Heterogeneity of Virulence Gene Expression in *Salmonella typhimurium*

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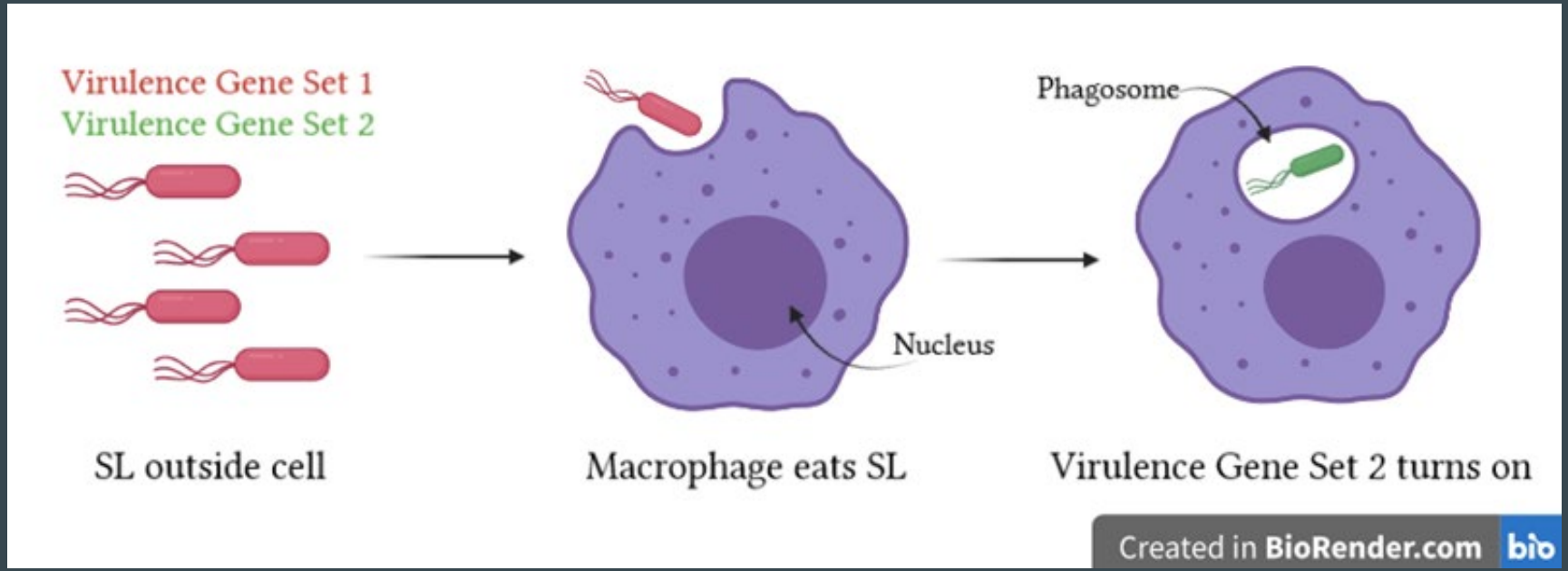
Lane Lab (Molecular Biosciences)
05.27.2021 Research Expo

