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Review Article

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 (Mtb)* 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

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).

Characteristic features of cell division and associated complexes	**Model organisms**	***Mycobacterium***
Growth pattern	• Lateral growth	• Polar growth
Cell duplication pattern	• Elongation and division sites are distinct. • MreB, actin like protein is present to direct lateral growth. Div1VA is present and functions in divisome. • Mixing of old and new cell wall material. • Symmetric growth and evenly placed septa result in homogeneous population.	• Absence of distinct elongation and division site. Division site becomes the elongation site for next cell division. • Wag31, homolog of Div1VA 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.
Absence or presence of unusual protein, LamA	• LamA protein is absent	• LamA protein is present. Depletion studies has demonstrated the role of LamA in maintenance of heterogeneous population.
Participating proteins, which do not allow septa formation over chromosome	• Noc, MinCDJ	• Min D homolog (ssd) is identified.
Early divisome complex	• FtsZ- FtsA- SepF-ZepF-EzrA	• SepF is identified.
Late divisome complex	• FtsK-FtsQLB-FtsW-FtsI	• FtsK, FtsQLB, FtsW, FtsI • FtsZ-FtsW-FtsI is known. • FtsZ-CrgA-PbpA-PbpB • CrgA-CwsA-Wag31 • FtsZ-FhaB-FtsQ (under oxidative stress)
Septal cell wall synthetic machinery	• MurA-G-Class A PBPs-RodA	• MurA-G-Class A PBPs-RodA is reported. However, the functional properties of these proteins have been found to be different.
Septal cell wall lysis machinery	• LytC-LytF, FtsEX	• RipA/B, ChiZ, Ami1, FtsX-RipC

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 cresecentus* 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

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 ($\tau_3\delta_1\delta'_1\chi_1\psi_1$) 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 topoisomerse 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]. SpoIIIE *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 govern 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 *Δspo0J* 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/SpoJ 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 (τ /γ, δ, δ '), 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 ($GyrA_2B_2$), 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

Table 2. Represents comparative analysis of chromosome replication and segregation unit in *Mycobacterium* from other model organisms.

Chromosome replication unit	**Model organisms**	***Mycobacterium***
DNA replication initiator or helicase-primase complex	DnaA, DnaB, DnaC, DnaG, DnaT, DNA gyrase (Topoisomerase II), Topo IV (Topoisomerase II) DNA topoisomerase I, PriA, PriB, PriC	DnaA, DnaB, DnaG, SSB, DNA gyrase (Topoisomerase II, GyrA2B2), DNA topoisomerase I (TopA) , PriA • Reported to lack DnaC, DnaT, PriA PriC and TopoIV
DNA replication complex or core complex	DNA Polymerase IIIα with the exonuclease subunit, ε, and the small subunit, θ	DNA Polymerase IIIα with the exonuclease subunit, ε. • Reported to lack small subunit, θ
Clamp loader complex	$\tau_3\delta_1\delta'_1\chi_1\psi_1$	$\tau/\gamma\ \delta_1\delta'$ • Reported to lack $\chi_1\psi_1$
Chromosome segregation Unit	**Model organisms**	***Mycobacterium***
Chromosome segregation pattern	Symmetric or Asymmetric	Asymmetric
Chromosome segregation proteins	Topoisomerse IV, FtsK (SpoIIIE in *B. subtilis*), XerC and XerD site-specific recombinases, Par A, ParB and SMC etc	Par A, ParB, FtsK, SMC (EptC,MSMEG_370, and MSMEG_2423 in *Msmeg* and Rv2922c in *Mtb*)

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 the 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 *M.tb* 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 IIIα 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 3. Presents list of potential drugs with the potential to target mycobacterium chromosome segregation system.

S. No	Drug Name [Ref]	Drug Target
1	9-hydroxyphenylfluoron [30]	SSB (Single strand binding protein)
2	kaempferol and myricetin [31]	PriA
3	pyrido-thieno-pyrimidines, benzo-pyrimido-furans [32]	DnaG (Primase)
4	Flavonols [33,34]	DnaB (helicase)
5	Fluoroquinolones [35]	Topoisomerase IV, DNA gyrase (Topoisomerase II)
6	Aminocoumarin [37,38]	DNA gyrase
7	Hydroxycamptothecin, Imipramine, Norclomipramine and m-AMSA [38-41]	TopA (Topoisomerase I)
8	N substituted tetracyclic indole, glycosylamines [43-47]	LigA (DNA-ligase)
9	Nargenicin, Guanine inhibitors, Compound 251D [48-51]	DnaE1 (DNA polymerase)
10	Griselimycin [52]	DnaN (Clamp loader protein)
11	Phenoxybenzamine, octoclothepin [53]	ParA (Chromosome segregation protein)

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.

