Supporting Information

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SI Text

Bacterial Strains, Media, and DNA Manipulations. The media were supplemented with hygromycin (HYG) (75 μg/mL), KAN (5 μg/mL or 25 μg/mL), or sucrose (5%) when needed for cultivation of *M. tuberculosis* strains. For *Escherichia coli* strains, Luria media was supplemented with HYG (200 μg/mL), KAN (50 μg/mL), or carbenicillin (100 μg/mL) when needed. Antibiotics were purchased from Sigma. Standard protocols or the manufacturer's instructions were used for all DNA manipulations (New England Biolabs/Invitrogen/Finnzymes). All oligonucleotide primers (Table S3) were synthesized at the Biotechnology Core facility, National Center for Preparedness, Detection, and Control of Infectious Diseases, Centers for Disease Control and Prevention (CDC). Clinical isolates were obtained from the culture collection at the Mycobacteriology Laboratory Branch, CDC. Human subject information linked to the clinical isolates used in this study is protected by the protocol approved by the CDC institutional review board.

Construction and Screening of the K204 Cosmid Library. A cosmid library of Sau3AI partial digest products of *M. tuberculosis* K204 in pYUB178-hyg [a derivative of pYUB178 (1) in which the KAN^R cassette has been replaced with a HYG^R cassette] was constructed and electroporated into *M. tuberculosis* H37Rv bacteria using standard methods. Transformants were selected on 7H10 agar plates containing either HYG alone or HYG and KAN. Genomic DNA was extracted from individual KAN^R transformants and analyzed by RATE (2) using primers specific to the pYUB178-hyg plasmid.

Construction of Recombinant Cosmids pAZ10 (pYUB854::*eis*::*Rv2415c*) and pAZ19 (pYUB854::*eisC-14T*::*Rv2415c*) for Allelic Exchange. The *Rv2415c* gene was amplified from the H37Rv genome using primers AZ151 and AZ152 and cloned into the XhoI/SpeI sites of pYUB854 after digestion with XhoI and SpeI, forming pAZ09 (pYUB854::*Rv2415c*). The *eis* gene and 1 kb of upstream sequence were amplified from the H37Rv genome or the K204 genome using primers AZ149 and AZ150. The fragments were digested with StuI and XbaI and cloned into pAZ09 (pYUB854::*Rv2415c*) to generate pAZ10 (pYUB854::*eis*::*Rv2415c*) or pAZ19 (pYUB854::*eisC-14T*::*Rv2415c*). These cosmids harbor the *eis* and downstream gene, *Rv2415c* separated by a HYG^R cassette.

Construction of Specialized Transducing Phages phAlexWT and phAlexC-14T. The specialized transducing phages phAlexWT or phAlexC-14T were generated from pAZ10 (pYUB854::*eis*::*Rv2415c*) or pAZ19 (pYUB854::*eisC-14T*::*Rv2415c*) and phAE159 as previously described (1). *M. tuberculosis* H37Rv bacteria or K204 bacteria were infected with phAlexC-14T or phAlexWT at a multiplicity of infection of 10:1 and selected on 7H10 containing HYG. Genomic DNA from individual transductants was sequenced and analyzed by mismatch amplification mutation assay–PCR using primers optimized for either the *eis*C-14T (AZ199 and AZ87) or WT (AZ198 and AZ87) allele using Promega Master Mix Taq.

Construction of Mutants and Complemented Strains. An unmarked deletion of *eis* (bases −32 to 1204 were removed) was constructed using pYUB854, phAE159, and pYUB870 as previously described (1). The final unmarked deletion of *eis* was confirmed by PCR. To complement the knockout strain, the *eis* ORF and 520 bp upstream of *eis* were amplified by PCR from genomic DNA of representative clinical isolates using primers AZ107 and AZ108, cloned into pHIN-STOP, and electroporated into H37RvΔ*eis*. Complemented strains were confirmed by PCR using internal *eis* primers AZ80 and AZ87 and sequencing. Promoter truncation derivatives of the *eis* promoter were amplified by PCR from K204 genomic DNA using primers AZ167 to AZ174 and AZ108, cloned into pHIN-STOP, and electroporated into H37RvΔ*eis*. Integration of the truncated promoter constructs was confirmed by PCR using primers HYG9F and AZ87 and sequencing.

