Redox Homeostasis and Radical Detoxification Systems in Mycobacterium tuberculosis

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Outline

• Redox Homeostasis & Mtb
• Oxidative Stress in *Mtb* lifecycle
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• Redox Homeostasis in *Mtb*
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• Summary
Part I. Redox Homeostasis & Mycobacterium tuberculosis
Redox Homeostasis

• The balance of oxidative and reductive capacity within a biological system such as a single cell, organ, or organism

• The reactive species will produce in all aerobic respiration

• Oxidative stress: Reactive oxygen species (ROS) e.g. O2•-, HO2•, HO• and RO•; Reactive oxygen species; Reactive nitrogen species (RNS) e.g. NO•, NO2• and NO3•

• Antioxidant defense: Enzymatic; Non-enzymatic
Extra difficulty for *Mtb* Redox Homeostasis

- As a pathogen, need to evade most **immune stress** from host cell
- **ROS/RNS** is most significant immune stress in macrophage
- Redox imbalance might also affect **antimycobacterial drug efficacy**. For example, INH or ethionamide

A typical infection of Mtb

“captured → survive in macrophage (→ enter dormancy → → resuscitation) → active infection”
Standard redox potential of normal redox stress species

- Radical species damage microbial DNA, lipids, and proteins, as well as other susceptible cellular constituents.
- The higher of Redox potential, the higher ability to make damage
Part II. Redox Stress in \textit{Mtb} lifecycle
Endogenous ROS stress

- reduction of O2 by various components of the electron transport chain under normal aerobic conditions, resulting in the production of ROS as superoxide radicals (O2•-).
- O2•- also oxidises the 4Fe–4S clusters of enzymes, such as dehydratases, leading to enzyme inactivation and release of Fe2+.
Endogenous ROS stress

- The $O_2^\cdot -$ also turns into $H_2O_2$ by Superoxide dismutase (SOD)

$$O_2^\cdot^- + O_2^\cdot^- + 2H^+ \rightarrow H_2O_2 + O_2$$

- The released Fe$^{2+}$ can then reduce $H_2O_2$ to intracellular $HO^\cdot$ (**much higher reactive**) (Fenton reaction)

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^\cdot + HO^-$$
ROS stress from immune system

• On phagocytosis of Mtb, lung macrophages and neutrophils produce large quantities of ROS and RNS.

• NADPH oxidase in host cell catalyses the O2 using NADPH as electron donor, generating O2⁻⁻, as depicted in the following

\[ 2O_2 + \text{NADPH} \rightarrow O_2^{2-} + \text{NADP}^+ + H^+ \]

• Besides, hypochlorite ion (ClO⁻⁻) could be generated by myeloperoxidase; ClO⁻⁻ is an extremely reactive oxidant and can lead to oxidative damage of lipids, proteins and DNA

\[ \text{Cl}^- + \text{H}_2\text{O}_2 \rightarrow \text{ClO}^- + \text{H}_2\text{O} \]
RNS stress from immune system

• In response to mycobacterial infection, another major antimicrobial pathway that acts through inducible NO synthase is activated.

\[
\text{L-arginine} + \text{NADPH} + \text{H}^+ + \text{O}_2 \rightarrow \text{L-citrulline} \\
+ \text{NADP}^+ + \text{H}_2\text{O} + \text{NO}^* \\
\]

• Than NO• react with O2•-- to produce highly reactive OONO−, than leads to the generation of NO−,•NO2, NO2−, N2O3, N2O4. which are all effective in killing Mtb.
Hypoxia Stress in granuloma

• **Before granuloma formed**
  - Immune response (mononuclear cells and T lymphocytes)
  - Low pH
  - Oxidative stress

• **After granuloma matured (solid granuloma)**
  - Hypoxia
  - Low nutrition (foamy macrophage contains rich fatty acid in granuloma center)
Part III. Redox Homeostasis in *Mtb*
Redox Homeostasis in *Mtb*

- similar to other bacterial species, *Mtb* has evolved pathways to monitor redox signals (such as O₂, NO and CO) and the alterations in all mentioned intra- and extracellular redox stresses.
- There are two basic types of strategy to keep redox homeostasis in *Mtb*: *non*-enzymatic and enzymatic
Non-enzymatic: THIOLS as Redox Buffers

