Bacterial iron detoxification at the molecular level

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ABSTRACT

Iron is an essential micro-nutrient and, in the case of bacteria, its availability is commonly a growth-limiting factor. However, correct functioning of cells requires that the labile pool of chelatable 'free' iron is tightly regulated. Correct metalation of proteins requiring iron as a cofactor demands that such a readily accessible source of iron exists, but over-accumulation results in an oxidative burden that, if unchecked, would lead to cell death. The toxicity of iron stems from its potential to catalyze formation of reactive oxygen species (ROS) that, in addition to causing damage to biological molecules, can also lead to the formation of reactive nitrogen species (RNS).

In order to avoid iron-mediated oxidative stress, bacteria utilize iron-dependent global regulators to sense the iron status of the cell and regulate the expression of proteins involved in the acquisition, storage and efflux of iron accordingly. Here, we survey the current understanding of the structure and mechanism of the important members of each of these classes of protein. Diversity in the details of iron homeostasis mechanisms reflect the differing nutritional stresses resulting from the wide variety of ecological niches that bacteria inhabit. However, in this review we seek to highlight the similarities of iron homeostasis between different bacteria, whilst acknowledging important variations. In this way we hope to illustrate how bacteria have evolved common approaches to overcome the dual problems of the insolubility and potential toxicity of iron.

Introduction

A great deal of the biological importance of iron stems from facile redox transformations between the Fe^{2+} and Fe^{3+} oxidation states that underpin its function as a cofactor in many enzymes. Iron-containing proteins are grouped into three main classes. Iron-sulfur clusters are thought to represent the oldest class of iron-containing cofactors. They typically consist of 2-4 iron ions (though occasionally more), but occasionally also contain a heterometal such as nickel or

molybdenum, linked by inorganic sulfide and covalently attached to the protein via the thiol groups of cysteine residues. These versatile cofactors are involved in many processes including respiration, photosynthesis, nitrogen fixation, hydrogen evolution and the associated electron transfer chains (1). The simplest iron-containing cofactors are formed by the binding of discrete metal ion to sites composed from the sidechains of histidine and/or the carboxylates aspartate and glutamate. These are principally employed to harness the oxidizing power of O_2 for processes such as DNA synthesis and methane oxidation (2). Heme is formed by the incorporation of iron into the tetrapyrrole protoporphyrin IX. This chemically versatile cofactor is critical in many processes including respiration, cycling of nitrogen and sulfur and detoxification reactions in addition to also supporting electron transfer (3-5). As a result of this versatility the demand for iron is large in most organisms, including the majority of bacteria, with up to 25% of the proteome binding iron in some form (6).

However, the same redox chemistry required for these roles (Equation 1 and the Fenton reaction, Equation 2) allows iron to catalyze the Haber-Weiss reaction (Equation 3).

$$Fe^{3+} + O_2^{-\bullet} \leftrightarrow Fe^{2+} + O_2 \quad \text{(Eqn. 1)}$$

$$Fe^{2+} + H_2O_2 \leftrightarrow Fe^{3+} + {}^{-}OH + {}^{\bullet}OH \quad \text{(Eqn. 2)}$$

$$O_2^{-\bullet} + H_2O_2 \leftrightarrow {}^{-}OH + {}^{\bullet}OH + O_2 \quad \text{(Eqn. 3)}$$

The resulting hydroxyl radicals ($^{\bullet}OH$) are highly reactive, causing damage to lipids, proteins, carbohydrates and nucleic acids (7). Superoxide ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) are produced as by-products of aerobic respiration (8) and, therefore, any aerobically respiring organism faces the requirement not only to detoxify ROS but also to strictly regulate the concentration of iron in any form able to catalyze the Haber-Weiss reaction. This need is particularly acute in the case of bacteria since, in addition to endogenously produced ROS, they are often subjected to assault by ROS produced either by competitors in the environment or in phagocytes produced by the immune system of hosts during infection (9).

Nitric oxide is known to play an important role as a signaling molecule in biological systems, but is also produced in elevated concentrations for defense or competition in a similar manner to ROS. Combination of nitric oxide with superoxide generates the peroxynitrite ion that is susceptible to further oxidation to either nitrogen dioxide or dinitrogen trioxide. Collectively these RNS can cause damage to nucleic acids and modify the sidechains of amino acids such that protein structure and function is impaired (9). Furthermore, both ROS and RNS are known to lead to breakdown of iron-sulfur clusters, resulting in the displacement of iron from the cofactor. Thus, iron homeostasis and the generation of ROS and RNS are intimately connected, as are the regulatory networks for their management within bacterial cells.

