Science Advances

AAAS

advances.sciencemag.org/cgi/content/full/4/5/eaao1478/DC1

Supplementary Materials for

Posttranslational modification of a histone-like protein regulates phenotypic resistance to isoniazid in mycobacteria

Alexandra Sakatos, Gregory H. Babunovic, Michael R. Chase, Alexander Dills, John Leszyk, Tracy Rosebrock, Bryan Bryson, Sarah M. Fortune

Published 2 May 2018, *Sci. Adv.* **4**, eaao1478 (2018)
DOI: 10.1126/sciadv.aao1478

This PDF file includes:

Supplementary Materials

table S1. Bacterial strains used in this study.

Strain or plasmid	Relevant genotype and description	Source
mc^2155	Wild type M. smegmatis	Lab stock
E.coli DH5α	Host of plasmid used for cloning	Lab stock
WT_empty vector	mc^2155 strain with with pjeb vector inserted at attB site	This study
HupBΔ_empty vector	hupB gene deletion mutant of mc^2155 with pjeb vector integrated at attB site	This study
HupBΔ_pjeb::WT	*hupB* gene deletion mutant of mc^2155 complemented with WT *hupB* expressed under its native promoter (200 bp upstream of *hupB* gene) in the pjeb vector	This study
HupBΔ_pjeb::WT	hupB gene deletion mutant of mc^2155 complemented with K86R mutant of *hupB* expressed under its native promoter	This study

table S2. Genes down-regulated >10-fold in small versus large colonies.

Gene name	Product	Fold change	p-value
MSMEG_5568	Clavaldehyde dehydrogenase	35.41568388	6.72E-98
MSMEG_6451	ArsR, transcriptional regulator	33.52727279	1.85E-148
MSMEG_0538	MarR	18.09940587	1.12E-163
MSMEG_3362	Enoyl-CoA hydratase	18.07062619	8.26E-53
MSMEG_0894	dihydrodipicolinate reductase	16.61854973	1.58E-46
MSMEG_4334	flavoprotein	16.24766249	6.00E-54
MSMEG_4333	TetR transcriptional regulator	14.55094106	3.71E-26
MSMEG_0285	TetR transcriptional regulator	13.80766428	1.20E-129
MSMEG_1564	lignostilbene-alpha,beta-dioxygenase	13.04035087	7.84E-14
MSMEG_4057	GntR transcriptional regulator	12.31583883	4.78E-110
MSMEG_4026	hypothetical protein	11.66841714	1.93E-93
MSMEG_4034	NAD dependent epimerase/dehydratase family protein	11.39409212	3.17E-95
MSMEG_3012	acetyl-CoA acetyltransferases	10.9587545	2.36E-40

MSMEG_5819	pyridoxamine 5'-phosphate oxidase family protein	10.64027145	1.29E-12
MSMEG_3520	TetR- transcriptional regulator	10.24468663	1.73E-56
MSMEG_2532	dehydroquinase dehydratase, type II	10.02220734	1.46E-36
MSMEG_3318	oxidoreductase	10.00810306	1.09E-37
MSMEG_6505	NfnB protein	9.513810908	2.13E-47
MSMEG_2011	LacI transcriptional regulator	9.239361958	1.54E-12
MSMEG_5596	oxidoreductase	9.237059746	1.31E-21

table S3. Genes up-regulated more than fivefold in HupBΔ versus WT.

Gene name	Product	Fold change	p-value
MSMEG_2266	hypothetical protein	14.99065998	0.000897823
MSMEG_4141	hypothetical protein	9.150673323	7.33E-10
MSMEG_0643	extracellular solute-binding protein, family protein 5, putative	7.368960396	0.000789029
MSMEG_3325	hypothetical protein	6.296780421	0.033627484
MSMEG_2274	hydrogenase assembly chaperone HypC/HupF	5.968932095	2.23E-06
MSMEG_2659	alanine dehydrogenase	5.64584855	0.002833064
MSMEG_5083	hypothetical protein	5.642153607	0.000341015
MSMEG_0641	binding-protein-dependent transport systems inner membrane component	5.481512254	2.61E-19
MSMEG_5568	Clavaldehyde dehydrogenase	5.358667289	0.005390566
MSMEG_3199	quinolinate synthetase complex, A subunit	5.265245111	0.068910413

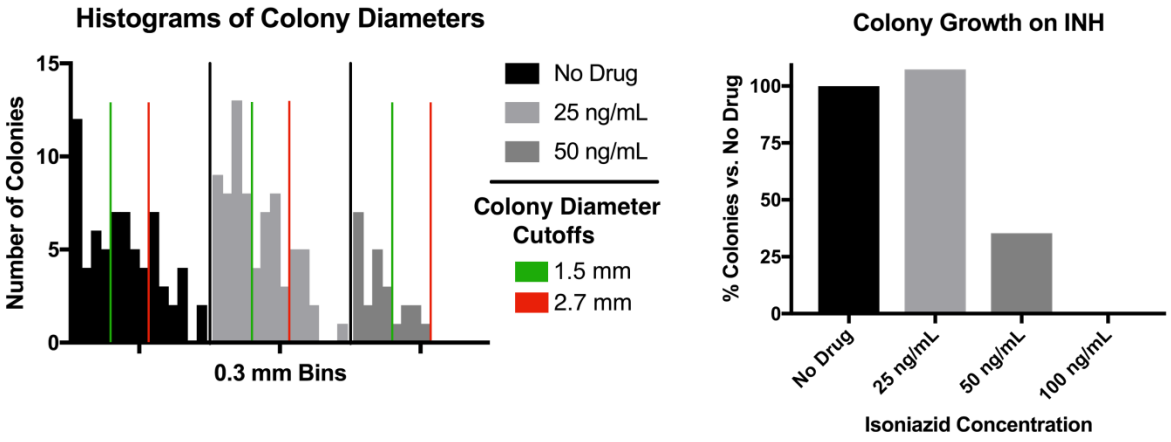


fig. S1. *M. tuberculosis* colony sizes and numbers plated on increasing concentrations of INH.

