Topoisomerases, topology modulatory proteins and topology – transcription coupling in mycobacteria

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DNA topoisomerases are attractive targets for lead molecule development by virtue of their essential role in replication, transcription and other DNA transaction processes. Our studies with mycobacterial DNA gyrase and topoisomerase 1 have led us to unravel many of their distinctive features. We have exploited the differences in their properties to design mycobacterial enzyme specific inhibitors. For example, gyrase specific peptide inhibitors derived from the inhibitory monoclonal antibodies jam the exit gate not allowing the enzyme to complete supercoiling cycle. Inhibition of DNA supercoiling activity of gyrase isolated from fluroquinolone resistant *M.tuberculosis* confirm the distinct mechanism of action. Different inhibitory antibodies developed against topoisomerase 1 also exhibit novel mechanism of action.

Next, I will discuss chromosomally encoded gyrase interacting protein that inhibits the enzyme in *in vitro* reactions. Glutamate racemase encoded by *murI* catalyses the essential function of conversion of L- glutamate to D form needed for cell wall biosynthesis. MurI binds gyrase A subunit and as a result, prevents gyrase binding to DNA affecting all the subsequent steps in the reaction cycle. Mutants with highly reduced racemase activity, inhibite DNA gyrase revealing the racemase activity independent moon lighting function of MurI.

Transcription relies on DNA topology at different steps of the process. The positive supercoiling ‘waves’ generated ahead of the advancing polymerase (RNAP) are resolved by the action of DNA gyrase. We have considered DNA gyrase and RNAP functioning in close proximity to each other instead of acting at a distance as conceived in the twin supercoiled domain model of transcription. We have isolated the physically interacting functional complex, termed ‘topotranscriptosome’, comprising of both DNA gyrase and RNAP activities. In the complex, the two essential enzymes enhance each other’s activities.