Deciphering a Sleeping Pathogen: Uncovering Novel Transcriptional Regulators of Hypoxia-Induced Dormancy in Mycobacterium Tuberculosis
**Background: Introducing Mycobacterium Tuberculosis**

**Tuberculosis (TB): Bacterial Infection**
- Spread through the inhalation of cough/sneeze droplets.
- As a result of this invasion...
  - Foreign bacteria invade host immune system.
  - Trigger airway inflammation in lungs.
  - Spread to other organs in the human body.

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**Project Premise:**

**Hypoxia-Induced Dormancy**
Purpose: Outlining the Research Problem

Deletion of TFs thought crucial to dormancy only conferred mild growth defects.

Several modeling techniques have been used to simulate oxygen depletion, which makes synthesizing findings considerably difficult.

Experimental attempts at directed gene disruption and protein localization give way to questionable results.

Current understanding of the MTB genetic architecture is highly insufficient.

Modeling TB infection can be more rigorously achieved with a computational approach.

Goal: To uncover transcriptional agents and regulatory mechanisms that control the transition of MTB in and out of dormancy.

#1: Literature Review  #2: Key Takeaways  #3: Research Objective
1. Compose an aggregate hypoxia dataset from several RNA-seq and microarray experiments in vivo.

2. Infer a gene regulatory network (GRN) based on these observations.

3. Apply downstream analyses to unearth interesting transcriptional dynamics.
Hypoxia TRIP Screen:
- Tracked 207 TFI Strains under several forms of environmental stress.
- Abundance Fold Change (Uninduced v. Induced).
- **Method:** Comparisons between log-phase abundance FCs to those at hypoxia and reaeration treatment.

**Goal:** Identify phenotypically relevant TFs that undergo significant growth abundances or defects in the transition from steady-state to hypoxic conditions.

*Log2FC >= 1.5 used as the cutoff for statistical significance.*

**TRIP Data Analysis**

*The set of growth abundance (GA) and growth defect (GD) TFs associated with hypoxia.*

<table>
<thead>
<tr>
<th>Regulator</th>
<th>UT_FC</th>
<th>HYP_FC</th>
<th>OVR_FC</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rv0767c</td>
<td>-6.02</td>
<td>1.909</td>
<td>7.929</td>
<td>GA</td>
</tr>
<tr>
<td>Rv2034</td>
<td>-2.31</td>
<td>1.88</td>
<td>4.19</td>
<td>GA</td>
</tr>
<tr>
<td>Rv1151c</td>
<td>-0.17</td>
<td>1.62</td>
<td>1.79</td>
<td>GA</td>
</tr>
<tr>
<td>Rv1776c</td>
<td>-6.09</td>
<td>-1.61</td>
<td>4.479</td>
<td>GD</td>
</tr>
<tr>
<td>Rv2642</td>
<td>1.13</td>
<td>-1.689</td>
<td>2.819</td>
<td>GD</td>
</tr>
<tr>
<td>Rv2009</td>
<td>0.6</td>
<td>-1.81</td>
<td>2.41</td>
<td>GD</td>
</tr>
<tr>
<td>Rv2359</td>
<td>0.45</td>
<td>-1.909</td>
<td>2.359</td>
<td>GD</td>
</tr>
<tr>
<td>Rv1152</td>
<td>0.49</td>
<td>-1.869</td>
<td>2.359</td>
<td>GD</td>
</tr>
<tr>
<td>Rv1473A</td>
<td>0.77</td>
<td>-1.57</td>
<td>2.34</td>
<td>GD</td>
</tr>
<tr>
<td>Rv0821c</td>
<td>0.56</td>
<td>-1.57</td>
<td>2.13</td>
<td>GD</td>
</tr>
<tr>
<td>Rv3291c</td>
<td>0.46</td>
<td>-1.609</td>
<td>2.069</td>
<td>GD</td>
</tr>
<tr>
<td>Rv0491</td>
<td>0.37</td>
<td>-1.63</td>
<td>2.0</td>
<td>GD</td>
</tr>
<tr>
<td>Rv0144</td>
<td>0.5</td>
<td>-1.5</td>
<td>2.0</td>
<td>GD</td>
</tr>
<tr>
<td>Rv3160c</td>
<td>0.23</td>
<td>-1.65</td>
<td>1.88</td>
<td>GD</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- UT = Untreated
- HYP = Hypoxia
- OVR = ABS(UT-HYP)
Visualizing the Hypoxia-Specific Gene Regulatory Networks

Edge Key:
- Blue (Positive Regulation)
- Red (Negative Regulation)
- Thickness (Network Confidence)
## Looking into the Functional Roles of Network Genes

