Activation of the Pro-drug Ethionamide Is Regulated in Mycobacteria*

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The anti-tuberculosis drug ethionamide (ETH), which is a structural analog of isoniazid (INH), is known to strongly inhibit mycolic acid synthesis in Mycobacterium tuberculosis. Although several targets have been identified for INH, only speculative information is available concerning ETH. Mutations within the promoter and the coding region of enoyl-acyl carrier protein reductase (InhA) were found to confer resistance to both drugs, thus leading to the impression that INH and ETH may share a common mode of action. However, a notable distinction between the two drugs lies in the lack of cross-resistance in clinical isolates. This may be attributed in part to the fact that the pro-drug INH must be activated via KatG, and no activation step for ETH has yet been described. Here we report the identification of an activator for ETH. The ETH activator (Rv3854c), which we have termed EthA, was found to be homologous to various monooxygenases and induced ETH sensitivity when overexpressed in mycobacteria. Interestingly, the neighboring open reading frame (Rv3855), which was found homologous to transcriptional repressors of the tetR family, led to ETH resistance when overexpressed. In addition, chromosomal inactivation of this gene by transposition led to ETH hypersensitivity. These data strongly suggest that Rv3855, which we have termed EthR, regulates the production of EthA, which subsequently activates the pro-drug ETH. This study opens up new avenues of research relating to ETH activation in mycobacteria, possibly leading to an improved efficacy of ETH and to the generation of new anti-mycobacterial agents.

The treatment of tuberculosis involves extremely lengthy and specialized chemotherapy regimens (1). The molecular composition and structural features of the mycobacterial cell envelope are thought to confer low permeability and thereby a basal resistance to most hydrophilic drugs (2). As a consequence, the lack of potency and protracted duration of drug administration are a major cause of rampant mutational drug resistance of Mycobacterium tuberculosis (3). An essential step in developing novel therapies for the treatment of M. tuberculosis infections is to determine why multidrug-resistant strains of M. tuberculosis are resistant to many existing anti-mycobacterial agents. Possible mechanisms of resistance include: alteration of the target enzyme that has become resistant to antibiotics; increased expression of the gene encoding the target enzyme; mutations causing impermeability of the mycobacterial cell to the antibiotic; and/or alterations of an activation mechanism. Thus, the urgent need to develop new therapies for treating M. tuberculosis infections requires the definition of the failures of existing treatments and the discovery of new drug targets.

The bacterial cell wall has been an effective target for many drugs (4). Many anti-tuberculosis agents, including ethambutol, cycloserine, isoniazid (INH),1 ethionamide (ETH), thioamide, and the thiosemicarbazones are known to inhibit cell wall biosynthesis. INH (see Fig. 1), which is one of the most efficient and the most widely used anti-tuberculosis drugs, has been the subject of intensive research during the past decade (5–7). Both M. tuberculosis and Mycobacterium bovis BCG are extremely susceptible to INH (minimum inhibitory concentrations (MIC), 0.02–0.2 μg/ml), and earlier evidence suggested that INH specifically inhibits the synthesis of mycolic acids in M. tuberculosis and M. bovis BCG (8–11). ETH (see Fig. 1), a structural analog of INH, is a useful second line anti-tuberculosis drug. The two drugs have almost identical effects in that both strongly inhibit the synthesis of mycolic acids (12, 13). Banerjee and colleagues (14) demonstrated that a single mutation in the inhA gene (NADH-specific, 2-trans-enoyl-acyl carrier protein reductase) confers resistance to INH and ETH, leading to the impression that the mode of action of both drugs is identical. In addition, mutations within katG, encoding a catalase-peroxidase led to the majority of INH-resistant isolates (6), demonstrating that INH is a pro-drug and that an activated metabolite is responsible for its mode of action (15, 16). However, the notable distinction between the actions of ETH and INH resides in the lack of cross-resistance (4, 17). The majority of

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1 The abbreviations used are: INH, isoniazid; ETH, ethionamide; ETHΔ, ETH-resistant; ETH*, ETH-susceptible; FAME, fatty acid methyl ester; MAME, mycolic acid methyl ester; TLC, Thin layer chromatography; ORF, open reading frame; TBAH, tetrabutylammonium hydroxide; hyg, hygromycin resistance gene; PCR, polymerase chain reaction; kb, kilobase(s); bp, base pair(s); MIC, minimum inhibitory concentration; BCG, bacillus of Calmette-Guérin.
strains resistant to ETH are sensitive to INH, whereas some strains resistant to INH show slightly increased sensitivity to ETH (18). Thus, there are subtle discrepancies between biochemical and genetic information on the modes of sensitivity and resistance in the cases of INH and ETH.

