efficient mutant of mc6, wild-type for drug-resistance</td>
<td>Snapper et al. (1990)</td>
</tr>
<tr>
<td>M. bovis BCG Pasteur</td>
<td>Wild-type</td>
<td>World Health Organization, Geneva</td>
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<tr>
<td>M. tuberculosis H37Rv TMC102</td>
<td>Wild-type, causes disease in guinea pigs</td>
<td>Trudeau Institute</td>
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<tr>
<td><strong>Plasmids</strong></td>
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<tr>
<td>pBluescript II KS (+)</td>
<td>E. coli–mycobacteria shuttle plasmid vector with blue/white selection, ~ 7 kb, derivative of pUC119</td>
<td>Donnelly-Wu et al. (1993)</td>
</tr>
<tr>
<td>pETd3</td>
<td>E. coli sequencing vector with blue/white selection</td>
<td>Stratagene</td>
</tr>
<tr>
<td>pETd3-mabA</td>
<td>E. coli expression vector with T7 promoter</td>
<td>Novagen</td>
</tr>
<tr>
<td>pYUB376</td>
<td>pETd3: 744 bp mabA PCR fragment from M. tuberculosis H37Rv</td>
<td>This work</td>
</tr>
<tr>
<td>pYUB630</td>
<td>pYUB376 derived, insert DNA 3' to inhA removed by digestion with XbaI and HindIII (Fig. 4)</td>
<td>This work</td>
</tr>
<tr>
<td>pYUB631</td>
<td>DpnI fragment carrying the CAT gene (Bartolome et al., 1991) inserted into the Sp61 site of mabA of pYUB630 (Fig. 4)</td>
<td>This work</td>
</tr>
<tr>
<td>pYUB632</td>
<td>Derivative of pYUB376 with a truncated inhA gene produced by digestion with BsaBI and HindIII (Fig. 4)</td>
<td>This work</td>
</tr>
<tr>
<td>pYUB633</td>
<td>As pYUB630 except that the XbaI– HindIII fragment is from M. bovis BCG</td>
<td>This work</td>
</tr>
</tbody>
</table>
recommendations. Purified DNA fragments were ligated into appropriate cloning vectors (Table 1) using T4 DNA ligase (New England Biolabs) to obtain the subclones. The CaCl2-facilitated transformations of DNA into E. coli DH5a were done following standard protocols (Sambrook et al., 1989). DNA was electroeluted into M. smegmatis following a previously published protocol (Snapper et al., 1990). Chromosomal DNA for PCR experiments and Southern blots was obtained from mycobacterial cells by chemical disruption of cell walls using the protocol of van Soolingen et al. (1994). DNA sequencing was performed on double-stranded DNA templates using the Automated Taq DyeDeoxy Sequencing kit ( Applied Biosystems) following the manufacturer’s protocols. A Perkin-Elmer model 480 Thermal Cycler was used for PCR reactions and Applied Biosystems PRISM 373 sequencing apparatus was used for DNA sequencing. All DNAs were sequenced on both strands. Nucleotide sequence was compiled and analysed using the Genetics Computer Group Sequence analysis software (Genetic Computer Group, 1995). mabA genes were translated using the translate program to obtain the amino acid sequences of the putative MabA proteins and the peptide sequences were aligned using the Megalign program of the Lasergene software package (DNastar).

Construction of the MabA-overproducing plasmid pET3d-mabA. The mabA gene product from M. tuberculosis was overproduced using the T7 polymerase system, as described previously (Studier et al., 1990). The mabA gene was subcloned into the pET3d vector by PCR using Taq DNA polymerase (Perkin Elmer). Restriction sites were introduced at both ends of the amplified sequence to facilitate cloning. PCR was performed on plasmid pYUB 376 template DNA. The N-terminal primer contained the first 24 bp of the mabA coding sequence and an NcoI restriction site overlapping the initiation codon. The C-terminal primer contained a HindIII restriction site, the termination codon and 20 bp of the mabA anti-codon sequence. The PCR reactions contained 6 ng template DNA, 1.5 μg each primer, 200 μM dNTPs, 10 μl 10× buffer for Taq polymerase, 5 units Taq polymerase, 7 mM MgCl2 and 10% DMSO (Perkin Elmer) in a total volume of 100 μl. The reactions were incubated at 94 °C for 1.5 min, 62 °C for 1:5 min and 72 °C for 1:5 min for 30 cycles. The products were separated on a 1% SEA KEM agarose (FMC) gel and a 744 bp PCR product was recovered using the GENE CLEAN II kit (BIO 101).

