A Comparison of Mutated versus Wild Type *Scc4* Phenotype in *Chlamydia trachomatis* Infected Cells

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Introduction

Chlamydia trachomatis is an obligate intracellular bacteria which needs a host cell in order to survive and replicate. Testing the effect of individual genes on chlamydial function by genetic approach has shown, in the past, to be difficult because *Chlamydia* has a small genome (~1 Mbps), where many of the genes coded for, including *scc4*, are essential for survival of the bacteria.

The study of Scc4, while still limited, indicates that Scc4 takes on a dual role in *Chlamydia* involved with both transcription and the Type III Secretion System (T3SS). Scc4 interacts with the RNA polymerase holoenzyme to modulate transcription, which may give the protein some control over gene expression. To participate in the T3SS, Scc4 forms a complex with Scc1 to chaperone the T3SS effector CopN, so that the CopN is exported effectively and timely into the host cells.

To examine the effect of *scc4* on *Chlamydia* infection, the plasmids with mutated *scc4* genes were created. The first mutant contained six alanine substitutions in the amino acid sequence, and in the second mutant, amino acid 49 was changed from isoleucine to valine. These plasmids were transformed into a *C. trachomatis,* resulting in strains, L2/6A and L2/I49V; that were used to infect HeLa epithelial cells. Changes in phenotype and gene expression of *C. trachomatis* were assessed using microscopy and PCR analysis. As expected, we observed the presence of plasmid-encoded *scc4* in the L2/I49V. Surprisingly, no plasmid-encoded genes were detected in L2/6A. Both mutant strains exhibit an altered phenotype during infection in HeLa cells.

Understanding the mechanisms of gene expression in *Chlamydia trachomatis* can lead to enhanced abilities to control infection and spread.

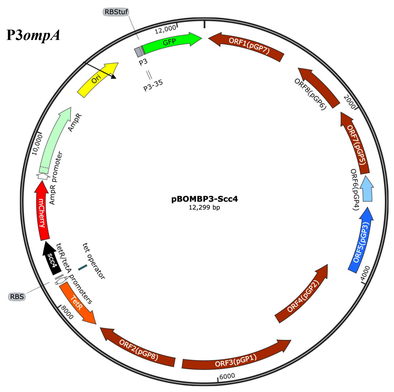
Mutation
- **Plasmid pBOMB-Scc4 used as a vector to create the new plasmids with mutated *scc4*.**

Transformation
- **The new mutated plasmids were transformed into *C. trachomatis* L2, resulting in strains L2/6A and L2/I49V.**

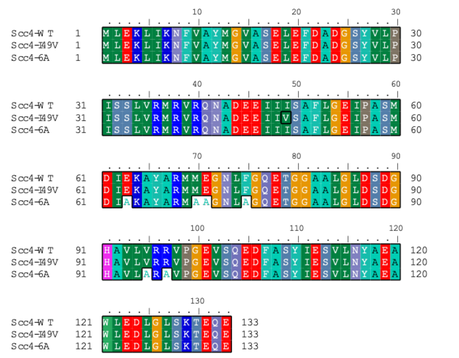
Genotype Analysis
- **PCR was used with select primers to amplify a section of plasmid and determine if gene was present.**

Phenotype Analysis
- **Microscopy was used to visually analyze bacteria and inclusions of mutated vs. wild type Scc4 in chlamydial infected cells.**

Design of Mutants



Plasmid map of backbone



Location of Mutations in Amino Acid Sequence

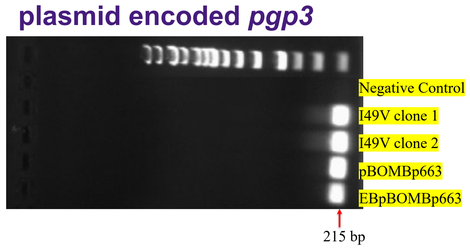
Results

PCR Analysis of L2/I49V Mutant

L2/I49V containing plasmid encoded *scc4*

L2/I49V containing plasmid encoded *ampR*

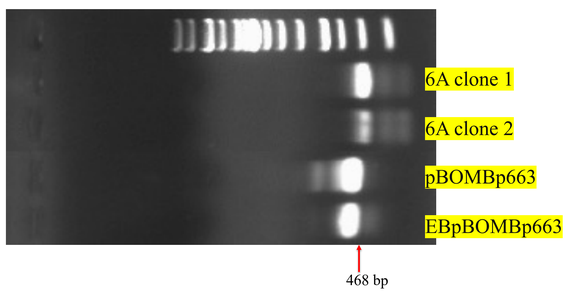
L2/I49V containing plasmid encoded *pgp3*



PCR analysis was able to show the correct fragment of mutated *scc4* in the plasmid, as well as the plasmid-encoded *pgp3* and *ampR*.

