Bacterial Two Hybrid Analysis of *Chlamydia trachomatis* Proteins

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ABSTRACT

Chlamydia trachomatis is an obligate intracellular bacterial pathogen which poses severe health problems throughout the world. There are over 90 million new cases annually, making it the most common sexually transmitted disease in the world. *Chlamydia* can pose significant problems during and after infection. It is imperative to understand how *Chlamydia* manipulates the host cell and potentially develop future treatments. The mechanisms by which *C. trachomatis* alters immune response is not well understood, but recent work has identified an interaction between the chlamydial inclusion membrane protein, CT226, and the potential interacting host proteins, Flightless homologue II (FLII), Leucine Rich-Repeat Flightless-Interacting Proteins 1&2 (LRRFIP1 and LRRFIP2) and TMOD3. FLII, LRRFIP1 and LRRFIP2 are known to interact as a complex and are upstream regulators of the inflammasome. Currently, it is unknown if CT226 interacts with one or all of the interacting partners and needs the actual interaction needs investigation. My hypothesis is that CT226 will directly interact with one of the 3 potential interacting partners (LRRFIP1, LRRFIP2, and FLII) and that we will be able to detect this interaction in the bacterial two hybrid system. Current efforts have focused on cloning CT226 into PUT18 and the three host proteins (FLII, LRRFIP1 and LRRFIP2) individually into PKNT25. PUT18 and PKNT25 are the bait and prey plasmids that have been adapted for use in the bacterial two hybrid system. Once each pair of plasmids are properly transformed (each *E. coli* strain will have two plasmids, one carrying CT226 and one carrying the potential interacting partner), they will be screened using traditional β-galactosidase assays or cAMP assays to determine the specific interactions.

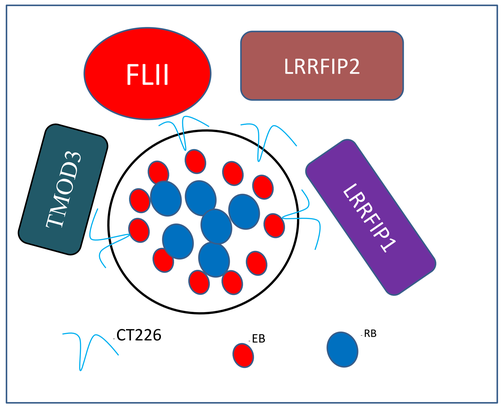

Figure 1. Schematic representation of CT226 interaction with TOMD3, FLII, LRRFIP1, and LRRFIP2.

INTRODUCTION

Chlamydia trachomatis is an obligate intracellular bacterial pathogen which poses severe health problems throughout the world. There are over 90 million new cases annually, making it the most common sexually transmitted disease in the world (1). Specific interactions between *C. trachomatis* and the host cell are important for survival due to its reduced genome (2). *Chlamydia* can pose significant problems during and after infection. It is imperative to understand how *Chlamydia* manipulates the host cell to obtain a better understanding of the pathogen and potentially develop future treatments. When *Chlamydia* first infects a cell, it does so as an elementary body (EB) which converts into a reticulate body (RB) during the replicative phase and then slowly transitions back to the EB infectious form at the end of the life cycle before infecting neighboring cells. The mechanisms by which *C. trachomatis* alters immune response is not well understood, but recent work done by my lab and other labs (3) have identified an interaction between the chlamydial inclusion membrane protein, CT226, and the potential interacting host proteins, Flightless homologue II (FLII), and Leucine Rich-Repeat Flightless-Interacting Proteins 1&2 (LRRFIP1 and LRRFIP2) and TMOD3. These three proteins (FLII, LRRFIP,1 and LRRFIP2) are known to work as a complex and are also known to interact with each other. My hypothesis is that CT226 will directly interact with one of the 3 potential interacting partners (LRRFIP1, LRRFIP2, and FLII) and that we will be able to detect this interaction in the bacterial two hybrid system.

METHODS

Cloning: A polymerase chain reaction (PCR) is then performed using sequence specific primers and Dreamtaq master mix. The PCR products are then visualized on an agarose gel. If the PCR is successful, then the PCR amplicons and undergo an overnight digest with restriction enzymes. The vector (PUT18C and pKT25) were cut using the same restriction sites. After digestion, the DNA is concentrated and cleaned with Zymo Clean and Concentrate kit. This concentrates the PCR products and purifies it from any contaminants/nucleotides/primers/enzymes. This product is then run on a final agarose gel before ligation. A ligation is then performed, which inserts the potential interacting partner DNA sequence into the corresponding vector.

