Manganese Utilization in \textit{Salmonella} Pathogenesis: Beyond the Canonical Antioxidant Response

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The metal ion manganese (Mn\textsuperscript{2+}) is equally coveted by hosts and bacterial pathogens. The host restricts Mn\textsuperscript{2+} in the gastrointestinal tract and \textit{Salmonella}-containing vacuoles, as part of a process generally known as nutritional immunity. \textit{Salmonella enterica} serovar Typhimurium counteract Mn\textsuperscript{2+} limitation using a plethora of metal importers, whose expression is under elaborate transcriptional and posttranscriptional control. Mn\textsuperscript{2+} serves as cofactor for a variety of enzymes involved in antioxidant defense or central metabolism. Because of its thermodynamic stability and low reactivity, bacterial pathogens may favor Mn\textsuperscript{2+}-cofactored metalloenzymes during periods of oxidative stress. This divalent metal catalyzes metabolic flow through lower glycolysis, reductive tricarboxylic acid and the pentose phosphate pathway, thereby providing energetic, redox and biosynthetic outputs associated with the resistance of \textit{Salmonella} to reactive oxygen species generated in the respiratory burst of professional phagocytic cells. Combined, the oxyradical-detoxifying properties of Mn\textsuperscript{2+} together with the ability of this divalent metal cation to support central metabolism help \textit{Salmonella} colonize the mammalian gut and establish systemic infections.

**Keywords:** manganese, \textit{Salmonella}, virulence, mismetallation, carbon metabolism, central metabolism, oxidative stress, nitrosative stress

INTRODUCTION

A rich microbiome, cellular and abiotic mucosal barriers, as well as cellular and humoral effectors of the innate and adaptive immune system may limit the colonization, growth and spread of pathogenic bacteria in mammalian hosts (Rosales and Uribe-Querol, 2017; Levinson et al., 2018; Uribe-Querol and Rosales, 2020). Hosts use metal transporters, calprotectins, siderocalins and a variety of other metal-sequestering proteins to limit the availability of Mg\textsuperscript{2+}, Fe\textsuperscript{2+}, Mn\textsuperscript{2+} and Zn\textsuperscript{2+} ions from bacteria, a phenomenon known as nutritional immunity (Bellamy, 2003; Loomis et al., 2014; Hennigar and McClung, 2016; Wang et al., 2018; Cunrath and Bumann, 2019; Monteith and Skaar, 2021). Bacteria display sophisticated transport systems that counteract metal restrictions imposed by hosts (Porcheron et al., 2013; Chandrangsu et al., 2017). Among the bioactive metal ions, Mn\textsuperscript{2+} plays a salient role in bacterial physiology and the adaptation of procaryotic cells to stress (Papp-Wallace and Maguire, 2006). Mn\textsuperscript{2+} is a cofactor of enzymes involved in carbon and nucleotide metabolism, DNA replication, and protein translation (Lovley and Phillips, 1988; Martin and Inlay, 2011; Culotta and Daly, 2013; Torrents, 2014; Kaur et al., 2017; Tong et al., 2017; Daniel et al., 2018; Li and Yang, 2018; Hutfilz et al., 2019). Mn\textsuperscript{2+} is also a cofactor of superoxide dismutase (SOD) and catalase (KatN) family members, and this divalent metal is utilized by carbon utilization enzymes such as
Manganese Regulates Salmonella Pathogenesis

Manganese limitation in the host

Hosts sequester transition metals in their fight against pathogenic organisms (Hennigar and McClung, 2016). Metal sequestration is mediated by calprotectin, lipocalin 2, metallothioneins, ferritin and transport systems including NRAMP1. Mn$^{2+}$ limitation is mostly mediated by calprotectin and NRAMP1.

Calprotectin

The host protein calprotectin, a member of the calcium-binding S100 family, limits Mn$^{2+}$ from extracellular Salmonella. Human calprotectin is a heterooligomer with a dedicated Zn$^{2+}$-binding site-1 and a versatile Mn$^{2+}$-, Fe$^{2+}$-, Zn$^{2+}$-, and Ni$^{2+}$-binding site-2 (Hayden et al., 2013; Zygiel and Nolan, 2018). Calprotectin is secreted by infiltrating neutrophils at sites of inflammation, reaching extracellular concentrations of about 40 μM, but is also expressed by epithelial cells and keratinocytes (Johne et al., 1997; Zygiel and Nolan, 2018; Jukic et al., 2021). Calprotectin limits metal bioavailability from bacteria, thus inhibiting bacterial growth, and its expression in epithelial cells diminishes binding of Salmonella to host cells (Nisapakultorn et al., 2001). Infection of gut mucosa by Salmonella attracts neutrophils, which secrete calprotectin in extracellular traps (Urban et al., 2009; Patel and McCormick, 2014). Fecal calprotectin levels in diarrheic children correlate with the severity of bacterial infection, and the concentration of calprotectin is increased in plasma during acute salmonellosis (Chen et al., 2012; De Jong et al., 2015). The acidic pH typical of infectious sites disrupts the tetramerization of calprotectin, not only attenuating its capacity to bind Mn$^{2+}$ but also impairing its growth-inhibiting properties (Menkin, 1956; Rosen and Nolan, 2020).

Although calprotectin inhibits Salmonella growth in vitro, its effectiveness in the intestinal lumen is severely limited by Salmonella’s Zn$^{2+}$ and Mn$^{2+}$ metal transporters (Liu et al., 2012; De Jong et al., 2015; Diaz-Ochoa et al., 2016).

NRAMP1

The integral membrane protein NRAMP1, which is also known as SLC11A1, transports divalent transition metals such as Fe$^{3+}$, Mn$^{2+}$, Co$^{2+}$ and Mg$^{2+}$ (Canonne-Hergaux et al., 1999; Forbes and Gros, 2003; Cellier et al., 2007; Cunrath and Bumann, 2019). The expression of NRAMP1 is restricted to lysosomal compartments of monocytes and macrophages, whereas the highly homologous NRAMP2 protein is widely distributed on most cells (Vidal et al., 1995; Gunshin et al., 1997). The expression of NRAMP1 is maximal at late stages in the maturation of phagosomes (Gruenheid et al., 1997). NRAMP1 can import or efflux metals into the phagosome (Atkinson and Barton, 1999; Kuhn et al., 1999; Zwilling et al., 1999; Jabado et al., 2000), although recent structural studies have suggested a strong unidirectional efflux movement under physiological conditions (Bozzi and Gaude, 2021). The directionality of NRAMP1-mediated ion transport is dependent on pH (Goswami et al., 2001). Although the affinity of human or mouse NRAMP1 to Mn$^{2+}$ has not been determined, a homologous transporter from Arabidopsis has a K$m$ of 28 nM, (i.e., about 4-fold higher than the affinity of the Salmonella transporters MntH and SitABC for Mn$^{2+}$) (Kehres et al., 2000; Kehres et al., 2002b; Caillatte et al., 2010).

