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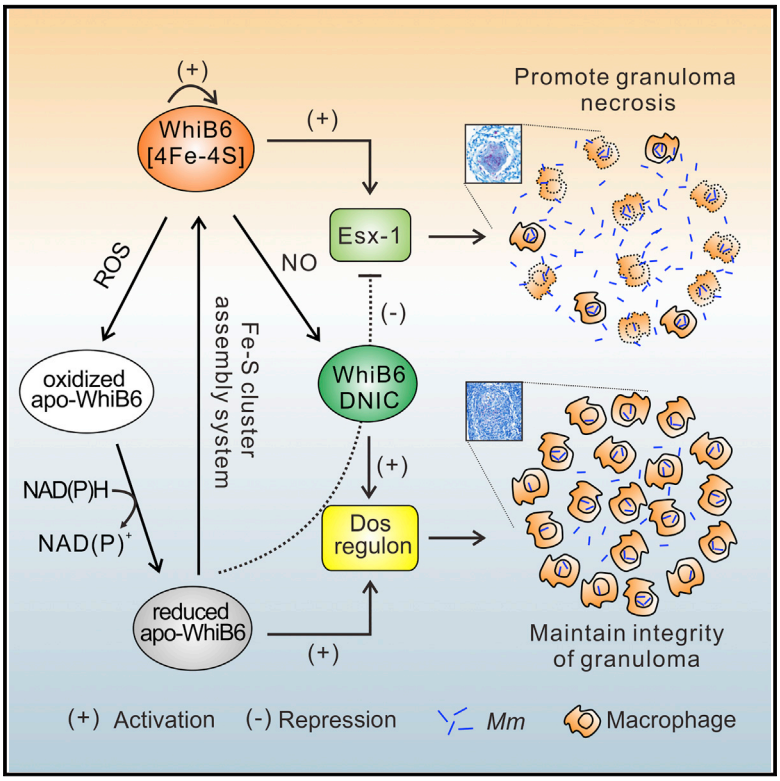
Mycobacterial WhiB6 Differentially Regulates ESX-1 and the Dos Regulon to Modulate Granuloma Formation and Virulence in Zebrafish

Graphical Abstract



Highlights

- The WhiB6 Fe-S cluster is essential for ESX-1 function in *M. marinum*
- Disruption of the *M. marinum* WhiB6 Fe-S cluster modulates transcription in *M. marinum*
- WhiB6 dynamically regulates ESX-1 and DosR gene expression upon exposure to NO
- Isoforms of WhiB6 modulate *M. marinum* virulence and granuloma formation in zebrafish

Authors

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In Brief

Chen et al. describe the cellular role of the WhiB6 protein in mycobacterial virulence, dissemination, sensing of extracellular stressors, and regulation of downstream genetic programs. WhiB6 regulation through its Fe-S cluster enables mycobacteria to establish persistent infection and maintain the integrity of granulomas.

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Mycobacterial WhiB6 Differentially Regulates ESX-1 and the Dos Regulon to Modulate Granuloma Formation and Virulence in Zebrafish

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SUMMARY

During the course of infection, *Mycobacterium tuberculosis* (*Mtb*) is exposed to diverse redox stresses that trigger metabolic and physiological changes. How these stressors are sensed and relayed to the *Mtb* transcriptional apparatus remains unclear. Here, we provide evidence that WhiB6 differentially regulates the ESX-1 and DosR regulons through its Fe-S cluster. When challenged with NO, WhiB6 continually activates expression of the DosR regulons but regulates ESX-1 expression through initial activation followed by gradual inhibition. Comparative transcriptomic analysis of the holo- and reduced apo-WhiB6 complemented strains confirms these results and also reveals that WhiB6 controls aerobic and anaerobic metabolism, cell division, and virulence. Using the *Mycobacterium marinum* zebrafish infection model, we find that holo- and apo-WhiB6 modulate levels of mycobacterial infection, granuloma formation, and dissemination. These findings provide fresh insight into the role of WhiB6 in mycobacterial infection, dissemination, and disease development.

INTRODUCTION

Mycobacterium tuberculosis (*Mtb*), the etiological agent of tuberculosis (TB), is one of the most successful intracellular pathogens. During infection, this pathogen is engulfed by resident (alveolar) macrophages and resides in the phagosome, whose environment has evolved to destroy invading agents (Flannagan et al., 2009). *Mtb* possesses a variety of mechanisms to survive within the phagosomal compartment or escape from it, ultimately leading to killing of the host cell (Ehrt and Schnappinger, 2009; Simeone et al., 2012). As infection progresses, infected macrophages recruit additional macrophages and other immune cells to form a granuloma, which is composed of mononuclear phagocytes, dendritic cells, and T and B lymphocytes (Philips and Ernst, 2012; Ramakrishnan, 2012).

It has been proposed that multiple stress factors inside the granuloma, such as NO, CO, low O_2, limited nutrients, and low pH, trigger *Mtb* to reprogram its metabolism to a non-replicating "dormant" state (Dutta and Karakousis, 2014; Schnappinger et al., 2003). The *Mtb* Dos dormancy regulon, consisting of the response regulator DosR and the heme-containing sensor kinases DosS and DosT, controls roughly 50 genes and is capable of responding to NO, CO, and hypoxia (Kumar et al., 2007, 2008; Shiloh et al., 2008; Voskuil et al., 2003). Studies have shown that the Dos dormancy regulon maintains energy levels and redox balance (Leistikow et al., 2010; Rustad et al., 2009). It is also believed that the Dos regulon is essential for *Mtb* to persist in lung lesions (Boon and Dick, 2012). WhiB5 has been characterized as a transcriptional regulator that controls the expression of genes involved in *Mtb* reactivation (Casonato et al., 2012). However, the genetic mechanism whereby *Mtb* enters, maintains, and emerges from a dormant state is poorly understood.

Recent studies have made progress in understanding how *Mtb* senses redox signals such as O_2 and NO via the family of Fe-S cluster-containing WhiB proteins in addition to the heme-based DosR/S/T system. Members of the WhiB family of redox sensor proteins exert diverse functions, including cell division (WhiB2), fatty acid metabolism and pathogenesis (WhiB3), and antibiotic resistance (WhiB7) (Burian et al., 2013; Konar et al., 2012; Singh et al., 2007, 2009). However, the mechanisms whereby these proteins sense and respond to signals to exert their regulatory effects are largely unknown. Noticeably, *whiB6* expression is highly induced upon treatment with NO and upregulated during infection of macrophages (Larsson et al., 2012; Saini et al., 2012a, 2012b). In another genome-wide expression study, *whiB6* (Rv3862c) expression was upregulated under prolonged hypoxia (Homolka et al., 2010). Using an ELISA, WhiB6 was detected as a reactivation-associated antigen in active TB patients to stimulate interferon-γ (IFN-γ) (Kassa et al., 2012).