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RESEARCH

Manganese transporters regulate the resumption of replication in hydrogen peroxide-stressed *Escherichia coli*

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Abstract Following hydrogen peroxide treatment, ferrous iron (Fe^{2+}) is oxidized to its ferric form (Fe^{3+}), stripping it from and inactivating iron-containing proteins. Many mononuclear iron enzymes can be remetallated by manganese to restore function, while other enzymes specifically utilize manganese as a cofactor, having redundant activities that compensate for iron-depleted counterparts. DNA replication relies on one or more iron-dependent protein(s) as synthesis abates in the presence of hydrogen peroxide and requires manganese in the medium to resume. Here, we show that manganese transporters regulate the ability to resume replication following oxidative challenge in *Escherichia coli*. The absence of the primary manganese importer, MntH, impairs the ability to resume replication; whereas deleting the manganese exporter, MntP, or transporter regulator, MntR, dramatically increases the rate of recovery. Unregulated manganese import promoted recovery even in the absence of Fur, which maintains iron homeostasis. Similarly, replication was not restored in *oxyR* mutants, which cannot upregulate manganese import following hydrogen peroxide stress. Taken together, the results define a central role for manganese transport in restoring replication following oxidative stress.

Keywords Manganese · DNA replication · Oxidative stress · Metal homeostasis

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Introduction

Ferrous iron is bound to mononuclear, heme and iron-sulfur enzymes and serves as an essential cofactor for a variety of metabolic pathways, including cellular respiration, DNA synthesis, and the tricarboxylic acid cycle (McHugh et al. 2003; Andreini et al. 2008). During oxidative stress, iron rapidly oxidizes to its ferric form, stripping this catalytic metal from iron-dependent proteins, rendering them inactive (for a review, see (Imlay 2014). High intracellular levels of free iron interact with hydrogen peroxide and superoxide to generate free radicals that can damage DNA, proteins, and lipids (Haber and Weiss 1934; Imlay et al. 1988; Touati et al. 1995; Tamarit et al. 1998; Daly et al. 2007). In contrast, manganese is far less reactive in the presence of oxygen and its byproducts, and can substitute for iron in iron-poor or oxidizing conditions (Kehres and Maguire 2003; Sobota and Imlay 2011; Anjem and Imlay 2012). Yet, manganese can also induce toxicity when cells accumulate too much of this metal (reviewed in (Kehres and Maguire 2003; Waters 2020)). Thus, cells tightly regulate the intracellular amounts of iron and manganese to

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manage both the functionality of metal-cofactored metabolic enzymes and the toxicity associated with excess transition metals.

The manganese-binding transcriptional regulator, MntR, controls intracellular manganese levels under normal growth conditions in *Escherichia coli* (Patzer and Hantke 2001; Kehres et al. 2002; Waters et al. 2011; Martin et al. 2015). Manganese-bound MntR functionally represses expression of the primary manganese importer, MntH, while also activating transcription of the manganese exporter, MntP. MntP expression levels are further modulated by a manganese-binding riboswitch found in the 5′-untranslated region of *mntP* mRNA (Dambach et al. 2015).

Iron homeostasis in *E. coli* is controlled by the global regulator Fur, which regulates expression of over 80 genes, including those involved in iron storage, utilization, and transport, through reversible binding to free ferrous iron (Bagg and Neilands 1987; Angerer and Braun 1998; McHugh et al. 2003; Seo et al. 2014) or a [2Fe–2S] cluster as has been suggested by recent work (Fontenot et al. 2020). In addition, Fur directly binds the promoter and regulatory regions of several genes encoding DNA synthesis and energy metabolism enzymes, coordinating cellular processes in response to iron availability (Seo et al. 2014). Curiously, the Fe^{2+}–Fur complex in *E. coli* also regulates intracellular manganese levels by repressing *mntH* expression (Kehres et al. 2000, 2002; Patzer and Hantke 2001).

Hydrogen peroxide rapidly reacts with ferrous iron to form hydroxyl radical and hydroxide anion with the concomitant production of ferric iron. This reaction both inactivates iron-dependent proteins and generates hydroxyl radicals that damage cellular targets, including DNA (Haber and Weiss 1934; Imlay et al. 1988; Touati et al. 1995; Tamarit et al. 1998; Daly et al. 2007; Sobota and Imlay 2011; Anjem and Imlay 2012). The redox sensor, OxyR, regulates the adaptive response of *E. coli* to peroxide stress, inducing expression of DNA repair enzymes, peroxide-scavenging enzymes, *fur*, and *mntH* (Aslund et al. 1999; Zheng et al. 1999, 2001; Kehres et al. 2002; Anjem et al. 2009; Sobota and Imlay 2011; Anjem and Imlay 2012). Hydrogen peroxide-activated OxyR thus modifies metal homeostasis through these latter two gene products to limit free iron in the cell, thus reducing free-radical formation due to Fenton chemistry, and increasing manganese concentrations to restore enzymatic activities depleted by the loss of iron (Kehres et al. 2000; Anjem et al. 2009; Faulkner and Helmann 2010; Seo et al. 2015).

In *E. coli*, manganese protection from hydrogen peroxide toxicity is mediated through its ability to restore ferro-metallated protein function either through Mn-remetallation of inactivated, mononuclear iron proteins or through induction of functionally redundant manganese-dependent enzymes (Hassan and Fridovich 1977; Beyer and Fridovich 1991; Anjem et al. 2009; Martin and Imlay 2011; Sobota and Imlay 2011). Consistent with this, although the presence of manganese in the growth medium does not affect the amount of oxidative DNA damage or its repair rate, it is required to restore replication following hydrogen peroxide treatment in cells (Hutfilz et al. 2019; Hoff et al. 2021). These results indicate an essential role in re-metallating iron-dependent enzymes or activating manganese-dependent counterparts that are required for DNA replication. Although adding manganese to media was shown to be sufficient to restore replication, whether manganese levels and homeostasis play a role in regulating replication following oxygen stress has not been examined. Here, we characterized the role manganese and iron transporters have on replication following hydrogen peroxide (H_2O_2) treatment and demonstrate that transporters and intracellular manganese levels play a critical role in regulating the time and rate that DNA replication recovers following oxidative stress.

Materials and methods

Bacterial strains

E. coli strain BW25113 (wild-type) and isogenic single gene deletion mutants in *oxyR* (JW3933), *soxR* (JW4024), *mntH* (JW2338), *yebN* (*mntP*, JW5830), *mntR* (JW0801) and *fur* (JW0669) belong to the Keio Collection and were obtained from the National BioResource Project at SHIGEN (Japan) (Baba et al. 2006). The kanamycin-resistance cassettes of JW0801, *mntR*::FRT-*kan*-FRT, and JW2338, *mntH*::FRT-*kan*-FRT, were excised following transformation with the temperature-sensitive, ampicillin-resistant pCP20 plasmid expressing FLP recombinase as described previously(Datsenko and Wanner 2000). Briefly, JW0801 and JW2338 were transformed with

pCP20 and selected for ampicillin resistance at 30 °C. Several colonies were purified on non-selective plates at 43 °C and tested subsequently for loss of all antibiotic resistance. BW25113 *mntR* kan^S (CL4444) derived in this manner was then transduced with the *fur*::FRT-*kan*-FRT allele from JW0669 using standard P1 transduction protocols to generate BW25113 *mntR fur*::FRT-*kan*-FRT (CL4460). BW25113 *mntH* kan^S (CL4462) was transduced with the *mntP*::FRT-*kan*-FRT allele from JW5830 to generate BW25113 *mntH mntP*::FRT-*kan*-FRT (CL5425).

DNA synthesis rate

Overnight cultures were diluted 1:100 and grown at 37 °C in Davis medium supplemented with 0.4% glucose, 0.2% casamino acids (DGC), and 200 µM $MnCl_2$ as indicated, to an OD_{600} of 0.25–0.35. At time zero, half of the cells were mock-treated, while the remaining half was treated with 10 mM H_2O_2 for 5 min. All cells were then filtered through 0.45 µm nitrocellulose membranes to remove H_2O_2 from the media, and resuspended in fresh DGC media either supplemented with $MnCl_2$ or without $MnCl_2$, corresponding to the initial growth conditions. Cultures were allowed to recover at 37 °C. At the indicated time points, duplicate 0.5-ml aliquots of culture were pulse-labeled with 0.5 µCi/ml [^{3}H]thymidine for 2 min at 37 °C. Cells were then lysed and DNA precipitated with cold 5% trichloroacetic acid and filtered onto Millipore glass fiber filters. The amounts of ^{3}H on each filter were determined by scintillation counting.