• Redox couples are present in all cells to keep the cytoplasm in a reduced, such as such as NAD+/NADH, NADP+/NADPH, FAD/FADH2
• The conventional redox couple glutathione (GSSG/2GSH) is absent in mycobacteria.
• Mycobacteria contain redox couples such as thioredoxin [TrxSS/Trx(SH)2], NADH/NAD+ and NADPH/NADP+, Rather, mycobacteria contain oxidised–reduced mycothiol (MSSM/2MSH) as the major redox buffer.
Enzymatic: Superoxide dismutases

- SODs produced by merely all cells to detoxify superoxide radicals. They catalyse the dismutation of $O_2\bullet-$ into $H_2O_2$ and molecular oxygen.

- *Mtb* contains two SODs, an iron-containing SOD called SodA and a Cu- and Zn-containing SOD called SodC.

- Its expression is enhanced by $H_2O_2$ exposure and on nutrient starvation, former study successfully showed that SodC protects *Mtb* against superoxide in vitro.
Enzymatic: Catalase peroxidase

- Catalase peroxidases (Kat) are enzyme systems used to detoxify H2O2 into H2O and O2.
- Mtb owns one catalase, KatG that shows catalase, peroxidase and peroxinitritase activity.
- KatG has been demonstrated to be a virulence factor (Ref. 110) that mediates resistance against the prodrug INH.
Enzymatic: Methionine sulfoxide Reductases

- MSRs use NADPH, Trx and TrxR as the system to reduce methionine sulfoxide to methionine.
- Mtb contain two MSRs, one active on both free and peptidyl methionine-(S)-sulfoxide, and one or more MSRs active on peptidyl, but not free, methionine-(R)-sulfoxide and to protect bacteria against ROS and RNS.
Change of Respiratory chain

- Lack of terminal electron acceptors (O2)
- **Nitrate becomes new main electron acceptors**
- The respiratory chain is also changed, different from **Quinol & cytochrome** transferring the electron in aerobic situation, a series of **nitrogen reductase** form the new anaerobic electron transfer chain
- Nitrate is reduced by a nitrate reductase (**narGHJ**) and is then excreted by a nitrite extrusion protein (**narK1, narK2, narK3**)
- **Alternate** electron carriers in the hypoxic: *fumarate reductase*; probable *NAD(P)H dehydrogenases*; *ferredoxin* (These three parts were upregulated in transcription analysis)
Part IV. Redox sensing in *Mtb*
Stringent response: response to hypoxia

- In *Mtb*, the **ratio** of amino-acylated tRNA to free tRNA was the first regulatory response to amino acid & carbon starvation by **RelA**.

- **ppGpp** is maintained in the cytosol by **RelA**.

- **ppGpp** inhibits polyphosphatase, resulting in the accumulating of **PolyP**. **PolyP** interacts with the **TA module**, finally **globally** affect **RNA polymerase**, then down-regulate gene expression.
DosT/DosS/DosR three component sensor & regulon: response Oxidative stress

- DosT is a gas sensor, activated by absence of oxygen or the binding of nitric oxide and carbon monoxide. DosS is a redox state sensor.

- Both DosT/DosS are Kinase to Phosphorylate DosR, resulting in downstream signaling.

- Expression of DosR was induced by DosT/DosS two component sensor.

- DosT & DosR activated in different time in hypoxia of granuloma.
DosR regulon

- Now 53 genes was found regulated by dosR. Including **4 transporters, 2 Nitrate respiratory chain, 2 regulator**

- Nearly 60% of the genes do not have an annotated function, by sequence & domain comparison, 11 involved in carbohydrate and fatty acid metabolism; 8 in electron transfer
WhiB proteins as iron–sulfur cluster-based sensors

- WhiB3 is an oxygen and NO sensor
- WhiB binds a [4Fe–4S]2+ cluster, which exposure to oxygen or NO leads to activate to a [2Fe–2S]2+
- This changes in WhiB proteins that enhance the DNA-binding activity of WhiB3
Summary:
Mycobacterial mechanisms of sensing and countering Oxidative stress.
Reference

Thank you!