Nitrogen metabolism in dormant *Mycobacterium tuberculosis*

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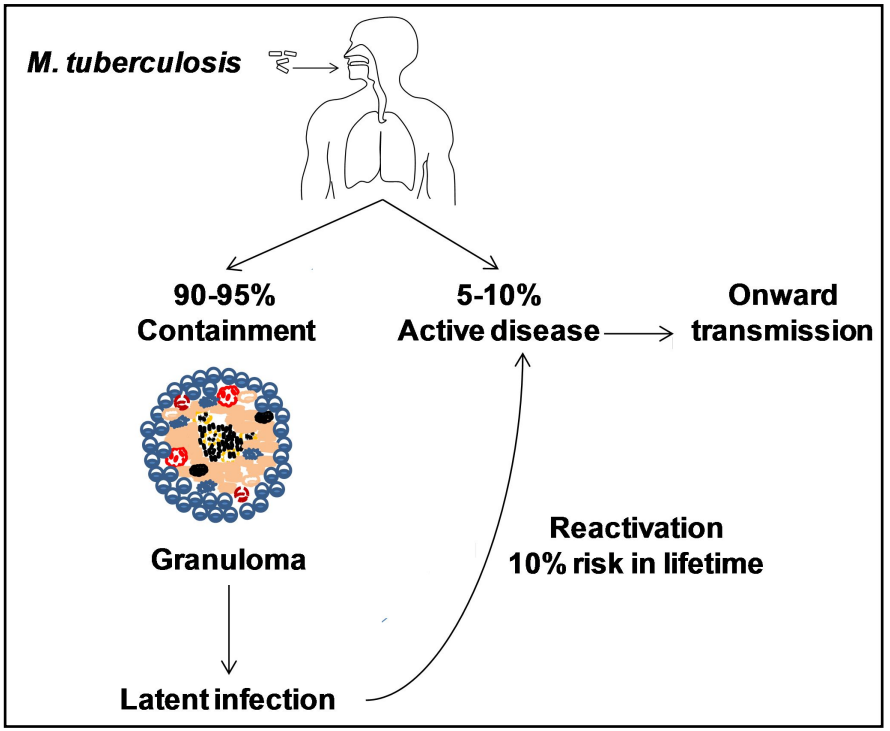
Outline

- Tuberculosis (TB) and Mtb
- Nitrogen metabolism in dormancy
    1. nitrate reduction
    2. ammonium assimilation
    3. regulatory mechanism
- Conclusions and perspectives

TB and Mtb

- Caused by aerobic bacteria Mtb
- Top infectious killing diseases.
  - HIV/AIDS 3 million
  - Tuberculosis kills 2 million
  - Malaria kills 1 million
- Widely spreaded world-wide
  - 1/3 carriers (latent infection),
  - among which 10% develop into disease (dormant Mtb >> active Mtb)



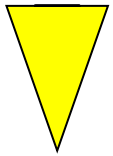
Most cases of active tuberculosis result from reactivation of latent infection

Latent infection and dormant Mtb

- Latent infection is ascribed to the tendency of Mtb to enter a dormant non-replicating state upon exposure to catastrophic stresses, such as anaerobiosis and host immunity.

Characteristics of dormant Mtb:

- still viable, re-activated
- phenotypic drug resistance
- resistent to the host defense factors: hypoxia, low pH, ROS, nutrient starvation
- respiratory nitrate reduction provide energy for dormant Mtb



Dormant Mtb relies on a specific nitrogen metabolism?

Owing to the limited availability of nutrients in host cell, how Mtb acquires nutrients to sustain its own metabolic demands is an intriguing question.

Why do we must know the nitrogen metabolism of dormant Mtb?

Because we want to know how to inhibit or stop the intracellular surival of dormant Mtb and eradicate it.

Nitrogen metabolism in dormancy

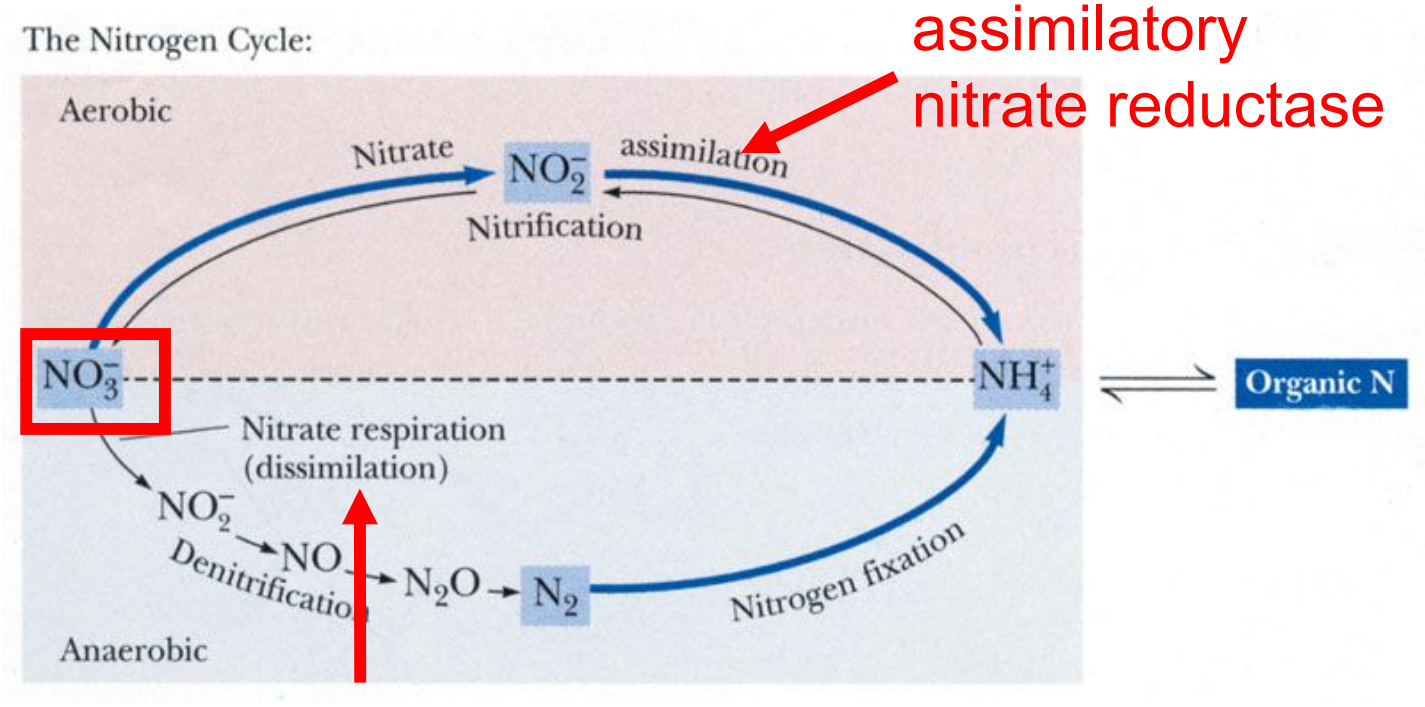
In addition to carbon, nitrogen is a fundamental constituent of biomolecules such as amino acids, nucleotides, cell wall components and organic cofactors.

