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The influence of the topoisomerase I (TopA) level on the cell cycle in *Mycobacterium smegmatis*.

Bacterial chromosome is a highly organised structure which is meticulously controlled at different stages of the cell-cycle. Negative supercoiling substantially contributes to compaction of chromosomal DNA and is essential for progression of the key cell processes such as chromosome replication and transcription. Hence, the variation in DNA superhelical density impairs basic cell functions. DNA topology is regulated by enzymes named topoisomerases. Topoisomerase I (TopA), which is categorized into type I family of topoisomerases, causes the relaxation of negative supercoils conducting the reaction through the transitional cleavage of single-stranded DNA. Topoisomerases as enzymes playing a pivotal role in the proper cell function appear to be a potential target for antibacterial drugs.

The tubercle bacilli are particularly problematic to combat pathogens due to their capacity to circumvent host defense system and survive in a dormant state for a prolonged time, in addition to their unusually impermeable cell wall. *Mycobacterial* TopA protein differs from its rod-shaped bacteria homologues with its high processivity and the presence of a long, C-terminal domain with unconserved structure. Although a growing body of evidence describes the activity of TopA in *Mycobacteriaceae*, as well as intracellular mycobacterial chromosome organization and spatial dynamics of DNA replication and segregation, still the biological consequences of TopA inhibition and changes of DNA topology have been scarcely studied in these bacteria.

Relatively rapidly-growing *M. smegmatis* may be regarded a convenient model to study the biology of *Mycobacteria* along with pursuing new targets for antituberculosis drugs. The doctoral project examined how the alterations in TopA level affect the growth and chromosomal dynamics of *M. smegmatis*. It was demonstrated that *topA* is essential for *M. smegmatis* and the significant reduction of TopA level lead to a decline in growth rate, however, interestingly the partial reduction of the protein level did not induce any growth inhibition. Overproduction of topoisomerase I, in turn, did not cause changes in the growth rate of bacteria. Furthermore, the time-lapse studies allowed the observation that reduction of TopA level disturbed the DNA replication process. It was found that TopA depleted *M. smegmatis* strain exhibited increased incidence of replication reinitiation, which caused uneven cell divisions, and indirectly affected the cell length. Moreover, it was determined that a TopA C-terminal domain is required for TopA function *in vivo*. Over-production of C-

terminal domain caused cell cycle disorder similar to observed at TopA depletion however, in a lesser extent. The biological consequences of the TopA C-terminal domain over-production may result from its involvement in the intermolecular interactions. Work performed under doctoral project confirmed that topoisomerase I could be a suitable target for antibiotics however suggested that only complete inhibition of its activity results in inhibition of *M. smegmatis* proliferiation indicating the involvement of other topoisomerases in topology maintenance. Moreover my studies elucidated the fundamental role of the TopA C-terminal domain for the cell function which suggest that it could be molecular target for antituberculosis drugs.