References

1. Hett EC, Rubin EJ. Bacterial growth and cell division: a mycobacterial perspective. Microbiology and Molecular Biology Reviews. 2008 Mar;72(1):126-56.

2. Bates D, Kleckner N. Chromosome and replisome dynamics in E. coli: loss of sister cohesion triggers global chromosome movement and mediates chromosome segregation. Cell. 2005 Jun 17;121(6):899-911.

3. Cooper S. Regulation of DNA synthesis in bacteria: analysis of the Bates/Kleckner licensing/initiation-mass model for cell cycle control. Molecular Microbiology. 2006 Oct;62(2):303-7.

4. Badrinarayanan A, Le TB, Laub MT. Bacterial chromosome organization and segregation. Annual Review of Cell and Developmental Biology. 2015 Nov 13;31:171-99.

5. Val ME, Soler-Bistué A, Bland MJ, Mazel D. Management of multipartite genomes: the Vibrio cholerae model. Current Opinion in Microbiology. 2014 Dec 1;22:120-6.

6. Mott ML, Berger JM. DNA replication initiation: mechanisms and regulation in bacteria. Nature Reviews Microbiology. 2007 May;5(5):343-54.

7. Johnson A, O'Donnell M. Cellular DNA replicases: components and dynamics at the replication fork. Annu Rev Biochem. 2005 Jul 7;74:283-315.

8. Berkmen MB, Grossman AD. Spatial and temporal organization of the Bacillus subtilis replication cycle. Molecular Microbiology. 2006 Oct;62(1):57-71.

9. Lemon KP, Grossman AD. Localization of bacterial DNA polymerase: evidence for a factory model of replication. Science. 1998 Nov 20;282(5393):1516-9.

10. Lau IF, Filipe SR, Søballe B, Økstad OA, Barre FX, Sherratt DJ. Spatial and temporal organization of replicating Escherichia coli chromosomes. Molecular Microbiology. 2003 Aug;49(3):731-43.

11. Wang X, Possoz C, Sherratt DJ. Dancing around the divisome: asymmetric chromosome segregation in Escherichia coli. Genes & Development. 2005 Oct 1;19(19):2367-77.

12. Kato JI, Nishimura Y, Imamura R, Niki H, Hiraga S, Suzuki H. New topoisomerase essential for chromosome segregation in E. coli. Cell. 1990 Oct 19;63(2):393-404.

13. Errington J. Septation and chromosome segregation during sporulation in Bacillus subtilis. Current Opinion in Microbiology. 2001 Dec 1;4(6):660-6.

14. Wu LJ, Errington J. Bacillus subtilis SpoIIIE protein required for DNA segregation during asymmetric cell division. Science. 1994 Apr 22;264(5158):572-5.

15. Kim HJ, Calcutt MJ, Schmidt FJ, Chater KF. Partitioning of the linear chromosome during sporulation of Streptomyces coelicolor A3 (2) involves an oriC-linked parAB locus. Journal of Bacteriology. 2000 Mar 1;182(5):1313-20.

16. Mohl DA, Easter Jr J, Gober JW. The chromosome partitioning protein, ParB, is required for cytokinesis in Caulobacter crescentus. Molecular Microbiology. 2001 Nov;42(3):741-55.

17. Harms A, Treuner-Lange A, Schumacher D, Søgaard-Andersen L. Tracking of chromosome and replisome dynamics in Myxococcus xanthus reveals a novel chromosome arrangement. PLoS Genetics. 2013 Sep 19;9(9):e1003802.

18. Lewis RA, Bignell CR, Zeng W, Jones AC, Thomas CM. Chromosome loss from par mutants of Pseudomonas putida depends on growth medium and phase of growth. Microbiology. 2002 Feb;148(2):537-48.

19. Madabhushi R, Marians KJ. Actin homolog MreB affects chromosome segregation by regulating topoisomerase IV in Escherichia coli. Molecular cell. 2009 Jan 30;33(2):171-80.