Nutritional immunity imposed by NRAMP1 is a significant defense determinant against Salmonella as vividly illustrated by the high resistance of Sv129S6 or C3H/HeN mice carrying an intact nramp1 locus and the propensity of BALB/c or C57BL/6 mice bearing the mutant allele nramp1G169D to develop severe Salmonella infections (Brown et al., 2013; Wendy P.; Loomis et al., 2014). Recent work argued that NRAMP1 contributes to Salmonella pathogenesis by depriving phagosomes of Mg$^{2+}$ (Cunrath and Bumann, 2019). However, the latter model appears to be in conflict with the observation that the attenuation of Salmonella bearing mutations in the Mn$^{2+}$ transport system SitABC or the Mn$^{2+}$-dependent SpoT enzyme are contingent on the expression of a functional NRAMP1 (see below). More investigations are needed to definitively identify the metal specificity of NRAMP1 in different tissues and different times in the course of the Salmonella infection.

Mn$^{2+}$ IMPORT PROMOTES SALMONELLA PATHOGENESIS

Entry of Mn$^{2+}$ into the periplasmic space is mostly mediated by non-specific porins on the outer membrane, whereas transporters
in the cytoplasmic membrane actively influx this divalent metal cation into the bacterial cytoplasm (Figure 1A). MntH, a proton-dependent NRAMP1 homolog, actively imports Mn\(^{2+}\) with a \(K_m\) of 0.1 \(\mu\)M (Kehres et al., 2000). Salmonella upregulate MntH expression in response to both low Mn\(^{2+}\) concentrations and oxidative stress (Kehres et al., 2002a; Cunrath and Palmer, 2021). MntH mutant Salmonella grow poorly in mouse macrophages, but replicate rather well in mice (Boyer et al., 2002; Zaharik et al., 2004; Diaz-Ochoa et al., 2016). The slight attenuation of mntH deficient Salmonella suggests the existence of redundant transport systems. Genetic analyses revealed the presence of a Mn\(^{2+}\) responsive element in the promoter of the sitABCD operon that is encoded within the Salmonella pathogenicity island-1 gene cluster (Janakiraman and Slauch, 2000; Kehres et al., 2002b). The SitABCD transporter is a typical ABC transporter consisting of a periplasmic-binding protein SitA, an ATP-binding protein SitB and two integral membrane permeases, SitC and SitD. Initially, the SitABCD transporter was thought to function as a Fe\(^{2+}\) transport system, as this locus is regulated by Fur under iron-limiting conditions (Janakiraman and Slauch, 2000; Ikeda et al., 2005). However, metal ion uptake studies have shown that SitABCD mediates influx of Mn\(^{2+}\) with an apparent affinity of 0.1 \(\mu\)M, transporting Fe\(^{2+}\) with 30–100 times lower efficiency (Kehres et al., 2002b). A third transporter with broad cation specificity, ZupT, has also been implicated in Mn\(^{2+}\) transport in Salmonella during nitrosative stress (Yousuf et al., 2020).

Low Mn\(^{2+}\) concentrations activate the expression of MntH and SitABCD (Kehres and Maguire, 2003; Chandrangsu et al., 2017; Bosma et al., 2021). The mntH gene is repressed by both MntR and Fur in response to high Mn\(^{2+}\) and iron replete conditions, respectively (Kehres et al., 2000; Kehres et al., 2002a; Troxell et al., 2011; Powers et al., 2021). Expression of mntH is also induced by peroxide via H\(_2\)O\(_2\)-sensing OxyR regulatory protein (Kehres et al., 2002a) (Figure 1C). The persistence of Mn\(^{2+}\)-dependent repression of mntH in ΔmntR Salmonella points to the existence of MntR-independent control. Analysis of the mntH 5' UTR identified an Mn\(^{2+}\)-sensing riboswitch that forms a Rho-independent terminator (Shi et al., 2014; Scull et al., 2020) (Figure 1C). The presence of several transcriptional breakpoints suggests dynamic expression of MntH in diverse host niches. MntR, Fur and OxyR elements are also present in the sitABCD promoter (Ikeda et al., 2005). The

**FIGURE 1 |** Manganese transport systems in Salmonella. (A) While the outer membrane is permeable to Mn\(^{2+}\) ions, the inner membrane imports this metal ion via specific and non-specific transporters like MntH, SitABCD and ZupT. A type VI secretion system dependent Mn\(^{2+}\) acquisition mechanism observed in pathogens such as Burkholderia, Vibrio, and Yersinia is proposed. (B) Neighbor-joining tree of MnoT-like proteins identified in Salmonella genome by Pattern Hit Initiated BLAST. (C) Genetic organization of mntH and sitABCD operons with regulatory elements in the promoter regions. (D) Clustal alignment of Burkholderia MnoT (WP_171466016) and Salmonella YncD (ACY88391.1) proteins by Clustal Omega aligner. The alignment results were extracted and reformatted in MView command line utility. All protein identities were normalized by aligned length and the residues are colored using the default built-in colormap.
sitABCD operon does not contain, however, a riboswitch-like sequence at the 5′ UTR. Despite the similarities in transcriptional control and their identical affinity for Mn2+, MntH and SitABCD have specialized functions. MntH primarily transports Mn2+ at acidic pH, whereas SitABCD preferentially works at alkaline pH (Kehres et al., 2002b), suggesting that Salmonella may preferentially utilize MntH or SitABCD at different anatomical locations or times during infection (Ikeda et al., 2005).