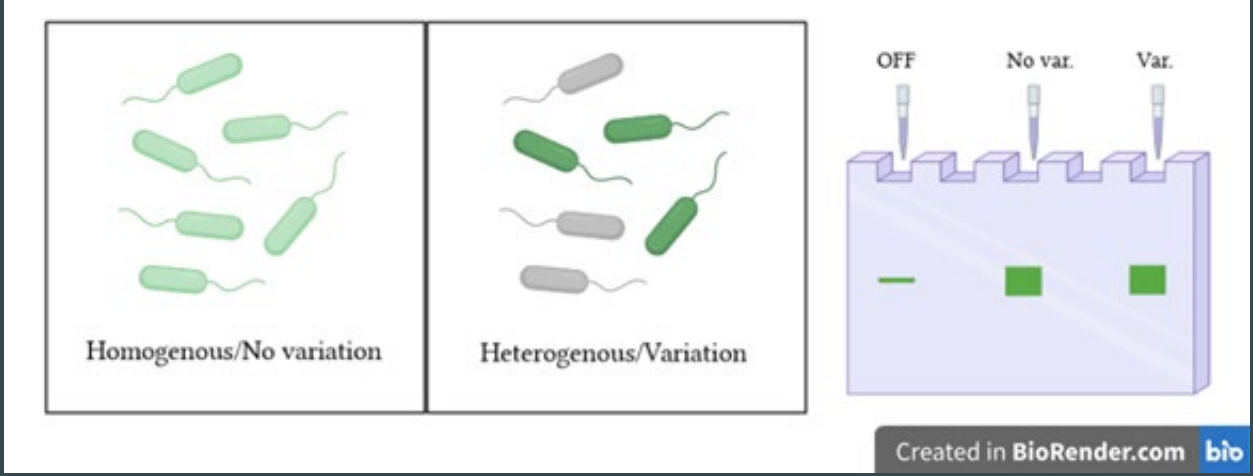
Virulence in *Salmonella Typhimurium*

- Virulence genes help bacteria like Salmonella (SL) invade and damage hosts



Cell-to-cell variation is found in virulence gene expression

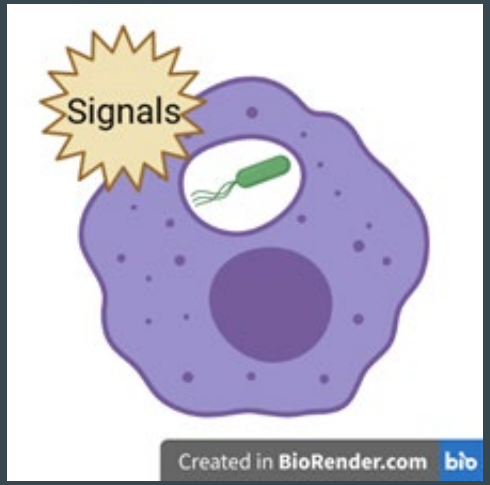
- Gene expression levels may vary among genetically identical bacteria

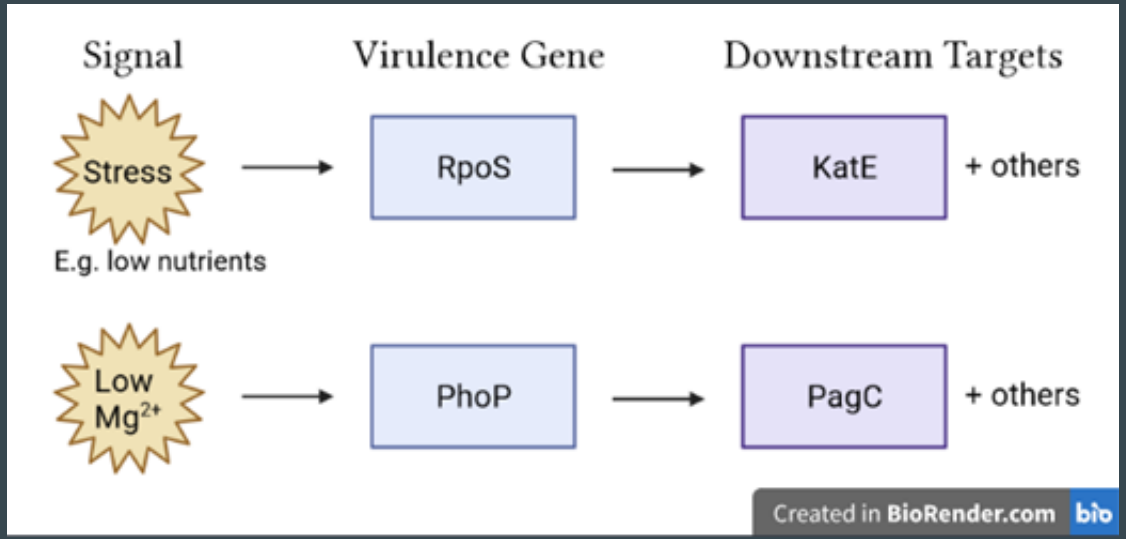


- This variation cannot be distinguished at the population level
- Single-cell variation found in one set of Salmonella virulence genes (SPI-1)