Because INH requires activation via KatG, it is tempting to postulate an activation process for ETH. Inactivation of such an ETH-specific process could account for the lack of cross-resistance between the two drugs. Interestingly, ETH undergoes oxidation by rat liver microsomes to generate a highly reactive S-oxide, possibly a sulfinate (Fig. 1) that exhibits greater activity in vitro against \textit{M. tuberculosis} than ETH itself (19–21). These observations, in addition to the fact that genetic alterations in \textit{katG} do not confer resistance to ETH, have led us to the hypothesis that ETH needs to be activated through a KatG-independent mechanism.

In this report, we describe the cloning and characterization of the gene \textit{Rv3855}, which we now term \textit{ethR}, that confers resistance to ETH, but not to INH when it is overexpressed in either \textit{Mycobacterium smegmatis}, \textit{M. bovis} BCG, or \textit{M. tuberculosis} on a multicopy vector. Furthermore, a transposon mutant of \textit{ethR} leads to ETH hypersensitivity in \textit{M. bovis} BCG. In addition, genetic and biochemical evidence suggests that \textit{ethR} encodes a transcriptional regulator that is not directly implicated in mycobacterial acid biosynthesis but plays an important role in the regulation of a second open reading frame (ORF), which is responsible for the activation of ETH. Analysis of the locus surrounding \textit{ethR} revealed the presence of an adjacent gene now termed \textit{ethA}, which encodes a putative monoxygenase, the predicted activator of ETH. Overexpression of \textit{ethA} led to hypersensitivity to ETH in mycobacteria. Thus, the data presented are compatible with the notion that EthR represses \textit{ethA}, which encodes the equivalent protein of KatG implicated in the activation of ETH.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Growth Conditions—**\textit{M. smegmatis} mc\textsuperscript{2}155, \textit{M. bovis} BCG, and \textit{M. tuberculosis} H\textsubscript{37}Ra and their transformants were grown on 7H11 agar supplemented with oleic-albumin-dextrose-catalase enrichment (Difco, Detroit, MI) or on Sauton medium (22) supplemented with 0.001‰ ZnSO\textsubscript{4} and 0.25‰ Triton WR1339 (Sigma). \textit{M. smegmatis} mc\textsuperscript{2}155 is an electro-transportation-efficient mutant of mc\textsuperscript{2}6 (23). \textit{M. smegmatis}, \textit{M. bovis} BCG, and \textit{M. tuberculosis} were transformed as described previously (24). Large scale cultures of mycobacteria were grown to mid-log phase (M. \textit{bovis} BCG, 10–14 days; \textit{M. smegmatis} mc\textsuperscript{2}155, 36 h; \textit{M. tuberculosis} H\textsubscript{37}Ra, 12–16 days), harvested, washed with phosphate-buffered saline and stored at −20 °C until further use. \textit{Escherichia coli} strains XL1-Blue (Stratagene, La Jolla, CA), NK5587, and their transformants were grown in Luria-Bertoni (LB) broth (Life Technologies, Inc.) and agar plates (25).

**Identification of pETH80 and Transposon y8 Mutagenesis of pETH80 in \textit{E. coli}—**A genomic library of \textit{M. tuberculosis} H\textsubscript{37}Rv, constructed by cloning 35- to 40-kb Sau3AI fragments of chromosomal DNA into the shuttle cosmid pYUB18 (27), was the source of cosmid pETH80 conferring ETH resistance to \textit{M. smegmatis}. The minimal region of pETH80 involved in ETH resistance was identified by y8 mutagenesis in \textit{E. coli} as described by Geyer (28). Briefly, a suitable \textit{E. coli} donor strain NK5587 (F\textsuperscript{−} lacY \textsuperscript{−}:Tn9) was transformed with pETH80. One isolated transformant was subsequently conjugated with \textit{E. coli} SH305 (recA1 srl::Tn10) with selection for kanamycin-resistant/tetracycline-resistant exconjugants. Plasmid DNA was extracted from 50 double resistant exconjugants, retransformed into \textit{M. smegmatis}, and scored for their ETH\textsuperscript{−} phenotype. Tn1000 (y8) insertions were localized by restriction mapping and sequencing using primers near the end of y8 (primer y8: 5′-CAACGAATTATCTCCTTT-3′; primer y62: 5′-TCAATAAGTTTATACCATC-3′).

