Chlamydia trachomatis: The key to human pathogen and host interactions

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Abstract

Chlamydia trachomatis is an obligate intracellular pathogen that is commonly sexually transmitted. While *Chlamydia* infections are easily treated with antibiotics, there are long-term health consequences such as pelvic inflammatory disease and ectopic pregnancy that may still occur in patients who received treatment and cleared the infection. The focus of this study is on these long-term effects and the reasons that they are occurring. Once the pathogen has entered the host cell, it forms an inclusion and manipulates the host from within. This project focused on the host kinases that were recruited to the inclusion by *Chlamydia* as well as what happened to the kinase substrates during the course of infection. Through immunofluorescence, it was determined that there are multiple host kinases and kinase substrates that are recruited to the *Chlamydia* inclusion. Western blotting was then used to identify which kinases and kinase substrates were being manipulated by the *Chlamydia* throughout the infection process. Western blotting identified PKA substrate phosphorylation changes. The other substrates tested, however, indicated minimal phosphorylation changes indicating that *C. trachomatis* has a strategy in which it selects certain host substrates to utilize in the inclusion. The manipulation of certain host kinases by the *C. trachomatis* inclusion is a key component in host-pathogen interactions and may lead to novel therapeutic targets in the future.

Introduction

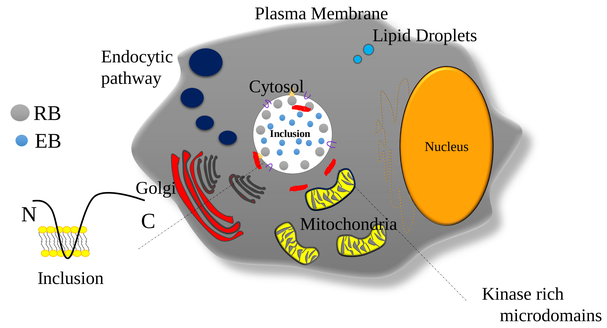

Figure 1. Life cycle of *C. trachomatis*

Chlamydia trachomatis is an obligate intracellular pathogen that is commonly sexually transmitted among humans (3). In fact, it is the most commonly reported sexually transmitted disease in the United States with an estimated three million *Chlamydia* infections each year (1). Of these approximately three million people, only about one half have been found to seek treatment because *Chlamydia* is often asymptomatic (1). Untreated infections can have short and long-term negative consequences. However, even after treatment and clearance of the infection, long term health problems such as pelvic inflammatory disease, scarring of the fallopian tubes, tubal factor infertility (4) and ectopic pregnancies are still of great concern (2). These long-term effects caused by *C. trachomatis* infections are the primary focus of this research project. This project focuses on identifying which kinase substrates in the cell signaling pathway are manipulated by *C. trachomatis*. One kinase and corresponding kinase substrates identified as being actively manipulated during infection was Protein Kinase A (PKA). PKA and its substrates are known to play a significant role in cell proliferation and have been linked to many different cancers (5). We hypothesize that *C. trachomatis* alters these substrates over the course of infection in order to aid in its survival.

Results

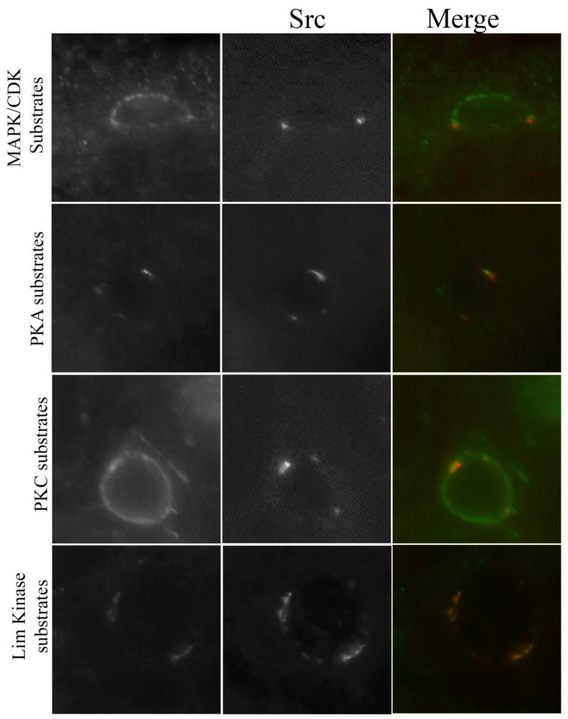

Figure 2. Immunofluorescence images showing recruitment of host kinases and kinase substrates to the Chlamydial inclusion. Map Kinases/CDK phosphorylated substrates, PKA phosphorylated substrates, PKC phosphorylated substrates and Lim Kinase are shown in green. Active Src Kinases (red) were used to visualize discrete microdomains. Merged images demonstrate colocalization with microdomains or peripheral staining of kinases and kinases substrates.