Sensing of iron and regulation of genes involved in iron-uptake/homeostasis

When considering the iron status of cells, it is important to distinguish between the quota, which is the total iron content of the cell, and that subset of the quota that is kinetically available for insertion into proteins and molecular cofactors, referred to as the 'labile iron pool' (10). The majority of the latter is likely in the Fe^{2+} oxidation state and coordinated by small molecules such as low molecular weight thiols (11,12). This represents the fraction of the quota available to fulfil metabolic requirement, but also that with the potential to catalyze unwanted ROS and RNS formation. Therefore the first requirement of any regulatory system for iron homeostasis is the ability to sense the concentration of the labile iron pool across the physiologically relevant range; 1-10 μM according to most estimates (13-15). As one might expect, this is achieved by transcriptional regulators whose affinities for target DNA are modulated by either binding directly to iron or by the binding of iron-dependent prosthetic groups. Often these are global regulators, controlling the expression of a great many genes, including those involved in the biosynthesis and import of siderophores, import of ferrous iron, and the storage and/or efflux of iron present in excess of cellular requirements. This balancing of metal trafficking to fulfil nutritional requirements whilst suppressing potential toxicity, shown schematically in Figure 1, is termed 'nutritional passivation' and is a common strategy that extends beyond iron metabolism (16).

Iron-sensing by Fur

Members of the Fur (ferric uptake regulator) superfamily are the most widespread

transcriptional regulators controlling iron homeostasis in bacteria. The first member of the Fur family was identified in *Escherichia coli* some 35 years ago (17) and, as the name suggests, was reported to regulate the intake of Fe^{3+} into the cell. This is achieved by the binding of the protein to 'Fur boxes', AT rich binding sites upstream of the regulated genes with the consensus sequence 5'-GATAATGATAATCATTATC-3'. It has been argued that the Fur box should be considered a 21-bp fragment containing two overlapping 7-1-7 inverted repeats that each bind a Fur dimer.

These are positioned such that the two copies of Fur bind to opposite faces of the DNA helix (18). Binding of Fur occludes access of RNA polymerase, thus repressing transcription of the responsive genes (19). However, despite the great deal of research effort directed at members of the Fur superfamily, an understanding of these processes at the molecular level has only recently been achieved.

Despite reports of both monomeric (20) and higher oligomeric (21) forms of Fur detected in solution, the physiologically relevant form of the protein is thought to be the homodimer. This is stabilized by a large buried interface between C-terminal dimerization domains (22) and, in most cases, the binding of a structural Zn^{2+} (23) ion by four conserved Cys residues (24). Occupancy of this structural site (S1) is required, but not sufficient, for DNA binding. The Fur family exhibits some structural variation and in certain examples the dimerization domain harbors a second structural site ligated by His and Glu residues (25). The dimerization domain is connected to the N-terminal DNA binding domain via a flexible hinge region containing a regulatory site comprising His and Glu sidechains that binds Fe^{2+} with a reported dissociation constant, K_d, of approximately 1 μM when determined *in vitro* (26). Whilst the regulatory site has been demonstrated to bind other di- and tri-valent metals it is thought that only Fe^{2+} is present at the concentration required to activate the protein *in vivo*. Occupancy of this site induces a rotation of the DNA binding domain relative to the dimerization domain, creating an increased void area between the two DNA binding domains such that they are able to accommodate double stranded DNA (25). It is thought that this conformational change forms the molecular basis of the increased affinity of Fur for DNA *in vitro* under elevated concentrations of the regulatory metal. *In vitro* studies utilizing gel-shift methods report K_d values of approximately 10 nM for complex formation between activated Fur and target DNA sequences (23).

The recently reported crystal structure of *Magnetospirillum gryphiswaldense* Fur (27) in complex with DNA has provided insight into the molecular basis for recognition of Fur binding sites (Figure 2). The AT rich composition of the Fur box results in a narrowing of the minor groove and consequent increase in negative charge density from the phosphate backbone that persists upon repressor binding. This facilitates shape recognition by Fur via a favorable electrostatic interaction between a conserved lysine residue (Lys15 in *M. gryphiswaldense* Fur numbering) and the minor groove. More specific interactions with bases in the major groove are facilitated by the rotation of the DNA binding domains induced by metal binding at the regulatory site. This involves van der Waals interactions between Tyr56 and consecutive thymine bases in the target sequences, and hydrogen bonding between the guanidinium group of Arg57 and the O6 and N7 atoms of a conserved guanine. A recent report suggests that Fur DNA-binding can be tuned by protein-protein interactions (28), in addition to the long recognized effect of iron binding. $EIIA^{Ntr}$, a component of the nitrogen metabolic phosphotransferase system, was shown to affect expression of Fur-regulated genes. *In vitro* gel shift measurements showed that this arises from formation of a protein-protein complex that lowers the affinity of holo-Fur for DNA. Consequently, repression of Fur-regulated genes requires a greater cytoplasmic Fe^{2+} concentration when $EIIA^{Ntr}$ is present. The K_d for the Fur-$EIIA^{Ntr}$ complex has not yet been determined, nor has the increase in K_d of the Fur-DNA complex in the presence of $EIIA^{Ntr}$.