20. Wu LJ, Errington J. RacA and the Soj-Spo0J system combine to effect polar chromosome segregation in sporulating Bacillus subtilis. Molecular Microbiology. 2003 Sep;49(6):1463-75.

21. Lee PS, Grossman AD. The chromosome partitioning proteins Soj (ParA) and Spo0J (ParB) contribute to accurate chromosome partitioning, separation of replicated sister origins, and regulation of replication initiation in Bacillus subtilis. Molecular Microbiology. 2006 May;60(4):853-69.

22. Ditse Z, Lamers MH, Warner DF. DNA Replication in Mycobacterium tuberculosis. Microbiol Spectr. 2017 Mar;5(2):10.1128/microbiolspec. TBTB2-0027-2016.

23. Boshoff HI, Reed MB, Barry CE, Mizrahi V. DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in Mycobacterium Tuberculosis. Cell. 2003 Apr 18;113(2):183-93.

24. Baños-Mateos S, van Roon AM, Lang UF, Maslen SL, Skehel JM, Lamers MH. High-fidelity DNA replication in Mycobacterium tuberculosis relies on a trinuclear zinc center. Nature Communications. 2017 Oct 11;8(1):855.

25. DeJesus MA, Gerrick ER, Xu W, Park SW, Long JE, Boutte CC, et al.Comprehensive essentiality analysis of the Mycobacterium tuberculosis genome via saturating transposon mutagenesis. MBio. 2017 Mar 8;8(1):e02133-16.

26. Ginda K, Bezulska M, Ziółkiewicz M, Dziadek J, Zakrzewska-Czerwińska J, Jakimowicz D. ParA of M ycobacterium smegmatis co-ordinates chromosome segregation with the cell cycle and interacts with the polar growth determinant DivIVA. Molecular Microbiology. 2013 Mar;87(5):998-1012.

27. Ginda K, Santi I, Bousbaine D, Zakrzewska-Czerwińska J, Jakimowicz D, McKinney J. The studies of ParA and ParB dynamics reveal asymmetry of chromosome segregation in mycobacteria. Molecular Microbiology. 2017 Aug;105(3):453-68.

28. Maloney E, Madiraju M, Rajagopalan M. Overproduction and localization of Mycobacterium tuberculosis ParA and ParB proteins. Tuberculosis. 2009 Dec 1;89:S65-9.

29. Panas MW, Jain P, Yang H, Mitra S, Biswas D, Wattam AR, et al. Noncanonical SMC protein in Mycobacterium smegmatis restricts maintenance of Mycobacterium fortuitum plasmids. Proceedings of the National Academy of Sciences. 2014 Sep 16;111(37):13264-71.

30. Glanzer JG, Endres JL, Byrne BM, Liu S, Bayles KW, Oakley GG. Identification of inhibitors for single-stranded DNA-binding proteins in eubacteria. Journal of Antimicrobial Chemotherapy. 2016 Dec 1;71(12):3432-40.

31. Huang YH, Huang CC, Chen CC, Yang KJ, Huang CY. Inhibition of Staphylococcus aureus PriA helicase by flavonol kaempferol. The Protein Journal. 2015 Jun;34:169-72.

32. Agarwal A, Louise-May S, Thanassi JA, Podos SD, Cheng J, Thoma C, et al. Small molecule inhibitors of E. coli primase, a novel bacterial target. Bioorganic & Medicinal Chemistry Letters. 2007 May 15;17(10):2807-10.

33. Griep MA, Blood S, Larson MA, Koepsell SA, Hinrichs SH. Myricetin inhibits Escherichia coli DnaB helicase but not primase. Bioorganic & Medicinal Chemistry. 2007 Nov 15;15(22):7203-8.

34. Lin HH, Huang CY. Characterization of flavonol inhibition of DnaB helicase: Real-time monitoring, structural modeling, and proposed mechanism. Journal of Biomedicine and Biotechnology. 2012 Oct 2;2012:735368.

35. Dwyer DJ, Collins JJ, Walker GC. Unraveling the physiological complexities of antibiotic lethality. Annual Review of Pharmacology and Toxicology. 2015 Jan 6;55:313-32.

36. Drlica K. Mechanism of fluoroquinolone action. Current Opinion in Microbiology. 1999 Oct 1;2(5):504-8.

37. Gillespie SH, Crook AM, McHugh TD, Mendel CM, Meredith SK, et al. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. N Engl J Med. 2014;371:1577-87.

38. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, et al. The transcriptional responses of Mycobacterium tuberculosis to inhibitors of metabolism: novel insights into drug mechanisms of action. Journal of Biological Chemistry. 2004 Sep 17;279(38):40174-84.

39. Ravishankar S, Ambady A, Awasthy D, Mudugal NV, Menasinakai S, et al. Genetic and chemical validation identifies Mycobacterium tuberculosis topoisomerase I as an attractive anti-tubercular target. Tuberculosis. 2015 Sep 1;95(5):589-98.`

40. Sandhaus S, Annamalai T, Welmaker G, Houghten RA, Paz C, et al. Small-molecule inhibitors targeting topoisomerase I as novel antituberculosis agents. Antimicrobial Agents and Chemotherapy. 2016 Jul;60(7):4028-36.

41. Godbole AA, Ahmed W, Bhat RS, Bradley EK, Ekins S, et al. Inhibition of Mycobacterium tuberculosis topoisomerase I by m-AMSA, a eukaryotic type II topoisomerase poison. Biochemical and Biophysical Research Communications. 2014 Apr 18;446(4):916-20.

42. Godbole AA, Ahmed W, Bhat RS, Bradley EK, Ekins S, et al. Targeting Mycobacterium tuberculosis topoisomerase I by small-molecule inhibitors. Antimicrobial Agents and Chemotherapy. 2015 Mar;59(3):1549-57.

43. Wall ME, Wani MC, Cook CA, Palmer KH, McPhail AA, et al. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from camptotheca acuminata1, 2. Journal of the American Chemical Society. 1966

Aug;88(16):3888-90.

44. Gong C, Martins A, Bongiorno P, Glickman M, Shuman S. Biochemical and genetic analysis of the four DNA ligases of mycobacteria. Journal of Biological Chemistry. 2004 May 14;279(20):20594-606.

45. Srivastava SK, Dube D, Kukshal V, Jha AK, Hajela K, et al. NAD+-dependent DNA ligase (Rv3014c) from Mycobacterium tuberculosis: Novel structure-function relationship and identification of a specific inhibitor. Proteins: Structure, Function, and Bioinformatics. 2007 Oct;69(1):97-111.

46. Srivastava SK, Dube D, Tewari N, Dwivedi N, Tripathi RP, et al. Mycobacterium tuberculosis NAD+-dependent DNA ligase is selectively inhibited by glycosylamines compared with human DNA ligase I. Nucleic Acids Research. 2005 Jan 1;33(22):7090-101.

47. Srivastava SK, Tripathi RP, Ramachandran R. NAD+-dependent DNA ligase (Rv3014c) from Mycobacterium tuberculosis: crystal structure of the adenylation domain and identification of novel inhibitors. Journal of Biological Chemistry. 2005 Aug 26;280(34):30273-81. `

48. Chengalroyen MD, Mason MK, Borsellini A, Tassoni R, Abrahams GL, et al. DNA-dependent binding of nargenicin to DnaE1 inhibits replication in Mycobacterium tuberculosis. ACS Infectious Diseases. 2022 Feb 10;8(3):612-25.

49. Wright GE, Brown NC, Xu WC, Long ZY, Zhi C, et al. Active site directed inhibitors of replication-specific bacterial DNA polymerases. Bioorganic & Medicinal Chemistry Letters. 2005 Feb 1;15(3):729-32.

50. Xu WC, Wright GE, Brown NC, Long ZY, Zhi CX, et al. 7-Alkyl-N2-substituted-3-deazaguanines. Synthesis, DNA polymerase III inhibition and antibacterial activity. Bioorganic & Medicinal Chemistry Letters. 2011 Jul 15;21(14):4197-202.

51. Chhabra G, Dixit A, Garg LC. DNA polymerase III α subunit from Mycobacterium tuberculosis H37Rv: Homology modeling and molecular docking of its inhibitor. Bioinformation. 2011;6(2):69-73.

52. Kling A, Lukat P, Almeida DV, Bauer A, Fontaine E, et al. Targeting DnaN for tuberculosis therapy using novel griselimycins. Science. 2015 Jun 5;348(6239):1106-12.

53. Nisa S, Blokpoel MC, Robertson BD, Tyndall JD, Lun S, et al. Targeting the chromosome partitioning protein ParA in tuberculosis drug discovery. Journal of Antimicrobial Chemotherapy. 2010 Nov 1;65(11):2347-58.