- Nitrogen sources: organic and inorganic compounds
  eg. ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-)

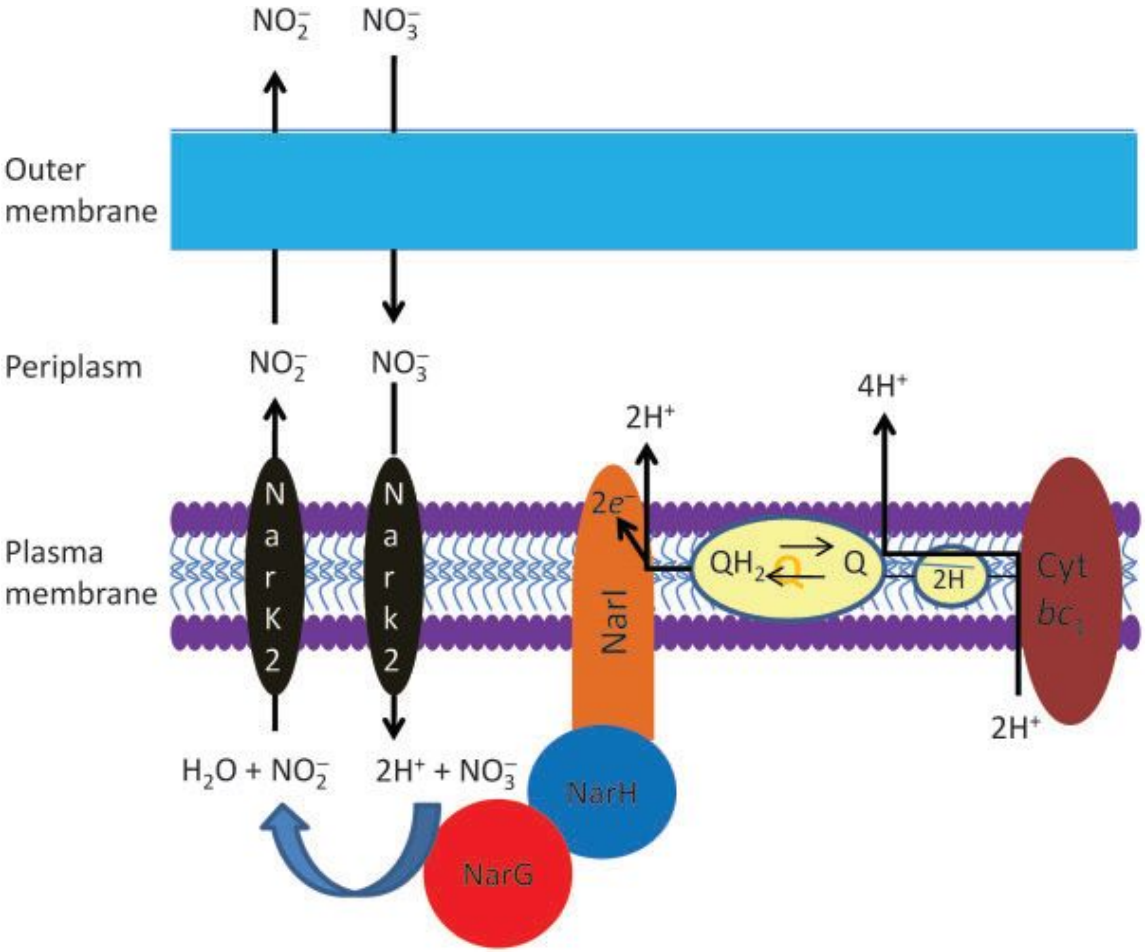
- Reduction of nitrate (NO_3^-)
- Assimilation of ammonium (NH_4^+)