Question/Hypothesis

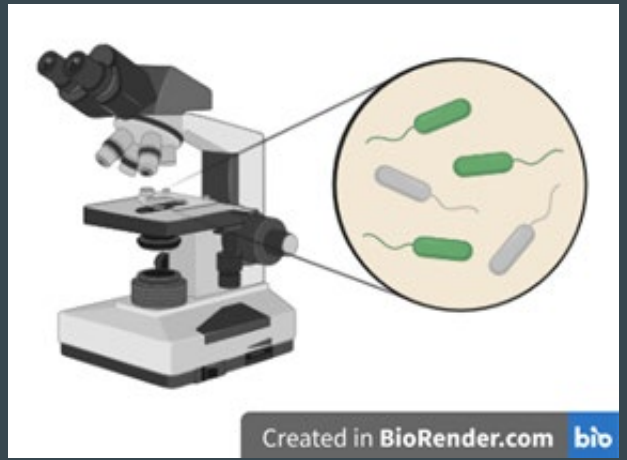
- To what extent do other virulence genes (beyond SPI) have variation in SL?
    - RpoS, PhoP





Fluorescent reporters to detect variation

- Promoters: Gene region for initiating transcription (gene expression)
- Fluorescent reporters using promoters
  - Attaching promoter to fluorescent protein lets us track gene expression
- Gene expression→ fluorescence visible under microscope



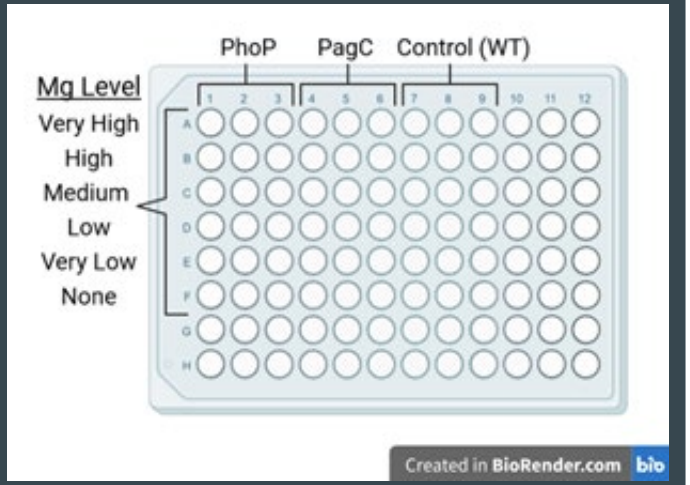
Automating Reporter Design

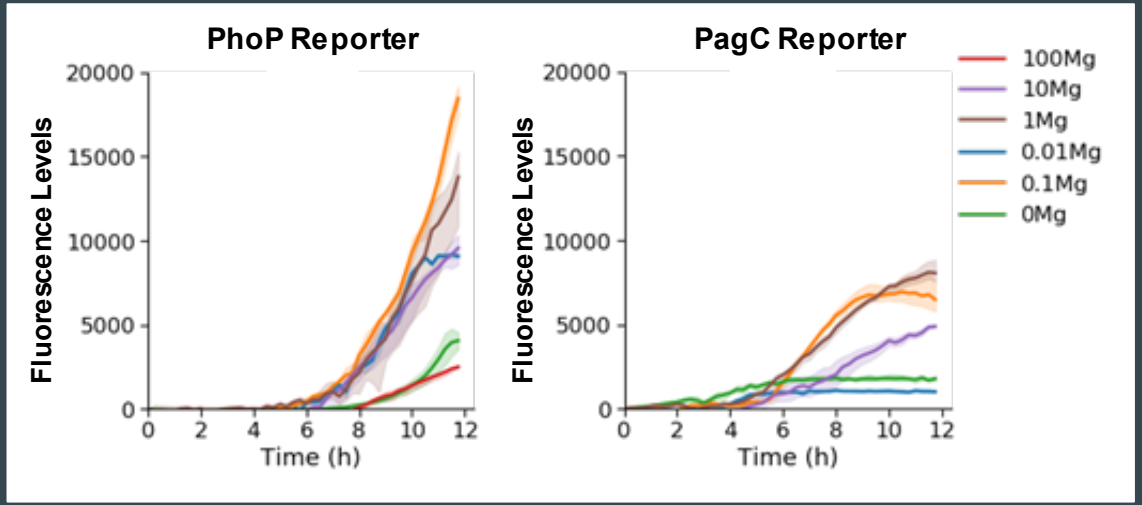
- Created Python code to help extract DNA sequence of promoters
- Prints 2000 DNA bases upstream of gene of interest where promoter region is
- Facilitates the reporter design process

