DevR–DevS is a bona fide two-component system of *Mycobacterium tuberculosis* that is hypoxia-responsive in the absence of the DNA-binding domain of DevR

Deepak Kumar Saini,[1] Vandana Malhotra,[1] Deepanwita Dey,[1] Neha Pant,[1] Taposh K. Das[2] and Jaya Sivaswami Tyagi[1]

Departments of Biotechnology[1] and Anatomy[2], All India Institute of Medical Sciences, New Delhi 110029, India

Correspondence
Jaya Sivaswami Tyagi
jstyagi54in@indiatimes.com

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Two-component systems play a central role in the adaptation of pathogenic bacteria to the environment prevailing within host tissues. The genes encoding the response regulator DevR (Rv3133c/DosR) and the cytoplasmic portion ($DevS_{201}$) of the histidine kinase DevS (Rv3132c/DosS), a putative two-component system of *Mycobacterium tuberculosis*, were cloned and the protein products were overexpressed, purified and refolded as N-terminally His_6-tagged proteins from *Escherichia coli*. $DevS_{201}$ underwent autophosphorylation and participated in rapid phosphotransfer to DevR in a Mg^{2+}-dependent manner. Chemical stability analysis and site-directed mutagenesis implicated the highly conserved residues His^{395} and Asp^{54} as the sites of phosphorylation in DevS and DevR, respectively. Mutations in Asp^{8} and Asp^{9} residues, postulated to form the acidic Mg^{2+}-binding pocket, and the invariant Lys^{104} of DevR, abrogated phosphoryl transfer from $DevS_{201}$ to DevR. DevR–DevS was thus established as a typical two-component regulatory system based on His-to-Asp phosphoryl transfer. Expression of the *Rv3134c–devR–devS* operon was induced at the RNA level in hypoxic cultures of *M. tuberculosis* H37Rv and was associated with an increase in the level of DevR protein. However, in a *devR* mutant strain expressing the N-terminal domain of DevR, induction was observed at the level of RNA expression but not at that of protein. DevS was translated independently of DevR and induction of *devS* transcripts was not associated with an increase in protein level in either wild-type or mutant strains, reflecting differential regulation of this locus during hypoxia.

INTRODUCTION

Intracellular signal transfer in bacteria is dominated by phosphoryl transfer between conserved transmitter and receiver domains in regulatory proteins of two-component systems. *Mycobacterium tuberculosis* contains eleven complete systems and seven orphan proteins belonging to sensor kinase and response regulator protein families. They are expected to modulate gene expression and direct the adaptation of tubercle bacilli to environmental challenges. In the first step, a sensor, which is usually a membrane-associated histidine protein kinase, is autophosphorylated at a conserved histidine residue in an ATP-dependent manner in response to an environmental stimulus such as O_2 tension, temperature, pH, ions, metabolites, etc. In the second step, the phosphoryl group is transferred to a highly conserved aspartic acid residue in the N-terminal domain of the response regulator (Parkinson & Kofoid, 1992; Stock *et al.*, 1995). Two-component regulatory proteins dephosphorylate response regulators in three different ways: through the phosphatase activity of sensor kinases; through reverse phosphorylation, which involves transfer of the phosphoryl group from response regulators back to sensor kinases; or by autodephosphorylation. Regulation may therefore take place by modulation of either the kinase activity or the phosphatase activity of these proteins. A change in the phosphorylation status of the response regulator protein in turn alters its affinity for DNA motifs present in the vicinity of target genes. These unique DNA–protein interactions typically modulate gene transcription, culminating in an appropriate adaptive response (Stock *et al.*, 1995, 2000).

The *devR–devS* genetic system was identified among genes differentially expressed in the virulent strain of *M. tuberculosis* (Kinger & Tyagi, 1993; Dasgupta *et al.*, 2000). DevR (Rv3133c) demonstrated homology to response regulator proteins of the NarL/UhpA/FixJ subfamily, and DevS (Rv3132c) to histidine sensor kinases. *devR* and *devS* are cotranscribed and conserved in *M. tuberculosis* and *Mycobacterium bovis* BCG (Dasgupta *et al.*, 2000); their nucleotide sequences are identical in *M. tuberculosis* (Cole *et al.*,