Bacterial transformation: For the cloning transformation, DH5α cells are used. The entire ligation mixture is put into the competent cell preparation, heat shocked at 42°C, mixed with 750µL of SOC media, and incubated in shaker for 1 hour at 37°C. The transformation is then plated onto LB agar plates with 100 ng/mL ampicillin and grown overnight at 37°C.

For the Bacterial-two hybrid, DHM1 or BTH101 competent cells are used for transformation. The same heat shock transformation method as DH5α is used. The final constructs in the PKT25 series (prey) and PUT18C series (bait) (one bait and one prey) are both transformed into the same bacterial strain.

RESULTS

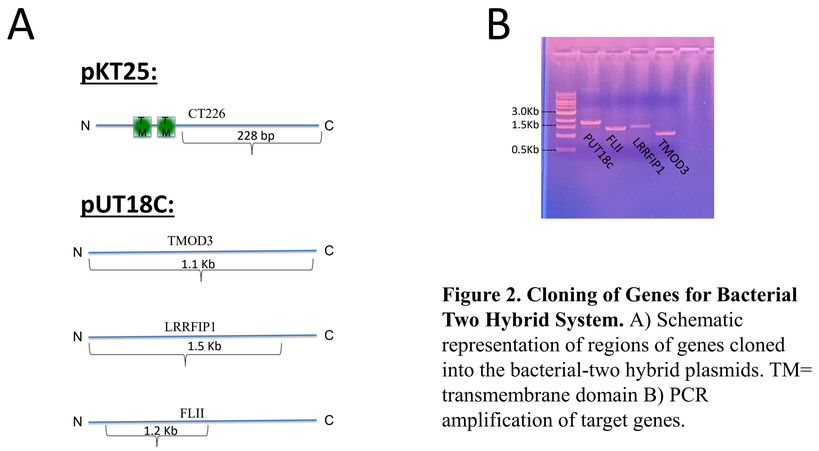

Figure 2. Cloning of Genes for Bacterial Two Hybrid System. A) Schematic representation of regions of genes cloned into the bacterial-two hybrid plasmids. TM= transmembrane domain B) PCR amplification of target genes.

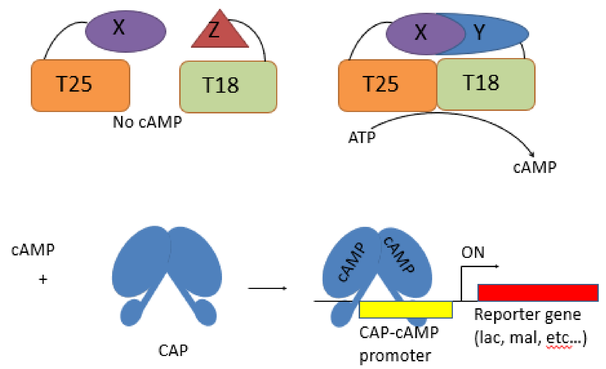

Figure 3. Mechanism of Bacterial-Hybrid interaction. When two proteins interact, cAMP is generated which interacts with CAP complex initiating transcription of target reporter genes including β-galactosidase and ability to utilize maltose for catabolism.

CONCLUSIONS and FUTURE DIRECTIONS

- CT226, TMOD3 and FL2 have successfully been cloned into the bacterial two hybrid
- LRRFIP1 are currently in the process of being cloned
- Cloning of LRRFIP2 will commence after cDNA clone has been obtained
- Once the final constructs are finished, we will assay for interactions between CT226, TMOD3, LRRFIP1 and FLII in the bacterial-two hybrid with growth on maltose and β-galactosidase expression

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REFERENCES

1. Robert C. Burnham and Jose Rey-Ladino. "Immunology of *Chlamydia* Infection: Implications for a *Chlamydia* Trachomatis Vaccine." Nature Reviews: Immunology 5 (2005): 149-161.

2. Gitsels A, Sanders N, Vanrompay D. Chlamydial Infection From Outside to Inside. Front Microbiol. 2019 Oct 9;10:2329. doi: 10.3389/fmicb.2019.02329. PMID: 31649655; PMCID: PMC6795091.

3. Olson MG, Widner RE, Jorgenson LM, Lawrence A, Lagundzin D, Woods NT, Ouellette SP, Rucks EA. Proximity Labeling To Map Host-Pathogen Interactions at the Membrane of a Bacterium-Containing Vacuole in Chlamydia trachomatis-Infected Human Cells. Infect Immun. 2019 Oct 18;87(11):e00537-19. doi: 10.1128/IAI.00537-19. PMID: 31405957; PMCID: PMC6803327.