H_2O_2 survival assays

Fresh overnight cultures were diluted 1:100 in DGC medium either with or without manganese supplementation as described above, grown at 37 °C to an OD_{600} of 0.3, and then treated with 10 mM H_2O_2. At the times indicated, 0.1 ml aliquots of each culture were removed and serially diluted in 10-fold increments into DGC medium with or without manganese according to initial subculture conditions. Triplicate 10 µl-aliquots of each dilution were then spotted on Luria–Bertani (LB) plates. Viable colonies were counted following overnight incubation at 37 °C.

Inductively coupled plasma-mass spectrometry (ICP-MS)

Fresh overnight cultures were diluted 1:100 in 10 ml DGC medium either with or without manganese supplementation as described above, grown at 37 °C to an OD_{600} of 0.5, and then cells were collected by centrifugation. ICP-MS was adapted from (Martin et al. 2015). Briefly, cells were washed twice with two-volumes of ice-cold 20 mM Tris, 1 mM EDTA (pH 7.4) and once with two-volumes of ice-cold 20 mM Tris (pH 7.4). Cells were resuspended in 1 ml ice-cold 20 mM Tris (pH 7.4), lysed by sonication and cell debris was cleared by centrifugation. The metal content in the cleared lysates was determined by the Trace Element Analytical Laboratory at Portland State University and intracellular concentrations were then calculated. Intracellular metal concentrations were normalized to the intracellular protein concentration value of ~300 mg/ml as derived from (Imlay and Fridovich 1991). Unsupplemented DGC medium contained 26 µM manganese and 268 µM iron as determined by ICP-MS.

Results

Active manganese import and retention is required for the rapid recovery of DNA synthesis after oxidative stress

mntH and mntP encode the primary manganese importer and exporter, respectively (Makui et al. 2000; Waters et al. 2011). Their activity and expression is regulated under iron-replete conditions or in the absence of oxidative stress by the regulator, MntR, which binds manganese and limits intracellular manganese concentrations by repressing *mntH* transcription and upregulating *mntP* expression (Fig. 1A and (Patzer and Hantke 2001; Waters et al. 2011; Yamamoto et al. 2011)). To determine how intracellular manganese concentrations regulate replication after oxidative stress, we compared the ability of cells to restore DNA synthesis when challenged with H_2O_2 in wild-type cells and mutants lacking the manganese importer, exporter, or regulator. Cultures grown in defined medium with or without manganese supplementation (200 µM manganese (II) chloride) were exposed to 10 mM H_2O_2 for 5 min. To

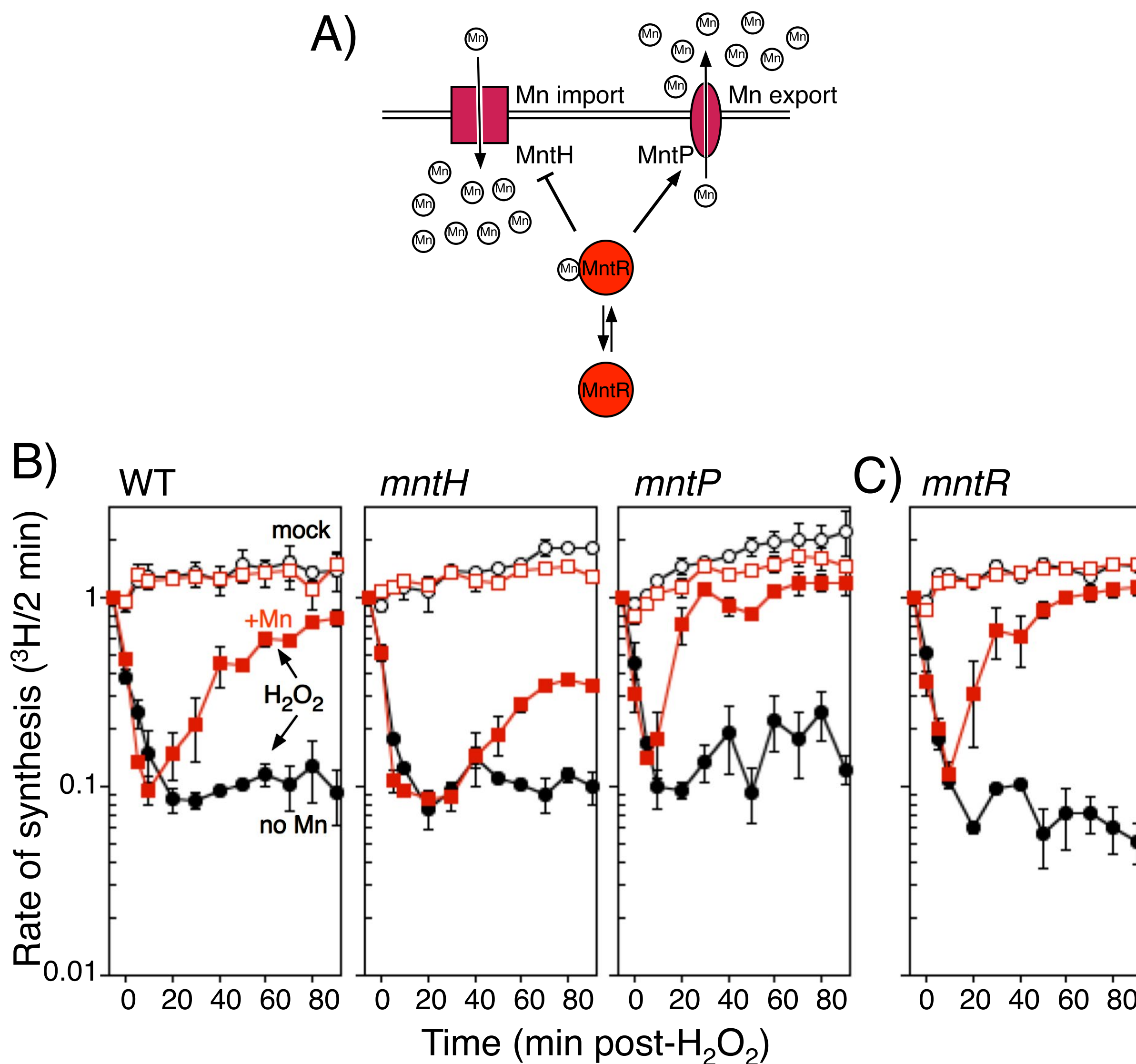


Fig. 1 Conditions that favor high intracellular manganese levels facilitate the restoration of DNA synthesis following oxidative stress. **A** MntR bound to Mn^{2+} represses MntH expression and upregulates MntP expression to limit intracellular manganese concentrations under manganese-rich conditions. (B and C) [^{3}H]thymidine was added to cultures for 2 min at the indicated times following treatment at time zero. The rate of synthesis (^{3}H incorporation/2 min) relative to the amount incorporated immediately prior to exposure are plotted for **B** wild-type (WT) cells, *mntH*, and *mntP* mutants, and **C** *mntR* mutants exposed to mock treatment (open symbols) or 10 mM H_2O_2 for 5 min (filled symbols) in manganese-supplemented (red squares) and -unsupplemented (black circles) media. Graphs show an average of at least two independent trials. Error bars represent one standard error of the mean. (Color figure online)

determine the rate of DNA replication (^{3}H incorporation in the DNA/2 min) during the period of recovery, aliquots were pulse-labeled for 2 min with [^{3}H] thymidine before the DNA was precipitated and the amount of ^{3}H incorporated was quantified. All experiments included a mock-treated control to ensure that any observed differences in the rate of replication were due to H_2O_2 treatment, and not due to changes in the overall growth phase of the cultures over the time course examined.

As shown Fig. 1B, wild-type cultures require the presence of manganese in the medium to restore

replication following H_2O_2 treatment. Immediately following treatment, replication was inhibited by greater than 90%. In the presence of manganese, we observed the rate of replication begin to recover at 20 min post-H_2O_2 exposure and approach pre-exposure levels within 90 min of treatment. However, in the absence of manganese, replication did not resume for the duration of the 90-min time course. Inactivation of either the importer, MntH, or exporter, MntP, affected the recovery of replication, even when manganese was present in the media. Deletion of *mntH* delayed and prevented replication from fully recovering after H_2O_2 exposure, with synthesis suppressed by ~70% throughout the time course.

By contrast, recovery occurred much more rapidly in *mntP* mutants, with DNA synthesis fully restored to pre-treatment rates within 30 min after H_2O_2 removal (Fig. 1B). The observation that the recovery of replication is affected in the absence of the manganese importer and exporter even when manganese is present in the media demonstrates that this metal must be actively transported and retained by the cell to restore replication, and argues that Mn regulation plays a critical role in restoring replication during oxidative stress.