The PCR product was cut with NcoI and HindIII and ligated into the previously cut and gel-purified pET3d (Table 1) at room temperature overnight. The ligation mixture was used to transform supercompetent Epicurian Coli SCS1 cells and transformant colonies were selected on kanamycin plates. The mabA plasmid was grown in M-OADC-TW broth, and dilutions were plated on 7H10 agar plates and 7H10 agar plates containing various concentrations of INH or ETH. Here, MIC was defined as the concentration of the drug that produced >99% inhibition of bacterial growth when inocula of ~1000 organisms were plated. Similar sized inocula were used in every assay because MICs to INH and ETH vary considerably with large variations in inoculum.

RESULTS

Cloning and characterization of mabA genes from M. smegmatis, M. tuberculosis and M. bovis BCG

The report of cloning the inhA gene indicated the presence of an ORF, orf1/mabA, upstream of inhA in M. tuberculosis, M. bovis and M. smegmatis (Banerjee et al., 1994). Also, in that report, subcloning analysis demonstrated that, unlike in M. smegmatis, these two genes form an operon in M. tuberculosis and M. bovis. Cosmid clones were identified at that time which contained both mabA and inhA from these bacteria. In this study, parts of these cosmid inserts containing mabA were subcloned into pBluescript II KS+(+) (Table 1) and the sequence upstream of inhA was determined (Fig. 1). We compared these sequences with each other, as well as with the recently released mabA sequence (accession no. AF002133) from Mycobacterium avium. The DNA region between the end of mabA and the
beginning of inhA from M. avium is more similar to that of M. smegmatis than M. tuberculosis. This strongly indicates that, unlike the M. tuberculosis/M. bovis mabA-inhA operon, mabA and inhA are independently transcribed in M. avium as in M. smegmatis. This is consistent with the previous observation that the M. avium mabA-inhA plasmids confer a similar level of resistance to INH and ETH as M. smegmatis mabA-inhA plasmids, which is considerably higher than the resistance conferred by the plasmids from M. tuberculosis and M. bovis (Banerjee et al., 1994). These gene organization data also correlate well with similar MICs for INH in M. smegmatis and M. avium, which are much higher than the MICs of M. tuberculosis complex. Moreover, like the M. smegmatis ORF, the M. avium mabA ORF was found to be longer (765 bp, 255 aa) than that of M. tuberculosis and M. bovis (741 bp, 247 aa). In M. smegmatis, the reading frame probably starts with an ATG, although there is a possible GTG start site present two codons downstream. The hypothetical ATG start codon gives a size conservation (255 aa) between the MabAs of M. smegmatis and M. avium. There are significant differences between the DNA sequences in the region upstream of the mabA ORFs as well as in the beginning of the ORFs.

Conservation of mabA and MabA among different mycobacteria

As in inhA (Banerjee et al., 1994), we found that the DNA sequence of the mabA gene from M. tuberculosis is identical to that of M. bovis BCG (Fig. 1); we will therefore refer to these two genes as M. tuberculosis mabA. Despite the difference in gene organization and translational start site, the mabA gene appears to be highly conserved between different mycobacteria. At the DNA level, the identity was approximately 80% (Fig. 1), which is in agreement with the high degree of homology indicated by the Southern blot using a fragment containing mabA. In all the 11 mycobacteria tested thus far, including important pathogens like Mycobacterium leprae, M. avium and Mycobacterium intracellulare, bands hybridizing with the M. smegmatis mabA probe were observed (data not shown). Such conservation of mabA among various mycobacteria is consistent with our repeated failure to produce a knockout of this gene in M. smegmatis. However, the chromosomal gene could easily be knocked out when an extra episomal copy was present (data not shown). These results indicate that MabA is likely to be essential, like InhA.

