In-Vitro Helix Opening of *M. tuberculosis* oriC by DnaA Occurs at Precise Location and Is Inhibited by IciA Like Protein

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Abstract

**Background:** *Mycobacterium tuberculosis* (*M.tb*), the pathogen that causes tuberculosis, is capable of staying asymptomatically in a latent form, persisting for years in very low replicating state, before getting reactivated to cause active infection. It is therefore important to study *M.tb* chromosomal replication, specifically its initiation and regulation. While the region between *dnaA* and *dnaN* gene is capable of autonomous replication, little is known about the interaction between DnaA initiator protein, oriC origin of replication sequences and their negative effectors of replication.

**Methodology/Principal Findings:** By KMnO₄ mapping assays the sequences involved in open complex formation within oriC, mediated by *M.tb* DnaA protein, were mapped to position −500 to −518 with respect to the *dnaA* gene. Contrary to *E. coli*, the *M.tb* DnaA in the presence of non-hydrolysable analogue of ATP (ATP₇S) was unable to participate in helix opening thereby pointing to the importance of ATP hydrolysis. Interestingly, ATPase activity in the presence of supercoiled template was higher than that observed for DnaA box alone. *M.tb* rV1985c, a homologue of *E.coli* IciA (Inhibitor of chromosomal initiation) protein, could inhibit DnaA-mediated in-vitro helix opening by specifically binding to A+T rich region of *oriC*, provided the open complex formation had not initiated. rlcA could also inhibit in-vitro replication of plasmid carrying the *M.tb* origin of replication.

**Conclusions/Significance:** These results have a bearing on the functional role of the important regulator of *M.tb* chromosomal replication belonging to the LysR family of bacterial regulatory proteins in the context of latency.

Introduction

Replication in eubacteria is initiated when DnaA, an initiator protein, binds to DnaA boxes located within the origin of replication (oriC) sequence [1]. Initiation of replication in *E. coli* proceeds with the binding of DnaA protein to oriC [2] and leads to opening of 13-mer region, which is followed by entry of DnaB helicase to form the prepriming complex [3]. In many bacteria either or both the 3’ and 5’ flanking regions of the *dnaA* gene exhibit oriC activity, thereby conferring the ability to replicate autonomously. In *Bacillus subtilis*, both the 5’ and 3’ flanking regions of *dnaA* act as oriC [4], whereas in *Mycobacterium tuberculosis* (*M.tb*), *M. bovis* [5] and *M. smegmatis* [6,7,8], only the 3’ flanking region provides oriC function. There are five DnaA-binding sites in the *oriC* region of *E. coli*, referred to as R boxes, to which both active ATP-DnaA and inactive ADP-DnaA proteins bind with equal affinity [9,10]. There are additional initiator binding sites in the *oriC* region referred to as I sites, to which only DnaA-ATP can bind [11].

DnaA protein binds with nearly equal affinity to ATP and ADP. In *E. coli* the function of ATP appears to be allosteric and the non-hydrolysable analogue ATP₇S can replace ATP in helix unwinding [12]. For opening of the DNA duplex multiple DnaA proteins, complexed with ATP, bind to *oriC* and melt the DNA unwinding element (DUE). ADP bound form of DnaA is inactive for replication initiation, forming an important level of regulation at the origin.

The *E. coli* IciA protein (Inhibitor of Chromosome Initiation) blocks initiation at very early stage in-vitro by binding specifically to A+T rich region of *oriC* [12,13]. Binding of IciA blocks the opening of A+T rich region mediated by DnaA and HU (Histone like protein) or integration host factor (IHF) protein and this inhibition of strand opening by IciA does not affect binding of DnaA and IHF (or HU) protein to their respective binding sites [14]. IciA contains helix turn helix motif at the N terminal region and shows homology to LysR family of prokaryotic transcription regulators [12]. IciA has also been implicated in binding to A+T rich regions within the plasmid *ori* sequence and the copy number of the F plasmid is increased in *iciA* deletion mutant [15]. IciA also shows higher binding preference for curved DNA [16]. Further, IciA is involved in regulation of *ndt* gene encoding ribonucleoside

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Mechanistic Insights into a Novel Exporter-Importer System of *Mycobacterium tuberculosis* Unravel Its Role in Trafficking of Iron

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**Abstract**

**Background:** Elucidation of the basic mechanistic and biochemical principles underlying siderophore mediated iron uptake in mycobacteria is crucial for targeting this principal survival strategy *vis-à-vis* virulence determinants of the pathogen. Although, an understanding of siderophore biosynthesis is known, the mechanism of their secretion and uptake still remains elusive.

**Methodology/Principal Findings:** Here, we demonstrate an interplay among three iron regulated *Mycobacterium tuberculosis* (*M.tb*) proteins, namely, Rv1348 (*IrtA*), Rv1349 (*IrtB*) and Rv2895c in export and import of *M.tb* siderophores across the membrane and the consequent iron uptake. *IrtA*, interestingly, has a fused N-terminal substrate binding domain (SBD), representing an atypical subset of ABC transporters, unlike *IrtB* that harbors only the permease and ATPase domain. SBD selectively binds to non-ferrated siderophores whereas Rv2895c exhibits relatively higher affinity towards ferrated siderophores. An interaction between the permease domain of *IrtB* and Rv2895c is evident from GST pull-down assay. In *vitro* liposome reconstitution experiments further demonstrate that *IrtA* is indeed a siderophore exporter and the two-component *IrtB*-Rv2895c system is an importer of ferrated siderophores. Knockout of *msmeg_6554*, the *irtA* homologue in *Mycobacterium smegmatis*, resulted in an impaired *M.tb* siderophore export that is restored upon complementation with *M.tb* *irtA*.

**Conclusion:** Our data suggest the interplay of three proteins, namely *IrtA*, *IrtB* and Rv2895c in synergizing the balance of siderophores and thus iron inside the mycobacterial cell.

**Introduction**

The survival of *Mycobacterium tuberculosis* (*M.tb*) within the hostile environment of the host macrophages depends upon a variety of mechanisms, including its ability to acquire essential nutrients from the host. Mycobacteria can acquire almost all the nutrients except iron that is sequestered within the host as an immune response against the invading pathogen [1]. In intracellular pathogens, assimilation of iron is an essential attribute to circumvent its deleterious effects of siderophore accumulation within the cells, have so far been identified only in few microorganisms.