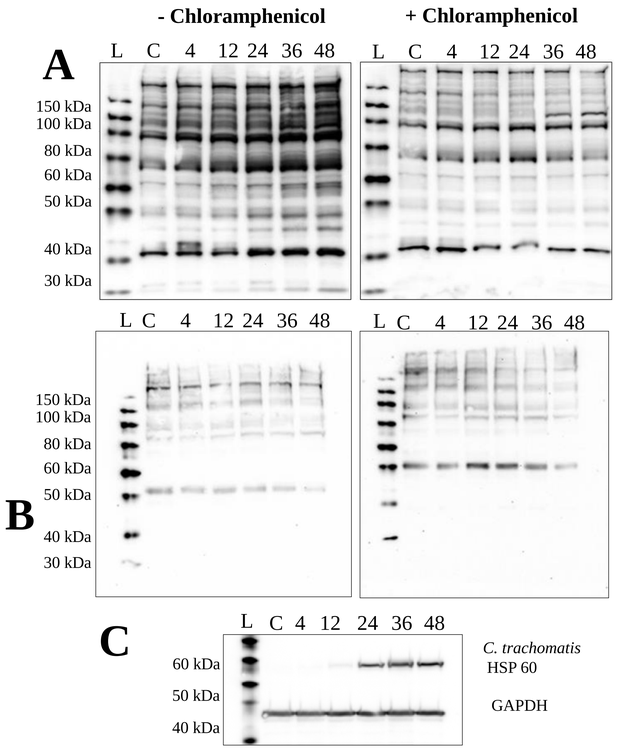

Figure 3. Western blot analysis of phosphorylated kinase substrates during *C. trachomatis* infection. A) P-PKA substrates, B) CDK kinase Substrate. C) GAPDH loading controls and HSP60 to detect *C. trachomatis* are shown. LEFT (-Chloramphenicol), RIGHT (+ Chloramphenicol). No changes were observed in substrates P-MEK, P-LIMK, and P-PLC gamma 2.

Conclusions

- Multiple host kinases and kinase substrates are recruited to the inclusion during *C. trachomatis* infection
- PKA substrate phosphorylation changes during infection
- Minimal changes in phosphorylation of kinase substrates are seen with multiple kinases suggesting that *C. trachomatis* kinase pathway manipulation is strategic

Future Directions

- Identify specific host substrates that are differentially phosphorylated through Mass Spec analysis
- Examine additional kinase pathways for changes in regulation
- Test to see if phosphorylation changes in specific pathways is altered *in vivo*

Acknowledgements

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Methods

Immunoflourescence

HeLa cells grown on round glass coverslips in 24 well plates were infected with *C. trachomatis* L2 for 24 hours, fixed in methanol for 10 minutes and blocked in PBS + 0.1% BSA overnight. Primary antibodies to kinases (described earlier) and active Src Kinases were diluted in block solution, added to cells, incubated at room temperature for 1 hour washed 3 times in PBS. Secondary antibodies anti-rabbit 488 and anti-mouse 594 (Jackson Immuno Research) were diluted in block, added to cells and incubated for 1 hour at room temperature. Cells were washed 3 times in PBS and coverslips mounted on microscope slides. *C. trachomatis* inclusions were imaged using a Nikon Eclipse 80i. Images were assembled in Photoshop.

Western Blotting

HeLa cells were infected with *C. trachomatis* L2 and lysed with SDS-PAGE sample buffer at various time points post infection (0hr, 4hr, 24hr, 48hr). Half the samples were then treated with chloramphenicol. Samples, both treated and not treated with chloramphenicol, were separated by SDS-PAGE at 125v for 1.5 hours. Gels were transferred to nitrocellulose membranes using Tris Glycine buffer at 100 V for 1 hour. Blots were blocked incubated with primary antibodies overnight at 4°C. All primary antibodies were purchased from Cell Signaling Technologies. Blots were incubated with anti-mouse or anti-rabbit secondary antibodies also made by Cell Signaling (Anti-rabbit IgG, HRP-linked Antibody or Anti-mouse IgG, HRP linked Antibody). Each gel was developed with Cell Signaling Signal Fire ECL reagent, visualized and imaged.

References

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