Understanding Chromosome Replication and Segregation Unit of *Mycobacterium* and Its Comparative Analysis with Model Organisms: From Drug Targets to Drug Identification

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Abstract

Bacterium maintains its pathogenicity in the host by continuing replication and adopting temporal and spatial coordination of cell division steps such as cell wall synthesis, DNA replication, chromosome segregation, Z ring assembly, septum formation and finally cytokinesis. This multistep process requires spatiotemporal assembly of macromolecular complexes and is probably regulated by redundant and multifunctional activities of cell replication and division proteins. Two macromolecular assemblies of peptidoglycan biosynthesis, known as elongasome and divisome are known to drive the division of mother cell into two daughter cells and are characterized by the presence of signature protein complexes. Though the exact composition of macromolecular complexes is yet to be defined in *Mycobacterium*, the presence of some conserved proteins demonstrates the preservation of elementary units. Along with elongasome and divisome complexes, chromosome replication and segregation proteins are very important to understand as these proteins are very essential for bacilli survival, sustenance, and pathogenesis. In this review, along with presenting the differential features of *Mycobacterium* cell division process, we are comparing chromosome replication and segregation proteins of *Mycobacterium* with other bacterial species as we aim to identify structural and functional differences between these proteins in different species. In this review, we have also listed the potential drugs that can be tested to target *Mycobacterium* chromosome replication and segregation proteins. We expect that based on these differences identified, researchers would be able to direct their research in the characterization of *Mycobacterium* specific drug.

Keywords: Cell division proteins, Elongasome, Divisome, Chromosome replication and segregation unit, Drug-Drug targets identification

Introduction

The rise of multi drug and extensively drug resistant variants of *Mycobacterium tuberculosis* (*Mtbc*) has emphasized the need to study every physiological event of this pathogen in detail. Cell division is one such event, which regulates bacterial survival and sustenance. Status of bacterial replication is disparate in latent or active forms of mycobacterial infection. While latent form contains non-replicating or dormant form of mycobacteria, active tuberculosis is characterized by the presence of actively replicating bacilli [1]. However, the regulatory mechanism of switch in cell division mode in host by pathogen has not yet been properly understood.

Cell division in mycobacteria is less extensively characterized as compared to other model organisms. However, published reports related to *Mycobacterium* physiology have shown preservation of few elementary proteins with multifunctional properties. Most of these reports are suggestive of elongasome and divisome complexes, whose function is in regulating the doubling of cell mass and division of cell mass into two daughter cells. In the current review, the comparative and unusual features of the cell division process in mycobacteria with other model organisms is presented (Table 1). However, the central focus of this review is to analyze comparative analysis of chromosome replication and segregation proteins in mycobacteria from other model organisms as the information available is very limited and sparse. In addition, reviewing and identifying potential drugs for targeting the proteins involved in chromosome replication and segregation...
of the mycobacteria is the aim of the current review. We expect that the identification of possible drug targets from this very essential process of cell division would pave the path to target *Mycobacterium* induced pathogenesis, which is the leading cause of 1.7 million deaths every year.

### Chromosome Replication and Segregation System in Model Organisms

Though partial interdependent, independence among cell division events suggests diversified, complex signals and molecular processes involved in establishing coordination between them. The concept of increased cell mass as a main driving force for DNA segregation is found nullified with observed faster movement of replication fork in comparison to the elongation rate [2,3]. This observation suggests independent regulation of cell mass and chromosome segregation events [3].

The pattern of chromosome segregation is found variable between different bacterial organisms due to differential localization of chromosomal origins (ori) and proteins involved. Symmetric (*Escherichia coli* and vegetative cell division of *Bacillus subtilis*) versus asymmetric segregation (*Caulobacter crescentus* and chromosome I of *Vibrio cholerae*) of chromosomes requires organized or proper positioning of oriC and nucleoid compaction with respect to cell cycle [4]. While in symmetric segregation, the duplicated origins move rapidly to one quarter or three-quarter positions, which mark the pre-divisional sites, in asymmetric segregation, one of the replication origins localizes at the old pole and the other duplicated origin is directed towards the new pole. Existence

### Table 1. Presents differential features of *Mycobacterium* cell division as compared to other model organisms. (Please note that proteins connected through dash in the table represents that these proteins are part of the same interacting complex).

