The Expression of OmcA in *Chlamydia muridarum* is Modified in Response to Environmental Stress

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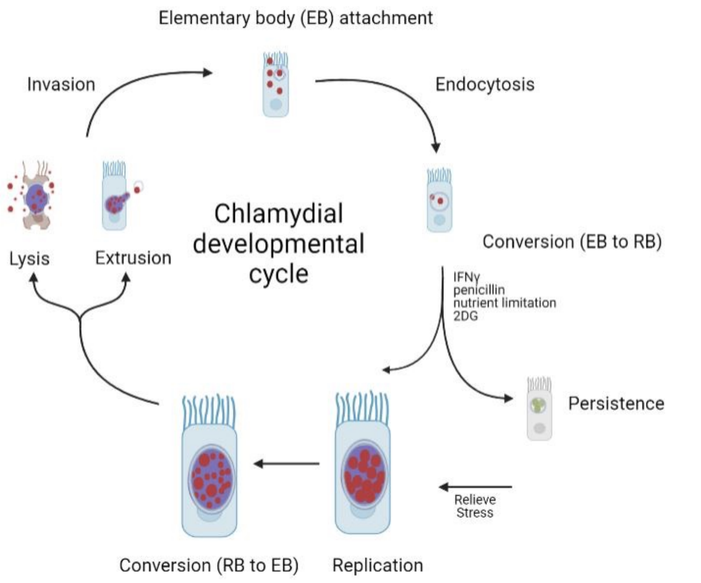
Introduction



GlgA: Virulence protein found to be downregulated under glucose stress in *C. trachomatis* but not *C. muridarum.*[1]

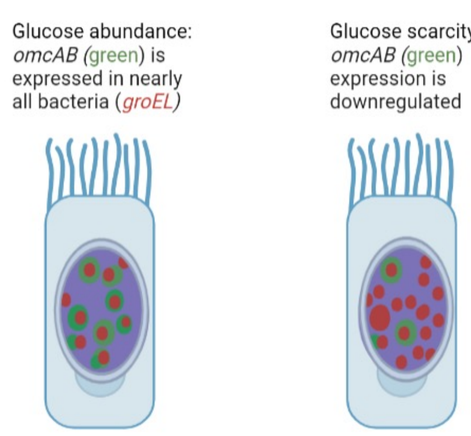


Figure 1. *Chlamydia trachomatis* lifecycle relating to development and glucose availability.[2]

Hypothesis

Unlike *Chlamydia trachomatis, Chlamydia muridarum* fails to modulate OmcA expression in response to environmental stress.

Methods

- Seed L929 cells and infect them with *C. trachomatis and C. muridarum* at 1 MOI and treat with stressors: 2-deoxyglucose (2DG), penicillin, 2,2' bipyridyl, deferoxamine, and mannitol
- Strains used in study: *C. trachomatis* E3024 and *C. muridarum*
- Live cell imaging at 18 hours post infection with *groEL::mCherry* and *omcA::gfp*
- Immunofluorescent staining at 40 hours post infection with anti-OmcB antibody to determine protein expression
- Infection forming unit assays (IFU)

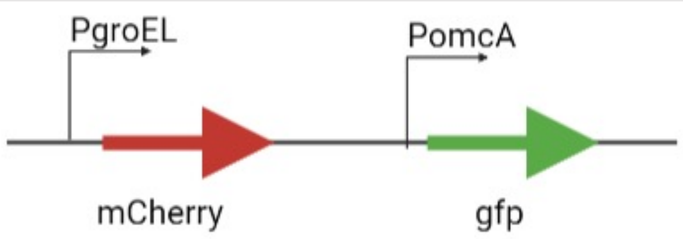


Figure 2. Fluorescence marker for *omcA* and *groEL*.

Results

C. trachomatis downregulates _omcA::gfp_ in response to multiple environmental stressors.

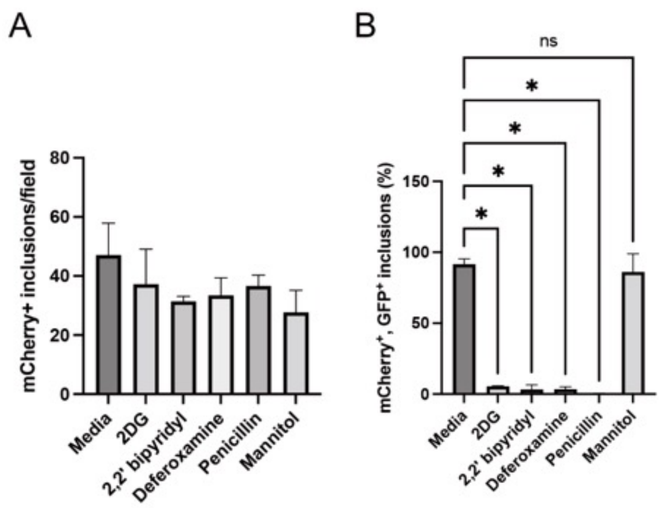


Figure 3. Inclusion formation (A), *omcA::gfp* expression (B) were assayed 24 hours post infection (PI), *P<0.05.

