Abstract

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, lifelong 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).

Reviews Microbiology, 48 (Research) 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.

Methods

Immunofluorescence

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 times 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.

Results

Figure 2. Immunofluorescence images showing recruitment of host kinases and kinase substrates to the Chlamydia 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 kinase substrates.

Figure 3. Western blot analysis of phosphorylated kinase substrates during C. trachomatis infection. A) 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.

Introduction

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).

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

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References