To further characterize how manganese regulation affects replication resumption, we also examined its recovery in the absence of MntR. When sufficient manganese is present in the cell, MntR-bound to Mn^{2+} suppresses the expression of the MntH importer and activates expression of the MntP exporter. Thus, in the absence of MntR regulation, manganese accumulates to higher levels in cells (Patzer and Hantke 2001; Kehres et al. 2002; Waters et al. 2011). Consistent with these roles, the rate of replication in *mntR* mutants grown in manganese-supplemented medium recovered more rapidly than in the corresponding parental strain, fully restoring replication to pre-exposure rates by 60 min post-H_2O_2 challenge (Fig. 1C). Together, these results indicate that active manganese import and exporter suppression facilitates the recovery of DNA synthesis following oxidative stress. We infer that the increased manganese levels allow for the remetallation of oxidized iron-cofactored proteins restoring cellular metabolism and DNA synthesis when strains are cultured in the presence of manganese.

Manganese, but not iron, promotes the recovery of replication after oxidative stress, and upregulated import of this metal can partially compensate for dysregulated iron uptake

Considering that both iron and manganese are used as enzymatic co-factors (Levin et al. 1991; Andreini et al. 2008; Cvetkovic et al. 2010; Cotruvo and Stubbe 2011), we wondered if upregulation of either metal could be utilized to restore replication. To examine this possibility we monitored the recovery of replication occurring in mutants lacking Fur. Fur represses the expression of iron uptake genes through a feedback loop involving reversible Fe^{2+} binding (Bagg and Neilands 1987; Angerer and Braun 1998; McHugh et al. 2003; Seo et al. 2014) and Fig. 2A). Thus, in the absence of Fur, intracellular levels of iron become unregulated and increase (Varghese et al. 2007; Martin et al. 2015). Following H_2O_2 challenge, the absence of Fur impaired, rather than promoted the resumption of replication (Fig. 2B), consistent with the idea that iron is not as effective a substitute for manganese in promoting replication after oxidative stress. We interpret the result to also suggest that the excess iron present in *fur* mutants leads to increased H_2O_2-induced DNA, protein, and membrane damage. Irrespective, this result suggests that iron, unlike manganese, is unable to promote the recovery of replication during oxidative stress. Consistent with this interpretation, increasing the manganese concentrations by deletion of *mntR*, partially compensated for the absence of Fur, allowing replication to resume in these mutants. Taken together, the results are consistent with the idea that manganese, but not iron, cofactors are required to restore replication following oxidative stress.

OxyR, but not SoxR, is required for replication to recover after H_2O_2 challenge

OxyR is a known redox sensor that is activated following H_2O_2 treatment and mediates the cellular adaptive response to oxidative stress by regulating genes involved in peroxide scavenging, DNA repair, as well as regulating both iron and manganese import (Aslund et al. 1999; Zheng et al. 1999, 2001; Kehres et al. 2002; Anjem et al. 2009; Sobota and Imlay 2011; Anjem and Imlay 2012) and Fig. 3A). SoxR, is another redox sensor regulator that upregulates

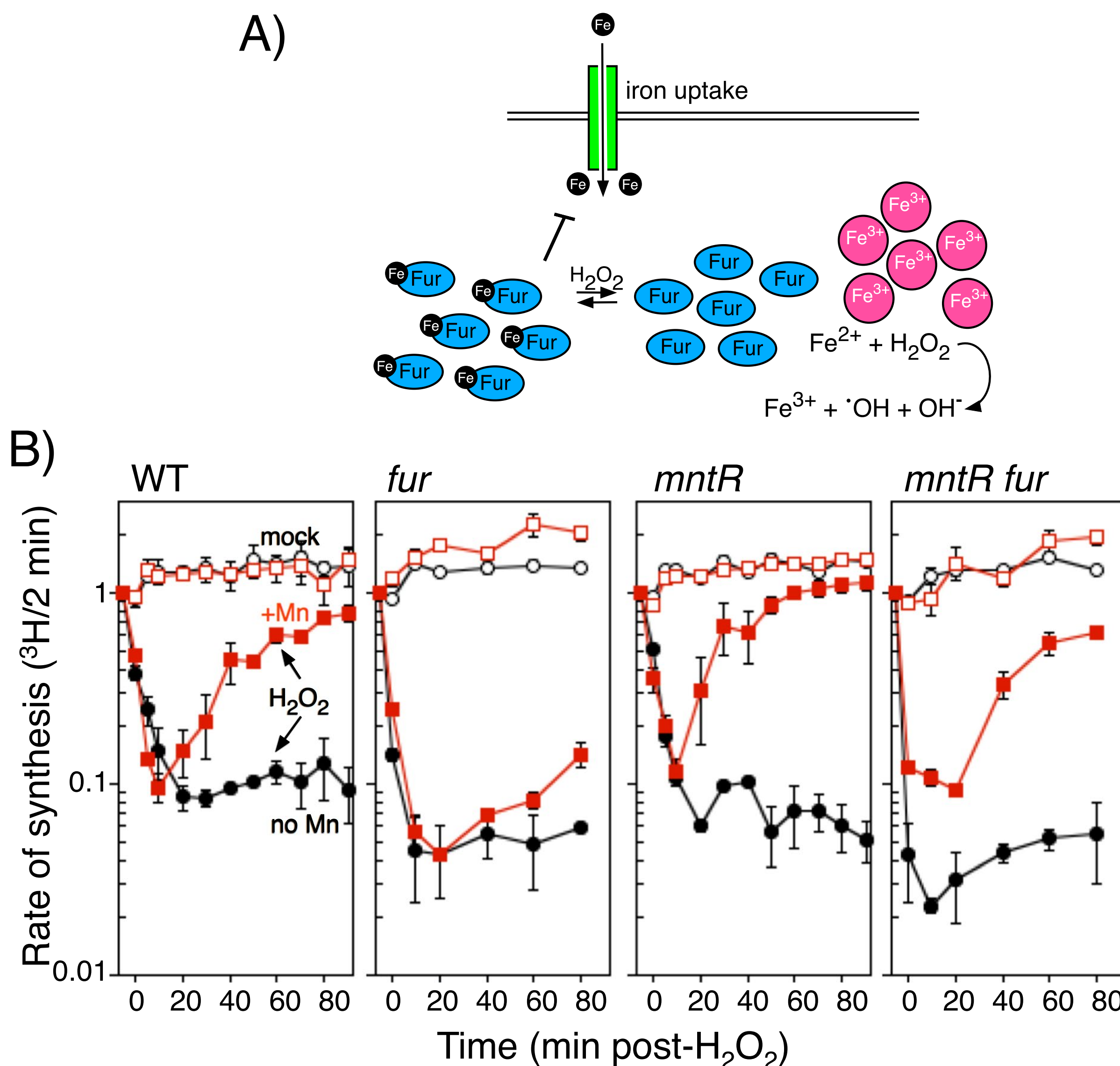


Fig. 2 Manganese import promotes replication recovery even in the absence of iron uptake regulation in H_2O_2-treated cells. **A** Fur limits iron uptake following oxidative stress. **B** Data were obtained and plotted as for Fig. 1. The rate of synthesis (^{3}H incorporation/2 min) relative to the amount incorporated immediately prior to exposure are plotted for WT, *fur*, *mntR*, and *mntR fur* cells grown in medium with (red squares) and without (black circles) manganese supplementation and exposed to mock treatment (open symbols) or 10 mM H_2O_2 for 5 min (filled symbols). Wild-type and *mntR* plots are reproduced from Fig. 1 for comparison. Graphs show an average of at least two independent trials. Error bars represent one standard error of the mean. An abbreviated time course was performed for *fur* and *mntR fur* strains. (Color figure online)

several DNA repair and scavenging genes in response to superoxide, but is not known to regulate manganese (Wu and Weiss 1992; Li and Demple 1994; Zheng et al. 1999, 2001). To examine the contribution of these sensors to the regulation of replication after oxidative challenge, we compared the DNA synthesis occurring in wild-type cells to that occurring in *oxyR* and *soxR* mutants following oxidative challenge in cells grown with or without manganese supplementation using the replication assay described above.