In addition to the classic gene repression mechanism described above, Fur has been shown to act as an activator of gene expression, both directly (29-32) and indirectly (33). Direct activation occurs through binding in the promoter region (29-32), while indirect regulation occurs via interaction with the non-coding RNA RhyB (see the section on ferritins below) (34), by the displacement of histone like proteins (35), or by blocking the binding of a

second repressor (36). Regulation of gene expression by apo-Fur has also been demonstrated in a limited number of cases (37,38), and genome-wide studies have demonstrated the Fur regulon to encompass dozens of transcription units, containing >100 genes in some cases (33,37,39-41). It is now apparent that Fur-like proteins constitute a superfamily with members identified that are responsive to other metals (Mur, the manganese uptake regulator (42) and Zur (43) , the zinc uptake regulator), and to peroxide-induced oxidative stress (Per) (23).

Genes identified as being regulated by Fur, such as that in *E. coli*, include those encoding iron-uptake systems such as *fhu*, *fec* and *feo*, the *suf* iron-sulfur cluster assembly system, iron-sulfur containing proteins such as *fumA*, *acnA*, *acnB* and *nuo*, the iron containing superoxide dismutase *sodB*, and the iron storage proteins *bfr* and *ftnA* (see below). Consistent with its role as a repressor of iron import systems, the transcriptional response of a Fur deletion mutant is similar to that evoked by iron limitation, even under iron replete conditions. This inability to correctly sense the iron status of the cell has been demonstrated to result in an increase in ROS production (44), suggesting that, in contrast to some other metals, cellular storage and efflux mechanisms are unable to compensate for the resulting elevated concentration of the labile iron pool. Fur has been shown to be involved in the re-modeling of cell metabolism away from iron-containing enzymes, management of ROS and reconfiguration of the cell membrane to protect against antibiotic attack, in addition to controlling cellular iron homeostasis (10,33,45).

Iron-sensing by DtxR/IdeR

Proteins of the DtxR/IdeR (Diphtheria toxin repressor/Iron dependent regulator) family are the global transcriptional regulators controlling iron-uptake in GC rich Gram-positive bacteria (46). Indeed, DtxR was first identified as an iron-dependent repressor of virulence factor expression in *Corynebacterium diphtheriae* and it is from this activity the name derives (47). Much effort has been devoted to the study of this group of bacteria as they include important human pathogens such as *C. diphtheriae* itself, *Mycobacterium tuberculosis* and *Staphylococcus aureus* and antibiotic producers such as *Streptomyces*. This included the demonstration that DtxR also regulates iron-uptake in *C. diphtheriae* and the identification of homologues in other organisms.

Proteins of this family exhibit similarities to Fur; they act primarily as repressors of transcription by occluding binding of RNA polymerase (48,49) but recognize a consensus sequence with greater GC content than that of Fur; 5'-TTAGGTTAGCCTAACCTAA-3' (50). The homodimers harbor multiple metal binding sites and undergo conformational change upon binding Fe^{2+} as corepressor. In the metal bound active form, double stranded DNA binds between two helix-turn-helix (HTH) N-terminal DNA binding domains that are linked via dimerization domains (51,52). *In vitro* DNA affinity of Fe^{2+}-sensing DtxR proteins is also activated by non-cognate divalent metal ions such as Ni^{2+}, Co^{2+}, Mn^{2+} and Cd^{2+}. Ni^{2+} and Fe^{2+} bind DtxR with the highest affinity, K_d being around 1 μM (53,54). However, distinct from Fur, these proteins also contain an SH3-like domain of unknown function as a C-terminal extension (52). They also differ in the molecular contacts leading to recognition of target DNA, and the nature of the conformational change induced by binding of the regulatory metal.