Acknowledgements: Elizabeth Hora, Baker Undergraduate Faculty Grant

Experiment 1 - Plate Reader

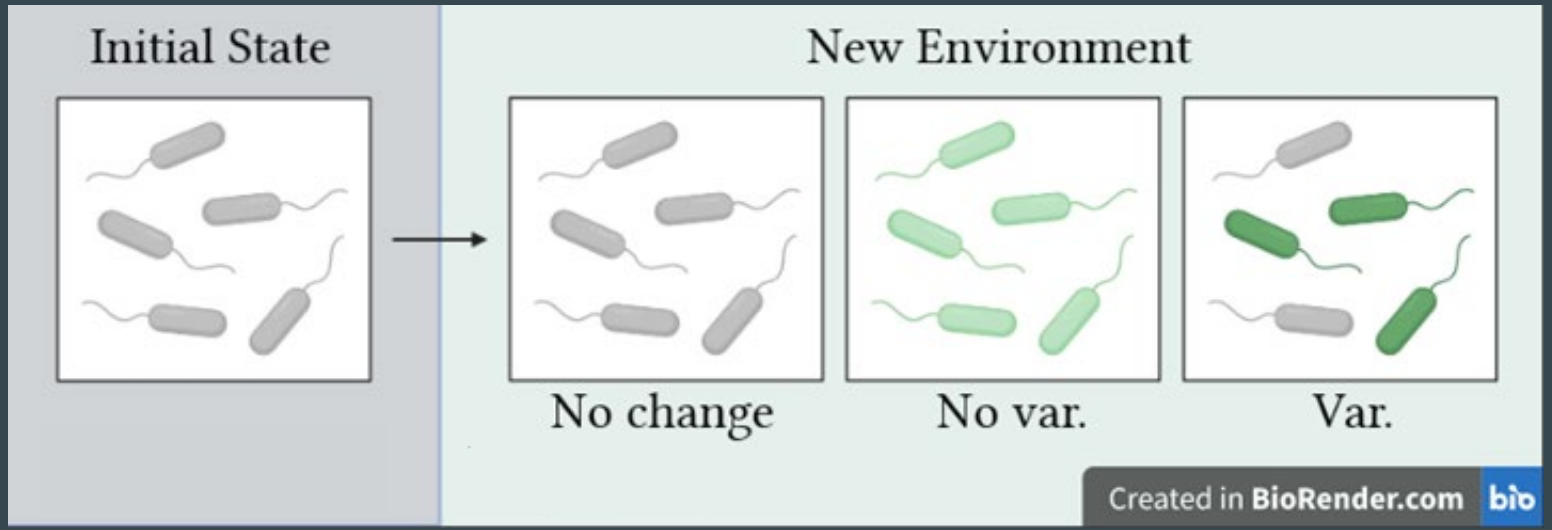
- Plate reader: Allows experiments using many media conditions
- Tested PhoP and PagC at different Mg^{2+} levels
- Highest activation for 0.1mM and 1.0mM Mg^{2+}