**Cloning and Expression of Rs355 and Rv3854c-Rv3855 was cloned into the mycobacterial overexpression vector pMV261 as follows.** PCR amplification was performed using the upstream primer 142: 5′-CCACCTCCGGCGGCATGCG-3′ and the downstream primer 143: 5′-TTGGCACTGGAATCTCCAGCACCC-3′, which contain an EcoRI restriction site (underlined). The 690-bp PCR product was digested with EcoRI and cloned into \textit{Mycobacterium ethionamide-resistant} pMV261, giving rise to pMV261-ethR, where ETHR is fused in-frame with the ATG initiation codon of hsp60. A similar strategy was used to construct the pMV261-based integration vector for Rs3854c. Rs3854c was amplified by PCR using the upstream primer 150: 5′-ACGACCTCAGCTGGTTGATC-3′ and the downstream primer 149: 5′-ACGGATCCCCGGAAGACGACCCA-3′, which contains a BamHI restriction site (underlined). The 1624-bp fragment was digested with BamHI and cloned into \textit{Mycobacterium ethionamide-resistant} pMV261, generating pMV261-ethA. The coding sequences of all amplified genes were verified by DNA sequencing after their cloning in pMV261.

**Determination of the in Vivo Effects of ETH and INH on Fatty Acid and Mycolic Acid Synthesis in Mycobacteria—**\textit{M. smegmatis} mc\textsuperscript{2}155, \textit{M. bovis} BCG, and \textit{M. tuberculosis} were grown to mid-log phase (\textit{A}\textsubscript{\textit{\text{endo}}−} \textsuperscript{−} 0.3). ETH or INH were added at various concentrations followed by further incubation for 4 h (for \textit{M. smegmatis}) or 24 h (for \textit{M. bovis} BCG and \textit{M. tuberculosis}). At this point, \textit{L.\textsuperscript{−}Acetate (1 µC/mmol) (Amer- sham Pharmacul Biotech) was added and the cultures were further incubated with gentle agitation at 37 °C for 4 h (for \textit{M. smegmatis}) or 24 h (for \textit{M. bovis} BCG and \textit{M. tuberculosis}). The resulting \textsuperscript{14}C-labeled cells from ETH- or INH-treated cultures were harvested, washed twice with phosphate-buffered saline, resuspended into 3 ml of 15% tetrabutylammonium hydroxide (TBAH), and saponified at 100 °C for 15 h. After centrifugation, the dihydroethionamide (4 ml), water (2 ml), and isododecanatrime (300 µl) were added, and the entire reaction mixture was agitated for 30 min. After centrifugation, the upper, aqueous phase was discarded and the lower, organic phase was washed twice with water, dried in a sand bath, and extracted twice with diethyl ether (3 ml) and the ethereal extracts were dried and resuspended into 500 µl of diethylmethane for radioactivity counting. Equal volumes of this extract, which is composed of fatty acid methyl esters (FAMEs) and mycolic acid methyl esters (MAMEs), were separated by thin-layer chromatography (TLC) on silica gel (Merck 5735 Silica Gel 60 F254, Darmstadt, Germany) and developed once in petroleum ether-acetone (95:5, v/v). Subsequent autoradiography revealed \textsuperscript{14}C-labeled fatty acid and mycolic acid methyl esters after overnight exposure of the TLC plates to Kodak BioMax MR film.

**Characterization of the M. bovis BCG Transposon Insertions—**Genomic DNA (200–500 ng) of the mutated genes was digested with \textit{MboI} (New England Biolabs). After heat inactivation of the restriction enzyme at 65 °C for 20 min, the digested DNA (40–100 ng) was ligated with T4 DNA ligase (New England Biolabs) at room temperature. PCR reactions were carried out in a total volume of 25 µl containing AmpliTag PCR buffer (Perkin-Elmer), 10% (v/v) dimethyl sulfoxide, 0.25 µM dNTPs, 0.4 µM 84L-F (5′-GTCATCCGGCAGTCTCCAGAG-3′), and 0.4 µM 84L-R (5′-AACTGGCCAAGTGGTCCTGCTGC-3′) primers, 4–10 ng of template DNA, and 0.5 unit of AmpliTag Gold (Perkin-Elmer). Thermal cycling was performed in a Perkin-Elmer 9600 machine with an initial denaturation at 94 °C for 10 min, followed by 40 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 90 s, and a final extension of 72 °C for 7 min.