<table>
<thead>
<tr>
<th>Characteristic features of cell division and associated complexes</th>
<th>Model organisms</th>
<th><em>Mycobacterium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth pattern</strong></td>
<td>Lateral growth</td>
<td>Polar growth</td>
</tr>
<tr>
<td><strong>Cell duplication pattern</strong></td>
<td>Elongation and division sites are distinct. MreB, actin like protein is present to direct lateral growth. DivIVA is present and functions in divisome. Mixing of old and new cell wall material. Symmetric growth and evenly placed septa result in homogeneous population.</td>
<td>Absence of distinct elongation and division site. Division site becomes the elongation site for next cell division. Wag31, homolog of DivIVA protein is present to direct polar growth. No mixing of old and new cell wall material. Asymmetric growth and unevenly placed septa result in heterogeneous population. Differential sized cells are reported to have different antibiotic sensitivities.</td>
</tr>
<tr>
<td><strong>Absence or presence of unusual protein, LamA</strong></td>
<td>LamA protein is absent</td>
<td>LamA protein is present. Depletion studies has demonstrated the role of LamA in maintenance of heterogeneous population.</td>
</tr>
<tr>
<td><strong>Participating proteins, which do not allow septa formation over chromosome</strong></td>
<td>Noc, MinCDJ</td>
<td>Min D homolog (ssd) is identified.</td>
</tr>
<tr>
<td><strong>Early divisome complex</strong></td>
<td>FtsZ- FtsA- SepF-ZepF-EzrA</td>
<td>SepF is identified.</td>
</tr>
<tr>
<td><strong>Septal cell wall synthetic machinery</strong></td>
<td>MurA-G-Class A PBPs-RodA</td>
<td>MurA-G-Class A PBPs-RodA is reported. However, the functional properties of these proteins have been found to be different.</td>
</tr>
<tr>
<td><strong>Septal cell wall lysis machinery</strong></td>
<td>LytC-LytF, FtsEX</td>
<td>RipA/B, ChiZ, Ami1, FtsX-RipC</td>
</tr>
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</table>
of symmetric versus asymmetric segregation is observed within a single organism possessing multipartite genome with each of the chromosomes is known to segregate differentially for e.g., *V. cholerae* [5].

Involvement of multiple proteins in mediating chromosome segregation events indicates preservation of mitosis like mechanisms to ensure correct positioning and segregation in bacterial species. Replisome, the multiprotein machinery responsible for chromosome replication is categorized into three catalytic complexes: the helicase-primase complex, the core complex, and the clamp loader complex. The function of helicase-primase complex (DnaA-DnaG) is to ensure the DNA unwinding and synthesis of short primer on DNA strands. The core complexes (DNA polymerase) comprising Pol IIIα, the exonuclease subunit, ε, and the small subunit, θ, function in synthesis of the new DNA strand on both primers bound leading and lagging strand templates. The function of the clamp loader complex (τδθψξ,ψζ) is to coordinate the replication of both leading and lagging strand biosynthesis.

Chromosome replication is initiated by a protein DnaA (AAA+ ATPase) that leads to strand separation for loading of replication machinery comprising DNA polymerase III and accessory proteins [6,7]. The multiple accessory proteins are involved in chromosome replication, which recruits in a sequential step starting with DnaA (initiator protein), histone like proteins HU and integration host factor (responsible for strand separation), DnaC delivering DnaB (helicase), DnaG (primase) and finally DNA polymerase holoenzyme Pol III. During initiation step, the origin of replication (oriC) localizes at the middle of the cell, which shifts towards poles after duplication [8]. Positioning at poles requires rapid bidirectional movement of replication fork. Positioned oriC at new cell poles can initiate a new round of replication upon sensing signal. In contrast to the previously thought hypothesis where replication machinery is to visualized as a mobile (tracking model) component, now DNA is thought to serve as a mobile component, which passes through the replication machinery for its duplication [9]. Duplicated termini are known to mark invaginating septum (division) and serve as a signal for disassembly of replication machinery [2,10,11].

