Treatment rapidly alters the physiologic state of *M tuberculosis* in sputum

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eHAT Collaboration

Makerere University | Stanford University | Yale University | UC Denver | UC San Francisco

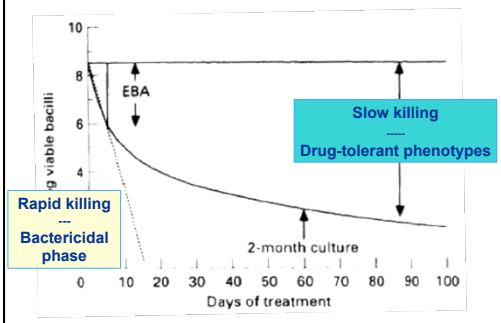
Mtb adapted for airborne transmission

- Molecular determinates of transmission fitness are unknown
- Drug exposure rapidly alters *Mtb* physiologic state

Mtb physiologic state

- Dynamic
- Adapted to human immunity & microenvironment
- Affects drug effectiveness
- Likely affects transmission fitness
- Humans may differ from experimental models

Altered physiologic state leads to drug tolerance



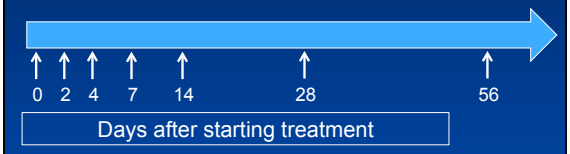
Mitchison DA Soc Appl Bacteriol Symp Ser 1996

Mtb-transcriptional profiling in sputum

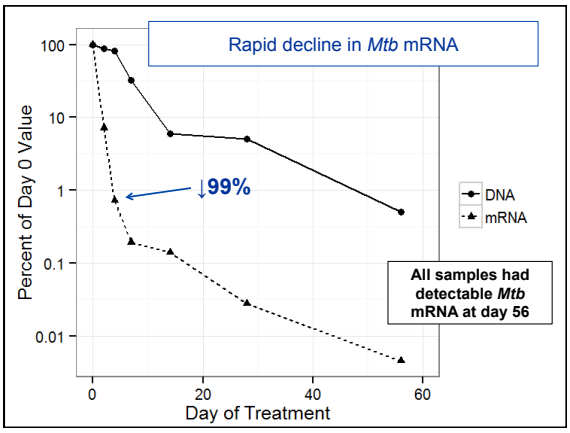
- Nested qRT-PCR for 2,400 *Mtb* transcripts
  - 60% of genome
- mRNA half-life is short
- "Biological snapshot" of *Mtb* physiologic state

Impact of treatment on *Mtb* transcriptome

- Ugandan adults with pulmonary TB
  - Sputum collected in RNA preservative



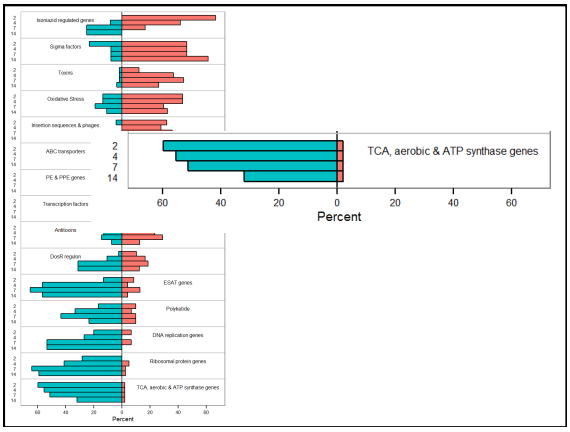
Walter J Infect Dis 2015;212:990-998.