**RESULTS**

**Identification of Cosmids Conferring ETH Resistance in \textit{M. smegmatis}—**The chromosomal region involved in ETH resist-
Ethionamide Resistance in M. tuberculosis

Identification by Transposon \( \gamma \delta \) (Tn1000) Mutagenesis of the Minimal Region of \( p\text{ETH80} \) Responsible for ETH Resistance—

The minimal region required for ETH resistance in \( M. \text{sme}-\)gnatiss was determined by transposon mutagenesis of \( p\text{ETH80} \). The method, adapted from Guyer et al. (28) is based on the transposition in \( E. \text{coli} \) of \( \gamma \delta \) during F-mediated conjugal mobilization. Briefly, the mutagenesis technique is based upon the observation that conjugal transmission of \( p\text{BR322} \) with the conjugal plasmid \( F \) is dependent on, or at least completely correlated with, the transposition of \( \gamma \delta \) from \( F \) to \( p\text{BR322} \) (28). The transposition of \( \gamma \delta \) has also proved efficient on plasmids other than \( p\text{BR322} \) (30). The cosmids \( p\text{ETH80} \) identified in this study is an \( E. \text{coli} \)–mycobacterial shuttle vector based on CoE1 origin of replication. Thus, the mapping process of the minimal region of \( p\text{ETH80} \) responsible for \( \text{ETHR} \) in mycobacteria was achieved in two steps. First, mutagenesis of \( p\text{ETH80} \) in \( E. \text{coli} \) before the re-introduction of the derivatized DNA into mycobacteria allowed the analysis of insertion mutations. Of the 50 clones analyzed, 38 \( \gamma \delta \) insertions were located in the inserted portion of \( p\text{ETH80} \). Three \( \gamma \delta \) insertions were able to abolish \( \text{ETHR} \) in \( M. \text{sme}-\)ognatiss. Restriction analysis of these clones revealed that these \( \gamma \delta \) insertions were located within 0.6 kb of DNA. Sequencing of the regions located left and right of \( \gamma \delta \) (with primers \( \gamma \delta \) and \( \gamma \delta \)) revealed that all three insertions disrupted the same ORF annotated \( \text{Rv}3855 \) in the \( M. \text{tuberculosis} \) \( \text{H37Rv} \) genome data base, which was thus termed \( \text{ethR} \).

Cloning and Overexpression of \( \text{ethR} \) (Rv3855) in Mycobacteria—To avoid possible interference of other ORFs of \( p\text{ETH80} \) in association with \( \text{ETHR} \), the coding region of \( \text{ethR} \) was amplified by PCR and cloned in-frame with \( \text{hsp60} \) into \( p\text{MV261} \). The resultant plasmid \( p\text{MV261-ethR} \) was transformed into \( M. \text{sme}-\)gnatiss, \( M. \text{bovis} \) BCG, and \( M. \text{tuberculosis} \), and the MICs of the transformed bacteria were compared with those of untransformed strains and with those of strains containing the original \( p\text{ETH80} \). The MICs were determined by plating serial dilutions onto medium containing kanamycin with plus 0–200 \( \mu\text{g/ml} \) ETH in increments of 10 \( \mu\text{g/ml} \) or 0–10 \( \mu\text{g/ml} \) INH in increments of 1 \( \mu\text{g/ml} \). The MIC was defined as the lowest concentration of ETH that inhibited the growth of 99% of the bacteria. Table I summarizes the results obtained, suggesting a direct correlation between the level of expression of \( \text{ethR} \) and ETH resistance.

| Strain          | MIC (\( \mu\text{g/ml} \)) of ETH |
|-----------------|-----------------------------------|
| \( M. \text{sme}-\)gnatiss | 15, 80, 250                      |
| \( M. \text{bovis} \) BCG | 2, 25, 35                         |
| \( M. \text{tuberculosis} \) | 1, 20, 30                         |

\( ^* \) Strain \( \text{mc}^{155} \). 2 \( ^* \) NT, not tested.

