Role of Internal Carbon Mobilization for an Optimal Re-Growth of Dormant *Mycobacterium tuberculosis*

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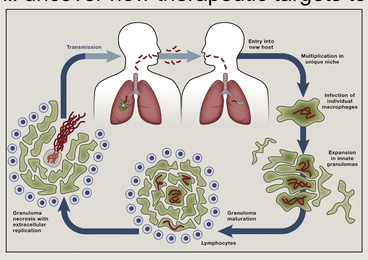
Abstract

Mycobacterium tuberculosis (Mtb) is the bacteria that results in the pathogenic infection of Tuberculosis, a serious pulmonary infection that is fatal if left untreated. In the presence of antibiotics. Mtb enters a persister state, in which the bacteria is phenotypically dormant and the infection subdues. However, upon the elimination of antibiotics, Mtb exits the persister state and returns back to a virulent state. We hypothesize that this reactivation process of Mtb include the internal consumption of upstream glycolytic metabolites as a source of downstream glycolytic intermediates as upstream ones are highly accumulated with reciprocal depletion of downstream ones during the dormant stage, and during re-activation these metabolites. This study examined the role of accumulated glycolytic intermediates as a source of internal carbon mobilization for an optimal re-growth of dormant Mtb. For this, we used hypoxia as this condition engendered Mtb into a dormant state, and metabolomic remodeling during re-growth was analyzed through LCMS mediated metabolomics. The outcome of this study will uncover new therapeutic targets to clear Mtb infection.



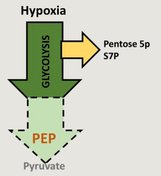
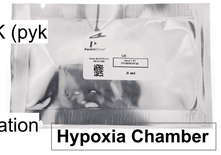


Fig. 1.1. Cycle of replication, dormancy, and re-activation in vivo of *Mycobacterium tuberculosis*

Method

1. Mtb Culture

a. 4 Strains of Mtb → RV (Wildtype), RV ATC, PYK (pyk knockdown), PYK ATC,
b. Utilized m7H9 or m7H10 growth media
c. Created mutant strains via Cripr—dCas9
d. Filter culture system to give hypoxia for re-activation



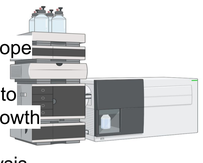
Hypoxia Chamber

2. Checking Reactivation Growth

e. Replicating culture:
   i. Bacteria set to optical density of 0.05 (day 0)
   ii. Optical density checked every 3-4 days to record replication growth
f. Reactivation culture:
   i. Re-activation culture created through disturbing Mtb filters upon 3 days of hypoxic conditions
   ii. Optical density checked every 3-4 days to record reactivation growth

3. LC-MS Metabolomics

g. Samples for metabolomics transferred to C13 isotope plates for detection of Internal vs External carbon
h. Liquid Chromatography Mass Spectrometry used to detect metabolites present in replication and re-growth samples
i. Principal component analysis and Heat Map analysis performed via Metaboanalyst V. 5.0



Growth kinetics of Mtb wild type (RV)

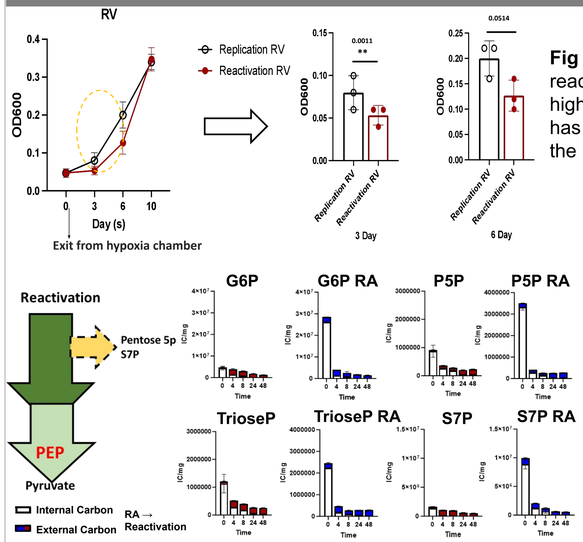


Fig 2.1. - Replication growth in comparison to the reactivation growth of mtb wild type strain (RV). As highlighted in the yellow circle, the replication growth has a substantially higher OD from days 3 to 6, as the re-activation growth is experiencing a lag phase.

Fig 2.2. - The metabolomic data of RV reactivation. The kinetic charts depict elevated levels of of G6P, P5P, S7P, and TrioseP at 0 hour time point, and upon exit from hypoxia depict significant depletion. This is resulting from Mtb accumulating these upstream glycolytic metabolites during persister state, and upon reactivation utilizing these metabolites for biosynthesis of PEP, thus triggering the Citric Acid cycle. The make up of the carbon source within 0 hour metabolite amongst G6P, P5P, S7P, and TrioseP is majority unlabeled, this indicates that it is an internal carbon source accumulation, compared to exit from hypoxia, in which majority of the metabolite is labeled indicating external C13 glucose as the carbon source.