Resolution of linked replicated chromosomes is necessary to complete chromosome partition. In *E. coli* decatenation is accomplished by topoisomerase IV (topo IV), FtsK (Spo111E in *B. subtilis*) and XerC and XerD site-specific recombinases [12]. Additionally, FtsK is known to possess Walker type ATP binding sites, which helps in translocation of residual DNA out of septum forming site [13]. SpoIIIIE Bs (FtsK orthologue) is shown to translocate the replicated chromosome into spores [14]. ParA/ParB system constitutes an important component of chromosome segregation machinery in other bacterial species [15]. The essentiality of this system is found to be variable. While deletion of the ParA/ParB system is not tolerable in *C. crescentus* [16] and *Myxococcus xanthus* [17], it is tolerable but results in altered chromosome segregation phenotype in *Pseudomonas putida* [18]. Movement of segrosomes (ParB bound with ParS sequence) is thought to be governed by ParA (Walker A ATPases). In substitution of ParA/ParB system, *E. coli* uses MreB (actin like homolog) to segregate chromosomes [19]. Sporulation, developmental process in *B. subtilis*, requires existence of Soj/Spo0J (a member of ParA/ParB families) system [20]. While Δsoj and Δsoj-spo0J deletion impairs the effective chromosome partition between daughter cells, Δsoj does not lead to any observed chromosome segregation defect [21]. The observed redundant function of SMC protein with Soj and Spo0J in *B. subtilis* indicates the importance of SMC/Soj/Spo0J factors in maintenance of chromosome segregation event [21].

**Chromosome Replication and Segregation System in Mycobacterium**

Like any other bacterial kingdom, *Mycobacterium* establishes perfect coordination of chromosome segregation events with elongation and division rate to attain viable progenies. Being able to show polar mode of growth, *Mycobacterium* segregates chromosomes asymmetrically. This organism is known to comprise most of the homologs of the replisome machinery; however, few replisome proteins are either not yet annotated or characterized. Table 2 presents comparative analysis of three replisome catalytic centers (helicase-primase, core and clamp loader complex) in *Mycobacterium* from other model organisms.

Most of the components of *Mycobacterium* DNA replication machinery have been reported essential. These include the DnaA replication initiator, PriA helicase loader, DnaB helicase, DnaG primase, single strand DNA binding proteins (SSB), clamp loader subunits (τδθψξ,ψζ), DNA polymerases I and III, DnaN β-clamp, DNA ligase I (LigA), and type I (TopA) and II (DNA gyrase) topoisomerases [22]. The homologs of few replication initiator proteins, DnaC, DnaT, PriB, or PriC protein have not been identified.

Not only, *Mycobacterium* chromosome replication machinery is reported to lack usual molecular constituents, but also there are structural differences in the molecular constituents of mycobacterium from other bacterium species. For e.g., DnaE1 encoded DNA Pol III is known to lack θ subunit [23,24]. These differential features would have the potential to be applied in future research for identification of new mycobacterial drugs. Along with structural differences, these constituents are present differentially between *Mycobacterium* and other model organisms. While *Mycobacterium* is characterized by the presence of only one Topoisomerase II, which is DNA gyrase (GyrA,B), two types of topoisomerase (DNA gyrase and TopoIV) are present in most of the model organisms. In contrast to the six subunits of clamp loader complex present in model organisms, *Mycobacterium* is known to comprise 4...
subunits; τ /γ , encoded by dnaX, and the δ and δ ’ ATPases, encoded by holA and holB, respectively and all four subunits are reported to be essential in pathogenic Mtb [25].

Like ParA, ParB, and FtsK proteins of other bacterial species, these proteins are also reported to be involved in mycobacteria. However, their role in chromosome segregation has not yet been elucidated. While ParAB genes are essential in Mtb, they are found to be non-essential in Msmeg (non pathogenic form of Mycobacterium) [26]. However, deletion of parA and parB in Msmeg has been found to result in chromosome segregation defects and accumulation of 10 or 30% anucleated cells in ΔparB and ΔparA deleted cells, respectively [27]. Interestingly, the movement of ParB complex is shown to be slower in Mtb in comparison to the Msmeg but covering 10% of the cell cycle in both cases suggests a correlation between segregation dynamics and growth rate.

