

Interactions between *Chlamydia trachomatis* and the Host Cell

With approximately 90 million cases occurring worldwide, the Gram-negative obligate intracellular pathogen, *Chlamydia trachomatis*, can be considered to be the most commonly transmitted bacterial sexually transmitted infections¹. After clearing of an infection, women can develop tubal infertility, pelvic inflammatory disease and have increased risk for the development of cervical cancer²³. In addition to being a sexually transmitted infection, *Chlamydia* is also the leading cause of infectious blindness worldwide, with most of the cases being concentrated in third world countries; such as those in Africa. * Clearly, *Chlamydia* is a common pathogen that can have severe medical consequences after an infection. As such, understanding what *Chlamydia* is doing to the host cell is imperative to prevent long term consequences of infection.

Chlamydia is an intercellular pathogen, meaning that it needs to gain entry into a host for the infection to continue. Once *Chlamydia* has entered the host cell, it forms an inclusion, a protective niche inside of which *Chlamydia* will grow and replicate. The membrane of the inclusion acts as an interface for communication between *Chlamydia* and the host. How is this made possible? *Chlamydia* secretes proteins by a special mechanism called the type three secretion system. Some of these proteins will insert themselves into the inclusion membrane, while others will be secreted and into the host cell and are called secreted effectors. So far researchers have identified 5 chlamydial secreted effector proteins. However, little is known about what these effectors do and what host proteins they interact with during an infection⁴.

The aim of this project is to clone 3 of these secreted effector proteins into a yeast-two hybrid (Y2H) system. The Y2H system is an established scientific method that can be used to identify potential interacting proteins. **The hypothesis is that these chlamydial effector proteins interact with unidentified host proteins.**

Before I can try to identify interacting partners for the *Chlamydial* effector proteins, they must first be cloned into the Y2H system. To do this, I will use gene specific primers to perform polymerase chain reaction (PCR) amplification using *Chlamydia* DNA as a template to generate many copies of the desired genes. Each gene will be amplified separately. To begin the process, the Y2H plasmid and each gene will be cut with complementary specific restriction endonucleases. This will create sticky DNA ends that can be temporarily stuck together due to the complementary sequences that each strand contains. Once generated, the DNA fragments (Y2H plasmid and chlamydial gene) will be sealed together with DNA ligase, which is an enzyme that will glue the sticky ends together, resulting in a plasmid. The final resulting plasmid will be then transformed into *E. coli* and sequenced to verify that the product has been successfully generated correctly. It is anticipated this production will take one semester. However, if this objective is completed early, I will proceed to start using the Y2H system to try and identify potential interacting host cell protein partners for each of these chlamydial proteins.

The diversity of infections and the long term consequences of chlamydial infections warrant further investigation of how *Chlamydia* manipulates the host cell. The cloning of these genes into the yeast-two hybrid system will provide an excellent platform to identify what host proteins *Chlamydia* interacts with during infection. Future implications of this research are huge as it can identify novel therapeutic targets or maybe even vaccine candidates.

References

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