Nitrate reduction in dormancy

- Nitrate reductase(NarGHJI): the assimilatory and respiratory nitrate reductase



Nitrate reduction: respiration of nitrate



The nitrate respiration pathway in mycobacteria

A. Khan and D. Sarkar. Microbiology, 2012

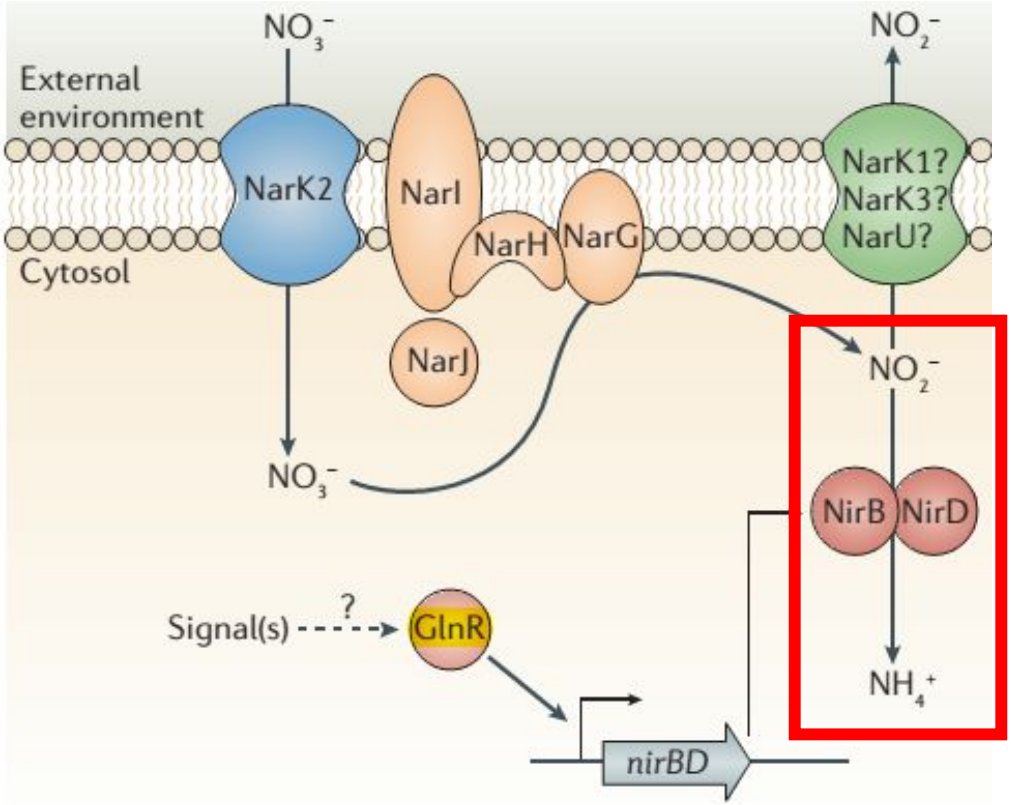
nitrate reductase: NarGHJI

Quinol (Q) pool: electron donor

proton motive force

Nitrate: the terminal electron acceptor

Nitrate reduction: assimilation of nitrate



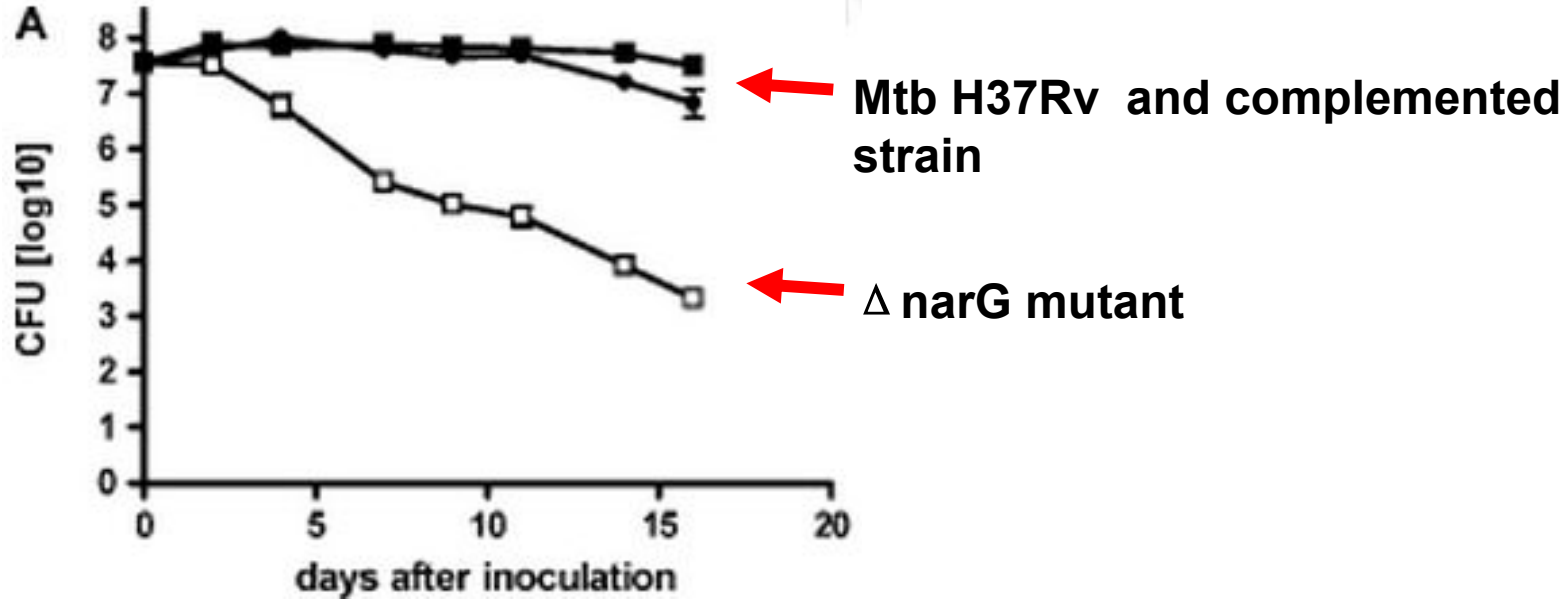
Mechanism of the nitrate assimilation

Downstream:
nitrate reductase: NarGHJI
nitrite reductase: NirBD
transcriptional regulator: GlnR

