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# Lag phase-associated iron accumulation is likely a microbial counter-strategy to host iron sequestration: Role of the ferric uptake regulator (fur)

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## HIGHLIGHTS

- Lag phase is characterized by rapid iron influx with oxidative hypersensitivity.
- Rapid iron accumulation could be microbial counter-strategy to host 'nutritional immunity'.
- Rapid iron accumulation could be mediated by the ferric uptake regulator (Fur) protein.
- This hypothesis may have particular relevance to *Helicobacter pylori* and *Mycobacterium tuberculosis*.

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## ABSTRACT

Iron is an essential metal for almost all forms of life, but potentiates oxidative stress via Fenton catalysis. During microbial lag phase there is a rapid influx of iron with concomitant oxidative hypersensitivity. How and why iron accumulation occurs remains to be elucidated. Iron homeostasis in prokaryotes is mediated by the ferric uptake regulator (Fur), an iron-activated global regulator that controls intracellular iron levels by feedback inhibition with the metal. Herein it is postulated, based on the expression profiles of antioxidant enzymes within the Fur regulon as observed in wild type and *Δfur* mutants, that iron accumulation is mediated by a transitively low concentration of the Fur protein during lag phase. Vertebrate hosts sequester iron upon 'sensing' an infection in order to retard microbial proliferation through a process known as 'nutritional immunity'. It is herein argued that the purpose of iron accumulation is not principally a preparative step for the replicative phase, as suggested elsewhere, but an evolved behavior that counteracts host iron sequestration. This interpretation is supported by multiple clinical and animal studies that demonstrate that iron surplus in hosts advances progression and susceptibility to infection, and vice versa. Contextualizing iron accumulation as a counter-immune behavior adds impetus to the development of antibiotics targeting pathogenic modes of iron acquisition.

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## 1. Introduction

### 1.1. Iron, an essential metal, potentiates oxidative stress

Reactive oxygen species such as hydrogen peroxide ($H_2O_2$), superoxide radical ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), and peroxyl radical ($^{\bullet}HO_2$), inflict genomic, proteomic, and cellular membrane damage, and have hence been implicated in numerous disease states as well as the process of aging (Cerutti, 1985; Halliwell, 1987; Imlay and Linn, 1988; Harman, 1991; Nunoshiba et al., 1999; Muller et al., 2007). These molecules are adventitiously formed from oxygen gas primarily through aberrant electron transfers within the mitochondrial electron transport pathway (Fridovich, 1978). That oxygen simultaneously grants life to aerobic organisms yet potentates cellular damage through the formation of radical molecules is known as the 'oxygen paradox' (Eze, 2006).

Iron is essential for the biochemistry of life, required by all organisms with exception to a few remarkable examples (e.g., see Posey and Gherardini, 2000). Iron potentiates oxidative stress because it catalyzes the formation of deleterious hydroxyl radicals from hydrogen peroxide via the Fenton reaction (Eq. (1))

$$Fe^{2+} + H_2O_2 \xrightarrow{\text{Fenton reaction}} Fe^{3+} + {}^{\bullet}OH + OH^- \quad (1)$$

Iron is a principle agent of oxidative damage and mutagenesis (Touati et al., 1995; Nunoshiba et al., 1999; Imlay and Linn, 1988).

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Indeed, much of the cytotoxicity of hydrogen peroxide is attributable to iron (Imlay et al., 1988). Such an analogous 'iron paradox' therefore necessitates the diligent regulation of the intracellular iron load in order to meet metabolic needs yet mitigate the formation of radical molecules (Halliwell and Gutteridge, 1984, 1997). Prokaryotic life has evolved sophisticated strategies for maintaining iron homeostasis through the coordination of proteins involved in the acquisition and storage of iron (Kuhn, 1998; Meneghini, 1997).

### 1.2. Iron homeostasis in prokaryotes

Iron homeostasis in prokaryotes is mediated by a global regulator known as the ferric uptake regulator (Fur). Fur is present in most species of bacteria, the exceptions being Gram-positive bacteria with high genomic GC-content such as *Corynebacterium* sp. and *Streptomyces* sp., for which the global regulator known as the diphtheria toxin regulator (DtxR; also known as IdeR and SirR) mediates iron homeostasis (Andrews et al., 2003). The Fur protein is post-translationally activated by binding of ferrous iron, inducing a conformational change that allows Fe(II)–Fur to recognize and bind DNA (Carpenter et al., 2009; Troxell and Hassan, 2013; Hantke, 2001; Escolar et al., 1999; Andrews et al., 2003). The majority of cases of transcriptional regulation by Fe(II)–Fur proceeds through promoter occlusion: Upon binding of Fe(II)–Fur to promoter sequences known as 'Fur boxes', gene transcription is repressed via blockage of RNA polymerase (Carpenter et al., 2009; Troxell and Hassan, 2013; Hantke, 2001; Escolar et al., 1999; Andrews et al., 2003). Hence, when intracellular iron levels are high, Fe(II)–Fur predominates, and when iron levels are lowered, *apo*-Fur predominates, consequently determining the expression of a host of proteins involved in the metabolism of iron (Carpenter et al., 2009; Troxell and Hassan, 2013; Hantke, 2001; Escolar et al., 1999; Andrews et al., 2003). Fe(II)–Fur may alternatively de-repress gene transcription by inhibiting the transcription of *ryhB*, a small regulatory RNA that binds to target mRNA and signals their degradation through recruitment of degradosomes (Massé and Gottesman, 2002; Massé et al., 2003). As *ryhB* inhibits the expression of multiple proteins, all of them iron-bearing, it is thought that *ryhB* serves as an 'austerity signal', reserving iron for the synthesis of only the most indispensable of proteins (Massé et al., 2005; Jacques et al., 2006). Intriguingly, *ryhB* also suppresses translation of *fur* mRNA, creating a negative feedback loop that is dependent on the intracellular iron concentration (Vecerek et al., 2007). Another mechanism of de-repression involves the microbial global gene repressor, H-NS (a nucleoid protein): Displacement of H-NS within the promoter region of the target gene by Fe(II)–Fur permits gene translation (Troxell et al., 2011b; Nandal et al., 2010; Stoebel et al., 2008).