mntH mutant Salmonella display little attenuation, whereas sitABCD mutants are attenuated in systemic models of infection (Janakiraman and Slauch, 2006; Boyer et al., 2002; Zaharik et al., 2004). Attenuation of sitABCD mutant Salmonella is contingent on the presence of host NRAMP1 (W. P. Loomis et al., 2014; Zaharik et al., 2004). Additionally, the non-specific transporter ZupT also competes with the host NRAMP1 for Mn2+ metal ions in phagosomes, contributing to Salmonella virulence (Karlinsey et al., 2010). By competing for Mn2+ with host cell calprotectin, MntH and SitABCD Mn2+ transport systems help Salmonella overgrow commensals in the inflamed gut (Diaz-Ochoa et al., 2016). Acquisition of Mn2+ also powers detoxification of ROS produced by inflammatory neutrophils in the gut mucosa (Diaz-Ochoa et al., 2016). The role of MntH and SitABCD may not be limited to extracellular bacteria, as genes encoding these Mn2+ transporters are transcribed in Salmonella residing in the cytosol of epithelial cells, and mntH and sitA Salmonella mutants grow poorly in the cytosol of epithelial cells (Powers et al., 2021). The acquisition of Mn2+ by cytosolic Salmonella may counteract oxidative stress, while facilitating utilization of sugars (Powers et al., 2021).

Salmonella deficient of MntH, SitA and ZupT transporters still acquire trace levels of Mn2+ in vitro, indicating the presence of other import systems (Karlinsey et al., 2010; Youusuf et al., 2020). The search for other modes of Mn2+ import into Salmonella is still underway, and examples from other bacteria may lead to the discovery of novel Mn2+ uptake systems in Salmonella. Burkholderia pseudomallei import Mn2+ via a type VI secretion system (T6SS) and the TonB cell envelope protein (Si et al., 2017; DeShazer, 2019). B. pseudomallei undergoing oxidative stress secrete the Mn2+-binding T6SS effector TseM, and low Mn2+ concentrations induce expression of the Mn2+ specific, TonB-dependent MnoT integral protein (Figure 1A). TseM scavenges extracellular Mn2+ and actively shuttles the metal via MnoT. Salmonella T6SS is activated by oxidative stress and our bioinformatics analysis has revealed a conserved locus in the Salmonella genome with high similarity to Burkholderia MnoT (Figures 1B,D) (Kroger et al., 2013). Additional T6SS substrates with Mn2+-scavenging capacities are still being uncovered, including the TssS micropeptide from Yersinia pseudotuberculosis that chelates Mn2+ and sabotages bacterial clearance by inhibiting STING-mediated innate immune response (Zhu et al., 2021).

**Mn2+ EXPORT IN SALMONELLA PATHOGENESIS**

Paradoxically, excessive Mn2+ evokes oxidative stress in E. coli, affecting protein stability, interfering with envelope biogenesis, disrupting iron homeostasis and diminishing both tricarboxylic acid cycle and electron transport chain functions (Kaur et al., 2017). Bacteria excrete excessive Mn2+ using both the LysE superfamily MntP protein, and the cation diffuser facilitator (CDF) family member MntE (Martin et al., 2015). The Xanthomonas MntP homolog has two DUF204 domains that are conserved in Salmonella MntP protein (Li et al., 2011). MntP is regulated at transcriptional and posttranscriptional levels via MntR, mismetallated Fur and a yybP-ryoY riboswitch (Dambach et al., 2015; Bosma et al., 2021). The expression of the small RNA yybP, which encodes the small protein MntS, is repressed by MntR (Waters et al., 2011; Martin et al., 2015). The accumulation of apo-MntR in Mn2+ starved cells activates production of MntS, which represses MntP efflux activity and thus enlarges the intracytoplasmic pool of Mn2+. On the other hand, the yybP-ryoY riboswitch directly binds to Mn2+, stabilizing a secondary structure that prevents sequestration of the mntP ribosome-binding site during translation (Dambach et al., 2015). MntP mediates efflux of Mn2+ ions in Salmonella following nitrosative stress (Ouyang et al., 2022).

The second efflux pump MntE is widely distributed in Gram-positive bacteria (Chandransu et al., 2017; Lam et al., 2020). Streptococcus bearing mutations in mntE harbor excessive intracellular Mn2+ and experience attenuation of virulence (Rosch et al., 2009). Our bioinformatic analysis shows that Salmonella MntE/FiiY (YiiP) protein belonging to CDF family has around 26%–64% sequence similarity with Streptococcus MntE, although it mediates zinc and iron export (Grass et al., 2005; Huang et al., 2017).

**Mn2+ HELPS SALMONELLA ADAPT TO OXIDATIVE STRESS**

Salmonella are exposed to ROS generated in the innate host response. Sulfur-containing cysteine and methionine amino acids are primary targets of H2O2 (Bin et al., 2017). In addition, ROS carbonylates arginine, lysine, proline and threonine residues, and oxidize metal prosthetic groups and histidine residues (Ortiz de Orue Lucana et al., 2012; Chang et al., 2020). Salmonella have evolved diverse mechanisms to counter ROS generation, prevent formation of hydroxy radicals, inhibit delivery of ROS into Salmonella-containing vesicles, detoxify and scavenge ROS, or repair the resultant protein and DNA modifications (Buchmeier et al., 1995; De Groote et al., 1997; Vazquez-Torres et al., 2000a; Vazquez-Torres et al., 2000b; Gallois et al., 2001; Vazquez-Torres and Fang, 2001; Waterman and Holden, 2003; Halsey et al., 2004; Aussel et al., 2011; Bogomolnaya et al., 2013; Song et al., 2013; Rhen, 2019; Bogomolnaya et al., 2020; Shome et al., 2020). Of particular interest to this review, Mn2+ protects Salmonella from ROS-mediated cytotoxicity by serving as a cofactor for SOD and KatN enzymes, replacing Fe2+ in the active sites of mononuclear iron-containing enzymes, and acting as a nonproteinaceous antioxidant (Culotta and Daly, 2013; Imlay, 2014; Ighodaro and Akinloye, 2018).