At the amino acid level (Fig. 2) the homology was even greater (82–85% identity). Expectedly, M. tuberculosis MabA shows stronger homology to M. avium MabA than the M. smegmatis protein, since the former two organisms share a closer ancestry. Additionally, the similarity score, indicating amino acid residue conservation between the MabAs, is about 88–90%. Apart from the first few residues of the reading frames, homology is uniform throughout the protein sequence. All the MabA peptides contain an NADP-binding consensus sequence GXXXXXAXXXXAXG (Hanukoglu & Guthfink, 1989).
mabA gene from *Mycobacterium tuberculosis*

Fig. 2. Comparison of amino acid sequences of the mycobacterial MabA polypeptide with the KAR or fabG gene-product of *E. coli*, *H. influenzae* and *V. harveyi*. From top to bottom, the sequences presented are MabAs from *M. avium* (avMab), *M. tuberculosis* (tbMab) and *M. smegmatis* (smMab), and KARs from *E. coli* (ecFab), *V. harveyi* (vhFab) and *H. influenzae* (hiFab). The comparison was performed using the Jotun-Hein algorithm of the Megalign program of Lasergene software package from DNA-star. The boxes indicate identical amino acids. The putative NADPH-binding GXG-XXAXXXAXXXXXXG consensus sequence (Hanukoglu & Gutfinger, 1989) is indicated by a dashed overline.

Homology of MabA amino acid sequence with other KARs that are available in the database

A TFASTA (Genetics Computer Group, 1995) search was performed on the EMBL and GenBank databases, using both the amino acid sequences. The search revealed that the best homologies to MabA are KARs from *E. coli* (Rawlings & Cronan, 1992), *Vibrio harveyi* (Shen & Byers, 1996) and *Haemophilus influenzae* (Fleischmann et al., 1995). The sequence alignment of these three fatty acid biosynthesis enzymes with the three MabA sequences is shown in Fig. 2. The identity between the MabAs and the KARs is approximately 40% and the total similarity is about 60%. In addition, the KAR counterparts from plants, the β-ketoacyl-ACP reductases of *Cuphea lanceolata* (Klein et al., 1992), *Arabidopsis thaliana* and *Brassica napus* (Slabas et al., 1992), show strong homology (~35–40% identity) to the two MabA sequences.

Overexpression of MabA and biochemical determination of its activity

The 744 bp insert in the pET3d-ma6A construct was verified by sequencing and found to match the wild-type mabA sequence. To verify the function of the reading frame in the construct, pET3d-ma6A was transformed into BL21(DE3)pLysS, a lysogenic strain containing the gene for T7 RNA polymerase under the control of a lacUV5 promoter. Cell-free extracts made from cultures after 2.5 h with or without induction by 1 mM IPTG were examined by PAGE. The IPTG-induced cells harbouring pET3d-mabA showed moderate over-expression of a 28 kDa protein (data not shown) which matched the predicted molecular mass of 25665 Da. This protein band was not seen in extracts of uninduced cells or control cells without plasmids.

The soluble extracts from both induced and uninduced strains were assayed for KAR activity. The extracts were tested in the presence of either NADH or NADPH as cofactor in the presence or absence of IPTG induction. A_{490} is plotted as a function of reaction time for (1) induced culture with NADPH but without acetoacetyl-CoA, (2) uninduced cell extract+NADH, (3) uninduced cell extract+ NADPH, (4) induced cell extract+ NADH and (5) induced cell extract+ NADPH. The soluble extracts from both induced and uninduced strains were assayed for KAR activity. The extracts were tested in the presence of either NADH or NADPH. By a continuous scanning spectrophotometric assay, using both cofactors, the extracts from the induced cells were found to have considerable KAR activity (Fig. 3). However, the enzyme demonstrated a definite preference for NADPH, as seen by the sevenfold increase in activity in the presence of this substrate. This definite preference for NADPH agrees with the presence of an NADP-binding motif (Hanukoglu & Gutfinger, 1989) in MabA (Fig. 2). The extract from the uninduced cells showed no significant activity although there could be some back-
ground activity from the *E. coli* KAR. The host activity is probably not observed because the *E. coli* KAR, which preferentially uses acetoacetyl-ACP, has been reported to have very poor activity with acetoacetyl-CoA as substrate (Toomey & Wakil, 1966). In addition, induction of this activity by IPTG clearly distinguished the KAR of *M. tuberculosis* from any background contaminant from the *E. coli* host or other contaminating activities in the extract. The activity tests were then repeated with increasing concentrations of the induced extract and demonstrated a linear dependence (data not shown) of activity on the amount of the induced extract (i.e. MabA protein concentration) to further support the claim that the observed KAR activity is indeed from the induced MabA protein.