Alexandre Gouzy, et al. Nat Rev Microbiol, 2014

Nitrate reduction and virulence

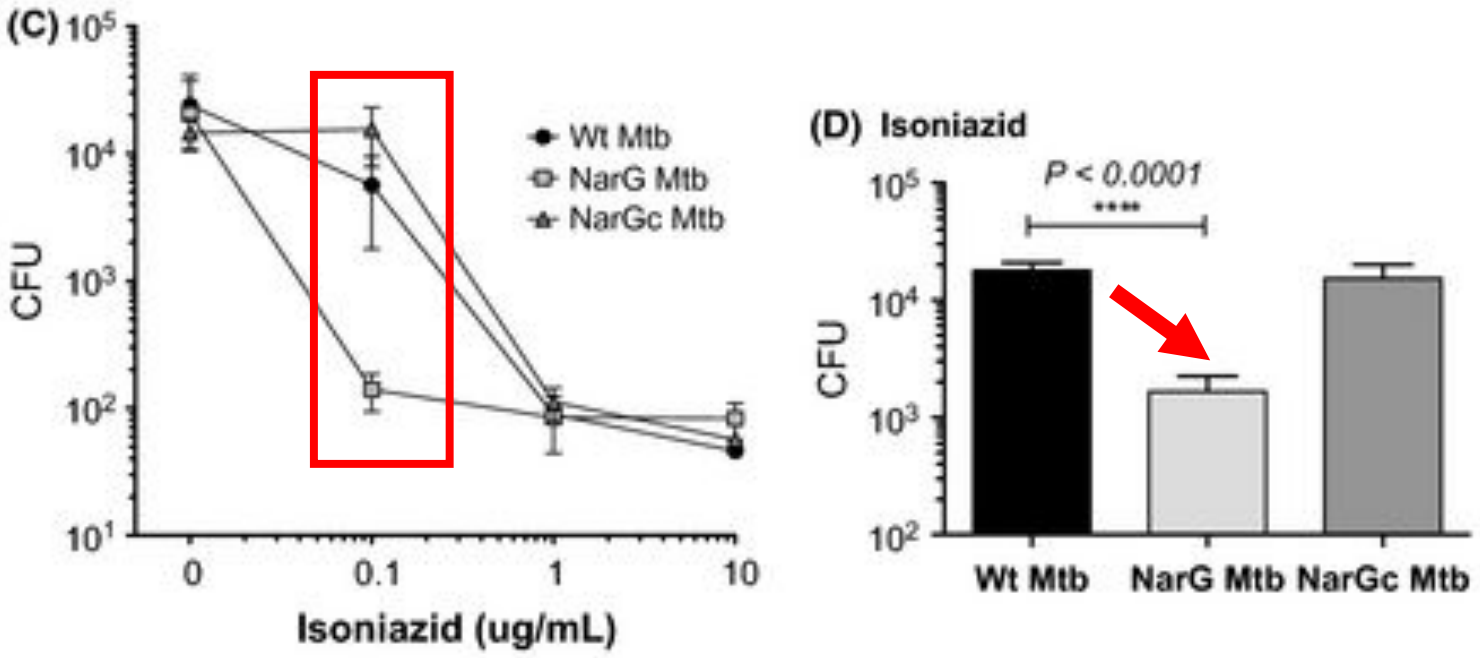
- Nitrate reductase(NarGHJI): mutant failes to persist under anaerobic conditions in vitro; complementation with Mtb *narG* restores the ability of the mutant to persist.



Aly S, et al. J Pathol, 2006

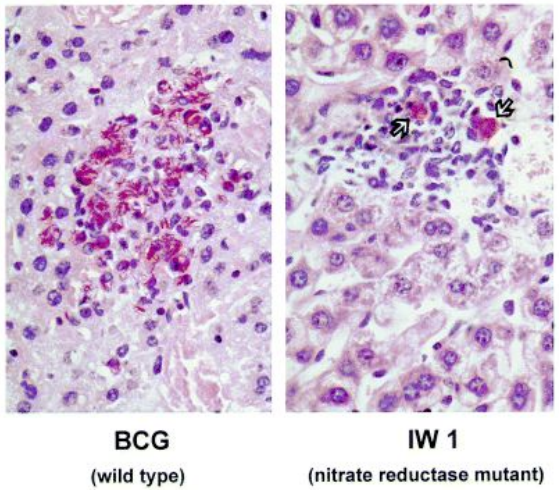
Nitrate reduction and virulence

- Nitrate reductase(NarGHJI): mutant is more susceptible than wild-type Mtb to treatment with isoniazid during infection of macrophages

