Elucidating virulence regulatory networks in Salmonella and Yersinia

Marcus Jones, JCVI
Jason McDermott, PNNL
Overview/Background

► Regulation of virulence in *Salmonella*
  - Infection of macrophages essential for virulence
  - *Salmonella* pathogenecity island (SPI) 2 is essential for infection
  - SPI-1 is involved in epithelial cell infection
  - 19 regulators with a significant impact on virulence

► Types of data
  - *In vitro*: culture conditions mimicking intracellular (LPM)
  - Intramacrophage (*ex vivo*): infection of cultured macrophages
  - *In vivo*: infection of mice
  - CHIPseq: transcription factor binding information
Spi1 & 2 in vitro expression
phoPQ ex vivo observations
phoPQ ex vivo observations
Spi2 expression *in vitro* LPM20hr

![Heatmap graph showing expression levels of various genes.](image-url)
ssrAB Spi2
ex vivo
expression
Spi2 expression FACS sorted infection model
ChIP-chip and ChIP-seq analysis of *rpoE*
Regulatory Network Modeling of *Salmonella Typhimurium*

- **Existing knowledge**
  - Mapped regulatory relationships
  - Salmonella literature
- **Network inference from transcriptomics**
  - Mutual information
  - Logical influence
- **Network inference from proteomics**
  - Logical influence
- **CHiPseq experiments**
Known* regulatory relationships in *Salmonella*

*or mapped from *E. coli* in REGPrecise
Inferred Regulatory Networks

- Logically inferred networks
  - “If regulator A is removed, what is its effect on other genes?”

- Statistically inferred networks
  - If regulator A and B have similar expression patterns then they are predicted to have a regulatory relationship
CHIPseq Regulatory Network Determination

- Crp
- SpvR
- FruR
- YqgE
- FisL
- Hnr
- EnvZ
- RpoS
- HrD
- IHF
- RpoE
- PhoP
- ArcA
- STM2078
- STM4783
- SsrB
ChIPSeq of RpoE in macrophages

4 hour 8 hour 18 hour

RpoE binding
No binding detected

Bar chart showing the comparison between rpoE (intragenic) and rpoEp3 at 4, 8, and 18 hours.
### Integrated regulatory network for *Salmonella* virulence

<table>
<thead>
<tr>
<th>Network</th>
<th>Genes</th>
<th>Relationships</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known*</td>
<td>924</td>
<td>1299</td>
</tr>
<tr>
<td>Logical</td>
<td>231</td>
<td>619</td>
</tr>
<tr>
<td>Inferred</td>
<td>827</td>
<td>1432</td>
</tr>
<tr>
<td>CHIPseq</td>
<td>1728</td>
<td>2495</td>
</tr>
<tr>
<td>Combined</td>
<td>2712</td>
<td>6197</td>
</tr>
</tbody>
</table>
Interpreting Cell-specific Expression with Regulatory Networks

► RNAseq of *Salmonella* in different white blood cell types
  - Macrophages
  - Monocytes
  - Natural killer cells
  - CD8+ T-cells
  - CD4+ T-cells
  - B cells
  - Dendritic cells
  - Polymorphonuclear neutrophil (PMN) cells

► Overlay expression on regulatory networks

► Interpretation by functional modules and genes of interest
Salmonella regulation in white blood cells *in vivo*

Functions not observed
- Amino acid biosynthesis (Ala, Asp, Gln, Gly, Ile, Leu, Lys, Met, Phe, Ser, Trp, Tyr, Val)
- Transposase (tnpA)
- Cytochrome C biogenesis (ccm operon)

Functions not in macrophages
- Amino acid biosynthesis (Arg, His)
- Propanediol utilization-related (pdu, cbi)
- Flagella (flg, flh, fli)
- T3SS (pagD, pagK, ssal, ssaP, sseA, sseB, ssel)

Functions in macrophages only
- Thiamine biosynthesis (thiJ, thiK, thiQ)
T3SS Regulation in Macrophages
T3SS Regulation in Neutrophils

SPI-1

SPI-2

SPI-1 & SPI-2

Unknown
T3SS Regulation in CD8+ T-cells
Host white blood cell response to *Salmonella* infection

- Monocytes
- Macrophage
- Neutrophils
- Dendritic cells
- CD4+ T-cells
- B cells
- CD8+ T-cells
- Natural killer
Inferred Macrophage Immune Response Network

Logic inferred regulatory relationships

Statistically inferred regulatory relationships

Next Steps

- Interpretation in genome-scale models of metabolism
- Biological implications of regulation
- Investigating host response to *Salmonella* infection
- Developing a predictive model of *Salmonella* regulation

- *In vitro* and intramacrophage expression differences

- Topological analysis for identification of critical nodes

Key Acknowledgements

**PNNL**
- Joshua Adkins-PI
- Richard Smith-Co-PI
- Matt Monroe-TL
- Charles Ansong, PM
- Jason McDermott-TL
- Thomas Metz-TL

- Heather Brewer
- Roslyn Brown
- Brooke Deatherage Kaiser
- Young-Mo Kim
- Hugh Mitchell
- Ernesto Nakayasu
- Chris Overall

**OHSU**
- Fred Heffron-TL
- Afshan Kidwai
- Jie Li
- George Niemann
- Hyunjin Yoon

**UCSD**
- Bernhard Palsson-TL
- Araash Bordbar
- Pep Charusanti
- Daniel Hyduke
- Josh Lerman
- Monica Mo
- Brian Schmidt

- Vladimir Motin-TL
- Sadhana Chauhan

**UTMB-Motin**
- Marcus Jones

**WSU**
- Kate McAteer

**JCVI**
- Mark Adams-TL

**NIAID Input**
- Valentina Di Francesco
- William Alexander
- Fred Cassels
- Peter Dudley
- Suman Mukhopadhyay
- Malu Polanski

**SB Working Group’s Service**
- Tom Braciale
- Pieter Dorrestein
- William Jacobs
- Thomas Kepler
- Reinhard Laubenbacher
- Douglas Lauffenburger
- Dennis Monack
- Virginia Miller
- Shelley Payne
- Stanley Perlman
- Marcelo Sztein
- Reid Townsend

**Sanford-Burnham**
- Scott Peterson