Quantitation of fluorescent chlamydial gene reporter expression by flow cytometry enables investigation of responses to immunological and environmental stress in real time

UNC SCHOOL OF MEDICINE

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INTRODUCTION

- *Chlamydia trachomatis* and *C. muridarum* are obligate intracellular bacteria so investigating gene expression by these pathogens can be technically challenging[1].
- An *omcA::gfp* reporter[2] facilitates real time detection of gene expression, enabling investigation of factors that prevent RB to EB transition in the chlamydial life-cycle.
- Flow cytometry is a highly sensitive technique that can be adapted to quantify chlamydial gene expression revealed by fluorescent reporters.

METHODS

Cell culture conditions. HeLa cells were passaged in 1X DMEM with 10% FBS and grown in a humidified atmosphere at 37°C with 5% CO_2.
Infection. Cells were infected with L2 *C. trachomatis* or *C. muridarum* expressing *groEL::mCherry*, *omcA::gfp*, or *glgA::gfp* reporters at multiplicity of infection 0.1-1.0 with centrifugation.
Imaging. Cells were live-imaged 24 hpi with an EVOS M7000 Microscope (ThermoFisher).
Flow cytometry. NucBlue was added to infected cells prior to harvest. Cells were fixed overnight in Cytofix buffer. Flow cytometry was performed on a LSRFortessa™ Cell Analyzer (BD) or an Attune NxT analyzer (ThermoFisher). VL1, BL1, and YL2 lasers were used to visualize DAPI, GFP, and mCherry, respectively.

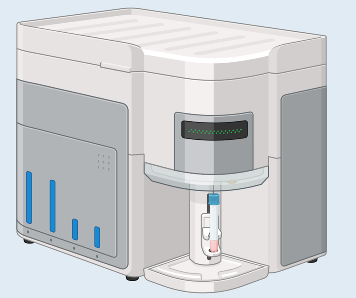

Figure 1. Flow Cytometry setup.

