Interplay of PhoP and DevR response regulators defines expression of the dormancy regulon in virulent *Mycobacterium tuberculosis*

Received for publication, June 8, 2018, and in revised form, August 15, 2018 Published, Papers in Press, September 4, 2018, DOI 10.1074/jbc.RA118.004331

Atul Vashist[‡1,2], **Vandana Malhotra**[§¶1,3], **Gunjan Sharma**[‡], **Jaya Sivaswami Tyagi**[‡4], **and Josephine E. Clark-Curtiss**[¶‖5]

From the [¶]*Department of Biochemistry, Sri Venkateswara College, University of Delhi, New Delhi 110021, India, the* [§]*Center for Infectious Diseases and Vaccinology, Biodesign Institute, and the* [‖]*School of Life Sciences, Arizona State University, Tempe, Arizona 85287, and the* [‡]*Department of Biotechnology, All India Institute of Medical Sciences, New Delhi 110029, India*

Edited by Chris Whitfield

The DevR response regulator of *Mycobacterium tuberculosis* is an established regulator of the dormancy response in mycobacteria and can also be activated during aerobic growth conditions in avirulent strains, suggesting a complex regulatory system. Previously, we reported culture medium–specific aerobic induction of the DevR regulon genes in avirulent *M. tuberculosis* H37Ra that was absent in the virulent H37Rv strain. To understand the underlying basis of this differential response, we have investigated aerobic expression of the *Rv3134c-devR-devS* operon using *M. tuberculosis* H37Ra and H37Rv *devR* overexpression strains, designated as LIX48 and LIX50, respectively. Overexpression of DevR led to the up-regulation of a large number of DevR regulon genes in aerobic cultures of LIX48, but not in LIX50. To ascertain the involvement of PhoP response regulator, also known to co-regulate a subset of DevR regulon genes, we complemented the naturally occurring mutant $phoP_{Ra}$ gene of LIX48 with the WT $phoP_{Rv}$ gene. $PhoP_{Rv}$ dampened the induced expression of the DevR regulon by >70–80%, implicating PhoP in the negative regulation of *devR* expression. Electrophoretic mobility shift assays confirmed phosphorylation-independent binding of $PhoP_{Rv}$ to the *Rv3134c* promoter and further revealed that DevR and $PhoP_{Rv}$ proteins exhibit differential DNA binding properties to the target DNA. Through co-incubations with DNA, ELISA, and protein complementation assays, we demonstrate that DevR forms a heterodimer with $PhoP_{Rv}$ but not with the mutant $PhoP_{Ra}$ protein. The study puts forward a new possible mechanism for coordinated expression of the dormancy regulon, having implications in growth adaptations critical for development of latency.

Despite global initiatives to control tuberculosis, it continues to be an enigma. *Mycobacterium tuberculosis*, the causative agent of tuberculosis has evolved into a highly efficient human pathogen due to its innate ability to adapt to the diverse host environments during infection. Although great strides have been made to elucidate the transcriptomic and proteomic profiles of *M. tuberculosis* in various cellular environments *in vitro* and *in vivo*, our understanding of the mechanisms that are responsible for growth adaptation are far from clear.

Long-term survival of mycobacteria in the host requires the bacteria to transition to a slow-growth phenotype resulting in dormant bacilli that may undergo reactivation when exposed to appropriate stimuli. The DevR (also called DosR) response regulator (RR)[6] of the DevR-DevS (DevRS) TCS has been extensively studied for its role in mycobacterial dormancy and virulence (1–10). A wide variety of cellular stimuli are known to activate the DevRS TCS (11–16), highlighting its central role in metabolic and growth-adaptive strategies of mycobacteria. The DevR RR is activated via phosphorylation by two histidine sensor kinases, DevS and DosT (17), and also by Ser/Thr protein kinases, PknH (18) and PknB (19), that facilitate fine tuning of the dormancy response. This, in addition to the growing list of *M. tuberculosis* RRs that interact with DevR and/or co-regulate the DevR regulon, such as MprA, NarL, and PhoP, indicate overlapping regulatory pathways as a means for expansion of signaling networks (20–23). Indeed, such a strategy will enable coordinated gene expression across the perpetually changing metabolic status of the cell.

The constitutive aerobic induction of the DevR regulon in the hypervirulent *M. tuberculosis* W/Beijing (24) and avirulent H37Ra strains (25) has sparked significant interest in the under-

The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The microarray data presented in this study have been deposited in the NCBI Gene Expression Omnibus (GEO) under the GEO series accession number GSE103551.

This article contains Figs. S1 and S2 and Tables S1–S3.

[1] Both authors contributed equally to this work.

[2] Supported by Department of Science and Technology, Government of India, Start-Up Research Grant YSS/2015/000562/LS.

[3] Supported by Department of Biotechnology, Government of India, Grant BT/Bio-CARe/07/9843. To whom correspondence should be addressed: Dept. of Biochemistry, Sri Venkateswara College, University of Delhi, New Delhi 110021, India. E-mail: vmal71@gmail.com.

[4] Supported by the Department of Science and Technology, Government of India, J. C. Bose National Fellowship.

[5] Supported by National Institutes of Health Public Health Service Grant AI46428 and Arizona State University start-up funding. Present address: Division of Infectious Diseases and Global Medicine, Dept. of Medicine, College of Medicine, University of Florida, Gainesville, FL 32610.

[6] The abbreviations used are: RR, response regulator; EMSA, electrophoretic mobility shift assay; M-PFC, mycobacterial protein fragment complementation; mDHFR, murine dihydrofolate reductase; TCS, two-component system; TRIM, trimethoprim; qRT-PCR, quantitative RT-PCR; LB, Luria broth; Kan, kanamycin; Hyg, hygromycin; EV, empty vector.