MIC Determination. The susceptibilities to KAN and AMK were determined according to guidelines and definitions stated by the CLSI (3), using 7H10 agar containing KAN (Sigma) at concentrations of 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 50, and 80 μg/mL or AMK (Sigma) at concentrations of 0.25, 0.5, 1, 2, 3, and 4 μg/mL. The MIC was defined as the lowest concentration of drug resulting in complete inhibition of growth or in growth of ≤1% of the initial inoculum after 4 weeks of incubation at 37 °C.

1. Bardarov S, et al. (1997) Conditionally replicating mycobacteriophages: A system for transposon delivery to *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 94:10961–10966.
2. Kim J, Carver EA, Stubbs L (1997) Amplification and sequencing of end fragments from bacterial artificial chromosome clones by single-primer polymerase chain reaction. *Anal Biochem* 253:272–275.
3. National Committee for Clinical Laboratory Standards (2003) Susceptibility testing of mycobacteria, norcardiae, and other aerobic actinomycetes; approved standard M24-A. (National Committee for Clinical Laboratory Standards, Wayne, PA).

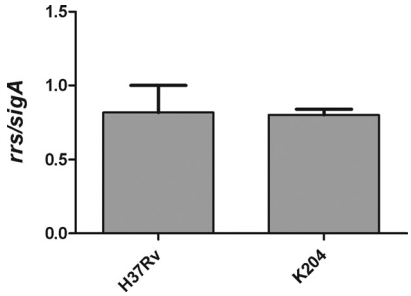

Fig. S1. *sigA* transcript levels in H37Rv and K204. The ratio of *sigA*/*rrs*(16S rRNA) transcripts was determined by qRT-PCR and normalized to H37Rv. Error bars represent the standard deviation of 3 independent experiments.

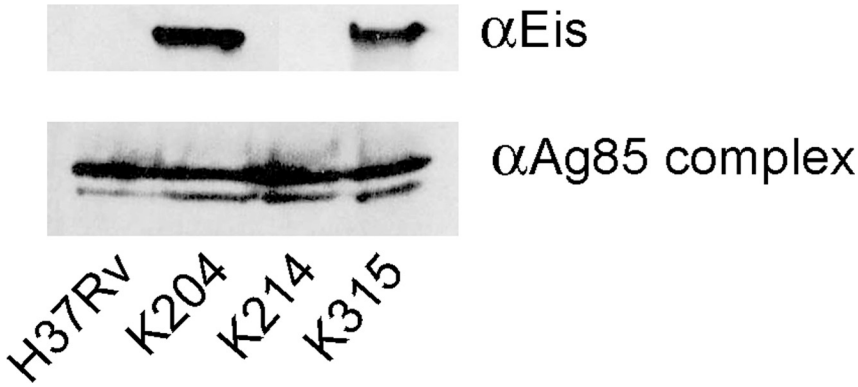

Fig. S2. Immunoblot analysis of cell filtrate proteins generated from strains harboring either WT (H37Rv, K214) or the C-14T *eis* allele (K204, K315) shows that Eis is present in the culture supernatants of C-14T promoter mutants. The lysates were probed with either anti-Eis or anti-Ag85 complex serum (Colorado State University).

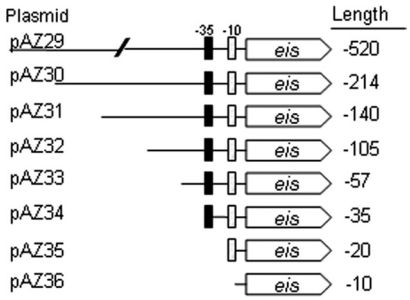

Fig. S3. Promoter truncation constructs in the pHIN-STOP plasmid backbone (Table S2). A plasmid containing each construct was used to complement the H37RvΔ*eis* strain. Eis expression and activity for each complement was analyzed by qRT-PCR and acetyltransferase activity analysis (data in Fig. 2). Length indicates the amount of sequence upstream from the *eis* start codon present on the complementing plasmid. Black and gray boxes represent the −35 and −10 promoter regions, respectively.