**Examination of the multiple copy effect of *M. tuberculosis* mabA on INH-ETH resistance**

New plasmids were constructed based on the mycobacteria-*E. coli* shuttle vector pMD31 (Donnelly-Wu *et al.*, 1993) to separate mabA from inhA (Fig. 4). The first of these new plasmids, pYUB630, is a derivative of a previously published plasmid, pYUB376 (Table 1). It contains the entire *mabA*-inhA locus but none of the extra DNA present 3' of plasmid pYUB376 and it confers the INH-ETH-resistance phenotype in the *M. smegmatis* mc²155 host by raising the MIC<sub>INH</sub> to 15 μg ml⁻¹ and MIC<sub>ETH</sub> to 30 μg ml⁻¹ from the wild-type values of 5 μg ml⁻¹ and 10 μg ml⁻¹ (Fig. 4). A chloramphenicol acetyltransferase (CAT) cassette (Bartolome *et al.*, 1991) was inserted into mabA in pYUB630. The CAT fragment is known to be nonpolar and does not carry any terminator of transcription. Hence, this insertion should not interfere with the expression of inhA. This construct with a defective mabA but a wild-type inhA was designated pYUB631. When introduced into mc²155, pYUB631 conferred the same resistance phenotype as pYUB630. pYUB632 contains a wild-type mabA and a truncated inhA. This plasmid failed to confer any drug resistance upon the host. The cloning vector pMD31 by itself did not confer any resistance to INH and ETH when introduced into mc²155. In addition, pYUB632 was tested in *M. bovis* BCG, where it also failed to show any enhanced resistance over the pMD31 vector control (MIC<sub>INH</sub> 0·2 μg ml⁻¹).

The results discussed above clearly show that interruption of *M. tuberculosis* mabA without affecting inhA does not interfere with the ability of the plasmid to confer INH-ETH resistance. However, a deletion of the inhA locus while keeping mabA intact causes a complete loss of the resistance phenotype. Therefore, unlike the overexpression of *M. tuberculosis* inhA, the overexpression of MabA from *M. tuberculosis* does not show the ability to titrate INH and ETH.

**DISCUSSION**

In *M. tuberculosis* and *M. bovis*, mabA and inhA are in one operon whereas in *M. smegmatis* (and probably in *M. avium*) these two genes are independently transcribed (Banerjee *et al.*, 1994). As discussed in the Introduction, MabA from *M. tuberculosis* was suggested as a possible target for INH, mainly due to frequent observations of mutations at the RBS of mabA in many clinical INH-resistant *M. tuberculosis* isolates (Musser, 1995). This hypothesis was further supported by the unique mabA-inhA operon arrangement in the INH-sensitive *M. tuberculosis* complex, the similarity in the degree of MabA conservation with InhA conservation among different mycobacteria and the probable essential nature of MabA activity. Additionally, this possibility was supported by a biochemical study on *M. avium* (Kikuchi *et al.*, 1989) which reported that INH inhibits a KAR in addition to an ER. This KAR, like
MabA, was demonstrated to prefer NADPH over NADH and was reported to have specific involvement in mycolic acid synthesis. Although a direct involvement of MabA in mycolate synthesis could not be tested due to our inability to procure or synthesize a long-chain substrate for the KAR assay, mabA probably synthesizes mycolic acid because it forms an operon with inhA, which has been shown to be involved in mycolate synthesis (Quemard et al., 1995; Wheeler & Anderson, 1996). Finally, in M. tuberculosis, there is room for the presence of other INH target(s) besides InhA because the MIC for the M. tuberculosis complex is considerably lower (25- to 100-fold) than that of M. smegmatis (Banerjee et al., 1994; Zhang et al., 1992) even though InhAs from these organisms are highly conserved. Hence, it became imperative to test the role of MabA in INH resistance.

