Investigating transcriptional regulators in *Mycobacterium tuberculosis*

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@asherichia

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Mycobacteria

- Member of Actinomycetales – diverse group
- *Mycobacterium* – split into two main groups
  - Fast Growing Mycobacteria (FG) and Slow Growing Mycobacteria (SG)

Notable members of FG:
- **M. smegmatis**
  - Non-virulent and often used as a ‘surrogate’ for TB due to faster growth
- **M. abscessus**
  - Often associated with immunocompromised patients and highly drug resistant

Notable members of SG (and MTBC):
- **M. bovis**
  - Primarily affects cattle but can infect humans, basis of BCG – limited protection
- **M. tuberculosis**
  - Causative agent of human tuberculosis
Tuberculosis

*M. tuberculosis*
- Leading cause of death by an infectious disease
- One death every 15 seconds, with ⅓ of world infected
- 2014 infections data (United Nations, 2015)
  - 9.6 million, ~500,000 multidrug resistant (increasing) = 1.5 million deaths
- Long treatment regimes

*M. bovis*
- Primary cause of tuberculosis in cattle
- Basis of BCG vaccine for TB
- Problematic in UK – in 2014, 9.4 million cattle tested and ~35,000 culled (APHA)
- Economic burden of *M. bovis*
  - Last decade: £500 million, next decade: +£1 billion
Mycobacteria

- *M. tuberculosis* and *M. bovis* grow extremely slow: ~24 hour doubling time (compared to *E. coli* – 20 minutes…)

- Different cell wall structure – Arabinogalactan/Mycolic acids

![Diagram of Mycobacterium, Gram negative (E. coli), Gram positive (Bacillus)]
Tuberculosis

- Primarily affects the lungs – Cavities and granuloma formation

- Hence – coughing, spluttering and presence of a bloody mucus
M. tuberculosis genome

- Sequenced 1998 - 4.4 million base pairs

- High GC content
  - M. tuberculosis – 65.6%
  - E. coli – 50%

- Strains exist and are usually localised
  - Beijing – Asia
  - Haarlem – Europe/S America/Caribbean

- Various transcriptional regulators
  - TetR
  - Sigma factors
Why TetR transcriptional regulators?
Transcriptional regulators

- Control the regulation of various genes encoding cytochromes, transporters etc.
- 52 TetR transcriptional regulators in *M. tuberculosis*
Transcriptional regulators

- Control the regulation of various genes encoding cytochromes, transporters etc.

- 52 TetR transcriptional regulators in *M. tuberculosis*
TetR regulators in *M. tuberculosis*

- Some are well studied:
  - *kstR*
    - Cholesterol utilisation (Kendall *et al.* 2007)
  - *kstR2*
    - Cholesterol utilisation (Kendall *et al.* 2010)
  - *bkaR*
    - Branched chain amino acid metabolism (Balhana *et al.* 2013)
  - *ethR*
    - Involved in ethionamide resistance (Baulard *et al.* 2000)
  - *inbR*
    - Isoniazid sensitivity (Yang *et al.* 2015)

- So what do the remaining do?
  = My PhD!
TetR genes

TetR locations within various *Mycobacterium* genomes
Basis of my PhD

Bioinformatics
- MEME, FIMO, BRIG

Expression of rTetR
- Expression in *E. coli*

DNA binding
Relatively easy
- EMSA, gel shifts, columns

Ligand binding
Difficult
- Identifying ligands that bind TetR

Function and regulation
- Mutagenesis and over/underexpression
  - RNA Seq/Microarray analysis
Bioinformatics

- **MEME**
  - Predicts DNA binding motif from multiple sets of genomic data
  - DNA in → Motif out

- **FIMO**
  - Takes motif from MEME and searches genome
  - Predicted binding sites
  - Helps with identifying regulatory genes

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GCACGTAAGGCACGAGAGTTGGTGCGGCCTAA
GAGCGCCACCACGACATCGGTTGATTGCCCGGGCAA
GCTGGCCGATTGCCGTTCCACCGGATCCCGGCAA
GTCCGAACGTATGTCTGAAAGGTGACTCGGCC
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Recombinant TetR and mutant

- Recombinant expression of TetR enables:
  - Determining DNA binding sites
  - Confirm bioinformatics predictions
  - Ligand binding
    - What ligand binds TetR?

- Functional studies in TetR mutant
  - Effect on susceptibility?
  - Increase/decrease growth rate?
  - Normal cell division?
  - Resistant to antibiotics?
So why?

“If you don’t go fishing, you won’t catch any fish”
Julian Parkhill

- Previous TetR transcriptional regulators control vital systems
  - Cholesterol utilisation – fundamental to growth - drug target?
  - Sridhar et al. (2016) recently described various TetR as drug targets

- Further understanding of *Mycobacterium*
- Aid in future studies and potential drug targets
  - For both *M. tuberculosis* and *M. bovis*