**Manganese-Based Detoxification of ROS**

Salmonella confronts exogenous ROS generated by either host NADPH oxidase in phagocytes or dual oxidase 2 in epithelial cells
(Vazquez-Torres and Fang, 2001; Behnse et al., 2014). Superoxide anion (O$_2^-$) formed by the vectorial transfer of electrons from flavoproteins and semiquinones to molecular oxygen is also a source of endogenous oxidative stress (Imlay, 2013). SODs and catalases expressed basally scavenge endogenously produced O$_2^-$ and H$_2$O$_2$, which accumulate at steady-intracellular concentrations of ~0.2 and ~50 nM, respectively (Imlay, 2013). Cytoplasmic membranes are semipermeable to exogenous H$_2$O$_2$, but at neutral pH prevent entry of O$_2^-$ (Bienert et al., 2006; Imlay, 2019). However, the HO$_2^-$ acid conjugate readily reaches the bacterial cytoplasm (Imlay, 2019). Salmonella synthesizes two structurally distinct classes of SOD enzymes that catalyze the disproportionation of O$_2^-+O_2$ to H$_2$O$_2$ (Perry et al., 2010; Imlay, 2019). Both Mn and Fe-dependent SODs (SodA and SodB, respectively) are cytoplasmic, whereas Cu,Zn-dependent SodC-I and SodC-II are periplasmic (Tsolis et al., 1995; Canvin et al., 1996; Taylor et al., 2009; Perry et al., 2010; Bismuth et al., 2021). Cu,Zn-SOD protect periplasmic or inner membrane targets from O$_2^-$ toxicity, and limit peroxynitrite formation from the reaction of O$_2^-+NO^-$ (De Groote et al., 1997; Gort et al., 1999). Mutants devoid of cytoplasmic Mn-SOD and Fe-SOD are auxotrophic for branched chain amino acids, sulfur-containing amino acids, and aromatic amino acids, and, due to defects in aconitate and fumarase, can only grow on fermentable carbon sources (Carlitz and Touati, 1986; Imlay and Fridovich, 1992). Salmonella lacking Mn-SOD are susceptible to early killing by J774 macrophages but are virulent in an acute mouse model of Salmonella infection, likely reflecting the existence of redundant antioxidant systems (Tsolis et al., 1995). However, a Salmonella strain deficient in Mn-SOD at a competitive disadvantage in the gut because of the Mn$^{2+}$ limitation imposed by calprotectin (Diaz-Ochoa et al., 2016). Collectively, these investigations indicate that the role played by Mn-SOD in Salmonella pathogenesis is tissue specific.

Salmonella degrade H$_2$O$_2$ with the aid of the catalase activity of KatG, KatE, and KatN, of which KatN uses Mn$^{2+}$ as cofactor. H$_2$O$_2$ induces transcription of katG in an OxyR-dependent manner, whereas katE and katN are members of the RpoS regulon (Buchmeier et al., 1995; Ibanez-Ruiz et al., 2000; Seaver and Imlay, 2001; Pardo-Este et al., 2018). Under oxidative stress and Mn$^{2+}$ deplete conditions, KatN seems to be dispensable for Salmonella growth, likely reflecting redundancy of multiple peroxide degrading enzymes such as the alkyl/thiol hydroperoxide reductases AhpC and TsaA as well as peroxiredoxin Tpx (Hebrard et al., 2009; Horst et al., 2010; Diaz-Ochoa et al., 2016). Independently, H$_2$O$_2$-induced protein damage can be effectively repaired by thioredoxin and glutathione systems (Agbor et al., 2014; Song et al., 2016). The redundancy of antioxidant defenses in Salmonella attest to the tremendous selective pressure this intracellular pathogen faces during the respiratory burst of professional phagocytes.

**Cambialistic Enzymes**

Because of the high binding affinity and ready availability in anoxic environments of the primitive Earth, Fe$^{2+}$ was incorporated as cofactor of many primordial metabolic enzymes (Imlay, 2014). However, Fe$^{2+}$ bound to a polypeptide can reduce H$_2$O$_2$, generating reactive hydroxyl and ferryl radicals in situ (Touati, 2000). This feature predisposes the Feox in [4Fe-4S] clusters of dehydratases and mononuclear Fe$^{2+}$ to H$_2$O$_2$ attack, and Fe$_2^+$-mediated reduction of H$_2$O$_2$ in proximity to DNA inflicts genotoxicity (Winterbourn, 1995; Henle et al., 1999; Park and Imlay, 2003; Anjem and Imlay, 2012). Iron and manganese exist in two interchangeable redox forms, 2+ and 3+. Due to the symmetry of half-filled d5 electron shells, Mn$^{2+}$ and Fe$^{3+}$ (3d5) are more thermodynamically stable than Mn$^{3+}$ (3d4) and Fe$^{2+}$ (3d6) (Lingappa et al., 2019). The Mn$^{3+}$/Mn$^{2+}$ and Fe$^{3+}$/Fe$^{2+}$ redox couples have potentials of 1.51 and 0.77 V, respectively. Therefore, Mn$^{2+}$ is less likely to donate electrons than Fe$^{2+}$ (Guillemet-Fritsch et al., 2005). It is for this reason that, under most biological conditions, Mn$^{2+}$ is less reactive than Fe$^{2+}$ (Nealson and Myers, 1992). The thermodynamic stability of Mn$^{2+}$, lower reactivity, and identical coordination geometry favor the mismellation of Fe$^{2+}$ by Mn$^{2+}$. Replacement of Fe$^{2+}$ with Mn$^{2+}$ prevents oxidative damage of metalloenzymes (Emerson et al., 2008; Puri et al., 2010; Hood and Skaar, 2012). Accordingly, members of the Enterobacteriaceae shift from an iron- to a manganese-centric metabolism following oxidative stress (Anjem et al., 2009; Aguirre and Culotta, 2012). Examples of cambialistic enzymes include Rpe in the pentose phosphate pathway as discussed below. Incorporation of Mn$^{2+}$ in place of Fe$^{2+}$ allows metabolic flow during exposure to oxidative stress.

**Mn$^{2+}$-Dependent Nonproteinaceous Antioxidants**

Manganese ions render bacteria resistant to oxidative stress, even in the absence of Mn-SOD (Horsburgh et al., 2002). This protection may be mediated by the Mn$^{2+}$-dependent degradation of O$_2^-+O_2$ (Archibald and Fridovich, 1981, 1982; Berlett et al., 1990; Yocom and Pecoraro, 1999). Mn$^{2+}$ reacts with O$_2^-+NO^-$ to form transient MnO$^{2+}$, which converts to manganous phosphate, H$_2$O$_2$, and H$_2$O (Barnese et al., 2012). In turn, Mn$^{2+}$ disproportionates H$_2$O$_2$ to H$_2$O and O$_2$ (Stadtman et al., 1990).