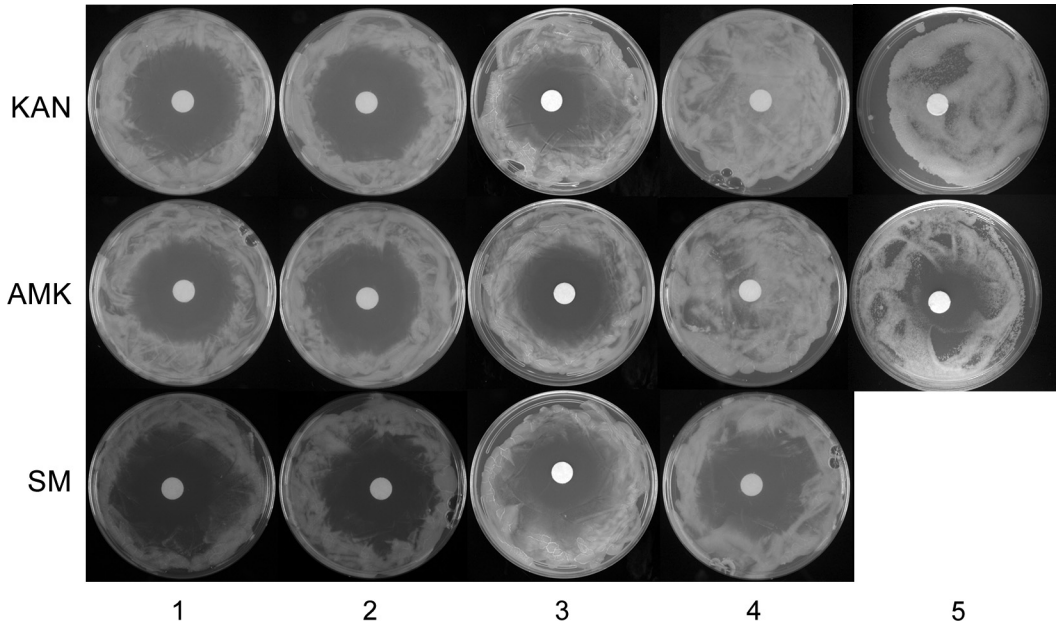

Fig. S4. Disk-diffusion assays for KAN, AMK, and SM on *M. smegmatis.* Twenty-microliter volumes of acetyltransferase assays were spotted onto Whatman filter disks on a lawn of *M. smegmatis* cells and incubated at 37 °C for 3 days; column 1: unmodified antibiotic; column 2: no acetyl-CoA control; column 3: no Eis control; column 4: 20 μL of complete acetyltransferase reaction; column 5: 40-μL volume of complete acetyltransferase reaction (not performed with SM). Similar results were obtained with *E. coli*.