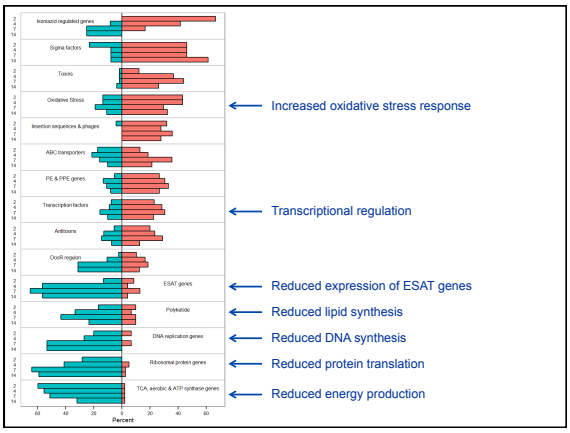


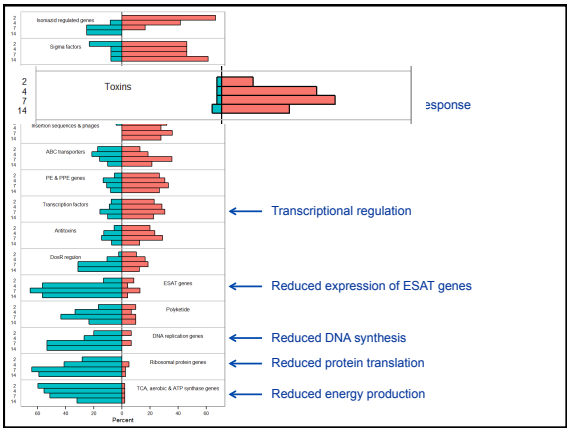
Impact of drug exposure on *Mtb* transcriptome

Massive alteration of MTB transcriptome

At least 20% of genes differentially expressed at each day




Isoniazid regulated genes
Sigma factors
Toxins
Oxidative Stress
Insertion sequences & phages
ABC transporters
PE & PPE genes
Transcription factors
Antitoxins
DosR regulon
ESAT genes
Polyketide
DNA replication genes
Ribosomal protein genes
TCA, aerobic & ATP synthase genes
Percent
Increased oxidative stress response
Transcriptional regulation
Reduced expression of ESAT genes
Reduced lipid synthesis
Reduced DNA synthesis
Reduced protein translation
Reduced energy production


Isoniazid regulated genes
Sigma factors
2
4
7
14
Toxins
esponse
Insertion sequences & phages
ABC transporters
PE & PPE genes
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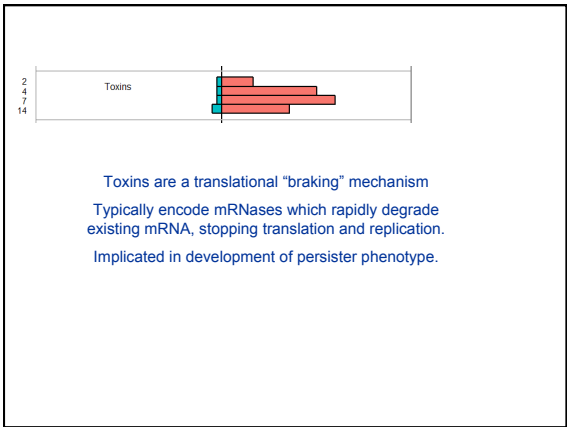
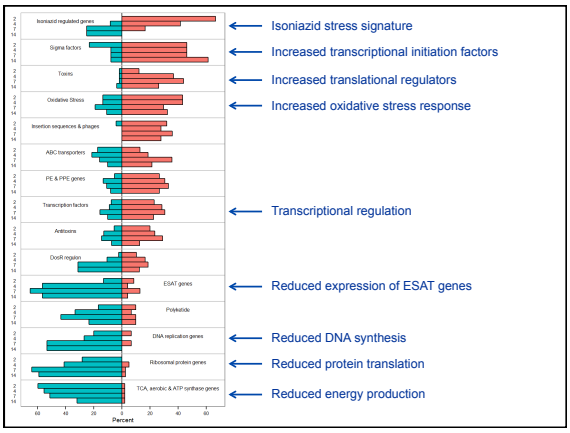
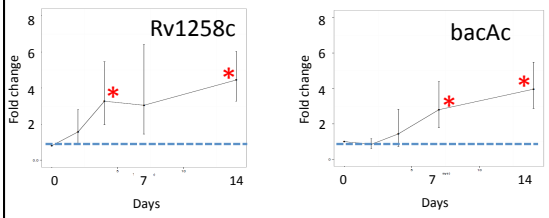
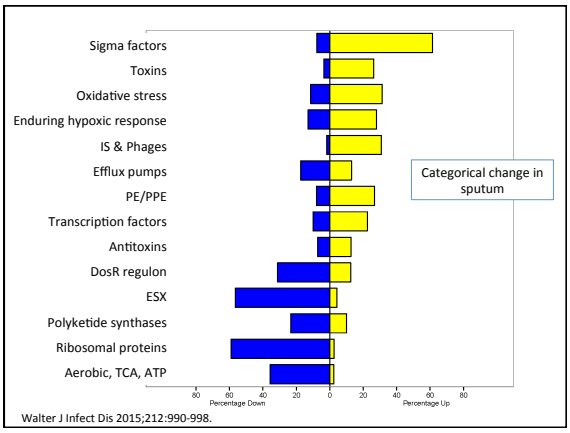

2
4
7
14
Toxins
Toxins are a translational “braking” mechanism
Typically encode mRNases which rapidly degrade existing mRNA, stopping translation and replication.
Implicated in development of persister phenotype.



