



Tuning the *Mycobacterium tuberculosis* Alternative Sigma Factor SigF through the Multidomain Regulator Rv1364c and Osmosensory Kinase Protein Kinase D

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ABSTRACT Bacterial alternative sigma factors are mostly regulated by a partner-switching mechanism. Regulation of the virulence-associated alternative sigma factor SigF of *Mycobacterium tuberculosis* has been an area of intrigue, with SigF having more predicted regulators than other sigma factors in this organism. Rv1364c is one such predicted regulator, the mechanism of which is confounded by the presence of both anti-sigma factor and anti-sigma factor antagonist functions in a single polypeptide. Using protein binding and phosphorylation assays, we demonstrate that the anti-sigma factor domain of Rv1364c mediates autophosphorylation of its antagonist domain and binds efficiently to SigF. Furthermore, we identified a direct role for the osmosensor serine/threonine kinase PknD in regulating the SigF-Rv1364c interaction, adding to the current understanding about the intersection of these discrete signaling networks. Phosphorylation of SigF also showed functional implications in its DNA binding ability, which may help in activation of the regulon. In *M. tuberculosis*, osmotic stress-dependent induction of *espA*, a SigF target involved in maintaining cell wall integrity, is curtailed upon overexpression of Rv1364c, showing its role as an anti-SigF factor. Overexpression of Rv1364c led to induction of another target, *pkas6*, involved in lipid metabolism. This induction was, however, curtailed in the presence of osmotic stress conditions, suggesting modulation of SigF target gene expression via Rv1364c. These data provide evidence that Rv1364c acts an independent SigF regulator that is sensitive to the osmosensory signal, mediating the cross talk of PknD with the SigF regulon.

IMPORTANCE *Mycobacterium tuberculosis*, capable of latently infecting the host and causing aggressive tissue damage during active tuberculosis, is endowed with a complex regulatory capacity built of several sigma factors, protein kinases, and phosphatases. These proteins regulate expression of genes that allow the bacteria to adapt to various host-derived stresses, like nutrient starvation, acidic pH, and hypoxia. The cross talk between these systems is not well understood. SigF is one such regulator of gene expression that helps *M. tuberculosis* to adapt to stresses and imparts virulence. This work provides evidence for its inhibition by the multidomain regulator Rv1364c and activation by the kinase PknD. The coexistence of negative and positive regulators of SigF in pathogenic bacteria reveals an underlying requirement for tight control of virulence factor expression.

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