Cells deficient in *oxyR* were impaired in their ability to restore replication following H_2O_2 exposure,

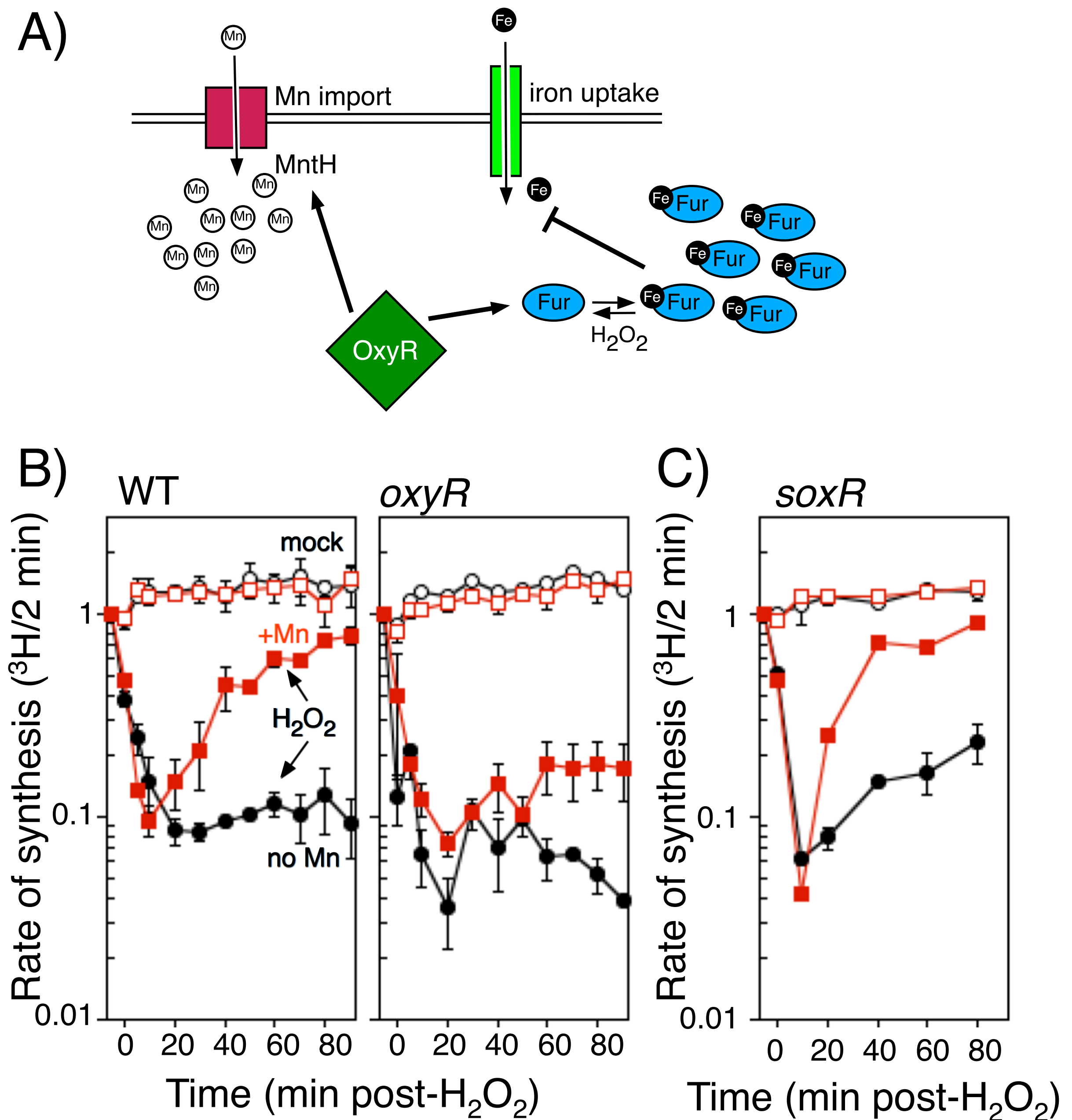


Fig. 3 OxyR, but not SoxR, is required for replication to recover after H_2O_2 challenge. Data were obtained and plotted as for Fig. 1. **A** OxyR upregulates MntH and Fur expression after H_2O_2 treatment to allow increased manganese import and decreased iron uptake, respectively. **B** The rate of synthesis (^{3}H incorporation/2 min) relative to the amount incorporated immediately prior to exposure are plotted for WT cells and *oxyR* mutants, and (C) *soxR* mutants grown in manganese-supplemented (red squares) or -unsupplemented (black circles) media exposed to 0 mM (open symbols) or 10 mM (filled symbols) H_2O_2 for 5 min. Wild-type plot is reproduced from Fig. 1 for comparison. Graphs represent an average from at least two independent experiments. Error bars represent one standard error of the mean. (Color figure online)

even in the presence of manganese supplementation (Fig. 3B). In contrast, *soxR* mutants cultured in the presence of manganese resumed replication with kinetics that were as rapid as wild-type cells (Fig. 3C). The impaired recovery of replication in *oxyR* mutants following H_2O_2 treatment indicated the sensor regulates the recovery of replication. Given that replication recovery only occurs in the presence of manganese and OxyR is known to upregulate MntH and Fur, it seems likely that OxyR's ability to restore replication likely occurs through direct regulation of these divalent metals.

Accumulation of intracellular manganese increases cell survival to acute oxidative stress

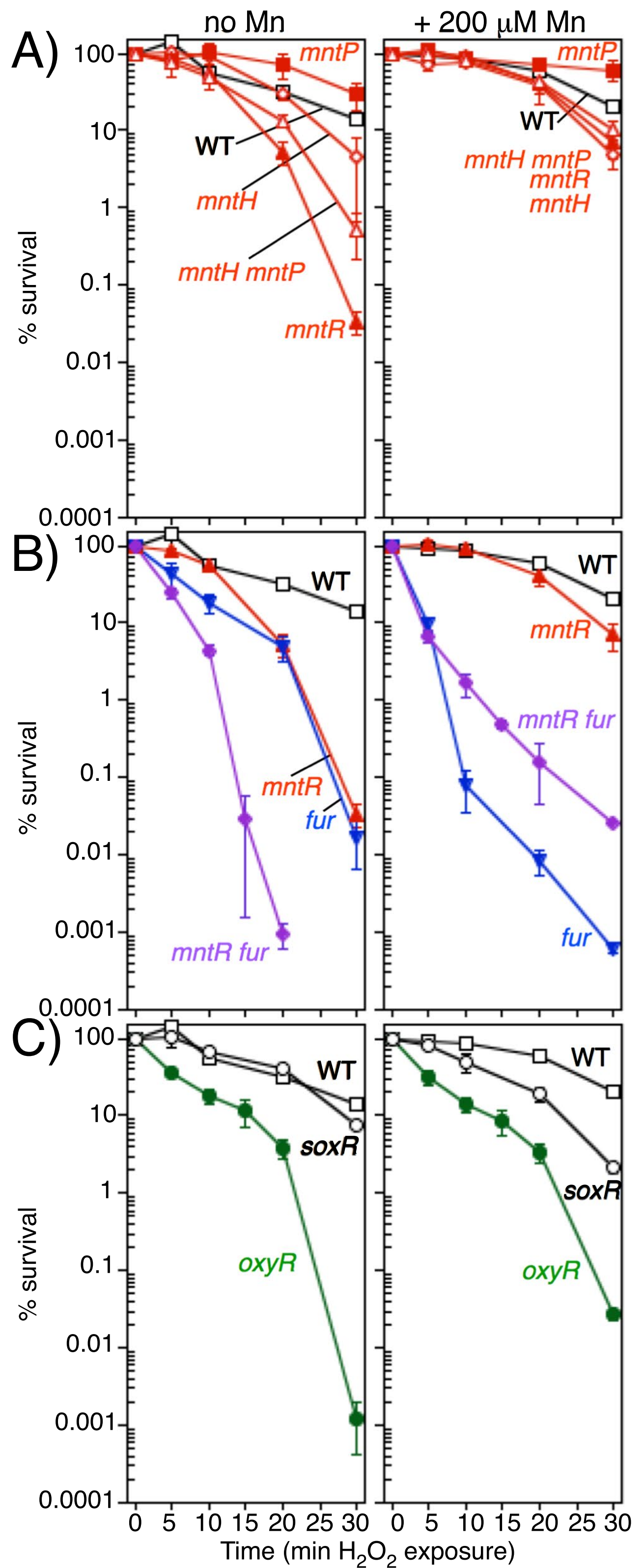
To examine how these manganese and iron transporters/regulators affected H_2O_2 survival, mutants were grown in defined medium either with or without manganese supplementation to early exponential phase, and exposed to 10 mM H_2O_2. The presence or absence of manganese, by itself, did not have a large effect on the survival of wild-type cells over the time period examined (Fig. 4A). Similarly, the absence of either the manganese importer MntH or exporter MntP did not have large effects on survival, with differences remaining within an order of magnitude over the time course examined irrespective of whether manganese was present in the media. However, in the absence of MntR regulation of these transporters, survival was noticeably reduced by ~100-fold after a 30 min exposure. Interestingly, manganese supplementation compensated for this and restored survival to near wild-type levels in *mntR* mutants. We found an additive effect of *mntH* and *mntP* mutations on H_2O_2 survival in the absence of manganese that generally mirrored that of *mntR* at shorter exposure times, but observed a ~10-fold difference in viability after 30-min of treatment. This result would suggest that additional *mntR*-regulated genes become essential for tolerance during prolonged exposure. Similar to what we observed with *mntR* cells, manganese supplementation allowed *mntH mntP* mutants to survive oxidative stress to near wild-type levels. The observations demonstrate that rapidly restoring or delaying replication does not directly correlate with viability. Consistent with what we've observed previously, cells that fail to restore replication when manganese is absent, remain viable for several hours (Hutfilz et al. 2019). The observation indicates that at least under some conditions, cells may remain static for growth until iron and manganese homeostasis can be restored, even if this occurs inefficiently.

