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Loss of kinase activity in *Mycobacterium tuberculosis* multidomain protein Rv1364c

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The alternative sigma factors are regulated by a phosphorylation-mediated signal transduction cascade involving anti-sigma factors and anti-anti-sigma factors. The proteins regulating *Mycobacterium tuberculosis* sigma factor F (SigF), anti-SigF and anti-anti-SigF have been identified, but the factors catalyzing phosphorylation–dephosphorylation have not been well established. We identified a distinct pathogenic species-specific multidomain protein, Rv1364c, in which the components of the entire signal transduction cascade for SigF regulation appear to be encoded in a single polypeptide. Sequence analysis of *M. tuberculosis* Rv1364c resulted in the prediction of various domains, namely a phosphatase (RsbU) domain, an anti-SigF (RsbW) domain, and an anti-anti-SigF (RsbV) domain. We report that the RsbU domain of Rv1364c bears all the conserved features of the PP2C-type serine/threonine phosphatase family, whereas its RsbW domain has certain substitutions and deletions in regions important for ATP binding. Another anti-SigF protein in *M. tuberculosis*, UsfX (Rv3287c), shows even more unfavorable substitutions in the kinase domain. Biochemical assay with the purified RsbW domain of Rv1364c and UsfX showed the loss of ability of autophosphorylation and phosphotransfer to cognate anti-anti-SigF proteins or artificial substrates. Both the Rv1364c RsbW domain and UsfX protein display very weak binding with fluorescent ATP analogs, despite showing functional interactions characteristic of anti-SigF proteins. In view of conservation of specific interactions with cognate sigma and anti-anti-sigma factor, the loss of kinase activity of Rv1364c and UsfX appears to form a missing link in the phosphorylation-dependent interaction involved in SigF regulation in *Mycobacterium*.

Despite global efforts to control tuberculosis, it remains an epidemic, with one-third of the world's population being infected by its etiologic agent, *Mycobacterium tuberculosis*, and over 1.5 million people dying from the disease each year. The notorious success of *M. tuberculosis* as a highly adapted pathogen rests upon its ability to establish a persistent infection in the hostile environment of the host cell through mechanisms involving transcriptional reprogramming, ensuring metabolic slowdown and upregulation of virulence and stress response pathways [1]. Switching of alternative sigma factors is known to regulate global gene expression to cope with the numerous environmental conditions encountered during the establishment of a successful infection [2]. The *M. tuberculosis* genome encodes 13 sigma factors, including 10 alter-

Abbreviations
GST, glutathione *S*-transferase; MBP, myelin basic protein; MursiF, multidomain regulator of sigma factor F; PAC, PAS domain-associated C-terminus; PAS domain, Per-Arnt-Sim domain; pNP, *p*-nitrophenol; pNPP, *p*-nitrophenyl phosphate; PP, protein phosphatase; SigA, sigma factor A; SigB, sigma factor B; SigF, sigma factor F; TNP-ATP, 2,4,6-trinitrophenyl ATP; UPD, RsbU/phosphatase domain; VSD, RsbV/substrate domain; WKD, RsbW/kinase domain.

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