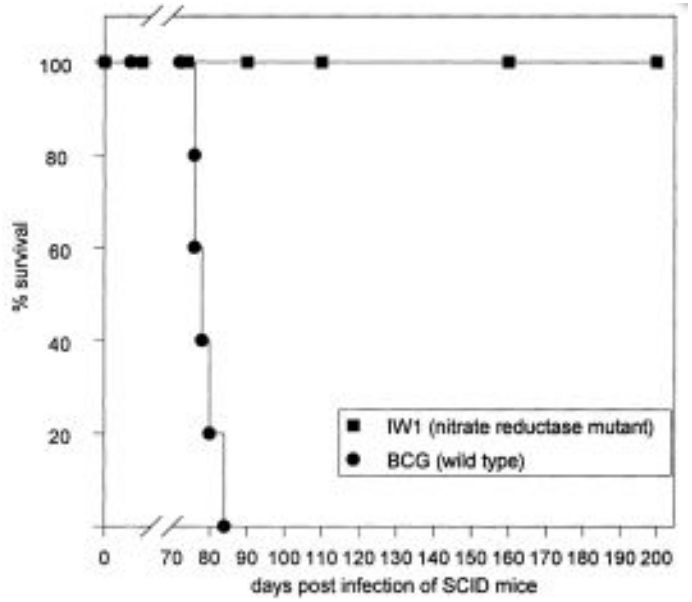


A. Cunningham-Bussel, et al. Microbiologyopen, 2013

Nitrate reduction and virulence



Isabel Weber, et al. Mol Microbiol, 2000



Nitrate reductase(NarGHJI):

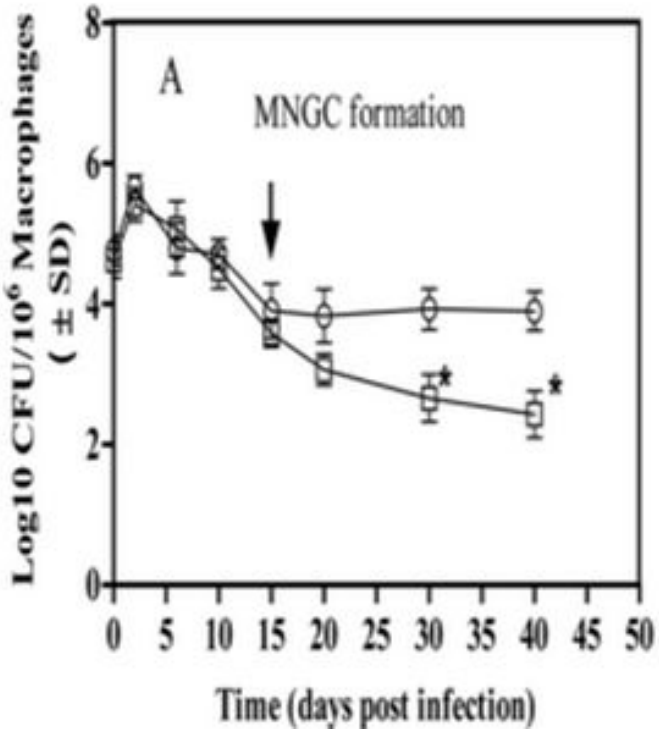
mice infected with the mutant had smaller granulomas containing fewer bacteria;

mice survival assay showed no signs of clinical disease after more than 200 days

M. bovis BCG

Nitrate reduction and virulence

- Nitrite reductase(NirBD): the number of viable nirBD mutants continue to decrease during intracellular dormancy.



MNGCs: multinucleated giant cells

Shamim Akhtar, et al. J Bacteriol, 2013

Nitrate reduction and virulence

- Nitrate reduction is proposed to play a role in the survival of Mtb during the dormant state.
- affect the virulence of Mtb

Assimilation of ammonium(NH_{4+})

Ammonium is assimilated into low molecular weight metabolites such as glutamate or glutamine.

GDH Pathway (glutamate dehydrogenase):

NH_4^+ + 2-oxoglutarate + NADPH+ H^+ <-GDH-> glutamate+$NADP^+$

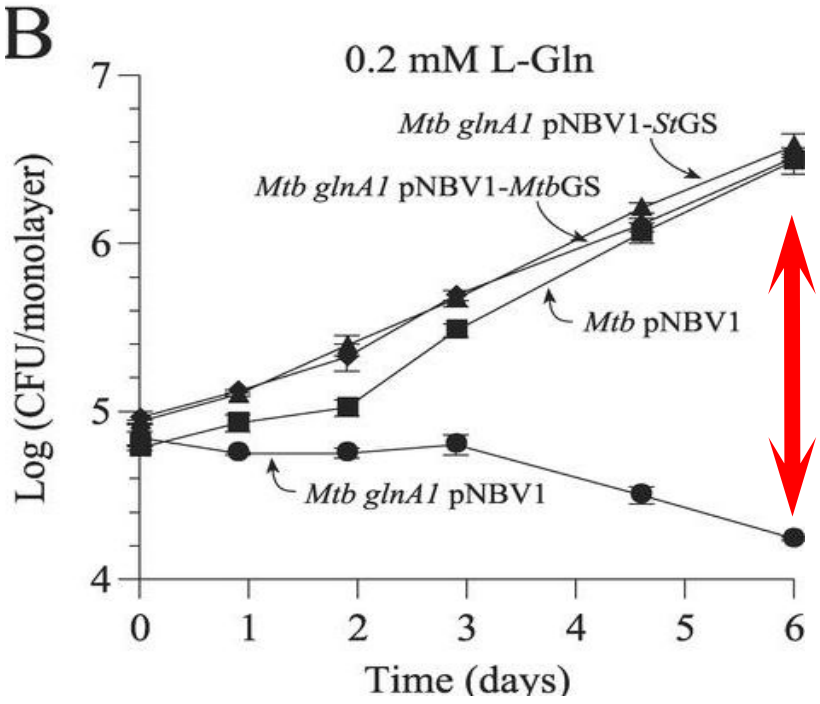
GS-GOGAT Pathway (Glutamine Synthetase-Glutamate Oxoglutarate Aminotransferase):