Selective Effects of ETH on Inhibition of Mycolic Acid Syn-
thesis—Untransformed and \( p\text{MV261-ethR} \)-transformed \( M. \text{bovis} \) BCG were grown in the presence or absence of ETH at various concentrations, after which cultures were labeled with 1,2-[\( ^{14}\text{C} \)]acetate. Combined MAMEs and FAMEs were extracted, resolved, and fractionated on TLC plates. Untransformed \( M. \text{bovis} \) BCG exhibited a clear decrease in the incorporation of radioactivity into MAMEs in the presence of ETH (from 0 to 100 \( \mu\text{g/ml} \)) (Fig. 2). Examination of the individual classes of mycolates revealed that the production of all species was specifically inhibited. In addition, the production of a yet to be described and identified product, possibly a mycolate-specific fatty acid precursor, was progressively inhibited after treatment with ETH (with and without INH) (Fig. 3). The characterization of this product may be crucial for the identification of the specific target for ETH, but has been hampered by its relatively low abundance in the mycobacterial cell wall. In contrast, treatment of \( M. \text{bovis} \) BCG (\( p\text{MV261-ethR} \)) with ETH (0–100 \( \mu\text{g/ml} \)) had no dramatic effect on the synthesis of MAMEs and FAMEs (Fig. 2). Possible mechanisms related to ETH resistance may include detoxification of the drug or repression of an activation process of ETH leading to its active metabolite, conceivably an S-oxide type derivative (19).

Construction of an \( M. \text{bovis} \) BCG \( \text{ethR} \) Knock Out Mutant—

Mutagenesis of \( M. \text{bovis} \) BCG NCTC 5692 was performed as described previously using the mycobacteriophage mini-transposon delivery system \( p\text{JSC84} \) (31).2 Individual mutants were isolated, and the transposon insertions were characterized by inverse PCR. A clone with a transposon insertion between nucleotides 4,327,971 and 4,327,972 in the \( M. \text{bovis} \) BCG genome was identified (Fig. 5). The insertion disrupted \( \text{ethR} \) between nucleotide positions 426 and 427, leading to the production of a truncated polypeptide of 142 amino acids (instead of 648 for the normal protein). The \( M. \text{bovis} \) BCG \( \text{ethR::hyg} \) strain was found to be extremely sensitive to ETH, with an MIC < 0.6 \( \mu\text{g/ml} \) (see Table I). The MIC for INH remained identical for \( M. \text{bovis} \) BCG and \( M. \text{bovis} \) BCG \( \text{ethR::hyg} \), confirming that \( \text{ethR} \) was specific for ETH resistance.

Inhibition of Mycolic Acid Synthesis in \( M. \text{bovis} \) BCG

(\( p\text{MV261-ethR} \))—The isolated \( M. \text{bovis} \) BCG \( \text{ethR::hyg} \) clone was grown in the presence or absence of ETH and examined for the relative inhibition of mycolic acid synthesis. Concentrations as low as 2.5 \( \mu\text{g/ml} \) ETH completely abolished mycolic

2 J. Cox, unpublished results.
acid synthesis in comparison to wild-type M. smegmatis (Fig. 7). These results strongly suggest that Rv3854c, which we have now termed ethA, plays a role equivalent to katG for INH, but in relation to ETH.