Regulation of iron by Fur had a much more pronounced effect on survival following H_2O_2, with the deletion of *fur* reducing survival several orders of magnitude after a 30-min exposure. This effect was further exacerbated in the presence of manganese (Fig. 4B). Additionally, the absence of both iron and manganese regulation (*mntR fur*) severely compromised survival in medium without manganese, reducing viability beyond the limits of detection in our assay after 30 min. However, addition of manganese restored survival in this mutant to levels similar to that of the *fur* single mutant.

Consistent with the regulation of MntH and Fur by OxyR, survival of the *oxyR* mutant matched that of the *mntR fur* mutants in most respects, with survival being severely compromised in the absence of manganese, but modestly improved when this metal was present in the media (Fig. 4C). A modest reduction in survival of *soxR* was observed when manganese was present in the media. The basis of this observation was not clear, but may relate to expression of adjacent genes in the *soxR* operon or in its regulon.

To determine whether intracellular manganese and iron concentrations correlated with the ability of cells to survive H_2O_2 exposure and restore replication after oxidative challenge, we measured the metal content of strains grown with and without manganese supplementation by ICP-MS. In the absence of manganese supplementation, total intracellular manganese concentrations were ~5–10 µM across all strains (Fig. 5A). An increase in intracellular levels of manganese was observed when strains were grown in medium supplemented with this metal. Wild-type, *mntH*, and *fur* cells accumulated approximately 3-fold more intracellular manganese when cultured in the presence of this metal, while *mntR*, *mntP*, and *mntR fur* mutants contained ~10-fold greater manganese concentrations compared to their unsupplemented counterparts. Intracellular iron concentrations in manganese-supplemented wild-type, *mntR*, *mntH*, *fur*, and *mntR fur* cells were within 10–15% of the corresponding cells cultured without manganese (Fig. 5B). Consistent with previous observations (Martin et al. 2015), we found that manganese supplementation reduced intracellular iron levels in *mntP*

Fig. 4 Accumulation of intracellular manganese increases cell survival to acute oxidative stress. The survival of **A** WT (open squares), *mntP* (filled squares), *mntH* (open diamonds), *mntR* (filled triangles), *mntH mntP* (open triangles), **B** *fur* (filled inverted triangles), *mntR fur* (filled diamonds), and **C** *soxR* (open circles), and *oxyR* (filled circles) cells grown in manganese-supplemented (right panels) and -unsupplemented media (left panels) following exposure to 10 mM H_2O_2 for the indicated times is shown. Graphs represent an average from at least three independent experiments. Error bars represent one standard error of the mean



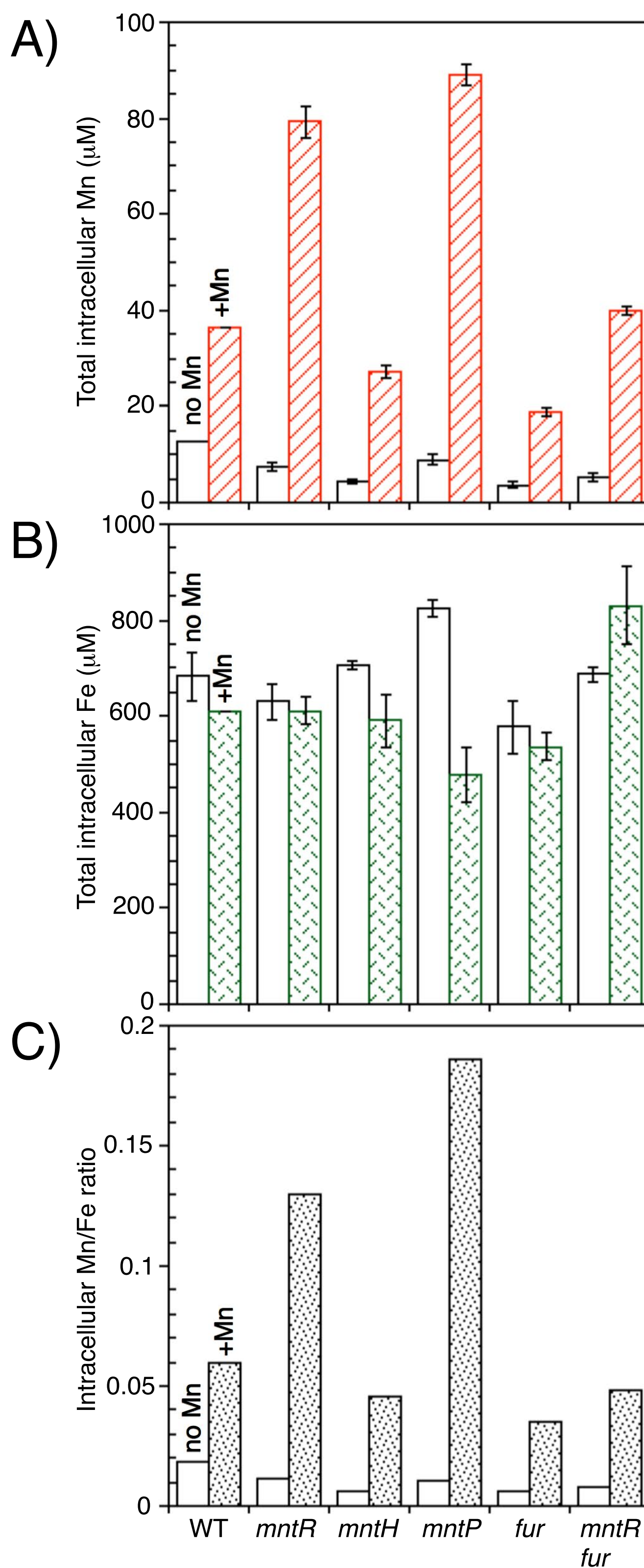


Fig. 5 The absence of MntR or MntP increases Mn/Fe ratio in manganese-supplemented medium. **A** Total intracellular manganese and **B** total intracellular iron measured by ICP-MS in WT, *mntR*, *mntH*, *mntP*, *fur*, and *mntR fur* strains cultured in the absence (unfilled bars) or presence (filled bars) of 200 µM manganese is plotted. Graphs represent an average of two independent measurements normalized to the total protein present in cell lysates. Error bars represent one standard error of the mean. **C** The ratio of manganese/iron concentrations for WT, *mntR*, *mntH*, *mntP*, *fur*, and *mntR fur* strains cultured in the absence (unfilled bars) or presence (filled bars) of 200 µM manganese is plotted

cells, although we observed a more modest effect of approximately 1.7-fold, a change from ~825 to ~480 μM. We did not observe any significant changes in iron concentration in *fur* cells compared to wild-type irrespective of manganese supplementation, but were only able to determine total intracellular iron bound to protein by ICP-MS and not free iron levels. Manganese supplementation increased the intracellular manganese/iron ratios for all strains, however this effect was much greater in *mntR* and *mntP* strains, increasing by 11- and 17-fold over unsupplemented cultures, respectively, compared to 3-fold in wild-type cells (Fig. 5C). Taken together, our data support the idea that increased intracellular manganese, but not iron, levels play a protective role in replication recovery and survival when cells face oxidative challenge.

Discussion

Previous work has shown that *E. coli* cells require manganese in the growth media to restore replication following oxidative stress (Hutfilz et al. 2019; Hoff et al. 2021). Here, we demonstrate that active transport of manganese is necessary and regulates the recovery from oxidative challenge. Taken together with earlier work showing that manganese import is needed for proper enzyme metallation following oxidative stress (Anjem et al. 2009), our results define a central role for manganese homeostasis in cellular tolerance to oxygen toxicity.