**Mn$^{2+}$-Driven Central Metabolism in Salmonella Virulence**

Growth of Salmonella in host cells relies on a versatile metabolism. Relevant to this review, Mn$^{2+}$ impacts glycolysis, reductive TCA and the pentose phosphate pathway in Salmonella sustaining oxidative stress.

**Metabolism of Mn$^{2+}$ in Glycolysis and Reductive TCA During Oxidative Stress**

The electron transport chain is a source of ATP and a dominant pathway for balancing NADH/NAD$^+$ redox. The oxidative inhibition of NDH-I NADH dehydrogenase in Salmonella undergoing oxidative stress decreases the energetic and redox
FIGURE 2 | Manganese dependent metabolic adaptations in Salmonella. (A) Schematic representation of central metabolites and enzymes involved in glycolysis and TCA cycle. Enzymes in red are Mn²⁺ dependent. Glycolytic conversion of 3PG to 2PG is catalyzed by GpmA, a Mn²⁺-independent protein or its non-homologous isofunctional Mn²⁺-dependent GpmB and GpmI. During oxidative stress induced Mn²⁺ limitation, Salmonella utilizes the GpmA isoform to synthesise 2PG. The Mn²⁺-dependent phosphoenolpyruvate carboxylase (Ppc) shunts PEP into the reductive TCA cycle. Ribulose-5-PO₄, 3-epimerase (Rpe), which catalyzes the conversion of ribulose-5-PO₄ to xylulose-5-PO₄, is mismetallated during oxidative stress. As a result, Salmonella metabolism shifts into the production of reductive intermediates of the TCA cycle. (B) Phylogenetic analysis of Salmonella GpmA, B and I enzymes reveal that GpmI is similar to S. aureus GpmI. Differences between sequences are estimated by the scale shown at the bottom of the panel. (C) Clustal alignment of Salmonella GpmA (ACY87394.1), GpmB (ACY91834.1) and GpmI (ACY90848.1) with S. aureus GpmI (WP_001085507). Same scheme as in Figure 1D was followed to represent the alignment.
outputs of the respiratory chain (Husain et al., 2008; Chakraborty et al., 2020). Thus, *Salmonella* experiencing oxidative stress favor glycolysis and fermentation (Figure 2A) to rescue ATP homeostasis and to balance redox. Glycolysis and associated fermentation generate ATP via substrate-level phosphorylation, produce intermediates for a variety of biosynthetic pathways, and balance redox (Figure 2A). Glycolysis is indispensable for the successful survival of *Salmonella* in host cells and is an essential component in resistance of *Salmonella* to the phagocyte NADPH oxidase (NOX2) (Bowden et al., 2009; Paterson et al., 2009; Götz and Goebel, 2010; Fitzsimmons L. et al., 2018; Chakraborty et al., 2020). *Salmonella* activate overflow metabolism in macrophages, partially to utilize the glycolytic products 3-phosphoglycerate (3PG) and 2-phosphoglycerate (2PG) as carbon sources (Jiang et al., 2021). Phosphoglycerate mutase (PGM) plays a unique role partially to utilize the glycolytic products 3-phosphoglycerate and 2-phosphoglycerate as carbon sources (Jiang et al., 2021). Phosphoglycerate mutase (PGM) plays a unique role in controlling the overflow metabolism that mitigates oxidative stress in *Salmonella*. PGM, the third enzyme in the payoff phase of glycolysis, converts 3PG to 2PG. Many bacteria encode two analogous PGM enzymes with no sequence or structural similarity (Foster et al., 2010; Radin et al., 2019). The dPGM analogues PGM enzymes with no sequence or structural similarity. Our bioinformatic analysis revealed the importance of anaplerotic reactions imposed by Mn2+ limitation, the NOX2-dependent oxidase (NOX2) (Bowden et al., 2009; Paterson et al., 2009; Götz and Goebel, 2010; Fitzsimmons L. et al., 2018; Chakraborty et al., 2020). *Salmonella* activate overflow metabolism in macrophages, partially to utilize the glycolytic products 3-phosphoglycerate (3PG) and 2-phosphoglycerate (2PG) as carbon sources (Jiang et al., 2021). Phosphoglycerate mutase (PGM) plays a unique role in controlling the overflow metabolism that mitigates oxidative stress in *Salmonella*. PGM, the third enzyme in the payoff phase of glycolysis, converts 3PG to 2PG. Many bacteria encode two analogous PGM enzymes with no sequence or structural similarity (Foster et al., 2010; Radin et al., 2019). The dPGM isoform utilizes the cofactor 2,3-bisphosphoglycerate, whereas the iPGM isoform requires Mn2+. While dPGM functions as a dimer or trimer, iPGM is active as a monomer (Foster et al., 2010). These Non-homologous I Sofunctional Enzymes (NISE) evolved independently to undertake the crucial metabolic conversion 3PG and 2PG, and bacteria may have accrued them via lateral gene transfer or non-orthologous gene displacement (Omelchenko et al., 2010). The genome of *Salmonella enterica* encodes two Mn2+-dependent iPGMs (GpmB and GpmI) and one Mn2+-independent dPGM (GpmA) (Figures 2B, C). The two Mn2+-dependent iPGMs are unique in sequence and structure. Our bioinformatic analysis revealed that *Salmonella* GpmB, which is structurally similar to Bacillus stearothermophilus phosphatase, PhoE, is conserved among major Enterobacteriaceae members, while two-domain monomer GpmI has around 50% sequence similarity with Staphylococcus aureus orthologue (Figure 2C), suggesting a common evolutionary origin (Figure 2B). Strikingly, *Salmonella* exposed to ROS produced in inflammation preferentially utilize the Mn2+-independent GpmA isoform over the Mn2+-cofactored GpmB enzyme (Chakraborty et al., 2020). The preferential utilization of the Mn2+-independent GpmA by *Salmonella* during resistance to NADPH oxidase-mediated host defense may be explained by the high demand for Mn2+ during periods of oxidative stress as hinted by the negative selection of mntH mutants after H2O2 treatment (Chakraborty et al., 2020). In addition to the constraints imposed by Mn2+ limitation, the NOX2-dependent acidification of the cytoplasm of intracellular *Salmonella* may also explain the preferential utilization of the acid phosphatase family member GpmA over its alkaline GpmB counterpart.