<table>
<thead>
<tr>
<th>GO Term</th>
<th>Overlap</th>
<th>P-Value</th>
<th>Genes</th>
<th>Phenotypic Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptidoglycan Biosynthetic Process</td>
<td>8/15</td>
<td>0.003567</td>
<td>Rv2154c; Rv1086; Rv3682; Rv3794; Rv2152c; Rv0483; Rv0050; Rv1018c.</td>
<td>The peptidoglycan layer is essential for maintaining cellular integrity and forming a permeability barrier.</td>
</tr>
<tr>
<td>Proton-Transporting ATP Synthase Activity</td>
<td>6/8</td>
<td>0.034982</td>
<td>Rv1309; Rv1311; Rv1307; Rv1310; Rv1308; Rv1306.</td>
<td>Protonmotive force is required for maintaining ATP homeostasis and viability of hypoxic MTB.</td>
</tr>
<tr>
<td>Cell Redox Homeostasis</td>
<td>5/12</td>
<td>0.002969</td>
<td>Rv1470; Rv1471; Rv0688; Rv1324; Rv1677.</td>
<td>Preservation of an appropriate redox balance is critical to the persistence of MTB.</td>
</tr>
<tr>
<td>Fatty Acid Biosynthetic Process</td>
<td>7/17</td>
<td>0.048612</td>
<td>Rv3825c; Rv1484; Rv2524c; Rv0533c; Rv1094; Rv2244; Rv2246.</td>
<td>Macrophage fatty acid metabolism is needed to supplement MTB survival in hypoxia.</td>
</tr>
<tr>
<td>Response to Stress</td>
<td>8/14</td>
<td>0.013853</td>
<td>Rv3223c; Rv2028c; Rv3134c; Rv2374c; Rv2624c; Rv0576; Rv0982; Rv2035.</td>
<td>An indicator that bacteria are sensing and adapting to the anaerobic environment.</td>
</tr>
</tbody>
</table>

*Enrichment analysis was performed with the Enrichr API of GSEAPy; an adjusted P-Value cutoff of <= 0.05 was used to determine statistical significance.*
Shared Target Components Between Rv0821c and Rv0144

<table>
<thead>
<tr>
<th>Target</th>
<th>Functional Description</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rv1384c</td>
<td>Essential for PAT lipid biosynthesis, which is a significant constituent of the mycobacterial cell wall.</td>
<td>Cell Wall and Cell Processes</td>
</tr>
<tr>
<td>Rv2026c</td>
<td>MmpL3 protein is a transmembrane transporter of mycolic acid; long chain fatty acids found in the lipid-rich cell walls of tuberculosis bacterium.</td>
<td>Cell Wall and Cell Processes</td>
</tr>
<tr>
<td>Rv3804c</td>
<td>Refers to proteins of the antigen 85 complex that contribute to the biogenesis of trehalose dimycolate, a dominant structure required for cell wall integrity.</td>
<td>Lipid Metabolism</td>
</tr>
<tr>
<td>Rv3478c</td>
<td>Lipolytic enzyme LipF involved in cellular metabolism.</td>
<td>Intermediary Metabolism and Respiration</td>
</tr>
<tr>
<td>Rv2219</td>
<td>Probable conserved transmembrane protein.</td>
<td>Cell Wall and Cell Processes</td>
</tr>
<tr>
<td>Rv1832</td>
<td>Glycine cleavage system that catalyzes the degradation of glycine, which has been implicated in the biosynthesis of peptidoglycan and other cell wall structural components.</td>
<td>Intermediary Metabolism and Respiration</td>
</tr>
<tr>
<td>Rv1196</td>
<td>Resembles PPE18, a cell wall associated protein that is involved in inflammatory response and cytokine manipulation.</td>
<td>PE/PPE</td>
</tr>
</tbody>
</table>

**Rv0821c (PhoY2):** Inactivation leads to antibiotic resistance; maintains inorganic phosphate homeostasis; stress response.

**Rv0144:** Shown to be regulated by RelA, critical for establishing persistent infection in mice.

**Takeaway:** A dual mechanism of mycobacterial persistence linked to cell wall synthesis and intracellular transport.
Characterizing the Rv2359-Rv1152 Relationship

The Rv1152-Rv2359 connection could function as a **metal ion-responsive homeostasis mechanism** that is effectively downregulated during hypoxia.

As a result, pathogen would have more time to make **anticipatory adaptations** to future host immune response and build resistance to oxidative stress.

**Strong expression- and phenotypic-based correlations, along with GRN connectivity, indicate a potential relationship.**
Discussion & Conclusion

- MTB dormancy in hypoxia shown to be functionally associated with stress response, cell redox homeostasis, metal ion cycling, and cell wall metabolism – all of which modulate critical host-pathogen interactions.

- Unraveling Transcriptional Regulatory Mechanisms
  - **Rv0821c-Rv0144**: Dual System of Persistence (Via Cell Wall Synthesis)
  - **Rv1152-Rv2359**: Delayed Zinc Limitation Enables Anticipatory Adaptations

- Investigating Key Factors of Interest
  - Nutritional Immunity
  - Defense Antioxidants Counter Pro-Inflammatory Cytokines

Results

- **Incorporate reaeration data** (7D to 12D) to catalog other physiological adjustments during reintroduction to the stationary phase.
- Apply the **DREM 2.0 approach**, which identifies bifurcation points that track transitions between coordinated regulatory programs and gene states.

Future Directions

Limitations

Experimental Data Restricted to the Defined Hypoxic Model

Lack of Gold Standard Data to Supplement GRN Construction
References


