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“Interplay between topoisomerase I and RNA polymerase of *Chlamydia trachomatis*”

Chlamydia trachomatis (Ct) is a gram-negative, obligate intracellular bacterium that is the leading cause of sexually transmitted bacterial infection worldwide. Ct has a unique developmental cycle, alternating between an infectious elementary body (EB) and a non-infectious reticulate body (RB). Precise signals that trigger changes in Ct development remain unclear, but two notable aspects are relevant: temporal gene expression and varied DNA supercoiling. The latter is sustained by the DNA topoisomerases (Topos). Previously, our lab utilized the recently-developed CRISPR technique to knock down Ct *topA* encoding topoisomerase I (TopA), which relaxes negative supercoils in bacteria. We observed that *topA* repression impaired EB-to-RB transition; conversely, expression of late genes was downregulated and early genes maintained their expression, highlighting the important link of DNA supercoiling and the developmentally regulated gene expression. RNA polymerase (RNAP) is responsible for transcription and consists of $\alpha_2\beta\beta'\omega$ subunits (core enzyme) and a promoter-specific σ factor in bacteria. We hypothesize that by directly interacting with the RNAP, TopA participates in the regulation of transcription during the chlamydial developmental cycle.

To begin to test this hypothesis, the objective of my study was to determine whether chlamydial TopA binds to the full-length chlamydial β subunit of RNAP, RpoB, *in vitro.* Two different strategies were employed. First, we co-transformed *E. coli* cells with two different expression vectors, one cloned with *topA* and a sequence of 6-histidine to its 3’ and the other one cloned with *rpoB*. Second, the plasmid vector cloned with *topA* and a sequence of 6-histidine to its 5’ or cloned with *rpoB* was individually transformed into *E. coli*. The expression of proteins of interested were induced by addition of proper inducers, followed by assessing the protein expression levels, purification of proteins, and the characterization of the complex of TopA and RpoB using chromatography, binding assay, SDS-PAGE, and Western blot analysis.

We confirmed the inducible expression of His6-TopA, TopA-His6, or RpoB in *E. coli*. Interestingly, we found that co-expression of TopA-His6 with RpoB resulted in a high level of RpoB expression compared to that in *E. coli* expressing RpoB only, implying the potential action of TopA in modification of gene expression likely via changing DNA supercoiling. Moreover, we provide evidence that His6-TopA is efficient in binding to RpoB and produces a stable protein complex *in vitro.*

Our results indicate a direct interaction between chlamydial TopA, and the RNAP mediated by its core subunit, RpoB, consistent with the role of TopA in affecting transcription elongation. How such direct interaction may affect the expression of highly transcribed genes at the site of transcription in *C. trachomatis* will be further studied.