Cross talk between DevS sensor kinase homologue, Rv2027c, and DevR response regulator of *Mycobacterium tuberculosis*

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Abstract **Rv2027c is a putative orphan histidine sensor kinase that bears strong homology to DevS of the hypoxia-responsive DevR–DevS two-component system in *M. tuberculosis*. The cytosolic C-terminal domain of Rv2027c protein ($Rv2027c_{194}$) was overexpressed in *E. coli* and biochemically characterized. $Rv2027c_{194}$ underwent autophosphorylation at a conserved His^{392} residue and engaged in phosphotransfer with DevR response regulator. The rates of autophosphorylation and the stabilities of the phosphorylated species were broadly similar in Rv2027c and DevS. However, unlike DevS, Rv2027c utilized Ca^{2+} as an alternative divalent ion during autophosphorylation. In contrast to DevS which completed phosphotransfer to DevR in 5–10 min, phosphotransfer from Rv2027c∼P was only partial at 30 min. Unlike *devS* transcription that was hypoxia-responsive, Rv2027c transcript levels were not upregulated from basal levels during hypoxia. The differential regulation of *devS* and *Rv2027c* genes, the ability of Rv2027c to utilize Ca^{2+} as a divalent cation in autophosphorylation at physiological concentrations and to engage in phosphotransfer with DevR suggests that the DevR regulon could be modulated by more than one environmental cue relayed through DevS and Rv2027c.**

Keywords: Two-component system; DevS and Rv2027c sensor kinase; DevR response regulator; Cross talk; Calcium; *M. tuberculosis*

1. Introduction

Two-component systems of bacteria play a major role in their adaptation to the environment. In its most basic form, the two-component system consists of a histidine sensor kinase and a response regulator [1]. These systems use transient phosphorylation of the sensory and regulatory proteins at conserved residues for transducing extracellular or intracellular signals, thereby leading to an appropriate cellular response [2]. Such phosphorylation-based signalling has been established for five of eleven systems in *M. tuberculosis*, RegX3–SenX3 [3], TrcR–TrcS [4], MprA–MprB [5], PrrA–PrrB [6] and DevR–DevS (Rv3133c–Rv3132c/DosR–DosS) [7]. The genes encoding two-component systems are generally located adjacent to each other and are also coexpressed. The presence of seven 'orphan' genes coding for histidine kinases or response regulators but without the genes coding for the other component of proteins of two-component systems adjacent to them was an intriguing finding derived from determination of the genome sequence of *M. tuberculosis* H37Rv [8]. For instance, the gene encoding a potential sensor kinase, Rv2027c, is not adjacent to a gene encoding a response regulator. Since Rv2027c bears strong homology with DevS [8], there exists a possibility that diverse signals sensed by DevS and Rv2027c could generate a similar genetic response by signalling to a common response regulator protein, DevR. In this paper, we report for the first time the likely function of the orphan histidine kinase, Rv2027c. We have established Rv2027c to be a bonafide sensor kinase that undergoes autophosphorylation at a conserved histidine residue and communicates with DevR through a phosphotransfer reaction. Its unique metal ion requirement and physiological relevance are discussed.

2. Materials and methods

2.1. Bacterial strains, media, growth conditions and plasmids

Escherichia coli DH5α (Invitrogen Inc., USA) and XL1 Blue (Stratagene Inc., USA) were used for routine cloning and *E. coli* BL23 (DE3) (gift from Dr. S. Vijaya, Indian Institute of Science, Bangalore) for protein production. Antibiotics, when required, were used at the following concentrations: ampicillin at 100 μg/ml, hygromycin at 200 μg/ml in *E. coli* and 25 μg/ml in *M. smegmatis*. *E. coli* was grown in LB media, while *M. tuberculosis* H37Rv (kindly provided by Dr. R.F. Silver, Case Western Reserve University, Cleveland, USA) and *M. smegmatis* LR222 (gift from Dr. J. Crawford, CDC, Atlanta, USA) were cultured in Dubos–Tween Albumin broth (DTA). All routine recombinant DNA work was performed as described [9]. The cytosolic domain-coding portion of $Rv2027c_{194}$ gene (amino acids 380–573) of *M. tuberculosis* H37Rv was amplified using gene-specific primers, 2027bamf (5′ GCGAGAAGTGGAGGATCCTGACC 3′) and 2027bamr (5′ GGATTGCGCGGATCCGTCGAC GCC 3′, engineered *Bam*HI restriction site is underlined), and inserted in pPROEx-HTc expression vector as described [7] to generate plasmid pDSH194. The reporter vector pFPVH was generated by inserting a hygromycin resistance cassette from pGEMT-Hyg (obtained as a gift from Dr. A. Ranganathan, ICGEB, New Delhi) in the green fluorescent protein (GFP) reporter vector, pFPV27 at the *Nsi*I site [10].

2.2. Overexpression of recombinant protein

$Rv2027c_{194}$ protein was overexpressed, purified and refolded using an established matrix-assisted protein renaturation protocol as described [11].

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Abbreviations: cGMP, cyclic GMP; GAF, cyclic GMP (cGMP) – regulated cyclic nucleotide phosphodiesterases, adenyl cyclases and bacterial transcriptional regulator FhlA; GFP, green fluorescent protein; USP, Universal stress protein

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