Structures of DtxR in complex with DNA were available before those of Fur and revealed two homodimers bound to each nucleotide fragment (51) (Figure 3). Each of the monomers harbors two metal binding sites (presumed to be iron *in vivo*) and, in further analogy to Fur, binding of divalent metal to the high affinity ancillary site imparts stability to the protein fold, whilst affinity for target DNA sequences is increased by the occupancy of the lower affinity primary site (46,55,56). However, in contrast to Fur, occupancy of the primary metal binding site results in only a small rotation of the DNA binding domains relative to the dimerization domains (52). Comparison of apo- and holo-structures of DtxR suggest that metal ion binding induces a helix to coil transition in the 6 N-terminal residues (51,56) and relieves what would, in the apo protein, be an unfavorable steric interaction with DNA. This, together with a small 'caliper' like movement of the N terminal domains, which brings them into better alignment with the major groove, results in the increased DNA affinity for the holo form of the repressor over the apo form (51). Residues 27-50 make up the helix-turn-helix DNA-binding motif containing helices B and C. Each monomer contributes a total of 9 favorable

interactions with nucleotide phosphate groups: Arg27, Ala28 and Arg29 of helix B, Thr40, Ser42, Arg47 and Arg50 of helix C together with Glu36 and Ser37 of the intervening loop. In further contrast to Fur, formation of the protein-DNA complex causes distortion of the nucleotide from the *B*-form conformation. This results in the opening up of the minor groove such that it can accommodate the guanidinium group of Arg60, located on the loop 57-61 connecting two strands of an antiparallel β-sheet (51).

Examples of the DtxR/IdeR family lacking the C-terminal SH3-like domain have been reported, but these are not responsive to Fe^{2+} *in vivo* (57,58). Given the recent discovery that DNA binding by Fur is modulated by formation of a complex with $EIIA^{Ntr}$ it is possible that the SH3 domain modulates the iron response of DtxR via protein-protein interactions. The suite of genes regulated by DtxR includes those involved in siderophore production and translocation, heme degradation, Fe^{2+}-import, iron-sulfur cluster assembly and iron storage (59), demonstrating similar regulatory activity to Fur despite there being no evolutionary link between the two protein families.

Iron-sensing by RirA and Irr

The genomes of the α-proteobacteria contain homologues of Fur but, where these have been characterized, they have been shown either to have a diminished role in iron regulation compared to other examples of Fur, or to be responsive to other metals, such as Mn^{2+} (60,61). Global regulation of iron is performed by two novel transcriptional regulators found, with few exceptions, only within the α proteobacteria (62): Iron Response Regulator (Irr) (63) and Rhizobial Iron Regulator A (RirA) (64). These are currently less well characterized than either Fur or DtxR, with no crystal structures of either the proteins or protein/DNA complexes available to date. However, significant progress in understanding these proteins has been made recently and both are known to sense the availability of intracellular iron not by binding the metal itself, but instead by binding iron-containing prosthetic groups.

RirA is unique among bacterial iron-sensing transcriptional regulators in that it belongs to the Rrf2 family (64). As with many members of this family, affinity of RirA for DNA is modulated by the binding of an iron-sulfur cluster (65). Again, the protein exists as a homo-dimer in solution and homology modeling based on the recently reported structures of other Rrf2 regulators predicts that each monomer contains a DNA-binding domain, featuring a winged helix-turn-helix motif, connected to a dimerization helix via a loop containing three conserved Cys residues (66).

Under iron replete conditions the protein contains a $[4Fe\text{-}4S]^{2+}$ cluster, coordinated by the three conserved Cys residues (67) (with a likely additional, but unknown, ligand), and binds to *cis*-acting Iron-Responsive Operator sequences (68) (IRO boxes) in the promoter region of genes involved in iron uptake acting as a repressor of transcription in a manner analogous to Fur and DtxR. The apo-protein lacks any specific high-affinity interaction with DNA *in vitro* whilst a meta-stable $[2Fe\text{-}2S]^{2+}$ cluster-containing form has been shown to exhibit intermediate binding affinity (69). RirA has also been shown to promote transcription of genes (70,71), including those involved in iron storage under iron-replete conditions, via an indirect mechanism involving small non-coding RNA (72) in analogy to Fur.