Table S1. Strains used in this study

Strain	Genotype/relevant characteristics	Reference or source
E. coli		
HB101	F-Δ(gpt-proA)62, leuB6, glnV44, ara-14, galK2, lacY1, Δ(mcrC-mrr), rpsL20(Str^r), xyl-5, mtl-1, recA13, thi-1	NEB
BL21 Gold	F– ompT hsdS(rB– mB–) dcm + Tet^r gal (DE3) endA Hte	Novagen
M. smegmatis		
LR222	Easily transformable strain, used for phage preparation	Miller et al. (1)
M. tuberculosis		
H37Rv	Pansusceptible laboratory strain	
K204	Spontaneous Kan^r mutant of H37Rv	Maus et al. (2)
K315, K317	H37Rv with *eis*C-14T allele, Hyg^r introduced by allelic exchange	This study
K214, K215	K204 with *eis* WT allele, Hyg^r introduced by allelic exchange	This study
RvΔ*eis*	Unmarked *eis* deletion strain of H37Rv	This study
RvΔ*eis*+WT	RvΔ*eis* with an integrated copy of pAZ38 *eisWT*::*attB*	This study
RvΔ*eis*+C-14T	RvΔ*eis* with an integrated copy of pAZ29 *eisC-14T*::*attB*	This study
RvΔ*eis*+G-10A	RvΔ*eis* with an integrated copy of pAZ39 *eisG-10A*::*attB*	This study
RvΔeis + G-37T	RvΔ*eis* with an integrated copy of pAZ40 *eisG-37T*:*attB*	This study
RvΔ*eis*+C-12T	RvΔ*eis* with an integrated copy of pAZ41 *eisC-12T*::*attB*	This study
RvΔ*eis*+A-13G	RvΔ*eis* with an integrated copy of pAZ42 *eisA-13G*::*attB*	This study
RvΔ*eis*+pHIN-STOP	RvΔ*eis* with an integrated copy of pHIN-STOP ::*attB*	This study
RvΔ*eis* 520	RvΔ*eis* with an integrated copy of pAZ29 *eisC-14T*::*attB*	This study
RvΔ*eis* 214	RvΔ*eis* with an integrated copy of pAZ30 *eisC-14T*::*attB*	This study
RvΔ*eis* 140	RvΔ*eis* with an integrated copy of pAZ31*eisC-14T*::*attB*	This study
RvΔ*eis* 105	RvΔ*eis* with an integrated copy of pAZ32*eisC-14T*::*attB*	This study
RvΔ*eis* 57	RvΔ*eis* with an integrated copy of pAZ33*eisC-14T*::*attB*	This study
RvΔ*eis* 35	RvΔ*eis* with an integrated copy of pAZ34*eisC-14T*::*attB*	This study
RvΔ*eis* 20	RvΔ*eis* with an integrated copy of pAZ35*eisC-14T*::*attB*	This study
RvΔ*eis* 10	RvΔ*eis* with an integrated copy of pAZ36*eisC-14T*::*attB*	This study

Hyg^r, hygromycin resistant; Kan^r, kanamycin resistant.

1. Miller LP, Crawford JT, Shinnick TM (1994) The *rpoB* gene of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 38:805–811.
2. Maus CE, Plikaytis BB, Shinnick TM (2005) Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 49:3192–3197.

Table S2. Plasmids and phage used in this study

Plasmid/phage	Relevant characteristics	Reference or source
Plasmid		
pET19b	pET vector containing N-terminal His tag, Amp^r	Novagen
pET19b-eis	*eis* open reading frame cloned into pET19b, Amp^r	This study
pYUB178-hyg	Cosmid vector, Hyg^r	This study
pYUB854	Cosmid vector, with res sites flanking the Hyg^r gene	Bardarov et al. (1)
pEIS-KO	pYUB854 + 900 bp flanking sequence of *eis*	This study
pYUB870	Helper plasmid, *tnpR, sacB*, Kan^r	Bardarov et al. (1)
pAZ09	*Rv2415c* cloned into pYUB854 for allelic exchange, Hyg^r	This study
pAZ10	pAZ09 + *eis*WT and 1.2 kb upstream sequence	This study
pAZ19	pAZ09 + *eis*C-14T 1.2 kb upstream sequence	This study
pHIN-STOP	Integrating mycobacterial vector, Hyg^r, transcription stop sequence, used for complementation experiments	This study
pAZ29	pHIN-STOP + *eis*C-14T and 520 bp upstream sequence	This study
pAZ30	pHIN-STOP + *eis*C-14T and 214 bp upstream sequence	This study
pAZ31	pHIN-STOP + *eis*C-14T and 140 bp upstream sequence	This study
pAZ32	pHIN-STOP + *eis*C-14T and 105 bp upstream sequence	This study
pAZ33	pHIN-STOP + *eis*C-14T and 57 bp upstream sequence	This study
pAZ34	pHIN-STOP + *eis*C-14T and 35 bp upstream sequence	This study
pAZ35	pHIN-STOP + *eis*C-14T and 20 bp upstream sequence	This study
pAZ36	pHIN-STOP + *eis*C-14T and 10 bp upstream sequence	This study
pAZ38	pHIN-STOP + *eis*WT and 520 bp upstream sequence	This study
pAZ39	pHIN-STOP + *eis*G-10A and 520 bp upstream sequence	This study
pAZ40	pHIN-STOP + *eis*G-37T and 520 bp upstream sequence	This study
pAZ41	pHIN-STOP + *eis*C-12T and 520 bp upstream sequence	This study
pAZ42	pHIN-STOP + *eis*A-13G and 520 bp upstream sequence	This study
Phage		
phAE159	Mycobacterial phage	Bardarov et al. (1)
phΔ*eis*	pEIS-KO cloned into phAE159	This study
phALEX-WT	phAE159 + pAZ10, hyg^r	This study
phALEX-C-14T	phAE159 + pAZ19, hyg^r	This study