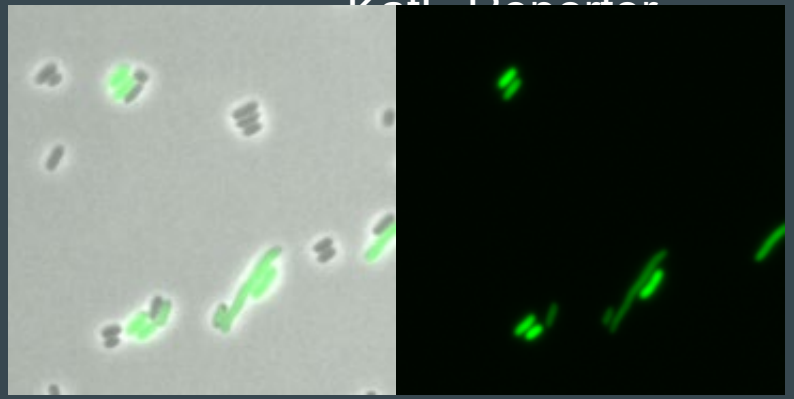
Experiment-2 Validation

- Validate reporters with known signals
  - Nutrient deprivation activates RpoS, low Mg^{2+} activates PhoP

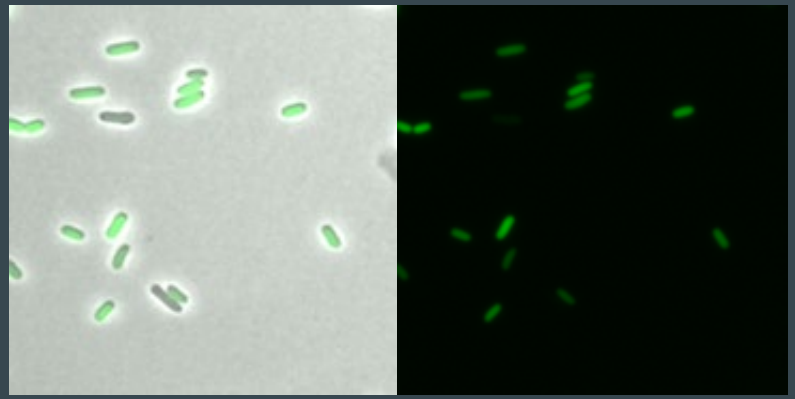


Experiment-2 Validation

RpoS Reporter



Phase



GFP

Phase

GFP

- SL with RpoS and KatE reporters after 12 hours in low nutrient environment
- Gene expression levels vary noticeably among cells