Additionally, ParA localization at poles and transiently at septum indicates its participation during a stage of division [28]. Localization of ParB at quarter cell positions and its abrogation in ΔparA background indicates ParA is important for ParB localization [26]. Moreover, demonstration of ParA interaction with Wag31 indicates ParA functionality as an anchor protein and for maintenance of dynamics between elongasome and chromosome segregation unit [26]. However, it is difficult to detangle the exact function of chromosome segregation units because of presence of abundant factors and pleiotropic nature of mutants. Mycobacterium is reported to have structural maintenance of chromosome (SMC) paralogs, wherein Msmeg is known to possess three SMC paralogs EptC, MSMEG_370, and MSMEG_2423, Mtb contains only one SMC (Rv2922c) protein [29]. These molecules are believed to play an important role in chromosomal organization, compaction, and partitioning. However, the mechanical insights of all these molecules need further investigation.

### Targeting Chromosome Replication and Segregation Event of Mycobacteria: Possible Drug-targets and Drugs

Identifying a possible cognate set of drug-drug targets is difficult for many human pathogens and it becomes more difficult for mycobacteria as the pathogen is difficult to handle. Moreover, constructing the knockout strains for its essential proteins is another laborious task, which makes such study difficult. In addition, the drugs which have been proved earlier to target purified Mycobacterium protein in vitro, are shown to lose efficacy when screen is performed with whole cell based and in vivo infection assays. Despite these challenges, efforts by the researchers have resulted in characterizing novel drug target-drug pairs in mycobacteria.

### Inhibitors Targeting Initiator and Helicase-primase Proteins of Chromosome Replication

Reports of potential drugs that can target molecular constituents of helicase-primase complex in model organisms are demonstrated. However, the potential utility of these drugs remains to be elucidated in the context of Mycobacterium. By disrupting the interaction of SSB (single strand binding protein) with PriA (helicase loader), many inhibitors targeting bacterial SSB have been identified. 9-hydroxyphenylfluoron...
is reported as the most potential one as it is reported to be associated with minimal activity against the human SSB homolog [30]. Similarly, kaempferol and myricetin, the PriA inhibitors have been identified in Streptococcus aureus (S. aureus) [31]. Efforts have identified pyrido-thieno-pyrimidines and benzo-pyrimido-furans as DnaG inhibitors [32], flavonols as DnaB inhibitors [33,34] and fluoroquinolones as topoisomerase II poisons [35]. Researchers are trying to understand the role of these inhibitors against mycobacterial proteins. Fluoroquinolones are reported to block the process of transcription and replication, which eventually results in damaged DNA. These inhibitors exert its DNA damaging activity via acting on DNA bound gyrase and topoisomerase IV proteins. [36].

Different classes of fluoroquinolones have been reported to exhibit differential efficacy and associated side effects in the treatment of Mycobacterium induced pathogenesis. In comparison to other fluoroquinolones, clinical trials conducted with moxifloxacin, in combination with standard antituberculosis agents for 4 months is reported to be associated with less or acceptable side effects [37]. Lack of any evidence of hypo or hyperglycemia or tendinopathies in this clinical trial has suggested its potential to serve as an antituberculosis agent [36]. Aminocoumarin (i.e. novobiocin), another topoisomerase inhibitor, is reported to act differentially from fluoroquinolones [37,38]. It is demonstrated to work by inhibiting the ATPase activity of DNA gyrase and present a potential drug candidate to be tested against Mycobacterium infection.

In a similar line, Topoisomerase I (TopA) inhibitor hydroxycamptothecin is screened against both drug susceptible Mtb and drug resistant XDR strains. This compound is found to demonstrate significant bactericidal activity against XDR form of TB in comparison to the drug susceptible TB [38]. Similarly, other small molecules inhibitors i.e., imipramine, norclomipramine, and m-AMSA have been reported against Mtb TopA [39-42]. Two additional hits, amasacrine and tryptanthrin, which is reported against bacterial TopA need to be tested against Mycobacterium infection [43]. Similarly, purified M.tb LigA, demonstrated to show NAD dependent DNA sealing activity, is reported to be inhibited by several compounds i.e., N substituted tetracyclic indole, glycosylamines [44-47]. In vitro and in vivo screens have successfully demonstrated the functional significance of these LigA inhibitors as bactericidal [44-47].