Manganese has been implicated in protection against oxidative stress in multiple bacterial species and high intracellular manganese/iron ratios are associated with increased resistance to oxidative stress (Kehres et al. 2000; Tseng et al. 2001, 2002; Daly et al. 2004; Seib et al. 2004; Anjem et al. 2009). However, under iron-replete or non-oxidative growth conditions, intracellular manganese concentrations are kept low (~5–15 μM) in *E. coli* by the activity of manganese-bound MntR to prevent mismetallation of iron- and other metal ion-cofactored enzymes (Fig. 6A and (Patzer and Hantke 2001; Kehres et al. 2002; Waters et al. 2011; Martin et al. 2015). Mn^{2+}-bound MntR represses expression of the primary manganese importer MntH, while activating export through increased *mntP* transcription. Manganese concentrations are further controlled by Fe^{2+}-bound Fur acting on MntH expression. When sufficient iron is present in the growth environment and cells are not undergoing oxidative stress, Fur activity maintains iron homeostasis through transcriptional control of iron transport and utilization to allow the multiple cellular processes that are co-factored by ferrous iron, including DNA synthesis and energy metabolism, while also limiting toxicity that can arise from free radical formation (Fig. 6A and (Bagg and Neilands 1987; Touati et al. 1995; Angerer and Braun 1998; McHugh et al. 2003; Seo et al. 2014)).

At immediate early times following hydrogen peroxide stress, ferrous iron becomes oxidized to its ferric form and disassociates from iron-centered enzymes (Fig. 6B and (Imlay 2014). The stripping of ferrous iron from mononuclear iron, heme, and iron-sulfur proteins results in rapid inactivation of these proteins and the inhibition/stalling of cellular metabolism, consistent with the rapid inhibition in replication that we observe. The Fe^{2+} or [2Fe–2S] bound to Fur itself could also be a target of oxidation (Varghese et al. 2007; Fontenot et al. 2020) and result in inactivation of Fur and de-repression of iron uptake, leading to increased intracellular levels of iron that could then induce further Fenton chemistry and cellular damage. In the absence of Fur, the influx of iron into the cell (Touati et al. 1995; Keyer and Imlay 1996; Varghese et al. 2007) would also be predicted to further exacerbate the effects of oxidative stress and this is what we observe with the increased replication inhibition and H_2O_2 sensitivity in *fur* mutants.

The dedicated reactive oxygen species sensor OxyR is activated following hydrogen peroxide stress through the formation of an intramolecular disulfide bond in each subunit of a working OxyR tetramer (Storz et al. 1990; Aslund et al. 1999; Zheng and Storz 2000). The resulting conformational change enhances interactions between OxyR and RNA Polymerase, thereby activating expression of genes in the OxyR regulon including those involved in peroxide scavenging, DNA repair, and iron and manganese import. The effects of this upregulation are seen at late times after peroxide challenge and result in increased intracellular manganese levels (~150 μM), allowing remetallation of iron-cofactored enzymes by manganese, and the restoration of replication and cellular metabolism (Fig. 6C) and (Anjem et al. 2009). In addition to controlling manganese levels in the presence of hydrogen peroxide, OxyR upregulation of Fur expression is required to restore levels of both

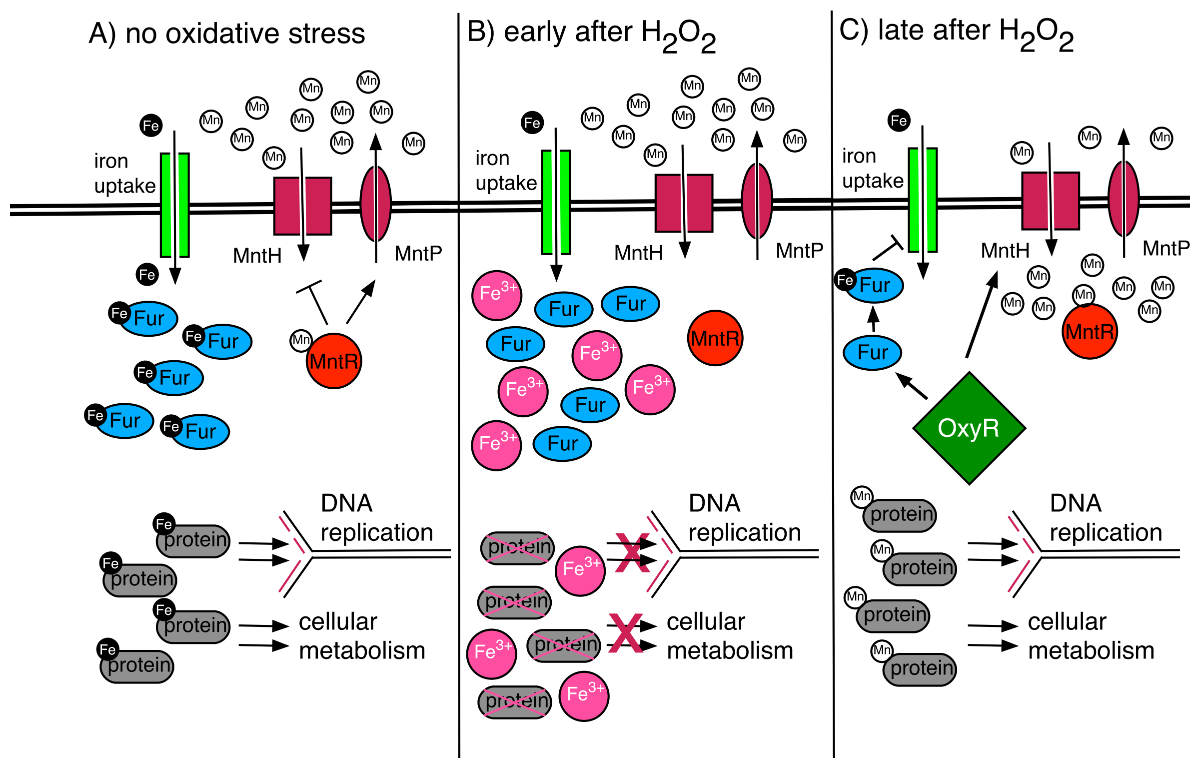


Fig. 6 Model for the replication response to H_2O_2 treatment under manganese-rich growth conditions. **A** In manganese-rich environments where oxidants are absent, both Mn^{2+}-bound MntR and Fe^{2+}-bound Fur inhibit expression of the primary manganese importer, MntH. At the same time, MntP expression is upregulated by Mn^{2+}-bound MntR and binding of free manganese to a riboswitch in the *mntP* promoter to export excess manganese from the cytoplasm. The dual activity of Mn^{2+}-bound MntR on manganese import and export prevents toxicity from excess intracellular manganese. **B** Exposure to H_2O_2 results in rapid oxidation and inactivation of ferrous iron-cofactored enzymes, inhibiting cellular metabolism and hence DNA replication immediately following oxidative stress. **C** H_2O_2 also oxidizes a sensory cysteine residue in OxyR, activating this transcription factor. OxyR upregulates expression of MntH and Fur, resulting in increased manganese import and Fur-mediated repression of further iron uptake, respectively. In cells under oxidative stress, rapid remetallation of oxidized mononuclear iron enzymes occurs using available manganese as the active metal cofactor, thus restoring metabolism

iron-bound and -unbound Fur to control the presence of free intracellular iron in peroxide-stressed cells and limit damage (Zheng et al. 1999; Varghese et al. 2007). In the absence of OxyR, both Fur and MntH expression remain at basal levels after hydrogen peroxide stress (Zheng et al. 1999; Varghese et al. 2007; Anjem et al. 2009), resulting in poor recovery of replication and decreased viability in *oxyR* cells.

Our results are consistent with the idea that manganese transport promotes hydrogen peroxide survival overall. Manganese supplementation has been shown to rescue iron overload in cells chronically exposed to endogenous sources of peroxide by replacing an oxidation-susceptible divalent metal with one that is stable (Anjem et al. 2009). Similarly, we find that the absence of MntR and consequently manganese transport regulation rescues the ability of a *fur* mutant to restore replication and survive oxidative challenge when sufficient manganese is present in the growth medium. Unlike manganese-centric organisms that encode both an active primary ABC (ATP-binding cassette) transporter, e.g. MntABC and SitABC, and a secondary NRAMP (natural resistance-associated macrophage protein) family importer, MntH, iron-centric *E. coli* encodes only *mntH* containing distinct manganese-, iron-, and oxidative species-responsive elements within its promoter (for a review, see (Bosma et al. 2021; Martin and Waters 2022). Despite this tight regulation of manganese import, *E. coli* cells deficient in MntH have been shown to be

able to use a secondary, low-affinity mechanism of import in the presence of manganese concentrations greater than 50 µM (Anjem et al. 2009). The identity of this transporter remains to be determined, however a few candidates include the broad specificity divalent metal ion transporter, ZupT, and zinc transporter, ZnuABC. A *zupT Salmonella* mutant completely abolishes both residual manganese import in a *mntH sitABC* background and all resistance to nitrosative stress (Yousuf et al. 2020). On the other hand, *znuA* and *znuC* mutants suppress the manganese sensitivity of *hflX E. coli* cells, which accumulate manganese in an MntR-independent manner, by inhibiting both zinc and manganese import (Kaur et al. 2014). Whatever this secondary pathway, the small amount of manganese that can be non-specifically imported into the intracellular space could account for the partial replication recovery we observe at late times in manganese-supplemented *mntH* cells. In the same way, while *oxyR* mutants only produce basal levels of MntH after oxidative stress, manganese-rich conditions could potentially allow for enough of this metal to accumulate, leading to the slight increase in survival of manganese-supplemented *oxyR* cells that we observe following prolonged exposure to hydrogen peroxide. Consistent with this interpretation, Imlay and others have found that unsupplemented rich medium, which contains more manganese than defined medium, supports growth in the presence of oxidative or nitrosative stress even when primary manganese import is impaired (Anjem et al. 2009; Yousuf et al. 2020).

