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Identification of Ser/Thr kinase and Forkhead Associated Domains in *Mycobacterium ulcerans:* Characterization of Novel Association between Protein Kinase Q and MupFHA

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Abstract

Background: *Mycobacterium ulcerans*, the causative agent of Buruli ulcer in humans, is unique among the members of *Mycobacterium* genus due to the presence of the virulence determinant megaplasmid pMUM001. This plasmid encodes multiple virulence-associated genes, including *mup011*, which is an uncharacterized Ser/Thr protein kinase (STPK) PknQ.

Methodology/Principal Findings: In this study, we have characterized PknQ and explored its interaction with MupFHA (Mup018c), a FHA domain containing protein also encoded by pMUM001. MupFHA was found to interact with PknQ and suppress its autophosphorylation. Subsequent protein-protein docking and molecular dynamic simulation analyses showed that this interaction involves the FHA domain of MupFHA and PknQ activation loop residues Ser^{170} and Thr^{174}. FHA domains are known to recognize phosphothreonine residues, and therefore, MupFHA may be acting as one of the few unusual FHA-domain having overlapping specificity. Additionally, we elucidated the PknQ-dependent regulation of MupDivIVA (Mup012c), which is a DivIVA domain containing protein encoded by pMUM001. MupDivIVA interacts with MupFHA and this interaction may also involve phospho-threonine/serine residues of MupDivIVA.

Conclusions/Significance: Together, these results describe novel signaling mechanisms in *M. ulcerans* and show a three-way regulation of PknQ, MupFHA, and MupDivIVA. FHA domains have been considered to be only pThr specific and our results indicate a novel mechanism of pSer as well as pThr interaction exhibited by MupFHA. These results signify the need of further re-evaluating the FHA domain –pThr/pSer interaction model. MupFHA may serve as the ideal candidate for structural studies on this unique class of modular enzymes.

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Introduction

Buruli ulcer is a disease of skin and soft tissues caused by the bacteria *Mycobacterium ulcerans* [1]. It is the third most important mycobacterial disease after tuberculosis and leprosy [2], and the prevalence continues to increase in tropical and sub-tropical countries [3]. *M. ulcerans* evolved from an *Mycobacterium marinum* ancestor through reductive evolution and acquired a large virulence determinant plasmid (pMUM001) [4]. This plasmid encodes genes for mycolactone synthesis that are required to circumvent the host immune response, as a strain lacking this plasmid is avirulent. Therefore, the pMUM001 plasmid is considered to be a key determinant of *M. ulcerans* pathogenesis [5,6].

Pathogenic species of mycobacteria require stringent control on cell division for survival in their host, and thus are likely to acquire specialized mechanisms through evolution to achieve this control. Signaling proteins that sense environmental changes and mediate cell response are important for regulating cell division. For instance, bacterial Serine/Threonine Protein Kinases (STPKs) are known to regulate cell division by sensing and responding to specific signals in the host environment [7]. Moreover, according to phospho-proteome analysis, numerous Ser/Thr phosphorylated proteins have been identified in *Mycobacterium tuberculosis*, suggesting that STPKs may regulate multiple cellular processes [8–12]. Indeed, eleven STPKs have been identified in *M. tuberculosis* and the majority of them have been shown to be involved in pathogenesis and drug resistance [13–15].

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