Metabolomics analysis of replication and re-growth bacilli

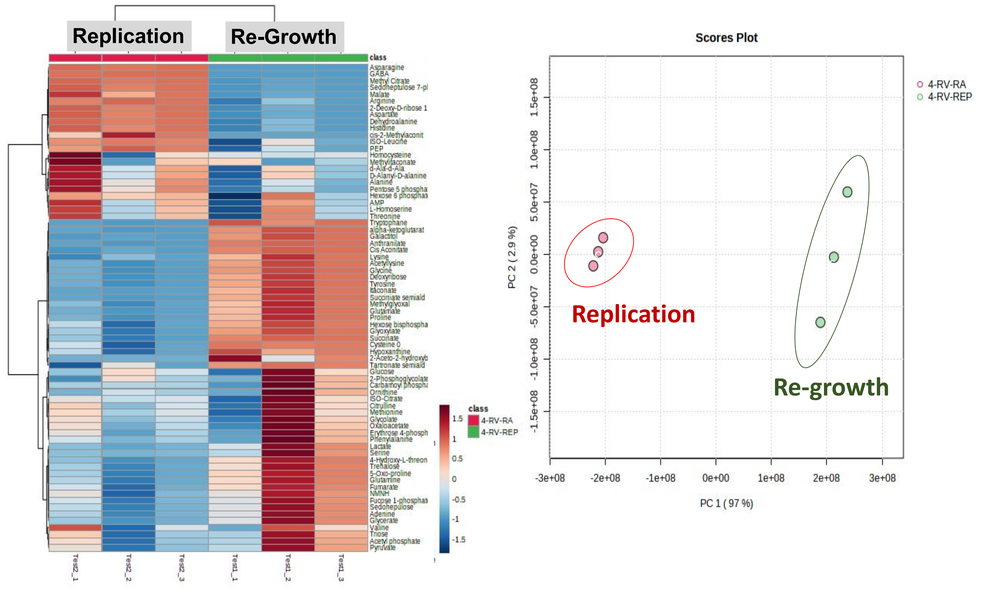


Fig 3.1
Left – Central carbon metabolism (e.g., glycolysis and pentose phosphate pathway) and cell wall glycolipid precursor metabolism (e.g., trehalose or alpha glucan) belong to high ranked pathways that were different between the two conditions.
Right – PCA analysis showed the Mtb metabolic networks in a replication and a re-growth condition were clearly different.

Growth kinetics of Mtb lacking pyruvate kinase (PYK)

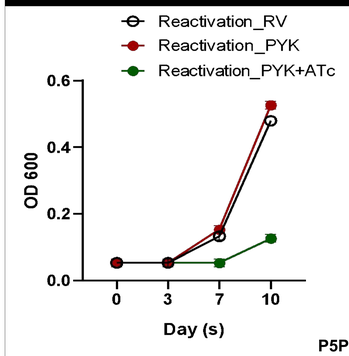


Fig 4.1. The reactivation growth of mtb (PYK) in comparison to mtb (PYK +ATC) with reactivation of RV as a control. The reactivation of PYK+ATC experienced similar optical density to RV and PYK until day 3. Between days 3-10 the optical density of PYK+ATC was significantly lower than that of RV and PYK.

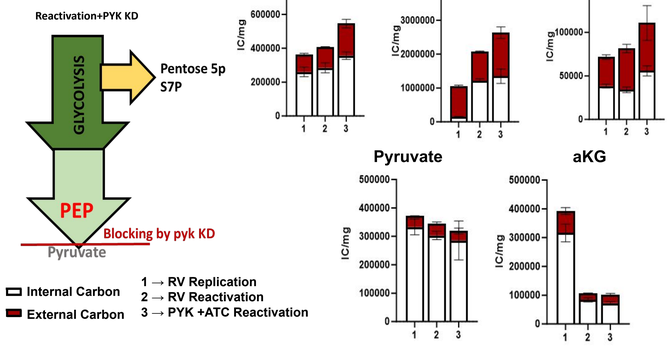


Fig 4.2. PYK blocks the conversion of PEP into pyruvate, shown by the depletion of pyruvate and build up of PEP in PYK reactivation. Mtb PYK re-directs the upstream glycolytic metabolites to the PPP pathway, this is shown as P5P and S7P - key metabolites in the PPP pathway - exhibit elevated levels within the PYK reactivation. Additionally, metabolites within the Citric Acid cycle, specifically succinate and alpha ketoglutarate depict depleted levels within PYK reactivation. This explains the significant latency of the PYK reactivation growth in comparison to the RV reactivation growth.

Summary

1. Mtb requires a prolonged period of lag phase to resume the re-growth (exit from a dormant state as compared to that of replication growth kinetics
2. Mtb in a dormant state maintains its viability and metabolism by using cell wall glycolipid as a new carbon source (Lee et al. 2019. Nat. Communication; Eoh et al. 2017 Nat. Microbiology).
3. The upper glycolytic intermediates (e.g. glucose 6P) were decreased during regrowth to fuel downstream glycolysis especially PEP as an alternate energy source. This reciprocally matched to the downregulation of carbon flux towards the biosynthesis of PPP, a source of NADPH and antioxidant.
4. Restored abundance of downstream glycolytic intermediates such as PEP is required to fuel downstream pathways such as amino acids to resume the replication.
5. PYK catalytic activity is required for optimal regrowth exit from a dormant state because PYK consume PEP to biosynthesize pyruvate and ATP, an initial energy to restore metabolic networks required for growth resumption.
6. Metabolic remodeling required for regrowth serves as a potential source of new drug targets to cure dormant Mtb infection.

References

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2. Transient drug-tolerance and permanent drug-resistance rely on the trehalose-catalytic shift in Mycobacterium tuberculosis. Lee et al. Nat Commun. 2019
3. Phosphoenolpyruvate depletion mediates both growth arrest and drug tolerance of Mycobacterium tuberculosis in hypoxia. Lim J et al. Proc Natl Acad Sci U S A. 2021