A knowledge-based and mathematical model of carbon flux in *Salmonella* revealed the importance of anaplerotic reactions around phosphoenolpyruvate (PEP) to oxaloacetate (OAA) conversion (Thiele et al., 2011; Dandekar et al., 2012). When modeled with glucose as sole C-source, the Mn2+-dependent PEP carboxylase enzyme (Ppc) seemed essential for fluxing glycolytic substrates into the reductive TCA cycle (Matsumura et al., 1999). *Salmonella* deficient of Ppc is virulent in BALB/c mouse model of infection (Tchawa Yinga et al., 2006). However, the combination of mutations in Ppc, acetate kinase (AckA) and phosphotransacetylase (Pta) results in a dramatic attenuation of *Salmonella* virulence (Chakraborty et al., 2020). Thus, generation of ATP via substrate-level phosphorylation together with the balancing of redox in the reductive TCA that is facilitated by fluxing PEP to oxaloacetate by the Mn2+-dependent Ppc contribute to *Salmonella* pathogenesis.

### Pentose-Phosphate Pathway

The mononuclear iron in ribulose-5-phosphate 3-epimerase (Rpe) in the pentose-phosphate pathway can be poisoned by submicromolar H2O2 concentrations (Sobota and Imlay, 2011). The inactive form of Rpe, however, can rapidly metallate with Mn2+ ions and revert to its active form (Sobota and Imlay, 2011). Metallation of Rpe with oxidative stress-resistant Mn2+ may allow for carbon flow through the pentose phosphate pathway, thereby generating NADPH reducing power that is needed to maintain antioxidant defenses such as glutathione or thioredoxin reductase (Song et al., 2016).

### Mn2+-Based Antinitrosative Defenses

Reactive nitrogen species synthesized by inducible nitric oxide (NO) synthase are bacteriostatic against *Salmonella* (Vazquez-Torres et al., 2000a; Thiele et al., 2011; Henard and Vázquez-Torres, 2012; Fitzsimmons L. F. et al., 2018). RNS modify biomolecules containing radicals, heme prosthetic groups, mononuclear iron, [Fe-S] clusters or redox active thiols in cysteine residues (Mikkelsen and Wardman, 2003; Forman et al., 2004; Poole, 2005; Husain et al., 2008; Pearce et al., 2009; Crawford et al., 2016; Jones-Carson et al., 2016; Jones-Carson et al., 2020). *Salmonella* mutants lacking Mn2+ transporters are more sensitive to RNS (Frawley et al., 2018; Yousuf et al., 2020; Ouyang et al., 2022). The intracellular concentrations of Mn2+ increase in *Salmonella* undergoing nitrosative stress, likely reflecting the upregulation of Mn2+ importers (Richardsen et al., 2011; Yousuf et al., 2020). Regulation of Mn2+ transport systems is under the control of the transcription factor DksA (Crawford et al., 2016). Maintaining the homeostasis of intracellular Mn2+ in *Salmonella* after exposure to NO also involves the MntP and FieF efflux pumps (Ouyang et al., 2022). It remains unknown if these Mn2+ efflux systems contribute to the antinitrosative defenses of *Salmonella*.

Transient drops in intracellular amino acids during NO stress induce RelA-catalyzed synthesis of the (p)ppGpp alarmone, and the hydrolytic activity of SpoT is essential for reestablishing ppGpp homeostasis (Richardson et al., 2011; Fitzsimmons L. F. et al., 2018). A Mn2+ ion is coordinated by at least two carboxylates from aspartate and glutamate residues in the hydrolase domain of SpoT (Hogg et al., 2004). Histidine along with H2O molecules coordinate the rest of the four electrons of...
Mn\(^{2+}\) homeostasis plays a poorly understood, but vital role in Salmonella pathogenesis. The elaborate transcriptional and posttranscriptional regulation of expression of diverse Mn\(^{2+}\) uptake and efflux systems help Salmonella navigate different metal-restricted anatomical sites in the vertebrate host. Historically, Mn\(^{2+}\) has been recognized as a cofactor of critical antioxidant defenses of Salmonella. Mn\(^{2+}\)-dependent SOD protects Salmonella from oxidative stress; a function for Mn\(^{2+}\)-dependent catalase in Salmonella virulence remains to be determined. Recent investigations have revealed the intricate relations between Mn\(^{2+}\) homeostasis, central metabolism, and antioxidant defenses of Salmonella. Salmonella rely on Mn\(^{2+}\) independent glycolysis during their adaptations to oxidative killing, but use Mn\(^{2+}\) to power anaplerotic pentose phosphate pathway reactions involved in redox balance necessary for central metabolism and synthesis of reductive power that fuels classical antioxidant defenses, such as glutathione reductase. Many unanswered questions still exist about the involvement of Mn\(^{2+}\) in the adaptations that promote Salmonella pathogenesis, providing a myriad of opportunities for future research.

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SRU and AV-T conceived the structure of this review and wrote the manuscript. SRU elaborated figures. All authors revised the manuscript and approved the final version.

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Aussel, L., Zhao, W., Hébrard, M., Guilhon, A.-A., Viala, J. P. M., Henri, S., et al. (2011). Salmonella Detoxifying Enzymes Are Sufficient to Cope with the Host Oxidative Burst. *Mol. Microbiol.* 80 (3), 628–640. doi:10.1111/j.1365-2958.2011.07611.x

Barnese, K., Gralla, E. B., Valentine, J. S., and Cabelli, D. E. (2012). Biologically Relevant Mechanism for Catalytic Superoxide Removal by Simple Manganese Compounds. *Proc. Natl. Acad. Sci. U.S.A.* 109 (18), 7692–7697. doi:10.1073/pnas.1203051109

Behnson, I., Jellbauer, S., Wong, C. P. E., Edwards, R. A., George, M. D., Ouyang, W., et al. (2014). The Cytokine IL-22 Promotes Pathogen Colonization by Suppressing Related Commensal Bacteria. *Immunity* 40 (2), 262–273. doi:10.1016/j.immuni.2014.01.003