### Inhibitors Targeting DNA Polymerase Complexes

The ability of genotoxic, nargenicin to bind between the DNA terminal base pair and the DnaE1 polymerase indicates its potency to act as a DNA replication inhibitor in Mycobacterium [48]. However, the observations in this study are based on in vitro screens. DNA polymerases in gram (+) and gram (-) bacteria is reported to be targeted by guanine inhibitors, which prevents dGTP binding [49,50]. Compound 251D, a hybrid molecule comprising 6-(3-ethyl4-methylanilino) uracil and fluoroquinolone moieties is another bacterial Pol IIIa inhibitor identified. Based on homology modeling and molecular docking studies, compound 251D has been reported as an inhibitor for Mtb DnaE1 [51]. The functional significance of these compounds as potential anti-mycobacterial agents needs to be discovered.

### Inhibitors Targeting Clamp Loading Complex and Chromosome Segregation Proteins

The natural product, Griselimycin isolated from Streptomyces has been identified as the novel inhibitor for DnaN encoded clamp protein. Along with in vitro studies, the bactericidal potency of this inhibitor against Mycobacterium has been

<table>
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<tr>
<th>S. No</th>
<th>Drug Name [Ref]</th>
<th>Drug Target</th>
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<tbody>
<tr>
<td>2</td>
<td>kaempferol and myricetin [31]</td>
<td>PriA</td>
</tr>
<tr>
<td>4</td>
<td>Flavonols [33,34]</td>
<td>DnaB (helicase)</td>
</tr>
<tr>
<td>5</td>
<td>Fluoroquinolones [35]</td>
<td>Topoisomerase IV, DNA gyrase (Topoisomerase II)</td>
</tr>
<tr>
<td>6</td>
<td>Aminocoumarin [37,38]</td>
<td>DNA gyrase</td>
</tr>
<tr>
<td>7</td>
<td>Hydroxycamptothecin, Imipramine, Norclomipramine and m-AMSA [38-41]</td>
<td>TopA (Topoisomerase I)</td>
</tr>
<tr>
<td>8</td>
<td>N substituted tetracyclic indole, glycosylamines [43-47]</td>
<td>LigA (DNA-ligase)</td>
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<tr>
<td>9</td>
<td>Nargenicin, Guanine inhibitors, Compound 251D [48-51]</td>
<td>DnaE1 (DNA polymerase)</td>
</tr>
<tr>
<td>10</td>
<td>Griselimycin [52]</td>
<td>DnaN (Clamp loader protein)</td>
</tr>
<tr>
<td>11</td>
<td>Phenoxybenzamine, octoclothepin [53]</td>
<td>ParA (Chromosome segregation protein)</td>
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identified in in vivo mice infection studies [52]. In addition, Par A is discovered as an interesting target for two drugs namely phenoxybenzamine and octoclothepin. These inhibitors have been reported to show bacteriostatic activity by disturbing the ATPase activity of Par A [53]. Other components of the chromosome segregation system are under investigation so that a series of suitable inhibitors against mycobacterium can be identified.

Conclusions and Future Perspectives

Continuous emergence of new MDR (multi drug resistant) and XDR (extensive drug resistant) drug resistant strains of Mtb and tuberculosis cases has forced the researchers to identify novel sets of drug targets and drugs. These new sets of drugs are expected to show their activities towards both drug sensitive and resistant tuberculosis cases. Moving forward in the direction of identifying novel combinations of drug target-drugs requires careful formulation of appropriate approaches. This formulated approach should result in identification of high affinity and selective multiple targets for a single drug. However, in cases where selectivity and sensitivity get compromised, identification of multiple substrates for a single drug can be disadvantageous.

Through this review, we are reporting that the process of cell division including chromosome replication and segregation is different in Mycobacterium from the other bacterial species. These differential features can be due to the presence of different molecular proteins and their altered structure and activities. We expect that these differential targets can serve as possible drug candidates, not only in drug sensitive cases but also in drug resistant cases. In this review, we have reported various potential drugs, which via targeting chromosome segregation systems can act as anti-mycobacterial agents. These potential drugs have been identified through either in vitro or murine infection experiments. Clinical trials conducted with some of these drugs have presented evidence, which supports the fact that these drugs have potential applications in strategizing combinatorial therapy against Mycobacterium pathogenesis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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