Data and Results - Validation

PhoP Reporter

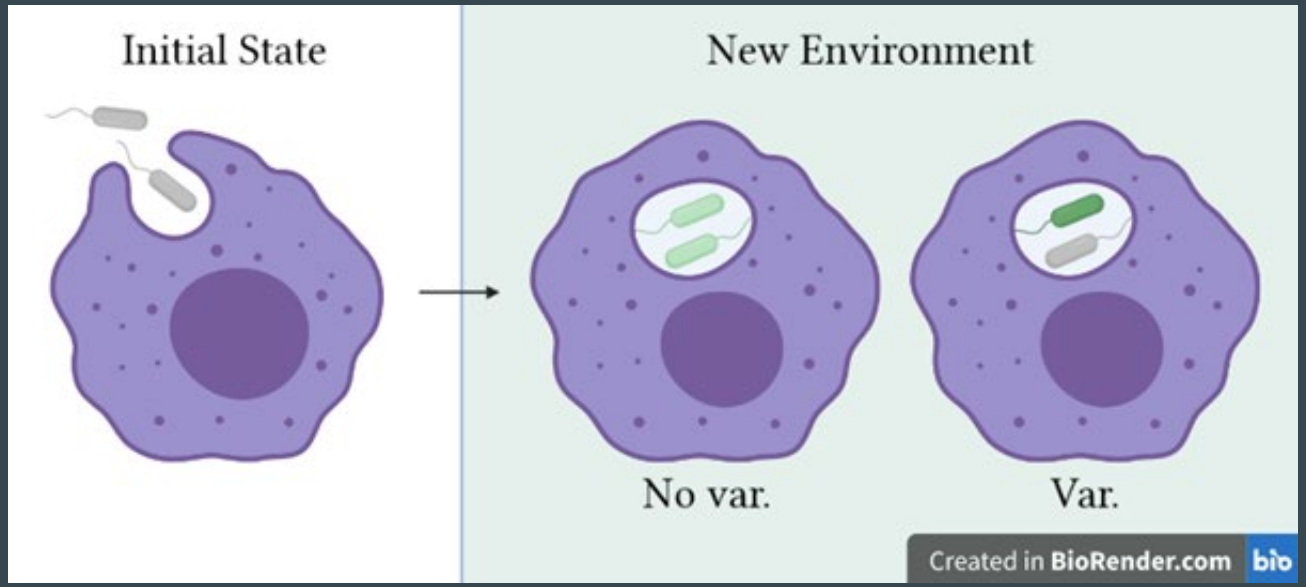
Phase GFP

Phase GFP

- SL with PhoP and PagC reporters after 8 hours in low Mg^{2+} environment
- Less pronounced gene expression variation - variability is more continuous

