Interplay between Topoisomerase I and RNA Polymerase of *Chlamydia trachomatis*

Amanda Baltar dos Santos, Li Shen.

Louisiana State University Health Sciences Center (LSUHSC) Department of Microbiology

Introduction

- ***Chlamydia trachomatis*** **is a gram-negative, obligate intracellular bacterium that is the leading cause of sexually transmitted infection (STI). In 2020, ~128.5 million new infections with *C. trachomatis* occurred worldwide among adults aged 15 to 49 years.**
- **All *Chlamydia* strains share a unique developmental cycle that alternates between an infectious elementary body (EB) and a non-infectious reticulate body (RB).**
- **During the developmental cycle, *C. trachomatis* expresses its genes in a temporal fashion.**
- **Transcription is the first and key step of gene expression and controlled by RNA polymerase (RNAP) composed by $\alpha_2\beta\beta'\omega$ subunits (core enzyme) and a sigma factor (σ).**

Chlamydial Topoisomerase I (TopA)

- **Topoisomerase I (TopA) is an essential enzyme and recognized target of antimicrobial and anti-cancer agent.**
- **It removes the hyper negative supercoils generated on the DNA template by the progressing RNAP complex during transcription elongation.**
- **Previously, our laboratory utilized the recently developed CRISPR technique to knock down *topA* encoding TopA.**
- **Repression of *topA* 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.**

Hypothesis

By directly interacting with the RNAP, TopA participates in the regulation of transcription during the chlamydial developmental cycle.

Methods

- **We constructed three different plasmids:**
  - **(1) pETHis6*topA* (in which *his$_6$-topA* is controlled by IPTG-inducible T7 promoter).**
  - **(2) pET28rpoB (in which *rpoB* is under the control of IPTG-inducible T7 promoter).**
  - **(3) pBOMBL-*topAH6* (*topA-his$_6$* is under the control of aTC-inducible P_{tet} promoter).**
- **Plasmids were transformed into the *E. coli* CodonPlus cells individually or in combination.**
- **Proteins of interest were expressed in the presence of appropriate inducer(s).**
- **Proteins were purified by chromatography techniques.**
- **SDS-PAGE and Western blot analysis were performed to verify the presence of TopA and RpoB.**
- **Binding Assay was performed to determine the ability of H6TopA interacting with the RpoB.**

Results

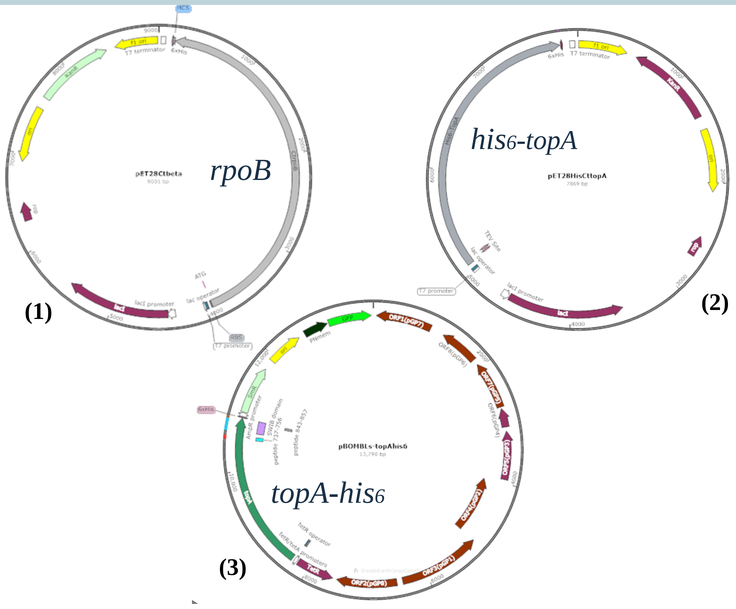

Figure 1. Map of the plasmids used.
(1)-(2) co-transformation of pET28rpoB and pETHis6topA.
(1)-(3) single transformation with pET28rpoB or pBOMBL-topAH6.

Inducible expression of proteins

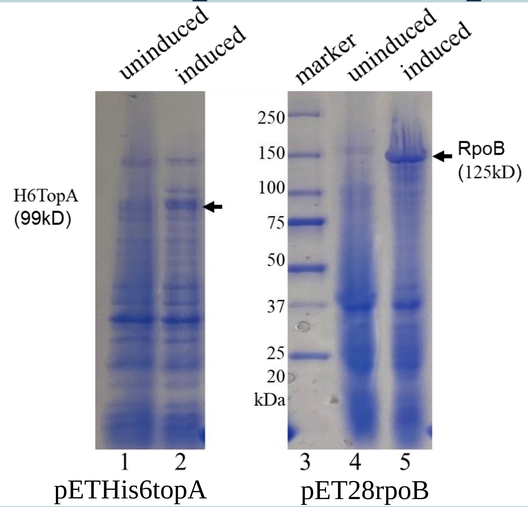

Figure 2. SDS-PAGE and Coomassie blue staining confirming inducible expression of topAH6 (left) and RpoB (right) in single transformed strains. Lane 1,4: uninduced; lane 2: aTC (200 µg/mL) induced; lane 3: marker; lane 5: IPTG (100mM) induced.

