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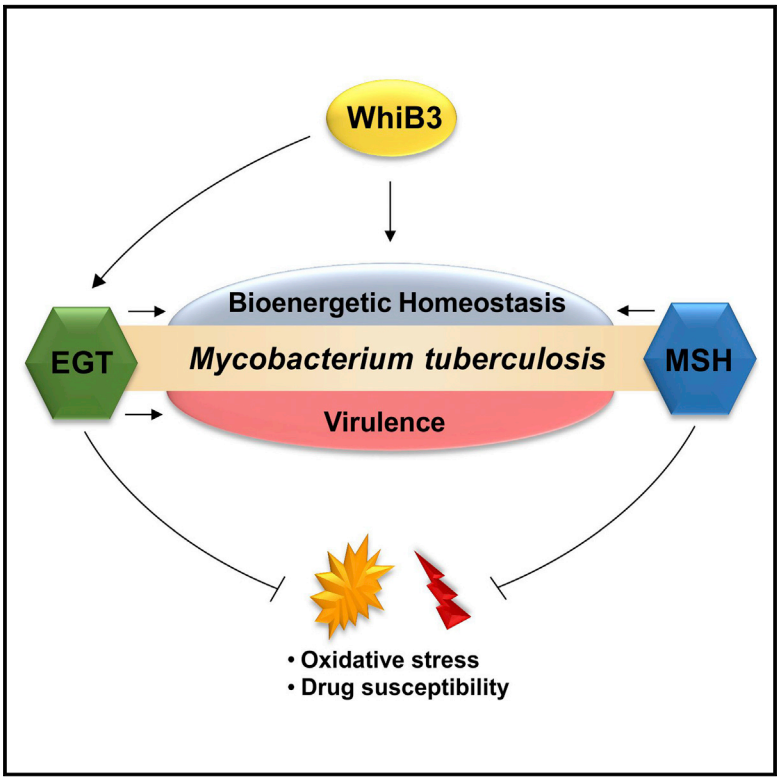
Ergothioneine Maintains Redox and Bioenergetic Homeostasis Essential for Drug Susceptibility and Virulence of *Mycobacterium tuberculosis*

Graphical Abstract



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In Brief

Saini et al. report that *Mtb* WhiB3, a 4Fe-4S redox sensor protein, regulates ergothioneine (EGT) production in a carbon-source-dependent manner and that EGT is required for *Mtb* survival in the host. The authors establish that EGT maintains redox and bioenergetic homeostasis in *Mtb,* which influences drug susceptibility and pathogenicity.

Highlights

- WhiB3 regulates EGT production and maintains bioenergetic homeostasis in *Mtb*
- EGT modulates drug sensitivity and protects *Mtb* from diverse oxidative stressors
- EGT is essential for *Mtb* survival in macrophages and in mice

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Ergothioneine Maintains Redox and Bioenergetic Homeostasis Essential for Drug Susceptibility and Virulence of *Mycobacterium tuberculosis*

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SUMMARY

The mechanisms by which *Mycobacterium tuberculosis* (*Mtb*) maintains metabolic equilibrium to survive during infection and upon exposure to antimycobacterial drugs are poorly characterized. Ergothioneine (EGT) and mycothiol (MSH) are the major redox buffers present in *Mtb*, but the contribution of EGT to *Mtb* redox homeostasis and virulence remains unknown. We report that *Mtb* WhiB3, a 4Fe-4S redox sensor protein, regulates EGT production and maintains bioenergetic homeostasis. We show that central carbon metabolism and lipid precursors regulate EGT production and that EGT modulates drug sensitivity. Notably, EGT and MSH are both essential for redox and bioenergetic homeostasis. Transcriptomic analyses of EGT and MSH mutants indicate overlapping but distinct functions of EGT and MSH. Last, we show that EGT is critical for *Mtb* survival in both macrophages and mice. This study has uncovered a dynamic balance between *Mtb* redox and bioenergetic homeostasis, which critically influences *Mtb* drug susceptibility and pathogenicity.

INTRODUCTION

Tuberculosis (TB) is the second most common cause of death from an infectious agent after HIV. This is largely due to the ability of *Mycobacterium tuberculosis* (*Mtb*) to remain in a dormant, drug-tolerant state for decades in humans before emerging to cause active disease in ~10% of those infected. *Mtb* is exposed to environments with a wide range of available carbon sources, reactive oxygen intermediates (ROIs), and reactive nitrogen intermediates (RNIs) inside the host that may cause cell death. Therefore, it is strongly anticipated that the ability of *Mtb* to maintain redox balance and metabolic homeostasis is critical to its pathogenicity and virulence (Kumar et al., 2011). In addition, some front-line TB drugs such as isoniazid are prodrugs that require bioreduction by *Mtb* for anti-mycobacterial activity (Lei et al., 2000). Thus, a fundamental challenge to global TB control is to understand the mechanisms by which *Mtb* adapts to diverse carbon sources and redox environments encountered in the host.

Mtb produces mycothiol (MSH; Figure 1A), which acts as a major redox couple to protect against various redox stressors and anti-TB drugs (Buchmeier et al., 2003; Rawat et al., 2007). *Mtb* also produces a second thiol couple, ergothioneine (EGT; Figure 1B), a sulfur-containing histidine derivative with potent antioxidant properties (Genghof, 1970; Hand and Honek, 2005). However, despite considerable effort, roles for EGT in *Mtb* and its potential involvement in redox homeostasis and pathogenesis remain unknown. Recently, we have shown that EGT levels in *Mtb* are modulated by protein phosphorylation during transition into late states of growth (Richard-Greenblatt et al., 2015), yet it is still unclear why mycobacteria produce both EGT and MSH to maintain redox homeostasis.

Redox balance is essential for energy metabolism, including glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation (OXPHOS). Despite this strong interdependence between redox homeostasis and energy metabolism, very few tools are available to investigate mycobacterial bioenergetics in real time and in a noninvasive manner. Since cellular respiration involves a complex interplay of biological factors, including the availability, nature, and concentration of oxidizable substrates as well as energy demand, methods for detecting such bioenergetic perturbations in *Mtb* will be of great value.

We previously demonstrated that WhiB3, an *Mtb* 4Fe-4S cluster redox sensor and virulence protein, maintains intracellular redox homeostasis of the mycobacterial cell to provide metabolic and cellular integrity (Singh et al., 2007, 2009; Steyn et al., 2002). In this study, we examined how WhiB3 controls