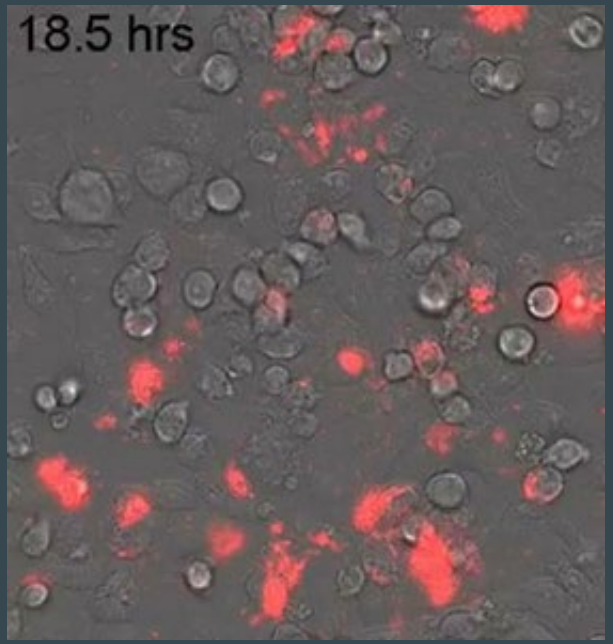
Experiment-3 Infection

- Infect macrophages with RpoS and KatE reporters

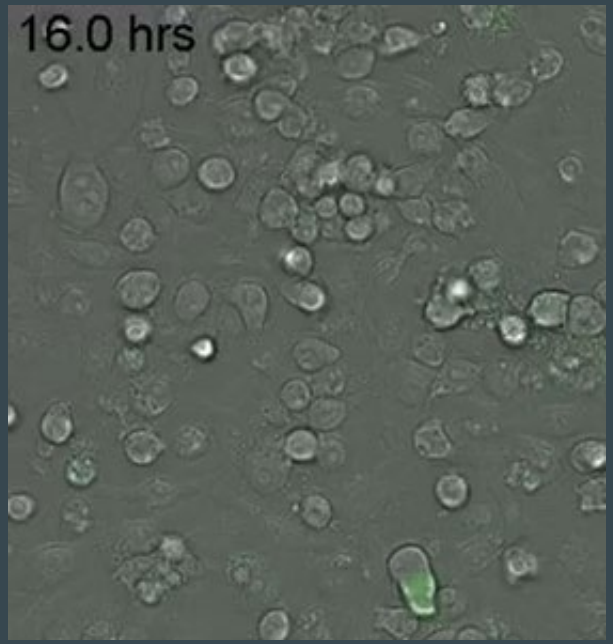


Experiment-3 Infection

- Timelapse of KatE reporters inside macrophages

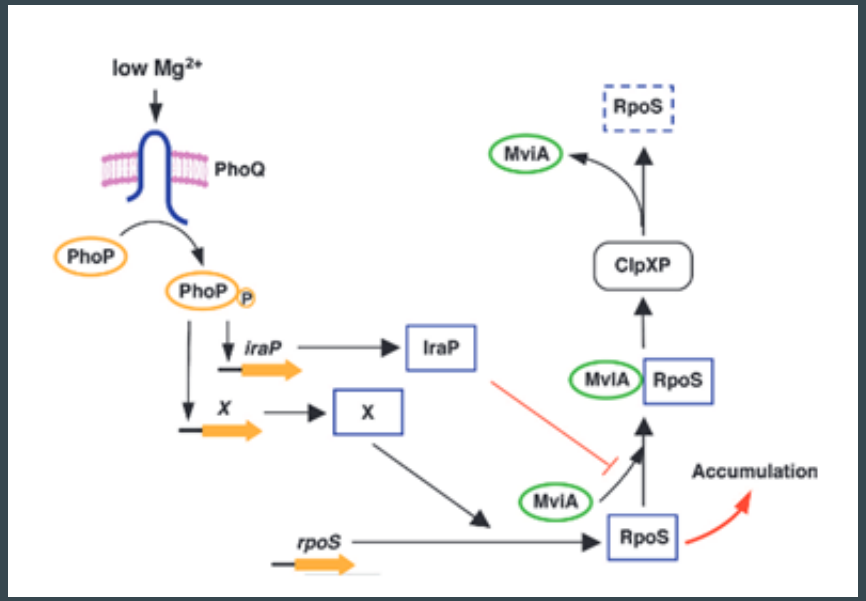


Phase + TRITC



Conclusions/Current Work

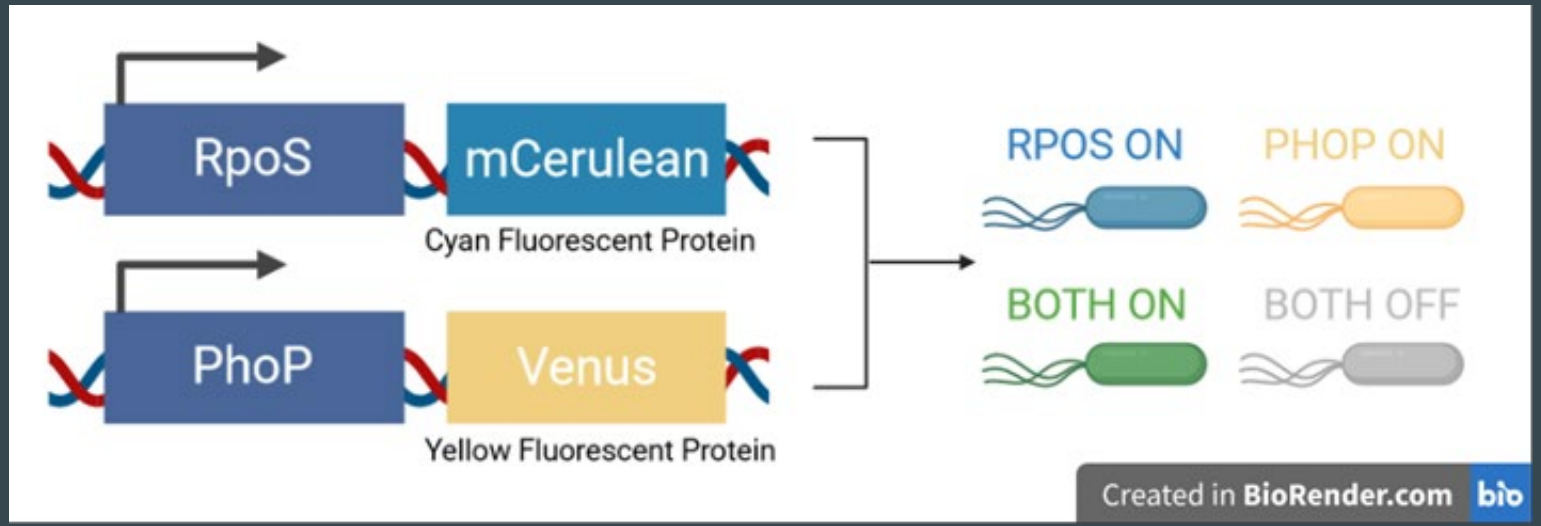
- Data thus far suggests single cell variation in RpoS and PhoP
- Next steps: investigate relationship between RpoS and PhoP activation