Co-expression of proteins

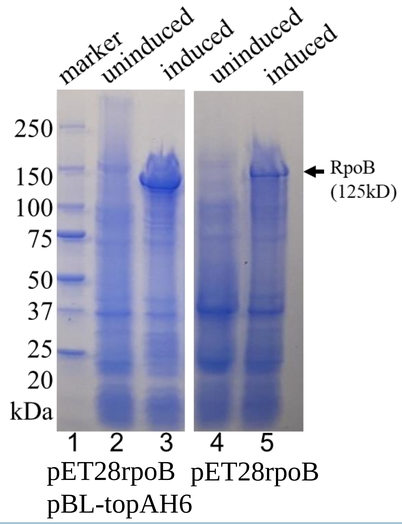

Figure 3. Comparing the levels of RpoB expression in co-transformed cells (left) to the single transformed cells (right) using SDS-PAGE and Coomassie blue staining. Plasmids used are as indicated.
Lane 1: Marker;
Lanes 2,3: co-transformation;
Lanes 4-5: single transformation.

Direct TopA-RpoB interaction

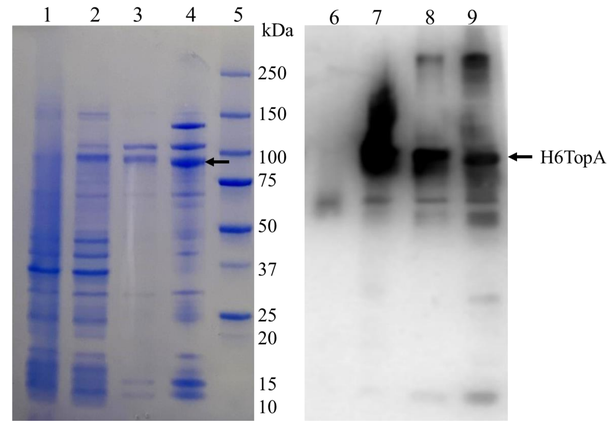

Figure 4. Verifying expression and presence of H6TopA using SDS-PAGE (left) and Coomassie blue staining, and Western blot (right). Lanes 1,6: uninduced bacterial lysate; lanes 2,7: aTC-induced bacterial lysate; lanes 3,8: H6TopA bound Ni-NTA beads; lanes 4,9: H6TopA and RpoB complex; Lane 5: marker.

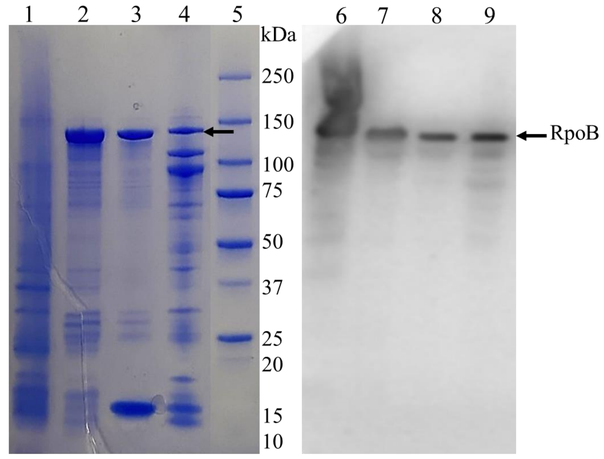

Figure 5. Verifying expression and presence of RpoB using SDS-PAGE and Coomassie blue staining (left), and Western blot (right). Lanes 1,6: uninduced bacterial lysate; lanes 2,7: IPTG-induced bacterial lysate; lanes 3,8: H6TopA bound Ni-NTA beads; lanes 4,9: H6TopA-RpoB complex; Lane 5: marker.

Conclusion and Future Research

- **We successfully expressed and purified RpoB and TopA from *E. coli.***
- **We observed higher expression of RpoB in the presence of TopA in *E. coli*.**
- **His6-TopA can efficiently bind to RpoB producing a stable protein complex *in vitro*.**
- **Future studies include to determine how direct interaction between TopA and RNAP may affect expression of highly transcribed genes in *C. trachomatis*.**

References

Shen, Li *et al.* "Targeted repression of DNA topoisomerase I by CRISPRi reveals a critical function for it in the *Chlamydia trachomatis* developmental cycle." mBio vol. 15,2 (2024): e0258423. doi:10.1128/mbio.02584-23

This research project was supported by Award Number: DBI-2349224 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program, National Institutes of Allergy and Infectious Diseases grants AI146454.