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Replisome dynamics during the cell cycle of *Mycobacterium smegmatis*.

Abstract

The genus *Mycobacterium* includes both human (*M. leprae* and *M. tuberculosis*) and animal (*M. bovis*) pathogens - causative agents of severe diseases, which have a profound impact on global health and economy. So far, mycobacteria have mainly been studied in the contexts of their pathogenicity. However, little is know about the cell cycle of these slow- growing bacteria. Recent studies revealed, that mycobacteria exhibit an unusual mode of cell elongation and division. In contrast to *Escherichia coli* and *Bacillus subtilis*, they exhibi polar cell extension and often divide asymmetrically generating daughter cells that differ in size and elongation rate. These substantial differences between mycobacteria and other model bacteria (*E. coli*, *B. subtilis*), as well as the relative paucity of knowledge about chromosome dynamics during the mycobacterial cell cycle, prompted me to study the dynamics of chromosome replication during life cycle of *M. smegmatis* – a nonpathogenic model organism that is often used to study the biology of tubercle bacilli. For this purpose, I constructed fluorescent reporter strains expressing different subunits of DNA polymerase III (catalytic subunit alpha and DnaN protein – the sliding clamp) fused with different fluorescent proteins. Time-lapse microfluidic microscopy analyses revealed that mycobacterial replisomes are highly dynamic, frequently splitting into two distinct replication forks. However, unlike in *E. coli*, the forks do not segregate toward opposite cell poles but remain in relatively close proximity. In addition, I show that the replisomes are slightly off-center, a feature that is likely correlated with the asymmetric mode of growth. Moreover, I show that mycobacterial population exhibits heterogeneity in the dynamics of DNA replication. In addition to cells with non-overlapping replication rounds, I unexpectedly observed cells with multifork replication. Thus, this study demonstrated for the first time, that reinitiation of another round of replication within the single cell cycle does also occur in slow-growing bacteria. Data presented here contributed to better understanding of cell biology of tubercle bacilli.