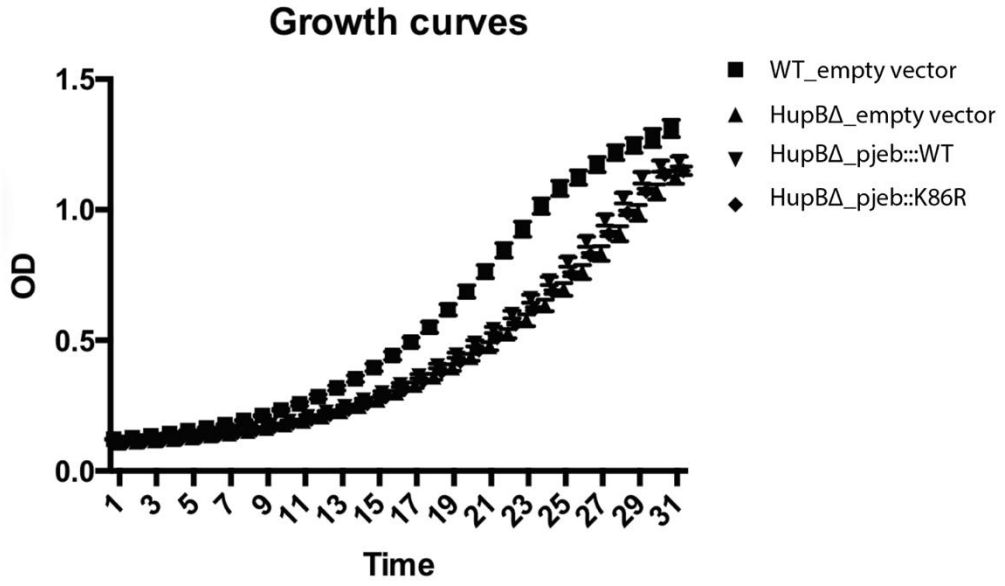


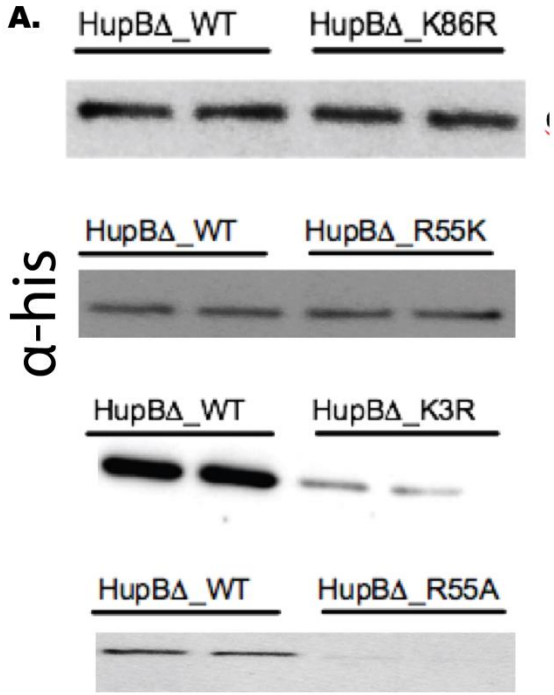
fig. S2. *M. smegmatis* strains expressing WT and mutant HupB alleles grown at WT rates. Indicated strains were grown in triplicate in 7H9 medium . There were no significant differences in growth rates between the *hupB* deletion mutant complemented with the WT and K86R alleles.

Residue	**Published** modification on *M.tuberculosis* HupB[9]	**Modification identified in this study on *M.smegmatis* HupB**
K3	acetylation	acetylation, methylation
R53, R54, or R55	none	methylation
K72	acetylation	acetylation
K86	acetylation	methylation
K94	none	acetylation, methylation
K103	Acetylation	acetylation, methylation

fig. S3. Modifications identified in previous study and current work.

Peptide Sequence	Mascot Ion Score	Mascot Identity Score	Modifications Identified by Spectrum
MNKAELIDVLTTK	46.9	28.6	Methyl (+14)
VKPTSVPAFRPGAQFK	40	31.2	Methyl (+14)
AVISGAQKLPADGPAVKR	26.7	28.8	Methyl (+14)
AVISGAQKLPADGPAVK	87.4	29.7	Acetyl (+42)
LPADGPAVKR	67.7	25	Acetyl (+42)
MNKAELIDVLTTK	26.9	28.7	Oxidation (+16), Acetyl (+42)

fig. S4. His-tagged HupB was purified from *M. smegmatis*, and modifications were identified by MS. Representative peptides are shown. Mascot Ion score is the calculated probability that the observed match between the experimental data and database sequence is a random event, reported in -10Log(P).



B.

HupB allele	HupB protein levels
WT	+++
K3R	+
K3Q	+
K72R	NA
K94R	NA
K86R	+++
K86A	++
K86L	++
R55A	+
R55K	no

fig. S5. Effects of disrupting HupB modification sites on HupB protein abundance. A) Protein levels of representative HupB point mutants tagged by western blot. **B)** Summary of all mutants assessed on HupB protein levels relative to wild-type as measured by western blot.