Hyg^r, hygromycin resistant; Amp^r, ampicillin resistant; Kan^r, kanamycin resistant.

1. Bardarov S, et al. (1997) Conditionally mycobacteriophages: A system for transposon delivery to *Mycobacterium tuberculosis*. *Proc Natl Acad Sci USA* 94:10961–10966.

Table S3. Primer sequences

Primer name	Sequence 5′-3′
178F	CTTTCCTGACAGTGACAGAC
178R	TACTGTTTATGTAAGCAGACAG
HYG9F	ACGACTTCGAGGTGTTCGAGGAG
AZ56	ATCCCGCGTTCTTCCCTC
AZ77	CCCCTTGTATTACTGTTTATGTAAG
AZ78	TTTGAGACACAACGTCGCTTTG
AZ80	GCGTAACGTCACGGCGAAATTC
AZ85	GCCTTCAGAACTCGAACG
AZ87	GTCAGCTCATGCAAGGTG
AZ109	CGCCATATGACTGTGACCCTGTGTAG
AZ120	ACAGGATCCACCGCGATCGAGAGTGTAC
AZ139	AGCTGGCCAAAGAGATGG A
AZ140	GGGCGTATTGCTGGATTTC
AZ141	GCGATCTACGAGCGATGG
AZ142	CTCGTCCCAGAGCACCTG
AZ143	CCGCAGATCCATGTACAG
AZ144	ACGTCACGGCGAAATTCG
AZ145	TCATGCAAGGTGGTAGCG
AZ146	GGGTTCTCTCGGATTGACG
AZ147	GGCTGCTGGCACGTAGTT
AZ149	CGAAGGCCTTGCAGAGCGAAGCGATGAGG
AZ150	CGATCTAGACACGGCCTTCAGAACTCGAAC
AZ151	CGACTCGAGGTGCTAGGCCGGCGCTAG
AZ152	CGAACTAGTTGTCTTAGGGCCTGTCGTG
AZ167	GCATCTAGATGTAGCGCGGTTGGACAATC
AZ168	ACGTCTAGAACCGGTACTTGCTCTGCACG
AZ169	GCATCTAGAACGTCACGGCGAAATTCG
AZ170	CGATCTAGAGCGTCACGCTGGCGGGGCTA
AZ171	GCATCTAGAACTGTCGTCGTAATATTCAC
AZ172	GCATCTAGAGCACGTGGCCGCGGCATATG
AZ173	AGCTCTAGAATATGCCACAGTCGGATT
AZ174	GCATCTAGAGTCGGATTCTGTGACTGT
AZ179	GTGACTGTGACCCTGTGTAG
AZ180	CATATGCCACAGTCGGATTC
AZ198	ACGTGGCCGCGGCATATGCC
AZ199	ACGTGGCCGCGGCATATGGT