NH_4^+ + glutamate + ATP --GS-> glutamine + ADP + Pi

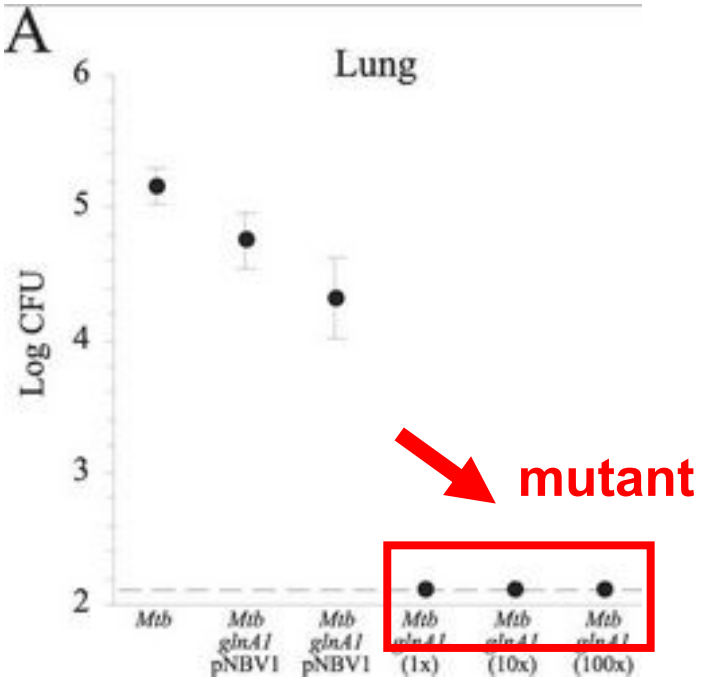
glutamine+2-oxoglutarate+NADPH+H^+ -GOGAT-> 2glutamate+ $NADP^+$

Ammonium assimilation and virulence

- Glutamine synthetase (GS) is essential for growth of Mtb in hman THP-1 macrophages and guinea pigs



bacterial viability in guinea pigs



Tullius MV1, et al. Infect Immun, 2003

Regulation of nitrogen metabolism

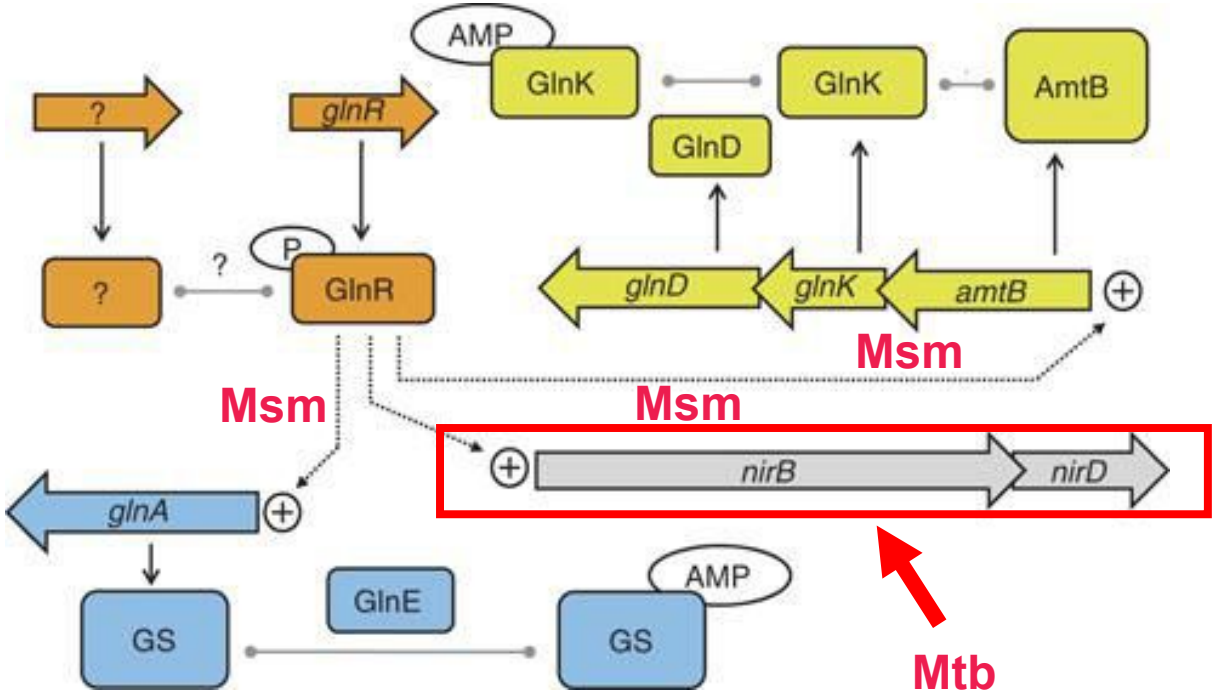
- Different molecular mechanisms are involved in the regulation of nitrogen metabolism.

  transcriptional level;

  post-transcriptional level: adenylylation; phosphorylation;