The disruption of replication by DNA damage is a primary cause of lethality in cells exposed to DNA damaging agents (Rupp and Howard-Flanders 1968; Howard-Flanders et al. 1969; Courcelle and Hanawalt 2001; Courcelle et al. 2001). Pausing replication to allow more time for repair to occur decreases the frequency of these potentially lethal events and has been demonstrated to be beneficial both for reducing mutations and increasing survival (Castellani et al. 1964; Ganesan and Smith 1969; Courcelle et al. 1997). Additionally, cells lacking RecF, a protein specifically required for replication resumption after disruption by DNA damage, are hypersensitive to UV-irradiation and other forms of damage (Courcelle et al. 1997, 1999, 2003; Chow and Courcelle 2004; Ona et al. 2009), yet are modestly resistant to hydrogen peroxide-induced DNA lesions (Hoff et al. 2021). Thus, one possibility is that the iron sensitivity of replication represents an adaptive response to prevent genomic replication from occurring during periods of oxygen stress when potentially lethal or mutagenic damage may be encountered. In cultures lacking manganese, this inhibition can last for several hours without compromising viability (Imlay and Linn 1986; Hutfilz et al. 2019).

The iron-dependent component required for replication in *E. coli* remains unknown. However, the results presented here demonstrate that manganese transport actively regulates the timing and ability to restore DNA synthesis after hydrogen peroxide treatment. Restoration may involve either direct remetallation of this protein or could involve a redundant manganese-specific counterpart. In eukaryotes, the polymerase responsible for leading strand DNA synthesis, Pol ε, has been shown to contain an iron-sulfur center required for replication (Netz et al. 2011; Lisova et al. 2022). It is not known, if Pol ε, plays a role in the oxidative response, or if this enzyme can utilize manganese during the recovery period. This and the identity of the proteins responsible for stalling replication after oxidative stress remain important questions to be addressed in future work for both bacteria and humans.

Conclusions

Reactive oxygen species generated by metabolic pathways or from exogenous sources damage DNA, proteins and lipids in both microbes and human cells. In this study, we show that regulation of manganese transport directly modulates the timing and ability of cells to resume DNA replication following oxidative challenge. In addition, we find that manganese transport promotes survival to hydrogen peroxide exposure particularly when intracellular free iron levels are perturbed. Taken together, our results define a role for manganese homeostasis in the bacterial response and recovery from oxidative stress. Identification of the iron-dependent replication component that is inactivated by acute oxidative challenge will be required to further our understanding of oxidative stress effects outside of DNA damage.

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Declarations

Conflict of interest The authors have no relevant conflicts of interest to disclose.

References

Andreini C, Bertini I, Cavallaro G, Holliday GL, Thornton JM (2008) Metal ions in biological catalysis: from enzyme databases to general principles. J Biol Inorg Chem 13:1205–1218

Angerer A, Braun V (1998) Iron regulates transcription of the Escherichia coli ferric citrate transport genes directly and through the transcription initiation proteins. Arch Microbiol 169:483–490

Anjem A, Imlay JA (2012) Mononuclear iron enzymes are primary targets of hydrogen peroxide stress. J Biol Chem 287:15544–15556

Anjem A, Varghese S, Imlay JA (2009) Manganese import is a key element of the OxyR response to hydrogen peroxide in *Escherichia coli*. Mol Microbiol 72:844–858

Aslund F, Zheng M, Beckwith J, Storz G (1999) Regulation of the OxyR transcription factor by hydrogen peroxide and the cellular thiol-disulfide status. Proc Natl Acad Sci U S A 96:6161–6165

Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H (2006) Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol 2:20060008

Bagg A, Neilands JB (1987) Ferric uptake regulation protein acts as a repressor, employing iron (II) as a cofactor to bind the operator of an iron transport operon in *Escherichia coli*. Biochemistry 26:5471–5477

Beyer WF, Fridovich I (1991) In vivo competition between iron and manganese for occupancy of the active site region of the manganese-superoxide dismutase of *Escherichia coli*. J Biol Chem 266:303–308

Bosma EF, Rau MH, van Gijtenbeek LA, Siedler S (2021) Regulation and distinct physiological roles of manganese in bacteria. FEMS Microbiol Rev 45:8468

Castellani A, Jagger J, Setlow RB (1964) Overlap of photoreactivation and liquid holding recovery in *Escherichia coli* B. Science 143:1170–1171

Chow KH, Courcelle J (2004) RecO acts with RecF and RecR to protect and maintain replication forks blocked by UV-induced DNA damage in *Escherichia coli*. J Biol Chem 279:3492–3496

Cotruvo JA, Stubbe J (2011) Class I ribonucleotide reductases: metallocofactor assembly and repair in vitro and in vivo. Annu Rev Biochem 80:733–767

Courcelle J, Hanawalt PC (2001) Participation of recombination proteins in rescue of arrested replication forks in UV-irradiated *Escherichia coli* need not involve recombination. Proc Natl Acad Sci U S A 98:8196–8202

Courcelle J, Carswell-Crumpton C, Hanawalt PC (1997) recF and recR are required for the resumption of replication at DNA replication forks in *Escherichia coli*. Proc Natl Acad Sci U S A 94:3714–3719

Courcelle J, Crowley DJ, Hanawalt PC (1999) Recovery of DNA replication in UV-irradiated *Escherichia coli* requires both excision repair and recF protein function. J Bacteriol 181:916–922

Courcelle J, Ganesan AK, Hanawalt PC (2001) Therefore, what are recombination proteins there for? BioEssays 23:463–470

Courcelle J, Donaldson JR, Chow KH, Courcelle CT (2003) DNA damage-induced replication fork regression and processing in *Escherichia coli*. Science 299:1064–1067

Cvetkovic A, Menon AL, Thorgersen MP, Scott JW, Poole FL, Jenney FE, Lancaster WA, Praissman JL, Shanmukh S, Vaccaro BJ, Trauger SA, Kalisiak E, Apon JV, Siuzdak G, Yannone SM, Tainer JA, Adams MW (2010) Microbial metalloproteomes are largely uncharacterized. Nature 466:779–782

Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, Venkateswaran A, Hess M, Omelchenko MV, Kostandarithes HM, Makarova KS, Wackett LP, Fredrickson JK, Ghosal D (2004) Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. Science 306:1025–1028

Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, Leapman RD, Lai B, Ravel B, Li SM, Kemner KM, Fredrickson JK (2007) Protein oxidation implicated as the primary determinant of bacterial radioresistance. PLoS Biol 5:e92

Dambach M, Sandoval M, Updegrove TB, Anantharaman V, Aravind L, Waters LS, Storz G (2015) The ubiquitous yybp-ykoy riboswitch is a manganese-responsive regulatory element. Mol Cell 57:1099–1109

Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S A 97:6640–6645

Faulkner MJ, Helmann JD (2010) Peroxide stress elicits adaptive changes in bacterial metal ion homeostasis. Antioxid Redox Signal. https://doi.org/10.1089/ars.2010.3682

Fontenot CR, Tasnim H, Valdes KA, Popescu CV, Ding H (2020) Ferric uptake regulator (Fur) reversibly binds a [2Fe–2S] cluster to sense intracellular iron homeostasis in. J Biol Chem 295:15454–15463

Ganesan AK, Smith KC (1969) Dark recovery processes in Escherichia coli irradiated with ultraviolet light. II.