Efflux Pumps



Comparison with existing experimental models of drug-tolerant phenotypes



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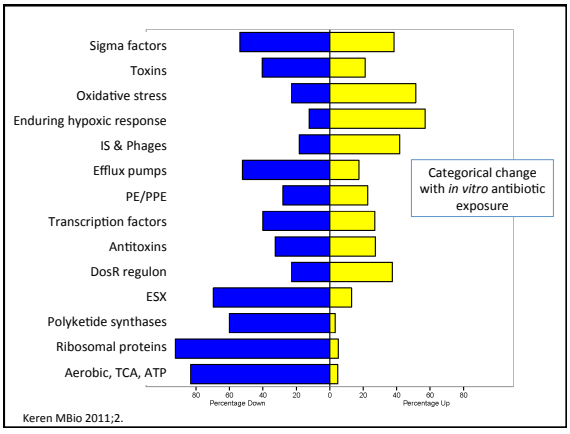
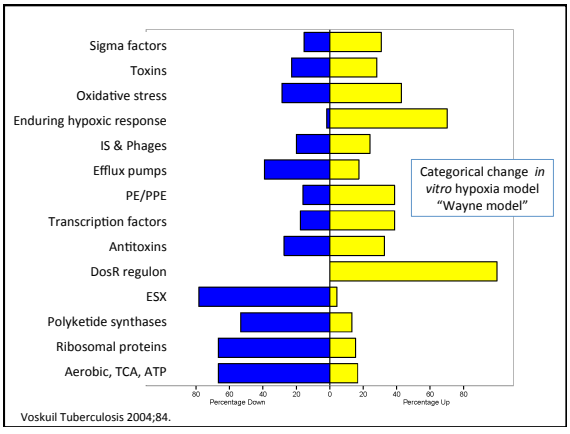
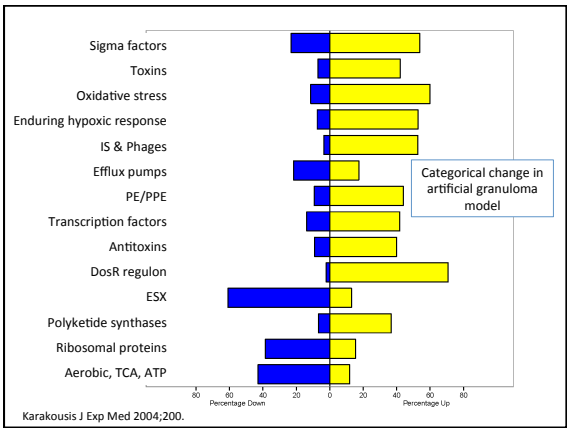


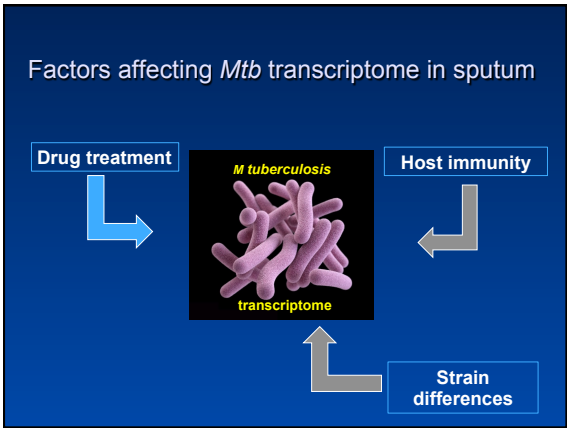
Keren MBio 2011;2.



Voskuil Tuberculosis 2004;84.



Karakousis J Exp Med 2004;200.



Take home message

- Treatment induces rapid & profound alteration in *Mtb* transcriptome in sputum
  - Down-regulation of metabolism, protein & lipid synthesis suggest slow growth
  - Up-regulation of stress responses, regulatory factors, key efflux pumps
- Implications for transmission fitness need further investigation
  - Are drug-treated phenotypes less fit for transmission?

Thanks

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