RESULTS

Flow cytometry is a sensitive quantitative method of measuring gene activation

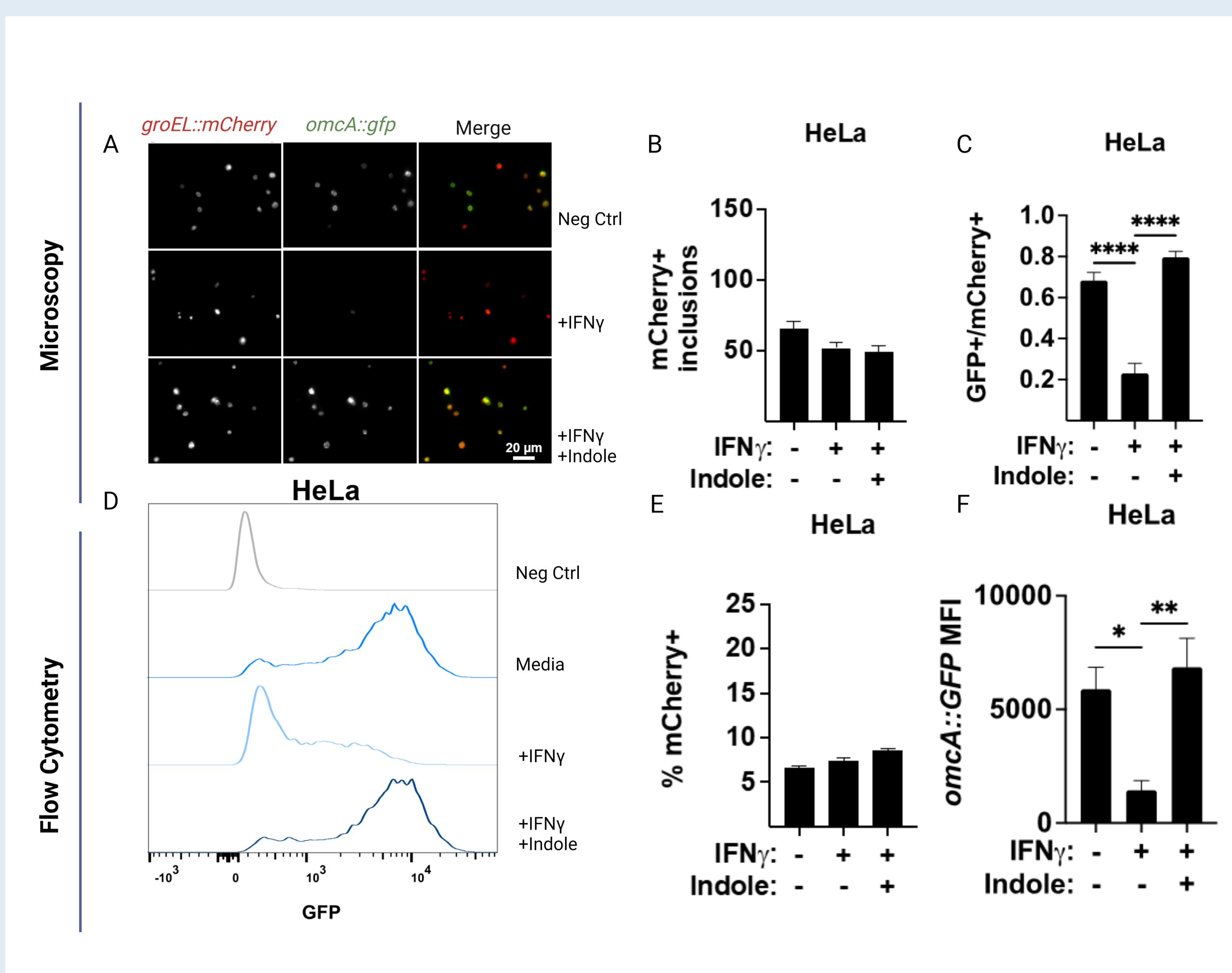

Figure 2. (A-C) Microscopy live-staining of *C. trachomatis* infection with *groEL::mCherry* and *omcA::gfp* reporters and quantitation of *omcA* expressed in infected cells. (D-F) Flow cytometry quantitation of *omcA* positive inclusions.

RESULTS

Flow cytometry quantifies differences in gene expression levels between chlamydial strains