Effect of uvr genes on liquid holding recovery. J Bacteriol 97:1129–1133
Haber F, Weiss J (1934) The Catalytic decomposition of hydrogen peroxide by iron salts. Proc R Soc London Ser A 147:332–351
Hassan HM, Fridovich I (1977) Enzymatic defenses against the toxicity of oxygen and of streptonigrin in *Escherichia coli*. J Bacteriol 129:1574–1583
Hoff CA, Schmidt SS, Hackert BJ, Worley TK, Courcelle J, Courcelle CT (2021) Events associated with DNA replication disruption are not observed in hydrogen peroxide-treated *Escherichia coli*. G3 (Bethesda). https://doi.org/10.1093/g3journal/jkab044
Howard-Flanders P, Theriot L, Stedeford JB (1969) Some properties of excision-defective recombination-deficient mutants of *Escherichia coli* K-12. J Bacteriol 97:1134–1141
Hutfilz CR, Wang NE, Hoff CA, Lee JA, Hackert BJ, Courcelle J, Courcelle CT (2019) Manganese is required for the rapid recovery of DNA synthesis following oxidative challenge in. J Bacteriol. https://doi.org/10.1128/jb.00426-19
Imlay JA (2014) The mismetallation of enzymes during oxidative stress. J Biol Chem 289:28121–28128
Imlay JA, Linn S (1986) Bimodal pattern of killing of DNA-repair-defective or anoxically grown *Escherichia coli* by hydrogen peroxide. J Bacteriol 166:519–527
Imlay JA, Fridovich I (1991) Assay of metabolic superoxide production in *Escherichia coli*. J Biol Chem 266:6957–6965
Imlay JA, Chin SM, Linn S (1988) Toxic DNA damage by hydrogen peroxide through the Fenton reaction in vivo and in vitro. Science 240:640–642
Kaur G, Sengupta S, Kumar V, Kumari A, Ghosh A, Parrack P, Dutta D (2014) Novel MntR-Independent mechanism of manganese homeostasis in *Escherichia coli* by the ribosome-associated protein HflX. J Bacteriol 196:2587–2597
Kehres DG, Maguire ME (2003) Emerging themes in manganese transport, biochemistry and pathogenesis in bacteria. FEMS Microbiol Rev 27:263–290
Kehres DG, Zaharik ML, Finlay BB, Maguire ME (2000) The NRAMP proteins of *Salmonella typhimurium* and *Escherichia coli* are selective manganese transporters involved in the response to reactive oxygen. Mol Microbiol 36:1085–1100
Kehres DG, Janakiraman A, Slauch JM, Maguire ME (2002) Regulation of *Salmonella enterica* serovar Typhimurium mntH transcription by H(2)O(2), Fe(2+), and Mn(2+). J Bacteriol 184:3151–3158
Keyer K, Imlay JA (1996) Superoxide accelerates DNA damage by elevating free-iron levels. Proc Natl Acad Sci U S A 93:13635–13640
Levin JD, Shapiro R, Demple B (1991) Metalloenzymes in DNA repair. *Escherichia coli* endonuclease IV and *Saccharomyces cerevisiae* Apn1. J Biol Chem 266:22893–22898
Li Z, Demple B (1994) SoxS, an activator of superoxide stress genes in *Escherichia coli*. Purification and interaction with DNA. J Biol Chem 269:18371–18377
Lisova AE, Baranovskiy AG, Morstadt LM, Babayeva ND, Stepchenkova EI, Tahirov TH (2022) The iron-sulfur cluster is essential for DNA binding by human DNA polymerase ε. Sci Rep 12:17436
Makui H, Roig E, Cole ST, Helmann JD, Gros P, Cellier MF (2000) Identification of the *Escherichia coli* K-12 nramp orthologue (MntH) as a selective divalent metal ion transporter. Mol Microbiol 35:1065–1078
Martin JE, Imlay JA (2011) The alternative aerobic ribonucleotide reductase of *Escherichia coli*, NrdEF, is a manganese-dependent enzyme that enables cell replication during periods of iron starvation. Mol Microbiol 80:319–334
Martin JE, Waters LS (2022) Regulation of bacterial manganese homeostasis and usage during stress responses and pathogenesis. Front Mol Biosci 9:945724
Martin JE, Waters LS, Storz G, Imlay JA (2015) The *Escherichia coli* small protein MntS and exporter MntP optimize the intracellular concentration of manganese. PLoS Genet 11:e1004977
McHugh JP, Rodríguez-Quinoñes F, Abdul-Tehrani H, Svistunenko DA, Poole RK, Cooper CE, Andrews SC (2003) Global iron-dependent gene regulation in *Escherichia coli*. A new mechanism for iron homeostasis. J Biol Chem 278:29478–29486
Netz DJ, Stith CM, Stümpfig M, Köpf G, Vogel D, Genau HM, Stodola JL, Lill R, Burgers PM, Pierik AJ (2011) Eukaryotic DNA polymerases require an iron-sulfur cluster for the formation of active complexes. Nat Chem Biol 8:125–132
Ona KR, Courcelle CT, Courcelle J (2009) Nucleotide excision repair is a predominant mechanism for processing nitrofurazone-induced DNA damage in *Escherichia coli*. J Bacteriol 191:4959–4965
Patzer SI, Hantke K (2001) Dual repression by Fe(2+)-Fur and Mn(2+)-MntR of the mntH gene, encoding an NRAMP-like mn(2+) transporter in *Escherichia coli*. J Bacteriol 183:4806–4813
Rupp WD, Howard-Flanders P (1968) Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J Mol Biol 31:291–304
Seib KL, Tseng HJ, McEwan AG, Apicella MA, Jennings MP (2004) Defenses against oxidative stress in *Neisseria gonorrhoeae* and *Neisseria meningitidis*: distinctive systems for different lifestyles. J Infect Dis 190:136–147
Seo SW, Kim D, Latif H, O'Brien EJ, Szubin R, Palsson BO (2014) Deciphering Fur transcriptional regulatory network highlights its complex role beyond iron metabolism in *Escherichia coli*. Nat Commun 5:4910
Seo SW, Kim D, Szubin R, Palsson BO (2015) Genome-wide reconstruction of OxyR and SoxRS transcriptional regulatory networks under oxidative stress in *Escherichia coli* K-12 MG1655. Cell Rep 12:1289–1299
Sobota JM, Imlay JA (2011) Iron enzyme ribulose-5-phosphate 3-epimerase in *Escherichia coli* is rapidly damaged by hydrogen peroxide but can be protected by manganese. Proc Natl Acad Sci U S A 108:5402–5407
Storz G, Tartaglia LA, Ames BN (1990) Transcriptional regulator of oxidative stress-inducible genes: direct activation by oxidation. Science 248:189–194
Tamarit J, Cabiscol E, Ros J (1998) Identification of the major oxidatively damaged proteins in *Escherichia coli* cells exposed to oxidative stress. J Biol Chem 273:3027–3032

Touati D, Jacques M, Tardat B, Bouchard L, Despied S (1995) Lethal oxidative damage and mutagenesis are generated by iron in delta fur mutants of *Escherichia coli*: protective role of superoxide dismutase. J Bacteriol 177:2305–2314

Tseng HJ, Srikhanta Y, McEwan AG, Jennings MP (2001) Accumulation of manganese in *Neisseria gonorrhoeae* correlates with resistance to oxidative killing by superoxide anion and is independent of superoxide dismutase activity. Mol Microbiol 40:1175–1186

Tseng HJ, McEwan AG, Paton JC, Jennings MP (2002) Virulence of *Streptococcus pneumoniae*: PsaA mutants are hypersensitive to oxidative stress. Infect Immun 70:1635–1639

Varghese S, Wu A, Park S, Imlay KR, Imlay JA (2007) Submicromolar hydrogen peroxide disrupts the ability of Fur protein to control free-iron levels in *Escherichia coli*. Mol Microbiol 64:822–830

Waters LS (2020) Bacterial manganese sensing and homeostasis. Curr Opin Chem Biol 55:96–102

Waters LS, Sandoval M, Storz G (2011) The *Escherichia coli* MntR miniregulon includes genes encoding a small protein and an efflux pump required for manganese homeostasis. J Bacteriol 193:5887–5897

Wu J, Weiss B (1992) Two-stage induction of the soxRS (superoxide response) regulon of *Escherichia coli*. J Bacteriol 174:3915–3920

Yamamoto K, Ishihama A, Busby SJ, Grainger DC (2011) The *Escherichia coli* K-12 MntR miniregulon includes dps, which encodes the major stationary-phase DNA-binding protein. J Bacteriol 193:1477–1480

Yousuf S, Karlinsey JE, Neville SL, McDevitt CA, Libby SJ, Fang FC, Frawley ER (2020) Manganese import protects *Salmonella enterica* serovar Typhimurium against nitrosative stress. Metallomics 12:1791–1801

Zheng M, Storz G (2000) Redox sensing by prokaryotic transcription factors. Biochem Pharmacol 59:1–6

Zheng M, Doan B, Schneider TD, Storz G (1999) OxyR and SoxRS regulation of fur. J Bacteriol 181:4639–4643

Zheng M, Wang X, Templeton LJ, Smulski DR, LaRossa RA, Storz G (2001) DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. J Bacteriol 183:4562–4570

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