Tu, X., Latifi, T., et al. (2006). The PhoP/PhoQ Two-Component System Stabilizes the Alternative Sigma Factor RpoS in Salmonella Enterica. *PNAS*, *103*(36), 13503-13508. https://doi.org/10.1073/pnas.0606026103

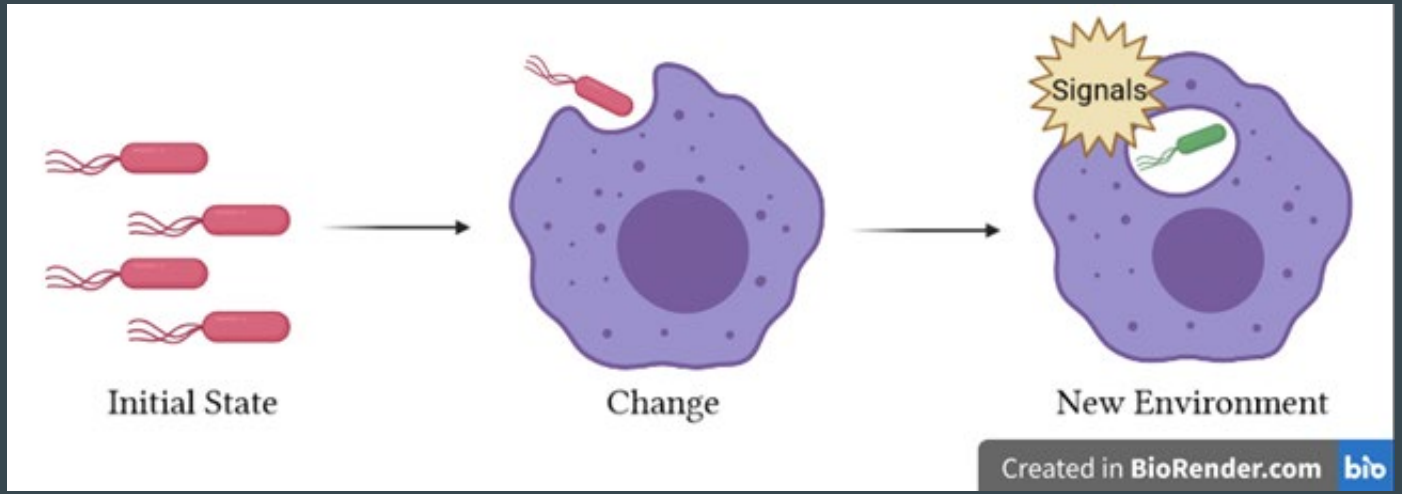
Conclusions/Current Work

- Data thus far suggests single cell variation in RpoS and PhoP
- Next steps: investigate relationship between RpoS and PhoP activation
- Studies suggest interregulation between RpoS and PhoP - do they vary together?



Overall Impact

- Leads to broader questions:
  - Why do genetically identical cells express different phenotypes?
  - How do bacteria survive and adapt in changing environments?
- Insight into infection strategies used by SL and other bacteria



Acknowledgements

- Lane Lab (Molecular Biosciences)
  - Dr. Keara Lane, Madison Smith, Elizabeth Hora, and others
- Baker Undergraduate Faculty Grant

https://sitn.hms.harvard.edu/seminars/2016/food-fights-back-exploring-salmonellas-journey-body/