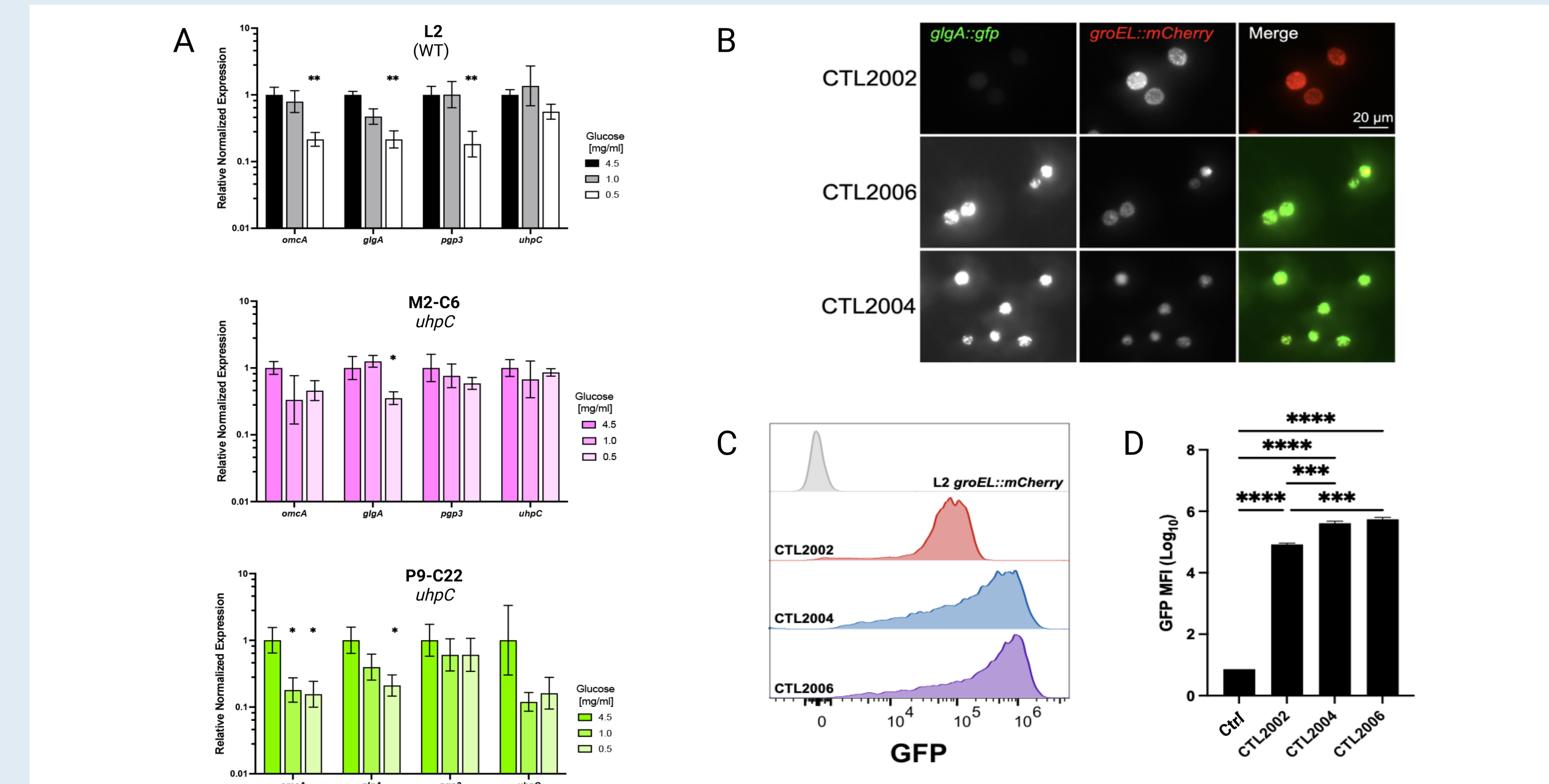

Figure 3. (A) Total RNA isolated from infected cells 24 h after infection, transcripts measured by qRT-PCR, graphed as relative normalized expression mean ±SEM. $P \leq 0.5^*$, $\leq 0.01^{**}$. Basal GFP expression within inclusions formed by CTL2002 (WT/*glgA::gfp*) is less intense than inclusions formed by CTL2004 (P9-C22 *glgA::gfp*) and CTL2006 (M2-C6 *glgA::gfp*) (B). Infected HeLa cells live imaged at 28 hours using an EVOS M7000 microscope or analyzed 24 hours after infection by flow cytometry (C-D). GFP fluorescence intensity for all mCherry+ cells is graphed. (C) Individual histograms represent the average of 3 experimental replicates for baseline levels of GFP expression at 4.5 mg/mL glucose. (D) GFP mean fluorescence intensity (MFI) for 3 experimental replicates. Data analyzed by one-way ANOVA with Holm-Sidak correction for multiple comparisons. $P < 0.05^*$, $P < 0.01^{**}$.

RESULTS

Flow cytometry facilitates studying chlamydial gene expression kinetics in real time