Bellamy, R. (2003). “The NRAMP Family: Co-evolution of a Host/pathogen Defense System,” in *Bacterial Evasion of Host Immune Responses*. Editors B. Henderson and P. C. F. Oyston (Cambridge University Press), 39–52. doi:10.1017/CBO9780511546266.004

Berlett, B. S., Chock, P. B., Yin, M. B., and Stadtman, E. R. (1990). Manganese(II) Catalyzes the Bicarbonate-dependent Oxidation of Amino Acids by Hydrogen Peroxide and the Amino Acid-facilitated Dismutation of Hydrogen Peroxide. *Proc. Natl. Acad. Sci. U.S.A.* 87 (1), 389–393. doi:10.1073/pnas.87.1.389

Bernal-Bayard, J., and Ramos-Morales, F. (2018). Molecular Mechanisms Used by the Gut Microbiota. *Mol. Microbiol.* 109 (16), 3572–3588. doi:10.1111/mib.13712

Bin, P., Huang, R., and Zhou, X. (2017). Oxidation Resistance of the Sulfur Amino Acid Metabolism: Methionine and Cysteine. *BioMed Res. Int.* 2017, 1–6. doi:10.1155/2017/9584932
Purcell, S. (2019). Salmonella and Reactive Oxygen Species: A Love-Hate Relation. *Front. Cell. Dev. Biol.* 7(2), 114. doi:10.3389/fcell.2019.00114

Perry, J. P. J., Shin, D. S., Getzoff, E. D., and Tainer, J. A. (2010). The Structural Biochemistry of the Superoxide Dismutases. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1804 (2), 245–262. doi:10.1016/j.bbadis.2009.11.004

Poole, R. K. (2005). Nitric Oxide and Nitrosative Stress Tolerance in Bacteria. *Biochem. Soc. Trans.* 33 (Pt 1), 176–180. doi:10.1042/BST0330176

Porcheron, G., Garénaux, A., Proulx, J., Sabri, M., and Dozois, C. M. (2013). Iron, Copper, Zinc, and Manganese Transport and Regulation in Pathogenic Porcine Enterobacteria: Correlations between Strains, Site of Infection and the Relative Importance of the Different Metal Transport Systems for Virulence. *Front. Cell. Infect. Microbiol.* 3, 90. doi:10.3389/fcimb.2013.00090

Power, T. R., Haerbele, A. L., Predeus, A. V., Hammarlöf, D. L., Cundiff, J. A., Saldarfa-Ahuactzi, Z., et al. (2021). Intracellular Niche-specific Profiling Reveals Transcriptional Adaptations Required for the Cytosolic Lifestyle of *Salmonella enterica.* *PLoS Pathog.* 17 (8), e1009280. doi:10.1371/journal.ppat.1009280

Puri, S., Hohle, T. H., and O’Brien, M. R. (2010). Control of Bacterial Iron Homeostasis by Manganese. *Proc. Natl. Acad. Sci. U.S.A.* 107 (23), 10691–10695. doi:10.1073/pnas.1002342107

Rhen, M. (2019). *Salmonella* and Reactive Oxygen Species: A Love-Hate Relationship. *Innate Immun.* 11 (3), 216–226. doi:10.1159/000496570

Radin, J. N., Kelliher, J. L., Solórzano, P. K. P., Grim, K. P., Ramezanifard, R., Nishimura, A., et al. (2019). Metal-independent Variants of Phosphoglycerate Epimerase in *Escherichia coli* Is Rapidly Damaged by Hydrogen Peroxide but Can Be Protected by Manganese. *Proc. Natl. Acad. Sci. U.S.A.* 108 (13), 5402–5407. doi:10.1073/pnas.1100410108

Song, M., Husain, M., Jones-Carson, J., Liu, L., Henard, C. A., and Vázquez-Torres, A. (2013). Low-molecular-weight Thiol-dependent Antioxidant and Antinitrosative Defences in *Salmonella* pathogenesis. *Mol. Microbiol.* 87 (3), 609–622. doi:10.1111/mmi.12119

Stadtmann, E. R., Berlett, B. S., and Chock, P. B. (1990). Manganese-dependent Disproportionation of Hydrogen Peroxide in Bicarbonate Buffer. *Proc. Natl. Acad. Sci. U.S.A.* 87 (1), 384–388. doi:10.1073/pnas.87.1.384

Tanner, J. R., and Kingsley, R. A. (2018). Evolution of *Salmonella* within Hosts. *Trends Microbiol.* 26 (12), 986–998. doi:10.1016/j.tim.2018.06.001

Taylor, C. M., Osman, D., and Cavet, J. S. (2009). Differential Expression of Two Iron-Responsive Promoters in *Salmonella enterica* Serovar Typhimurium Reveals the Presence of Iron in Macrophage-Phagosomes. *Microb. Path. Physiol.* 46 (2), 114–118. doi:10.1128/micpath.02008.11.001

Tchawa Yimga, M., Leatham, M. P., Allen, J. H., Laux, D. C., Conway, T., and Cohen, P. S. (2006). Role of Gluconecogenesis and the Tricarboxylic Acid Cycle in the Virulence of *Salmonella enterica* Serovar Typhimurium in BALB/c Mice. *Infect. Immun.* 74 (2), 1130–1140. doi:10.1128/iai.74.2.1130-1140.2006

Thiele, I., Hyduke, D. R., Steeb, B., Fankam, G., Allen, D. K., Bazzani, S., et al. (2011). A Community Effort towards a Knowledge-Base and Mathematical Model of the Human Pathogen *Salmonella Typhimurium* LT2. * BMC Syst. Biol.* 5, 8. doi:10.1186/1752-0509-5-8

Tong, Y., Zhai, Q., Wang, G., Zhang, Q., Liu, X., Tian, F., et al. (2017). System-wide Analysis of Manganese Starvation-Induced Metabolism in Key Elements of Lactobacillus Plantarum. *RSC Adv.* 7 (21), 12959–12968. doi:10.1039/C7RA0072C

Torres, E. (2014). Ribonucleotide Reductases: Essential Enzymes for Bacterial Life. *Front. Cell. Infect. Microbiol.* 4, 52. doi:10.3389/fcimb.2014.00052

Touati, D. (2000). Iron and Oxidative Stress in Bacteria. *Archives Biochem. Biophys.* 373 (1), 1–6. doi:10.1006/abbi.1999.1518

