OPEN ACCESS Freely available online

PLOS ONE

Mycobacterium tuberculosis Cyclophilin A Uses Novel Signal Sequence for Secretion and Mimics Eukaryotic Cyclophilins for Interaction with Host Protein Repertoire

Asani Bhaduri[1,2], Richa Misra[1], Abhijit Maji[1], Preetida J. Bhetaria[1], Sonakshi Mishra[1], Gunjan Arora[1], Lalit Kumar Singh[1], Neha Dhasmana[1], Neha Dubey[1], Jugsharan Singh Virdi[2], Yogendra Singh[1]*

1 CSIR-Institute of Genomics and Integrative Biology, Delhi, India, **2** Department of Microbiology, University of Delhi, Delhi, India

Abstract

Cyclophilins are prolyl isomerases with multitude of functions in different cellular processes and pathological conditions. Cyclophilin A (PpiA) of *Mycobacterium tuberculosis* is secreted during infection in intraphagosomal niche. However, our understanding about the evolutionary origin, secretory mechanism or the interactome of *M. tuberculosis* PpiA is limited. This study demonstrates through phylogenetic and structural analyses that PpiA has more proximity to human cyclophilins than the prokaryotic counterparts. We report a unique N-terminal sequence (MADCDSVTNSP) present in pathogenic mycobacterial PpiA and absent in non-pathogenic strains. This sequence stretch was shown to be essential for PpiA secretion. The overexpression of full-length PpiA from *M. tuberculosis* in non-pathogenic *Mycobacterium smegmatis* resulted in PpiA secretion while truncation of the N-terminal stretch obstructed the secretion. In addition, presence of an ESX pathway substrate motif in *M. tuberculosis* PpiA suggested possible involvement of Type VII secretion system. Site-directed mutagenesis of key residues in this motif in full-length PpiA also hindered the secretion in *M. smegmatis*. Bacterial two-hybrid screens with human lung cDNA library as target were utilized to identify interaction partners of PpiA from host repertoire, and a number of substrates with functional representation in iron storage, signal transduction and immune responses were detected. The extensive host interactome coupled with the sequence and structural similarity to human cyclophilins is strongly suggestive of PpiA being deployed by *M. tuberculosis* as an effector mimic against the host cyclophilins.

Citation: Bhaduri A, Misra R, Maji A, Bhetaria PJ, Mishra S, et al. (2014) *Mycobacterium tuberculosis* Cyclophilin A Uses Novel Signal Sequence for Secretion and Mimics Eukaryotic Cyclophilins for Interaction with Host Protein Repertoire. PLoS ONE 9(2): e88090. doi:10.1371/journal.pone.0088090

Editor: Volker Briken, University of Maryland, United States of America

Received June 22, 2013; **Accepted** January 3, 2014; **Published** February 4, 2014

Funding: This work was supported by grant BSC0104 from the Council of Scientific and Industrial Research (CSIR), India. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors declare that they have no conflict of interest.

* E-mail: mail: ysingh@igib.res.in

Introduction

Pathogenic microbiota, during evolution, have acquired strategic assets like pathogenicity islands, antigenic variation, quorum sensing ability, virulence factors and mimics to combat the eukaryotic host [1–3]. *Mycobacterium tuberculosis*, one of the oldest human pathogens, also possesses a complex machinery to subvert host defense mechanisms. Studies on *M. tuberculosis* virulence factors, specialized cell-wall lipids and novel secretory pathways have changed the earlier anthropocentric view of granuloma formation and mycobacterial transmission to a bacteriocentric interpretation [4–7]. This is also corroborated by reports on different clinical isolates of *M. tuberculosis* complex where modern lineages have been shown to be more aggressive and instrumental in rapid progression of tuberculosis [8]. A large number of secreted proteins of mycobacteria, some secretory pathways have been identified in recent times and with role of few of these secretory proteins established during infection process; the attention has now refocused on secretome [5,6,9,10].

Cyclophilins are one of the four structurally different groups of peptidyl-prolyl isomerases (the other families being FK506 binding proteins, parvulins and PP2A phosphatase activator), which modulate equilibration of *cis* or *trans* isomers of proline [11,12]. The cyclophilins belong to an ancient protein family ubiquitous throughout organismal hierarchy [13,14]. Unlike the eukaryotes, which encode multitude of cyclophilins, most prokaryotes possess a few cyclophilins [15,16]. The prokaryotic cyclophilins contain a single cyclophilin-like domain (CLD) unlike the large multi-domain eukaryotic counterparts and show a weaker cyclosporin A binding capacity than the eukaryotic cyclophilins [16,17]. The role of prolyl isomerases has been implicated in diverse vital processes which include, but are not restricted to, protein folding, chaperoning, RNA processing, signal transduction, stress responses and cell death [13,14,16]. The functions of these regulators have also been established in various pathological conditions like cancer, Alzheimer's disease, atherosclerosis, diabetes, asthma and in various viral diseases including HIV [14,18,19].

M. tuberculosis has two cyclophilins, namely cyclophilin A (PpiA) and cyclophilin B (PpiB). The former has been shown to be present in culture filtrates in several studies [10,20] and is reported to be upregulated in intraphagosomal niche during infection [21]. Although independent groups have partly characterized PpiA with respect to cyclosporin binding or structural moiety [22,23],

Richa Misra