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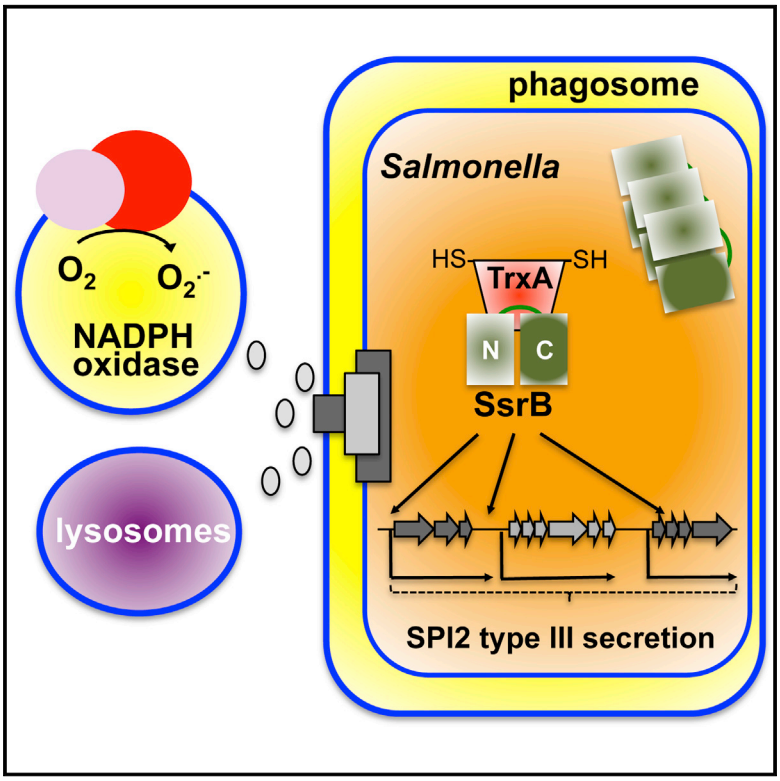
Antioxidant Defense by Thioredoxin Can Occur Independently of Canonical Thiol-Disulfide Oxidoreductase Enzymatic Activity

Graphical Abstract



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In Brief

Song et al. find that thioredoxin promotes expression of the SPI2 type III secretion to help *Salmonella* survive oxidative stress caused by NADPH phagocyte oxidase. Independent of its canonical thiol-disulfide oxidoreductase enzymatic activity, thioredoxin regulates SsrB post-translationally, thereby activating antioxidant defenses associated with SPI2.

Highlights

- Thioredoxin defends *Salmonella* against the NADPH phagocyte oxidase
- Thioredoxin promotes antioxidant defense by facilitating SPI2 transcription
- Thioredoxin binds to the SsrB linker, stabilizing this SPI2 response regulator
- Thioredoxin regulates SsrB independently of its CXXC catalytic motif

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Antioxidant Defense by Thioredoxin Can Occur Independently of Canonical Thiol-Disulfide Oxidoreductase Enzymatic Activity

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SUMMARY

The thiol-disulfide oxidoreductase CXXC catalytic domain of thioredoxin contributes to antioxidant defense in phylogenetically diverse organisms. We find that although the oxidoreductase activity of thioredoxin-1 protects *Salmonella enterica* serovar Typhimurium from hydrogen peroxide in vitro, it does not appear to contribute to *Salmonella's* antioxidant defenses in vivo. Nonetheless, thioredoxin-1 defends *Salmonella* from oxidative stress resulting from NADPH phagocyte oxidase macrophage expression during the innate immune response in mice. Thioredoxin-1 binds to the flexible linker, which connects the receiver and effector domains of SsrB, thereby keeping this response regulator in the soluble fraction. Thioredoxin-1, independently of thiol-disulfide exchange, activates intracellular SPI2 gene transcription required for *Salmonella* resistance to both reactive species generated by NADPH phagocyte oxidase and oxygen-independent lysosomal host defenses. These findings suggest that the horizontally acquired virulence determinant SsrB is regulated post-translationally by ancestrally present thioredoxin.

INTRODUCTION

All aerobic, and many anaerobic, organisms experience oxidative stress at some point in their lifetime. Univalent or divalent reduction of molecular oxygen in the electron transport chain or in the flavin prosthetic groups of cytosolic enzymes are sources of endogenous oxidative stress (Boveris and Chance, 1973; Husain et al., 2008; Korshunov and Imlay, 2010). Steady-state oxidative stress resulting from these metabolic processes is, nonetheless, overshadowed by the high flux of reactive oxygen species (ROS) synthesized by the multisubunit NADPH phagocyte oxidase during the respiratory burst in macrophages and neutrophils (Babior, 1999). *Salmonella enterica* are able to survive activity of this flavohemoprotein in polymorphonuclear and mononuclear phagocytes (Burton et al., 2014; Vázquez-Torres et al., 2000a). The respiratory burst produced by the NADPH phagocyte oxidase is essential to the host defense against salmonellosis, as demonstrated by the prevalence of *Salmonella* infections in chronic granulomatous disease patients bearing autosomal or X-linked mutations in cytosolic and membrane-bound components of this enzymatic complex (Mouy et al., 1989). Mice deficient in the gp91*phox* or p47*phox* subunits of the NADPH phagocyte oxidase recapitulate the hypersusceptibility of patients with chronic granulomatous disease to *Salmonella* infection (Burton et al., 2014; Mastroeni et al., 2000; van Diepen et al., 2002).

Salmonella employ multiple strategies to combat oxidative stress resulting from NADPH phagocyte oxidase activity. Periplasmic Cu-Zn superoxide dismutase SodCI, glutathione, and the ABC-type efflux pump MacAB defend this enteropathogen against cytotoxicity resulting from NADPH phagocyte oxidase (Bogomolnaya et al., 2013; De Groote et al., 1997; Song et al., 2013). In addition, the type III secretion system, encoded by the *Salmonella* pathogenicity island 2 (SPI2), reduces contact between *Salmonella* vacuoles and NADPH phagocyte oxidase-containing vesicles (Berger et al., 2010; Gallois et al., 2001; Vázquez-Torres et al., 2000b), thereby helping this bacterium maintain intracytoplasmic redox homeostasis in macrophages (van der Heijden et al., 2015). Despite the benefits associated with these antioxidant defenses, *Salmonella* suffer oxidative stress in phagocytic cells (Burton et al., 2014). Hydrogen peroxide (H_2O_2) is a critical effector of oxidative stress engendered in the respiratory burst of mononuclear phagocytes (Vázquez-Torres et al., 2000a). H_2O_2 leads to DNA double-strand breaks in a ferrous iron-dependent manner. In addition to this mode I killing, H_2O_2 oxidizes both Feα of [4Fe-4S] prosthetic groups in dehydratases and thiol groups in cysteine residues of target proteins (Imlay, 2003). Disulfide bond formation between neighboring cysteine residues is a common H_2O_2-mediated modification. Thioredoxins and cognate thioredoxin reductases help maintain thiol-disulfide redox homeostasis (Holmgren, 1989). Thioredoxin-1 increases *Salmonella* fitness in a murine model of salmonellosis, but it does not seem to protect this