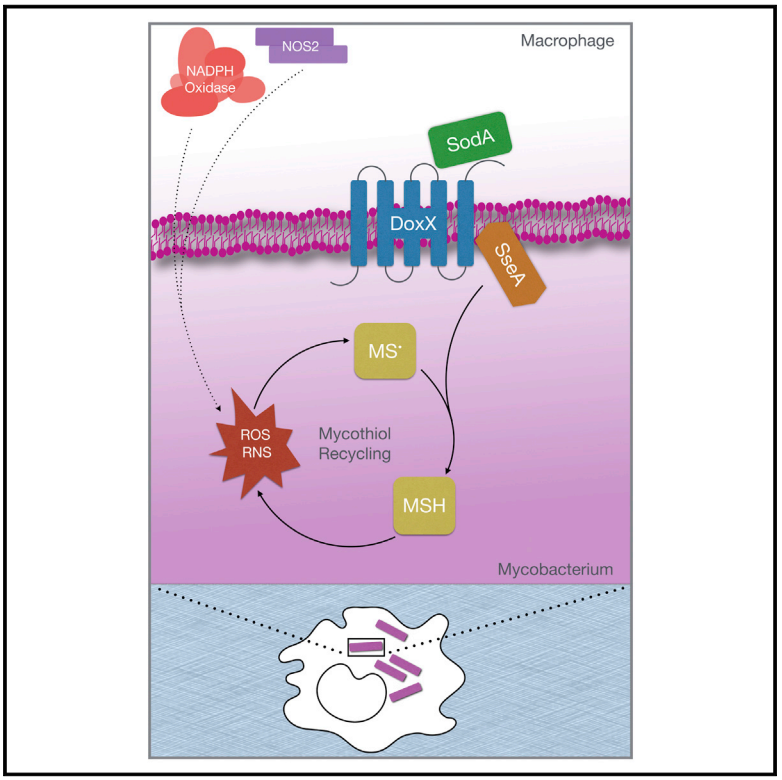
Short Article

Cell Host & Microbe

The Oxidative Stress Network of *Mycobacterium tuberculosis* Reveals Coordination between Radical Detoxification Systems

Graphical Abstract



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In Brief

Host-derived oxidants impose stress on *Mtb* and limit its growth. How *Mtb* coordinates resistance to oxidative stress remains unclear. Nambi et al. define the *Mtb* oxidative stress network during infection and identify the membrane-associated oxidoreductase complex, a three-protein complex that coordinates ROS detoxification with thiol homeostasis as required for infection.

Highlights

- In vivo *Mtb* screen identifies the membrane-associated oxidoreductase complex (MRC)
- The MRC coordinates ROS detoxification and thiol homeostasis during infection
- Loss of the MRC reduces thiol recycling and increases sensitivity to oxidative damage
- *Mtb* mutants of MRC components are highly attenuated

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The Oxidative Stress Network of *Mycobacterium tuberculosis* Reveals Coordination between Radical Detoxification Systems

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SUMMARY

***M. tuberculosis* (*Mtb*) survives a hostile environment within the host that is shaped in part by oxidative stress. The mechanisms used by *Mtb* to resist these stresses remain ill-defined because the complex combination of oxidants generated by host immunity is difficult to accurately recapitulate in vitro. We performed a genome-wide genetic interaction screen to comprehensively delineate oxidative stress resistance pathways necessary for *Mtb* to resist oxidation during infection. Our analysis predicted functional relationships between the superoxide-detoxifying enzyme (SodA), an integral membrane protein (DoxX), and a predicted thiol-oxidoreductase (SseA). Consistent with that, SodA, DoxX, and SseA form a membrane-associated oxidoreductase complex (MRC) that physically links radical detoxification with cytosolic thiol homeostasis. Loss of any MRC component correlated with defective recycling of mycothiol and accumulation of cellular oxidative damage. This previously uncharacterized coordination between oxygen radical detoxification and thiol homeostasis is required to overcome the oxidative environment *Mtb* encounters in the host.**

INTRODUCTION

A cornerstone of metazoan immunity is the production of anti-microbial oxygen and nitrogen radicals by phagocytes. In mammals, superoxide (O_2^-) is generated by the phagocyte NADPH oxidase and xanthine oxidase systems (Halliwell and Gutteridge, 2007). While this reactive species can interact directly with its targets, the superoxide radical is also converted into a number of chemically distinct oxidants, such as peroxide (H_2O_2), hypochlorite (HClO), hydroxyl radicals (OH·), and peroxynitrite ($ONOO^-$). Together, these species damage microbial DNA, lipids, and proteins, as well as particularly susceptible cellular constituents such as iron-sulfur (4Fe-4S) cluster proteins.

The complexity of the phagocyte oxidative burst is matched by the numerous strategies used by bacterial pathogens, such as *M. tuberculosis* (*Mtb*), to resist these insults. Virtually all cells protect themselves from oxidative stress using a cytosolic thiol redox buffer, such as the tripeptide, glutathione. In *Mtb*, the functional analog of glutathione is the cysteine glycoconjugate, mycothiol (Newton et al., 1996). In addition to this common redox buffering system, *Mtb* stress defense mechanisms also include dedicated antioxidant enzymes such as superoxide dismutase (SOD), catalase/peroxidase (KatG), thioredoxin reductase (Tpx), alkylhydroperoxide reductase (AhpC), and peroxiredoxin (AhpE) (Bryk et al., 2002; Edwards et al., 2001; Jaeger et al., 2004; Wilson and Collins, 1996).

Despite the identification of several enzymes that could protect *Mtb* from defined oxidative stresses, it remains unclear how the activities of these pathways are coordinated. Genetic interaction (GI) studies have the capacity to systematically define functional relationships between genes or pathways. A GI is defined by two mutations that modify the phenotype of the other. Aggravating interactions often result from loss-of-function mutations in redundant genes that produce a greater than additive effect. Alleviating interactions occur between genes in the same pathway that depend upon one another for their function and therefore produce a less than additive effect when simultaneously mutated. In order to understand the functional network that *Mtb* employs to resist the oxidative stresses produced during infection, we delineated a comprehensive GI network centered on SOD activity.

RESULTS

Delineating the Oxidative Stress Network during Infection

The primary oxidant produced by the phagocyte oxidative burst is superoxide. Defining a comprehensive oxidative stress interaction network required an *Mtb* mutant that is sensitive to this radical, as well as the array of additional superoxide-derived oxidants produced in vivo. The major SOD of *Mtb*, SodA, is essential for bacterial viability, and therefore not useful for this GI