It has recently been clearly demonstrated that INH reacts with NADH, the preferred cofactor for InhA, to form isonicotinic acyl-NADH inside the active site of InhA (Rozwarski et al., 1998). This reaction, catalysed by KatG and Mn²⁺, blocks the ER activity of the protein. ETH is proposed to bind InhA by a similar, but different, mechanism. These observations support the earlier hypothesis (Banerjee et al., 1994) that overexpression of InhA can titrate additional active INH (i.e. isonicotinic acyl-NADH) and thereby increase the MIC or the level of resistance. However, data discussed in this manuscript show that overexpression of MabA from M. tuberculosis does not confer the ability to titrate either INH or ETH. This rules out the possibility of MabA being a direct target in vivo for an active INH like isonicotinic acyl-NADH/NADPH. This conclusion is consistent with the fact that, until now, no mutations have been mapped to the MabA ORF in INH-resistant organisms. In contrast, mutations have been mapped to the mabA RBS with high frequency and also in the InhA ORF with lower, but significant, frequency (Banerjee et al., 1994; Ristow et al., 1995; Kapur et al., 1996, Rozwarski et al., 1998). Additionally, a report using single-copy integrating vectors demonstrated that the mutations in the mabA RBS actually led to overexpression of InhA (Mdluli et al., 1996). However, the authors concluded from their drug sensitivity assays that these mutations play no role in INH resistance. Nonetheless, on close scrutiny of their data, we see that these mutations conferred a clear increase in INH resistance, particularly at the level corresponding to the MIC of M. tuberculosis. Hence, we propose that mutations in the RBS of mabA mediate INH resistance in M. tuberculosis by overexpression of the InhA protein through transcriptional upregulation of the whole inhA operon and not by specific translational upregulation of MabA. This hypothesis is consistent with the fact that hundreds of INH-resistant strains from different parts of the world demonstrated mabA RBS mutations but none of many INH-sensitive strains screened have demonstrated these mutations (Musser et al., 1996).

In addition, the observation of INH-induced inhibition of both KAR and ER in the in vitro system of Kikuchi et al. (1989) is not necessarily contradictory to our hypothesis of MabA not being a direct and primary target of INH in vivo. These two systems are very different. In the in vitro system, KAR and ER were assayed for INH inhibition in semi-defined extracts. It is completely unknown whether those fractions contained some, or any, KatG and Mn²⁺. Moreover, the concentration of INH is much higher in the in vitro assay. All these important and many more subtle disagreements between the in vitro and the in vivo systems may make the mechanism of action of the in vitro system different from the in vivo model. Hence, given the complex nature of the INH mode of action, an undefined in vitro assay may not represent the actual in vivo environment.

Finally, cloning and characterization of MabA may also help in the future development of antimycobacterial therapeutics. Characterization of InhA activity and knowledge of its three-dimensional structure have generated a structure-function-based approach to design new antimycobacterial compounds (Rozwarski et al., 1998) and broad-spectrum antibiotics (Baldock et al., 1996). Similarly, the knowledge of the MabA activity together with the ability to overexpress this highly conserved protein presents the mabA-encoded KAR as a potential target for developing new drugs. Additionally, further biochemical and structural characterization of this KAR may also lead to greater insight into the as yet unknown but important biology of mycolic acid biosynthesis.

ACKNOWLEDGEMENTS

We thank Drs Emil C. Gotschlich and Vijay K. Pancholi for their critical review of this manuscript. This study was supported by a grant from the National Institute of Health (AI 27160).

REFERENCES