Regulation of nitrogen metabolism

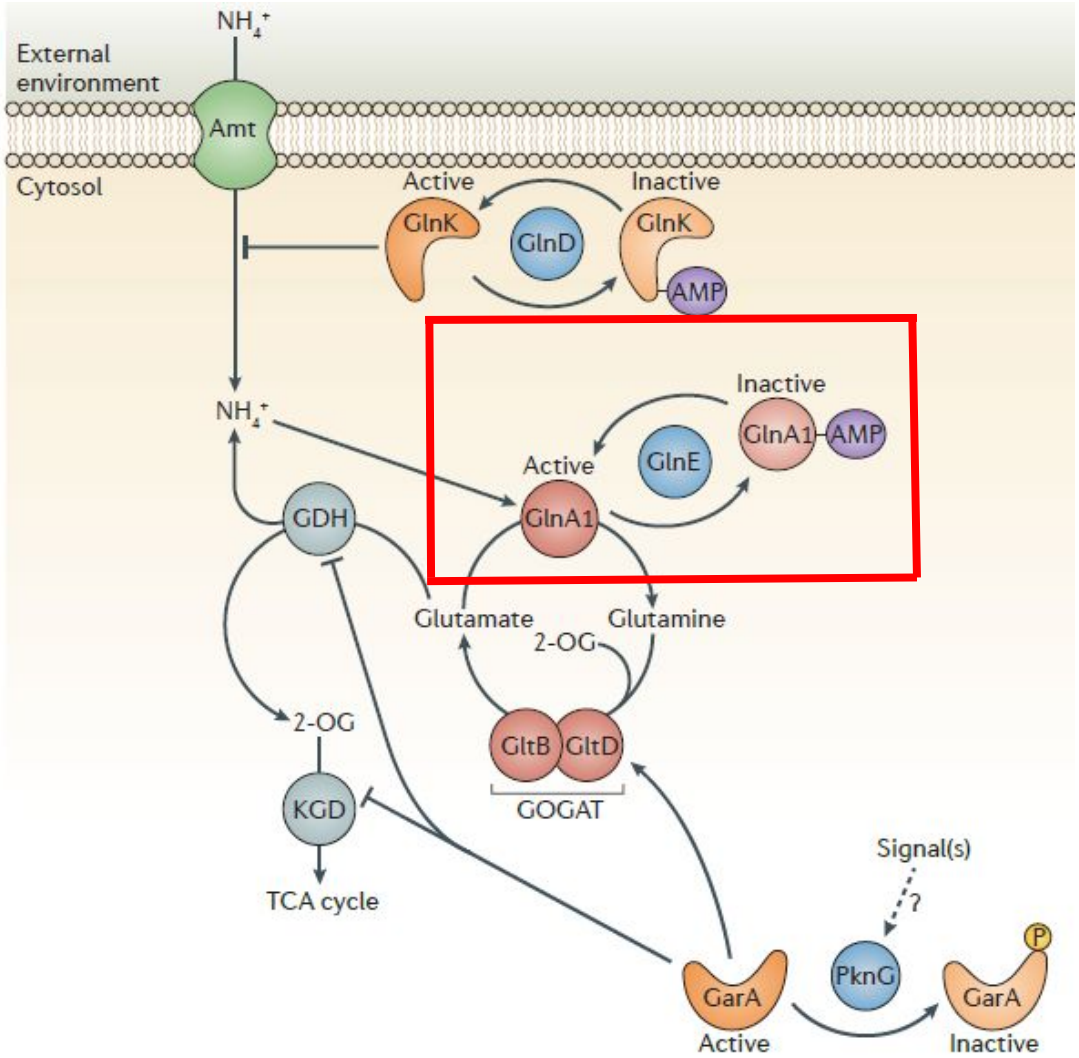
Transcriptional Regulation: GlnR activate transcription of the *nirBD* operon, which encodes a nitrite (NO_2^-) reductase