Results

C. trachomatis modifies inclusion phenotypes in response to environmental stress.

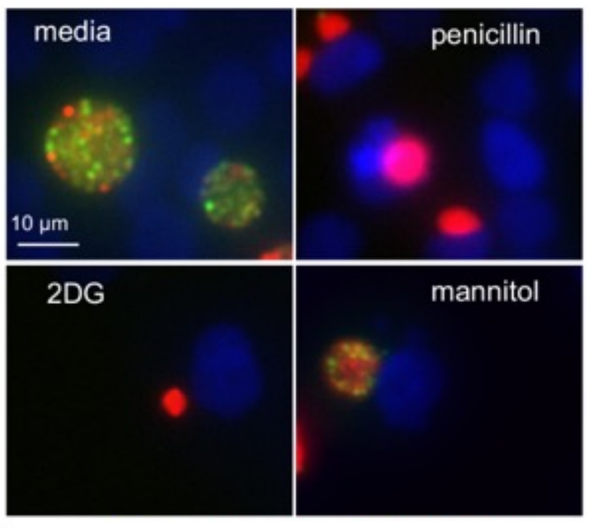


Figure 4. Live imaging of A2EN cells infected 24 hours post infection with CTE3024. NucBlu stained cell nuclei, *groEL::mCherry* is constitutively expressed while *omcA::gfp* is not.

C. muridarum expression of _omcA::gfp_ is unaltered in response to 2DG.

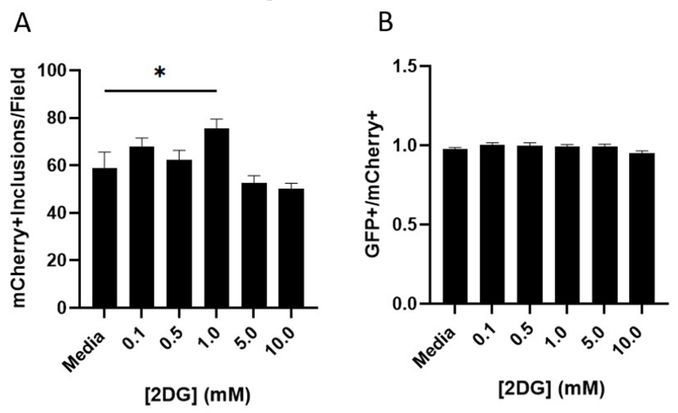


Figure 5. L929 cells infected with *C. muridarum* with increasing concentrations of 2DG 18 hours PI, *P<0.05. Inclusion formation (A) *omcA::gfp* expression (B).

C. muridarum expression of _omcA::gfp_ is unaltered in response to penicillin, iron limitation, and hyperosmotic stress.

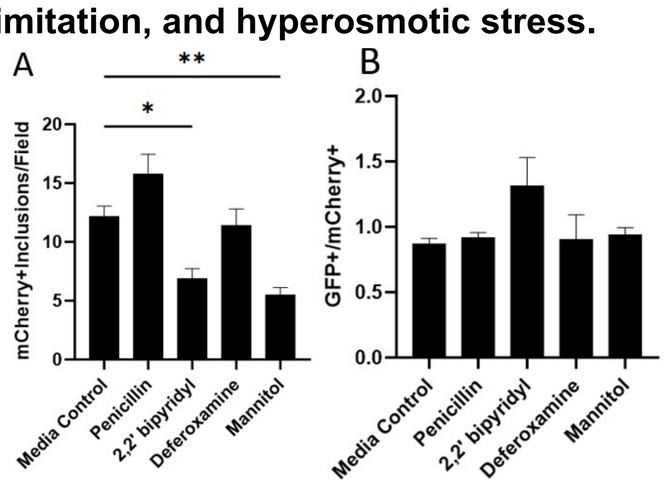


Figure 6. *C. muridarum* infected L929 cells with stressors. Images acquired 18 hours PI. *P<0.05, **P<0.01. Inclusion formation(A) *omcA::gfp* (B).

Results

2DG treated *C. muridarum* downregulates OmcB expression and alters RB morphology.