Troxell, B., Finn, R. C., Porwollik, S., McClelland, M., and Hassan, H. M. (2011). The Fur Regulator in Anaerobically Grown *Salmonella enterica* Serovar Typhimurium: Identification of New Fur Targets. *BMC Microbiol.* 11, 236. doi:10.1186/1471-2180-11-236

Tsois, R. M., Bäumler, A. J., and Heffern, F. (1995). Role of *Salmonella typhimurium* Mn-Superoxide Dismutase (SodA) in Protection against Early Killing by *Listeria Monocytogenes.* *Infect. Immun.* 63 (5), 1739–1744. doi:10.1128/iai.63.5.1739-1744.1995

Urban, C. F., Ermert, D., Schmid, M., Abu-Abed, U., Goossmann, C., Nacken, W., et al. (2009). Neutrophil Extracellular Traps Contain Calprotectin, a Cytosolic Protein Complex Involved in Host Defense against Candida Albicans. *PLoS Pathog.* 5 (10), e1000639. doi:10.1371/journal.ppat.1000639

Uribe-Querol, E., and Rosales, C. (2020). Phagocytosis: Our Current Understanding of a Universal Biological Process. *Front. Immunol.* 11, 1066. doi:10.3389/fimmu.2020.01066

Vazquez-Torres, A., and Fang, F. C. (2001). Salmonella Evasion of the NADPH Phagocyte Oxidase. *Microbes Infect.* 3 (14-15), 1313–1320. doi:10.1016/s1286-4579(01)01492-7

Vazquez-Torres, A., Jones-Carson, J., Mastroeni, P., Ischiropoulos, H., and Fang, F. C. (2000a). Antimicrobial Actions of the NADPH Phagocyte Oxidase and Inducible Nitric Oxide Synthase in Experimental Salmonellosis. I. Effects on Microbial Killing by Activated Peritoneal Macrophages *In Vitro.* *J. Exp. Med.* 192 (2), 227–236. doi:10.1084/jem.192.2.227

Vazquez-Torres, A., Xu, Y., Jones-Carson, J., Holden, D. W., Lucia, S. M., Dinauer, M. C., et al. (2000b). *Salmonella* Pathogenicity Island 2-dependent Evasion of the Phagocytic NADPH Oxidase. *Science* 287 (5458), 1655–1658. doi:10.1126/science.287.5458.1655
Uppalapati and Vazquez-Torres Manganese Regulation of Salmonella Pathogenesis

Vidal, S., Belouchi, A.-M., Cellier, M., Beatty, B., and Gros, P. (1995). Cloning and Characterization of a Second Human NRAMP Gene on Chromosome 12q13. *Mamm. Genome* 6 (4), 224–230. doi:10.1007/bf00352405

Wang, S., Song, R., Wang, Z., Jing, Z., Wang, S., and Ma, J. (2018). S100A8/A9 in Inflammation. *Front. Immunol.* 9, 1298. doi:10.3389/fimmu.2018.01298

Waterman, S. R., and Holden, D. W. (2003). Functions and Effectors of the Salmonella Pathogenicity Island 2 Type III Secretion System. *Cell Microbiol.* 5 (8), 501–511. doi:10.1046/j.1462-5822.2003.00294.x

Waters, L. S. (2020). Bacterial Manganese Sensing and Homeostasis. *Curr. Opin. Chem. Biol.* 55, 96–102. doi:10.1016/j.cbpa.2020.01.003

Waters, L. S., Sandoval, M., and Storz, G. (2011). The *Escherichia coli* MntR Miniregulon Includes Genes Encoding a Small Protein and an Efflux Pump Required for Manganese Homeostasis. *J. Bacteriol.* 193 (21), 5887–5897. doi:10.1128/JB.05872-11

Whittaker, J. W. (2012). Non-heme Manganese Catalase - the ‘other’ Catalase. *Archives Biochem. Biophysics* 525 (2), 111–120. doi:10.1016/j.abb.2011.12.008

Winter, S. E., and Baumler, A. J. (2011). A Breathtaking Feat. *Gut Microbes* 2 (1), 58–60. doi:10.4161/gmic.2.1.14911

Winterbourn, C. C. (1995). Toxicity of Iron and Hydrogen Peroxide: the Fenton Reaction. *Toxicol. Lett.* 82-83, 969–974. doi:10.1016/0378-4274(95)03532-x

Yocum, C. F., and Pecoraro, V. L. (1999). Recent Advances in the Understanding of the Biological Chemistry of Manganese. *Curr. Opin. Chem. Biol.* 3 (2), 182–187. doi:10.1016/S1367-5931(99)80031-3

Yousuf, S., Karlinsey, J. E., Neville, S. L., McDevitt, C. A., Libby, S. J., Fang, F. C., et al. (2020). Manganese Import Protects *Salmonella enterica* Serovar Typhimurium against Nitrosative Stress. *Metallomics Integr. biometal Sci.* 12 (11), 1791–1801. doi:10.1039/d0mt00178c

Zaharik, M. L., Cullen, V. L., Fung, A. M., Libby, S. J., Kuiat Choy, S. L., Coburn, B., et al. (2004). The *Salmonella enterica* Serovar Typhimurium Divalent Cation Transport Systems MntH and SitABCD Are Essential for Virulence in an Nramp1 G169 Murine Typhoid Model. *Infect. Immun.* 72 (9), 5522–5525. doi:10.1128/iai.72.9.5522-5525.2004

Zhu, L., Xu, L., Wang, C., Li, C., Li, M., Liu, Q., et al. (2021). T6SS Translocates a Micropeptide to Suppress STING-Mediated Innate Immunity by Sequestering Manganese. *Proc. Natl. Acad. Sci. U.S.A.* 118 (42). doi:10.1073/pnas.2103526118

Zwilling, B. S., Kuhn, D. E., Wikoff, L., Brown, D., and Lafuse, W. (1999). Role of Iron in Nrrmp1-Mediated Inhibition of Mycobacterial Growth. *Infect. Immun.* 67 (3), 1386–1392. doi:10.1128/iai.67.3.1386-1392.1999

Zygier, E. M., and Nolan, E. M. (2018). Transition Metal Sequestration by the Host-Defense Protein Calprotectin. *Annu. Rev. Biochem.* 87 (1), 621–643. doi:10.1146/annurev-biochem-062917-012312

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