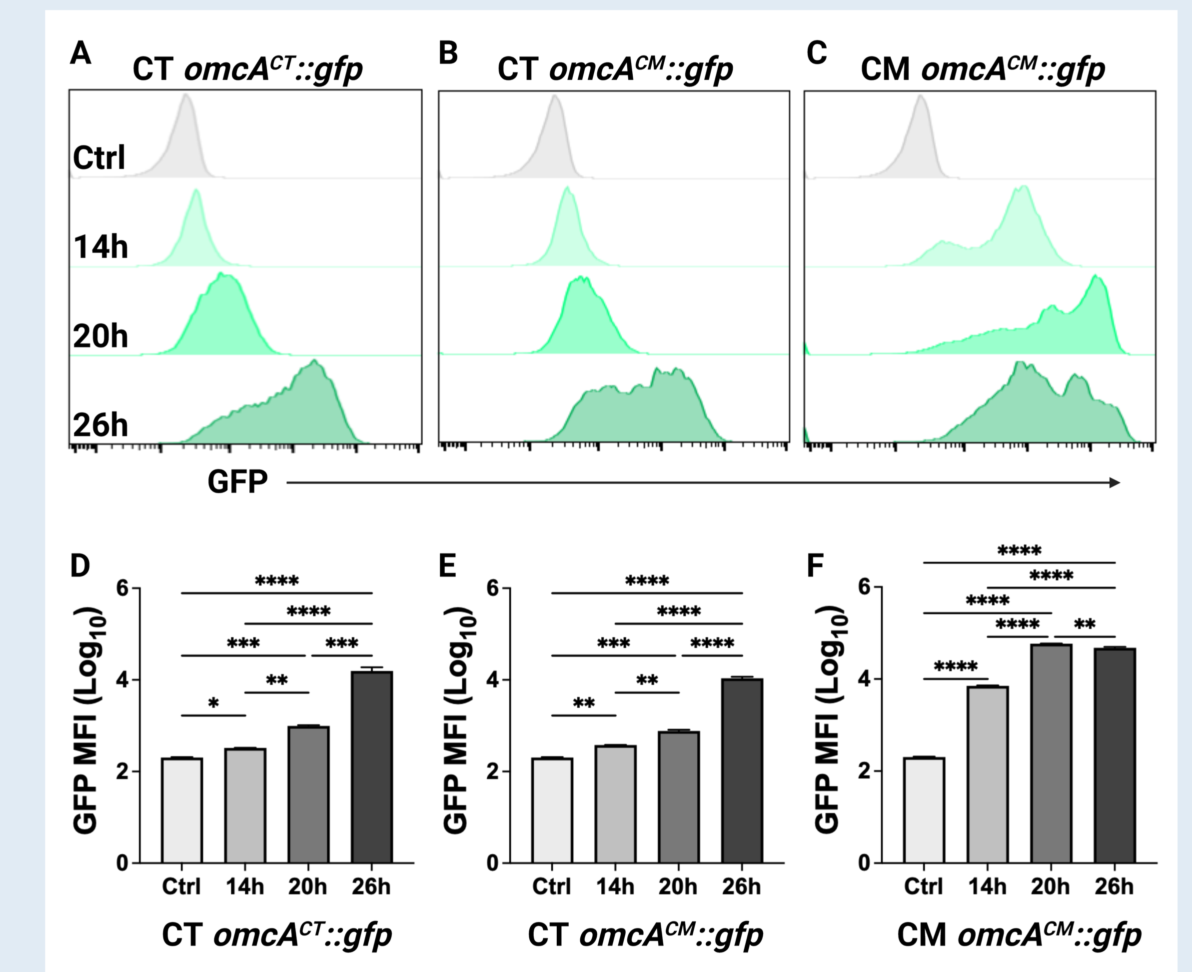

Figure 4. HeLa cells were infected with (A,D) *C. trachomatis* expressing native *omcA::gfp*, (B,E) *C. trachomatis* expressing *C. muridarum omcA::gfp*, or (C,F) *C. muridarum* expressing native *omcA::gfp*. Cells were harvested at indicated timepoints and analyzed by flow cytometry using an Attune (Thermo Fisher) instrument. (A-C) GFP fluorescence intensity over time for all mCherry+ cells. Individual histograms represent the average of 2 replicates (D-F) GFP mean fluorescence intensity (MFI) for 2 replicates. Data analyzed by one-way ANOVA with Holm-Sidak correction for multiple comparisons. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

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DISCUSSION/CONCLUSIONS

- Flow cytometry rapidly quantifies fluorescent expression in large numbers of cells
- Distinguishes variable levels of gene expression through interpretation of brightness levels (MFI)
- Supports high throughput of multiple, fixed samples enabling quantitative, kinetic studies of gene expression
- Limitations: flow cytometry is unable to distinguish signals from individual chlamydiae within an inclusion, data interpretation requires careful design of matched controls that mimic inclusion size and bacterial abundance
- Flow cytometry can support investigation of temporal gene regulation or other modulated gene expression pathways important for chlamydial development or virulence

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