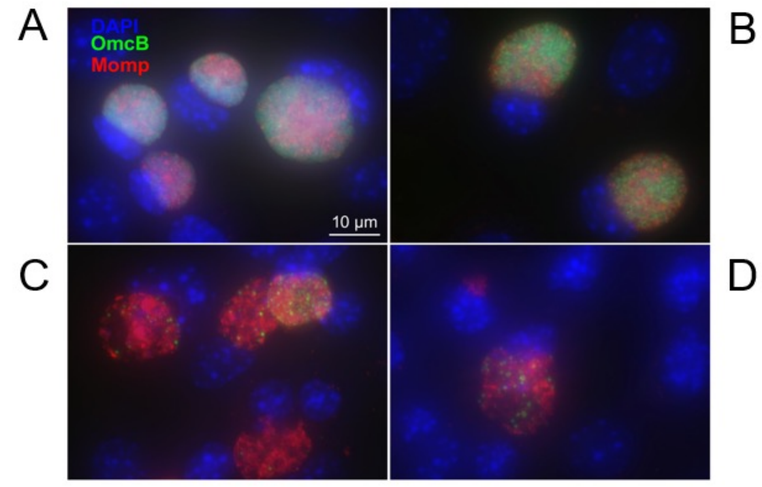


Figure 7. L929 cells infected with *C. muridarum* were treated with 2DG and were fixed and stained 40 hours post infection. (A) Media (B) 1.0 mM 2DG (C) 5.0 mM 2DG (D) 10.0 mM 2DG. Cells were stained with DAPI (blue), anti-MOMP (red), and anti-OmcB (green). Imaging done at 60x.

Stress results in fewer infectious *C. muridarum* progeny despite unaltered *omcA* transcription.

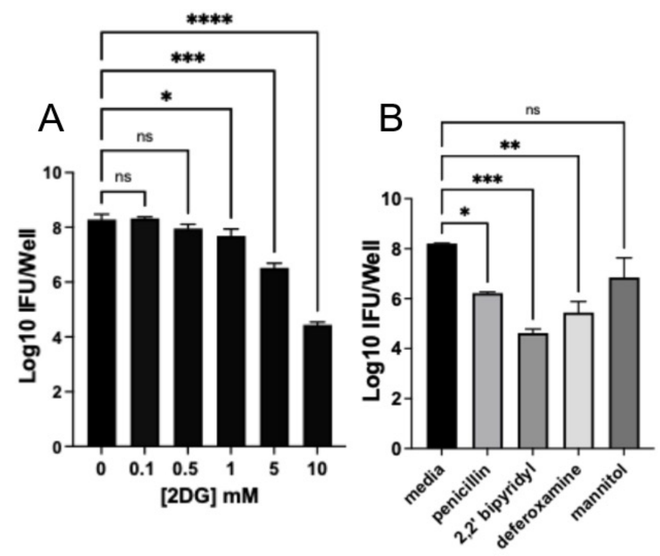


Figure 8. *C. muridarum* infected L929 cells 18 hours post infection. Cells were treated with 2DG (A) penicillin, iron, and mannitol (B). P*<0.5, P**<0.01, P***<0.001, P****<0.0001.

Discussion

- *omcA* transcription is unaltered in response to glucose limitation, penicillin, iron limitation, and hyperosmolality in *C. muridarum.*
- Reduced OmcB expression, generation of persistent RB, and reduced infectious progeny indicate that post-translational pathways leading to persistence[3,4,5] are active in *C. muridarum*.
- Our previous study[1] suggests that 2DG treated *C. muridarum* continue to be proinflammatory, indicating that virulence proteins are produced. Active recruitment of host responses in combination with low infectious yield could contribute to accelerated clearance in the murine genital tract.
- Future Investigations: determine if virulence is also modulated by environmental stress by monitoring the expression of *pgp3* by qPCR and secretion of Pgp3 by immunostaining with anti-Pgp3 antibody to observe phenotypic changes in protein expression.

References

1) O'Connell, C. M., et al. (2011). Toll-like receptor 2 activation by Chlamydia trachomatis is plasmid dependent, and plasmid-responsive chromosomal loci are coordinately regulated in response to glucose limitation by C. trachomatis but not by C. muridarum. Infection and Immunity, 79(3).
2) Cortina, M, E., et al. (2019). Chlamydia trachomatis and Chlamydia muridarum spectinomycin resistant vectors and a transcriptional fluorescent reporter to monitor conversion from replicative to infectious bacteria. PLoS, (14)6.
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4) Hatch ND, Ouellette SP. Inhibition of tRNA Synthetases Induces Persistence in Chlamydia. Infect Immun. 2020;88(4):e00943-19.
5) Ouellette Scot P, Hatch Nathan D, Wood Nicholas A, Herrera Andrea L, Chaussee Michael S, van Wezel Gilles P. Codon-Dependent Transcriptional Changes in Response to Tryptophan Limitation in the Tryptophan Auxotrophic Pathogens Chlamydia trachomatis and Streptococcus pyogenes. mSystems. 6(6):e01269-21.

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