**DISCUSSION**

INH and ETH are specific anti-tuberculosis drugs, which inhibit mycolic acid synthesis through InhA as suggested by resistance to both ETH and INH of an inhA mutant (14). Moreover, by the use of microarray hybridization, Wilson and coworkers (33) recently demonstrated that ETH treatment of M. tuberculosis induced the same genes that were induced by INH. However, Fattorini and coworkers (17) recently reported that of 46 INH-resistant strains of M. tuberculosis isolated from Italian patients only two were also ETH-resistant. Before INH exerts its lethal effect it must be converted to an active form, possibly an isonicotinic acyl anion (34) or an isonicotinic acyl radical (35) produced by the catalase-peroxidase KatG. ETH and other thioamides are sulfur-containing compounds, which are known to be substrates for oxidative catalysts, such as flavin-containing monoxygenases and cytochrome P-450 monoxygenases. An NADPH-dependent oxidation of ETH has previously been demonstrated in rat microsomes (20). More recently, Johnson et al. (34) suggested that in vitro oxidation of ETH produces electrophilic intermediates (S-oxides) capable of undergoing addition reactions to nucleophilic protein side chains (35). During the 1950s ETH and, subsequently, prothioamide were introduced in the treatment of tuberculosis and were deemed clinically as effective as dapsone in the treatment of leprosy (36). However, 25% of the patients suffered from various gastrointestinal symptoms and sometimes jaundice, especially when ETH was combined with rifampicin (37). The hepatotoxicity induced by administration of ETH and thionicotinamide was decreased by preadministration of methimazole. Preadministration of methimazole was also shown to decrease the levels of excretion of thionicotinamide S-oxide, indicating that thioamide S-oxidation, mediated by the flavin-containing monoxygenases, may be linked to the initiation of hepatotoxicity induced by these thioamides (38).

Here we describe evidence that ETH is activated by the monoxygenase homolog Rv3854c (EthA). EthA would then be the equivalent of KatG for INH. Thus, in a similar fashion by which mutations in katG abolish INH sensitivity without leading to ETH resistance, we would expect that mutations in ethA lead to ETH resistance without affecting INH sensitivity. Studies are currently in progress examining clinical isolates for point mutations within ethA. Overexpression of ethA led to a dramatic increase in ETH sensitivity of M. smegmatis and clearly indicated that only a small proportion of ETH is activated when mycobacterial cells are grown under laboratory conditions. Alternatively, when administered to humans, ETH may be activated by either eukaryotic oxidative processes as mentioned earlier or by EthA (or perhaps a combination of both). Thus, determining the respective contribution of the bacterial and the eukaryotic activation of thioamides to their ultimate necrogenic forms would be crucial to understand the impact of EthA on the efficacy of ETH in vivo and to help designing new improved versions of ETH.

ETH resistance may also be mediated by the overproduction of the putative repressor EthR. As a regulator, it is logical to assume that the production of EthR is also regulated, perhaps by signals external to the bacteria. Thus, any agent able to block EthR, or any physiological condition down-regulating ethR may favor the production of EthA and lead to the activation of substantial amounts of ETH, thereby increasing the sensitivity of the bacilli to ETH. Overexpression of ethA dramatically decreased the MIC of M. smegmatis for ETH to a level...
comparable to the MIC of M. tuberculosis (see Table I). This suggests that in part the innate relative resistance to ETH of M. smegmatis is associated with a low production of EthA and thus, indirectly, with EthR. Perhaps this mechanism may also account for the various susceptibility profiles to ETH displayed by other mycobacterial species.

Interestingly, although synthesis of α and α'-mycolates is insensitive to ETH in M. smegmatis overexpressing ethR, synthesis of epoxymycolates remained highly sensitive to the drug in this strain (data not shown). Thus, possible additional targets to InhA exist in relation to ETH and epoxymycolate synthesis, which has been previously shown to be highly sensitive to ETH (13).

It is interesting to note that EthA and EthR orthologs are also present in M. avium and M. leprae. The EthAR loci of M. avium and M. tuberculosis share a very similar architecture. In both cases, the two genes are oriented “head to head” and are separated by a putative 76-bp divergent promoter. In contrast, the two M. leprae orthologs are not located in the same locus. The initiation codons of the M. leprae EthA and EthR are found in the genome in positions 86,698 and 1,235,214, respectively. The genome of M. leprae seems to be reduced to a minimal pool of genes essential to the survival of this strictly intracellular pathogen. In contrast to many genes of M. leprae, EthA and EthR are apparently not truncated and are highly similar to their orthologs in M. tuberculosis and M. smegmatis. Altogether, these observations suggest that these genes have an important role in vivo. In addition, the M. tuberculosis genome possibly encodes more than 30 monooxygenases, and the relative role of each enzyme in the oxidation of ETH remains to be assessed in vivo.

The understanding of the mode of action of ETH is valuable for the design of improved versions of the drug, for the elaboration of potentiating agents, and for the understanding of drug resistance. Finally, an intriguing question, which awaits further experimentation, concerns the possibility of an equivalent mechanism of regulation of katG and INH resistance.
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