Mycobacterium tuberculosis Response Regulators, DevR and NarL, Interact *in Vivo* and Co-regulate Gene Expression during Aerobic Nitrate Metabolism*[S]

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Background: *M. tuberculosis* two-component systems converge to co-regulate gene expression during nitrate metabolism.
Results: Analysis of nitrate/nitrite-responsive gene expression reveals novel interaction between NarL and DevR response regulators.
Conclusion: Cooperative binding of NarL and DevR enables co-regulation of a subset of genes.
Significance: The findings establish endogenous nitrite as a signal and highlight interplay between *M. tuberculosis* NarLS and DevRS/DosT signaling systems.

***Mycobacterium tuberculosis* genes *Rv0844c*/*Rv0845* encoding the NarL response regulator and NarS histidine kinase are hypothesized to constitute a two-component system involved in the regulation of nitrate metabolism. However, there is no experimental evidence to support this. In this study, we established *M. tuberculosis* NarL/NarS as a functional two-component system and identified His^{241} and Asp^{61} as conserved phosphorylation sites in NarS and NarL, respectively. Transcriptional profiling between *M. tuberculosis* H37Rv and a Δ*narL* mutant strain during exponential growth in broth cultures with or without nitrate defined an ~30-gene NarL regulon that exhibited significant overlap with DevR-regulated genes, thereby implicating a role for the DevR response regulator in the regulation of nitrate metabolism. Notably, expression analysis of a subset of genes common to NarL and DevR regulons in *M. tuberculosis* Δ*devR*, Δ*devS*Δ*dosT*, and Δ*narL* mutant strains revealed that in response to nitrite produced during aerobic nitrate metabolism, the DevRS/DosT regulatory system plays a primary role that is augmented by NarL. Specifically, NarL itself was unable to bind to the *narK2*, *acg*, and *Rv3130c* promoters in phosphorylated or unphosphorylated form; however, its interaction with DevR~P resulted in cooperative binding, thereby enabling co-regulation of these genes. These findings support the role of physiologically derived nitrite as a metabolic signal in mycobacteria. We propose NarL-DevR binding, possibly as a heterodimer, as a novel mechanism for co-regulation of gene expression by the DevRS/DosT and NarL/NarS regulatory systems.**

Despite the availability of a vaccine and cost-effective antibiotics, tuberculosis continues to be a global health emergency. The remarkable ability of *Mycobacterium tuberculosis* to survive in nutrient-limited environments inside human macrophages and to persist for long periods of time necessitates studies to understand how *M. tuberculosis* coordinates adaptive responses that regulate growth, metabolism, and persistence.

Emerging evidence has revealed that highly integrated mycobacterial signaling networks play key roles in facilitating host-pathogen interactions and enabling intracellular survival strategies (1). The *M. tuberculosis* genome encodes 11 paired two-component signal transduction systems (TCSSs),[6] two orphan histidine kinases (HKs), and 6 orphan response regulators (RRs) (2). Notably, two RRs (MtrA and PrrA) are essential for intracellular survival of *M. tuberculosis* (3, 4), and several others (DevR, PhoP, and MprA) have been implicated in virulence and persistence (reviewed in Ref. 5). Of all of the systems, the DevRS/DosT (also called DosRS/DosT) system is the most well characterized and regulates a ~50-member regulon in response to hypoxia, nitric oxide (NO), carbon monoxide (CO), and ascorbic acid (6–13). More recently, studies have demonstrated a role for the DevR regulon in metabolic adaptation that is essential for *M. tuberculosis* to transition from an actively respiring to a latent non-replicating persistent state (14).

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The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE59063).
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[6] The abbreviations used are: TCSS, two-component signal transduction system; HK, histidine kinase; M-PFC, mycobacterial protein fragment complementation assay; mDHFR, murine dihydrofolate reductase; RR, response regulator; SPR, surface plasmon resonance; TRIM, trimethoprim; qRT-PCR, quantitative RT-PCR.