PCR Analysis of L2/6A Mutant

L2/6A containing plasmid encoded *scc4*



Strain L2/6A did contain *scc4* as evidenced by positive PCR results. We were unable to consistently confirm the presence of other genes on the plasmid.

References

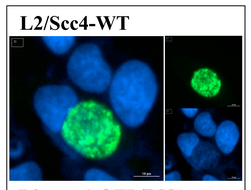
Gao, L., Cong, Y., Plano, G.V., Rao, G., Gisclair, L.N., Bartra, S.S., Macnaughtan, M.A., & Shen, L. (2020). Context-Dependent Action of Scc4 Reinforces Control of the Type III Secretion System. *American Society for Microbiology Journal of Bacteriology, 202.* (15), https://doi.org/10.1128/JB.00132-20.

Shen, L., Macnaughtan, M.A., Frohlich, K.M., Cong, Y., Goodwin, O.Y., Chou, C., LeCour, L., Krup, K., Luo, M., & Worthylake, D.K. (2015). Multipart Chaperone-Effector Recognition in the Type III Secretion System of *Chlamydia trachomatis*. *Journal of Biological Chemistry, 290.* (47), 28141-28155. https://doi.org/10.1074/jbc.M115.670232.

Wang, Y., Kahane, S., Cutcliffe, L.T., Skilton, R.J., Lambden, P.R., & Clarke, I.N. (2011). Development of a transformation system for Chlamydia trachomatis: restoration of glycogen biosynthesis by acquisition of a plasmid shuttle vector. *PLoS pathogens, 7*(9). E1002258 https://doi.org/10.1371/journal.ppat.1002258.

Microscopy Used to Visualize *Chlamydia* within the Inclusions

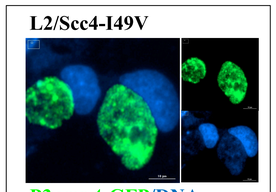
L2/Scc4-WT



P3*ompA*-GFP/DNA

Even and expected distribution of the GFP+ organisms (green). DAPI-stained DNA (blue)

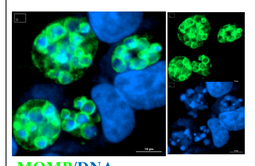
L2/Scc4-I49V



P3*ompA*-GFP/DNA

Uneven, irregular distribution of the GFP+ organisms. (green) DAPI-stained DNA (blue)

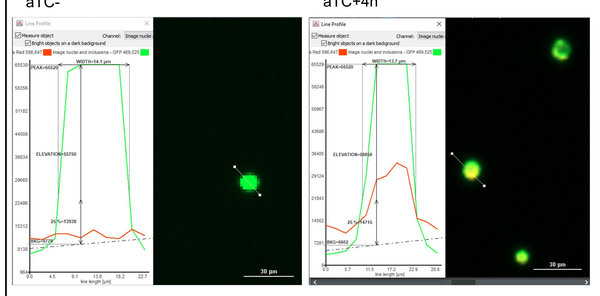
L2/Scc4-6A



MOMP/DNA

Enlarged reticulate bodies. Used antibody staining to visualize bacteria (green). DAPI-stained DNA (blue).

Inducible expression of P*tet* in *C. trachomatis*



The conditional induction of the P*tet* in *Chlamydia*. Left: minimal P*tet* function in the absence of aTC. Right: the aTC-induced expression of P*tet*-mCherry co-transcribed with the *scc4*.

aTC added 4 hours post infection. Imaging taken 30 hours post infection.

Summary

The L2/6A mutant exhibited the mutated fragment of *scc4*. We unexpectedly were not able to confirm the presence of any other genes in the plasmid via PCR. This could possibly mean that certain sections of the plasmid are incorrect or that the *scc4* fragment came from chromosome DNA.

The I49V mutant showed the correct mutated *scc4* fragment as well as *pgp3* and *ampR* genes using PCR. This indicates that the plasmid is present and correct.

Altering the amino acid sequence of *scc4* did in fact result in a change in the phenotype presented. Microscopy revealed that both mutants showed abrant distribution of inclusions, with the I49V mutant also showing enlarged reticulate bodies in the presence of ampicillin. Future studies would further explore the effect of *scc4* on phenotype in *Chlamydia trahcomatis* particularly on the growth rate on infected cells.

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