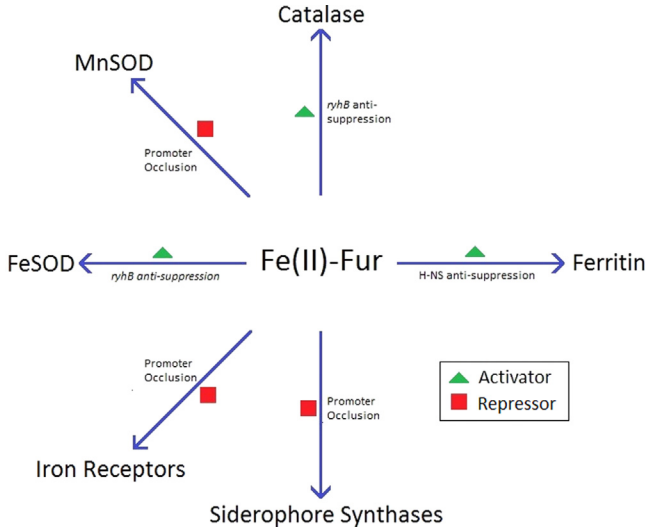


**Fig. 1.** Modes of regulation of activated (iron-bound) Fur, and the outcome on the expression of proteins involved in iron metabolism and oxidative homeostasis. See Sections 1.2, 3.1, and 4.1 for details.

Bacteria secrete iron chelators known as siderophores that bring iron into the cell. Examples of siderophores include enterochelin, areobactin, enterobactin, and ferrichrome (Saha et al., 2013). The majority of siderophores are produced by non-ribosomal peptide synthases. The expression of these biosynthetic enzymes increases when intracellular iron levels are low, and the transcriptional control of these genes is negatively regulated by Fe(II)–Fur via promoter occlusion (Troxell et al., 2011a; Bjarnason et al., 2003; McHugh et al., 2003; Tsolis et al., 1995; Bagg and Neilands, 1987; Brickman et al., 1990; De Lorenzo et al., 1988). Some evidence also suggests that Fe(II)–Fur negatively regulates the expression of siderophore transmembrane transport proteins as well as other iron receptors necessary for influx of iron (Miethke and Marahiel, 2007; Jacobsen et al., 2005; Ollinger et al., 2006; Ochsner et al., 1995). Ferritin is an archetypal iron-storage protein produced by both prokaryotes and eukaryotes (Harrison and Arosio, 1996). The expression of ferritin in prokaryotes is positively regulated by Fe(II)–Fur: As iron levels rises, expression of the ferritin gene, *ftnA*, is de-repressed via displacement of the H-NS repressor (Harrison et al., 2013; Nandal et al., 2010). Through these modes of regulation, Fe(II)–Fur regulates the expression of proteins related to iron metabolism as well as dozens of proteins of broader significance, including the iron- and manganese-containing isoforms of superoxide dismutase (FeSOD; MnSOD), and catalase, of which these two enzymes are necessary for ameliorating the oxidative impact of iron (Fig. 1).

### 1.3. Microbial lag phase is characterized by rapid iron accumulation and oxidative hypersensitivity

Research by Rolfe et al. (2012) profiling global gene expression during lag phase, the first phase of the microbial replication cycle, demonstrated that there is an unparalleled and transient increase in the intracellular concentration of iron. Using *Salmonella enterica* as a model species, the authors observed a maximal iron load of $4.1 \times 10^{-18}$ mol/cell at 4 min post-inoculation into fresh media, as compared to only $2.0 \times 10^{-18}$ mol/cell just 4 min prior. Iron accumulation was correlated with heightened expression of 20 genes that were only up-regulated during lag phase. Of these 20, the protein products of nine genes were iron uptake and storage proteins, and four were relevant to iron-sulfur cluster synthesis. As iron potentiates oxidative stress through Fenton catalysis, the authors examined whether cells in lag phase were hypersensitive to oxidative challenge. Lag phase *Salmonella* removed 4 min post-inoculum were treated with hydrogen peroxide. As compared to cells that were not in lag phase, these cells displayed an approximate 1000-fold decrease in viability. Of cells removed 20 min after inoculation into media, the viability was reduced by approximately 650-fold. Hypersensitivity was correlated with maximal intracellular iron concentration 4 to 20 min post-inoculation and was concomitant with the induction of almost every gene in the OxyR and SoxS oxidative stress regulons, including antioxidant enzymes superoxide dismutase and catalase. Hypersensitivity abated one hour post-incubation. The purpose of iron influx has yet to be elucidated, but has been suggested by the authors to be preparative step for transition into exponential phase, specifically, "for the assembly of iron cofactors and Fe–S clusters that are associated with essential metabolic machinery" (Rolfe et al., 2012; p. 696). Hence, lag phase is characterized as a period of oxidative hypersensitivity wrought by iron accumulation.

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