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Characterization of a two-component system, *devR-devS*, of *Mycobacterium tuberculosis*

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Summary By subtractive hybridization, we isolated genes, differentially expressed in virulent strain (*dev*), that are expressed at higher levels in the virulent *Mycobacterium tuberculosis* H37Rv strain in comparison to its avirulent counterpart, H37Ra, and consequently may be associated with the virulence phenotype of *M. tuberculosis*. A two-component system, *devR-devS*, was identified by DNA sequencing of a *dev* clone. DevR, the predicted gene product of *devR*, is a response regulator (RR) in the NarL/ UhpA subfamily of two-component systems. The *devS* gene product displayed homology with histidine protein kinases (HPKs) including UhpB, NarX and NarQ. The *devR-devS* locus is preceded by gene Rv3134c that encodes a putative alanine–valine- rich protein. This locus was conserved in *M. tuberculosis* and *M. bovis* BCG but not in other mycobacteria. A *devR -lacZ* transcription fusion demonstrated β-galactosidase activity in *M. smegmatis* and in *M. tuberculosis*. The *devR* and *devS* genes were cotranscribed and the levels of their transcripts were lower in two isolates of the avirulent H37Ra strain in comparison to the virulent H37Rv strain of *M. tuberculosis*. The level of DevR protein was also lower in one of the H37Ra strains in comparison to the H37Rv strain. However, in a third isolate of H37Ra, RNA and protein expression was equivalent to that in the H37Rv strain. Electron microscopic immunogold analysis of *M. tuberculosis* grown in laboratory medium and within human monocytes revealed specific labelling for DevR protein within the bacteria and the phagosomal lumen of infected monocytes. These findings collectively suggest a potential role for *devR-devS* in the regulation of genetic programmes unique to the tubercle bacillus. © 2000 Harcourt Publishers Ltd

INTRODUCTION

Mycobacterium tuberculosis is the causative agent of tuberculosis, which remains the single most frequent cause of mortality from an infectious disease in the world.[1] Intensive research efforts, worldwide, in conjunction with the application of modern biological tools, have begun to reveal the genetic programmes of *M. tuberculosis* that enable its adaptation to environmental challenges spanning from existence outside the human host to the establishment of dormancy with a possibility of reactivation upon the waning of host immunity.[2–7] An *M. tuberculosis* homologue of *smpB*, which has been implicated in intracellular survival of *Salmonella typhimurium*, has been identified.[8] Detailed characterization of the *smpB* gene is in progress.

Sequence analysis of the *M. tuberculosis* genome has unveiled the presence of 13 putative sigma factors, more than 100 regulatory proteins and eukaryotic-like serine/ threonine protein kinases, providing enormous opportunities for understanding its unique gene expression and regulation characteristics.[9] Since their initial discovery in bacteria a little over a decade ago, two-component systems have generated considerable excitement, as they are intimately involved in the regulation of bacterial physiological processes ranging from sugar uptake to virulence under a variety of environmental conditions.[10] Despite their ubiquitous presence, there is a wide variation in the prevalence of two-component systems in bacteria; the enteric organism *Escherichia coli* contains an abundance (~50), while only one system has been identified in the

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