Regulatory network of nitrogen metabolism in *M. smegmatis*

http://www.mikrobio.nat.uni-erlangen.de/research/burkovski/nitrogen.shtml

Regulation of nitrogen metabolism



Alexandre Gouzy, et al. Nat Rev Microbiol, 2014

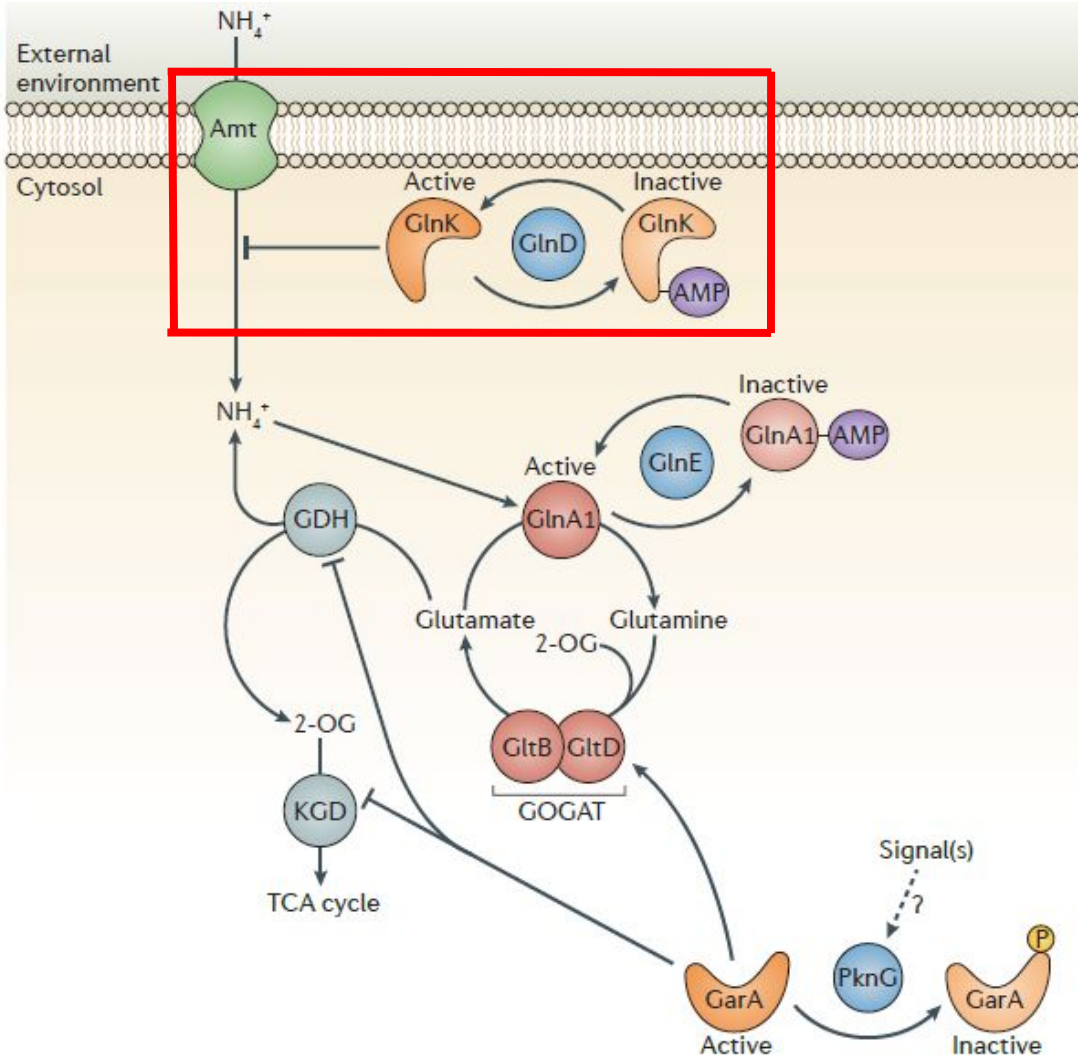
post-transcriptional level

- Adenylylation:

GlnE adenylylates GlnA1

GlnA1: glutamine synthetase
GlnE: adenylyl transferase

Regulation of nitrogen metabolism



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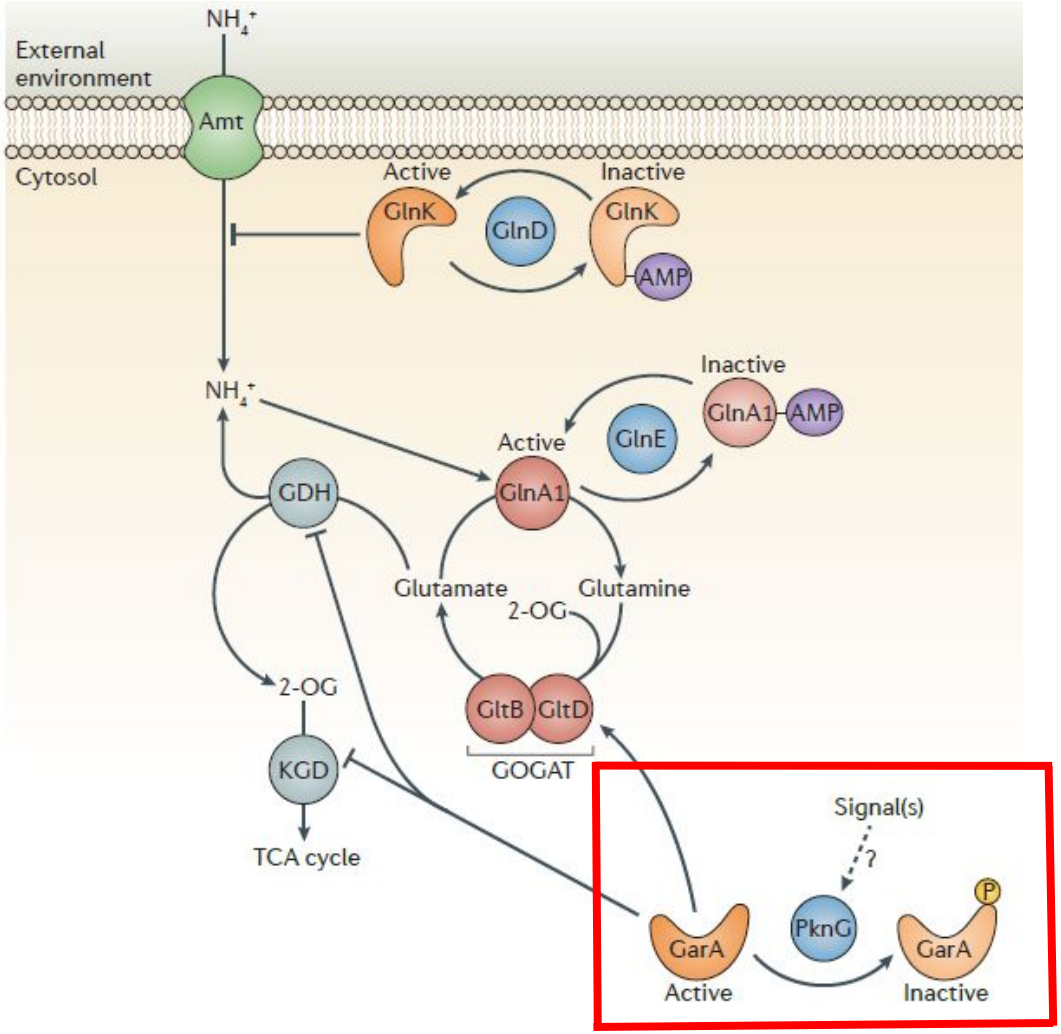
- Adenylylation:

GlnD adenylylates GlnK

Regulation of nitrogen metabolism



•<u>Phosphorylation</u>
PknG inactivates the regulator GarA

PknG: serine-threonine kinase
GarA: glycogen accumulation regulator A

Alexandre Gouzy, et al. Nat Rev Microbiol, 2014

Conclusions and perspectives

- In dormancy, nitrate metabolism is essential for the survival of dormant Mtb in macrophages.
- Nitrate metabolism and ammonium assimilation is individually involved in Mtb virulence during infection.

➢ Remaining question: GlnR

➢ need to be illustrated

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Thank you