Recent *in vitro* characterization of RirA from *Rhizobium leguminosarum* demonstrated that iron-sensing occurs via a reversible dissociation of a labile Fe^{2+} ion from the $[4Fe\text{-}4S]^{2+}$ cluster, with a K_d of 3 μM (66). Under iron replete conditions, the cluster remains stable in the $[4Fe\text{-}4S]^{2+}$ form. However, when iron is limiting, competition for the labile iron increases, yielding a $[3Fe\text{-}4S]^{0}$ cluster intermediate that is unstable to further breakdown to the apo-form, via a [2Fe-2S] form (as well as several other intermediates). Under low iron and in the presence of O_2, accelerated degradation to apo-RirA occurs. This results initially from the oxidation of the $[3Fe\text{-}4S]^{0}$ intermediate to a less stable $[3Fe\text{-}4S]^{1+}$ form, and is subsequently mediated by the oxidation of cluster sulfides. This susceptibility to O_2-mediated iron and sulfur oxidation is thought to underpin a dual Fe^{2+} and O_2 sensing role. RirA has been demonstrated to regulate iron-sulfur cluster biogenesis in *R. leguminosarum* and O_2 sensing by RirA may be important to ensure adequate cellular supply of iron-sulfur clusters under aerobic conditions even when iron is replete. An as yet unknown regulatory mechanism prevents upregulation of iron-uptake systems under these conditions (66).

Whilst RirA is restricted to the order *rhizobiales*, the Iron Response Regulator (Irr) is widely distributed among the α-proteobacteria (72,73). The protein is a homologue of Fur but senses the iron status of the cell not by binding Fe^{2+} from the free iron pool, but the iron containing prosthetic group heme (43). Due to the insolubility and potential cytotoxicity of heme, cells are unlikely to contain a 'free heme pool' akin to that of Fe^{2+}. Rather, it is thought that Irr is associated with ferrochelatase (74), the enzyme responsible for insertion of iron into protoporphyrin IX in the final step of heme biogenesis, and acquires the prosthetic group directly from it. Apo-Irr binds to Iron Control Element (67,75) sequences (ICE boxes) that are upstream of regulated genes and, like other Fur proteins, can act directly either as a repressor or an activator depending on the location of the ICE sequence (75). However, in the case of Irr, direct activation of regulated genes is far more common than for either Fur or DtxR. In further contrast to other examples of the Fur superfamily, Irr only binds to ICE sequences in the absence of its co-regulator. All examples characterized to date contain two heme binding sites. One of these is a conserved HxH motif (76,77), but studies have revealed significant diversity in the nature of the other. Possibly related to this, the mechanism by which de-repression occurs appears to differ markedly between members of the *rhizobiales* in which RirA also acts as an iron responsive global regulator, and other α-proteobacteria in which Irr is the only protein fulfilling this function. The best characterized examples are the Irr proteins from *R. leguminosarum* (belonging to the former class) and *Bradyrhizobium japonicum* (from the latter).

In organisms such as *R. leguminosarum*, *Agrobacterium tumefaciens* (78) and *Ensifer meliloti* (72), Irr forms part of a regulatory network involving RirA among other factors. These networks are interlinked, with Irr controlling expression of RirA whilst the two proteins regulate iron homeostasis in an antiparallel manner. Under high iron conditions, RirA represses the expression of iron uptake systems, whilst in low iron Irr represses the expression of iron storage systems but also RirA, thereby assisting in de-repression of RirA-regulated genes. The proteins are dimeric in solution and loss of DNA binding affinity is associated with the binding of heme at the HxH motif located close to the interface between the monomers (77). Disruption of this heme-binding motif by mutagenesis led not only to the abolition of heme binding but also high affinity binding of DNA by the apo-protein, thereby demonstrating the importance of this motif for the recognition of ICE box sequences. These observations led to a model in which a conformational change in the HxH motif upon binding of heme forms the molecular basis of the loss of DNA affinity. However, the detail of any such conformational change at the atomic level remains to be elucidated. Whilst the regulatory role, if any, of the second heme-binding site remains unclear, its occupancy has been shown to modulate the oligomeric state of the protein *in vitro* (79).

In organisms such as *B. japonicum*, in which Irr is the only global regulator of iron homeostasis, regulation is achieved via a different mechanism. These proteins have an HxH heme-binding motif similar to that identified in Irr from *Rhizobiales* (76,80), but this site preferentially binds heme with iron in the Fe^{2+} oxidation state. Furthermore, the binding of heme does not affect the affinity of the protein for DNA binding; rather, the protein has been shown to be conditionally stable with degradation initiated by the binding of ferric heme at a second site, the Heme Regulatory Motif (HRM). On binding to this site, the heme iron is five coordinate with the sulfur of a cysteine residue providing the axial ligand. Pulsed radiolysis studies demonstrated a ligand switch to axial ligation by histidine upon reduction followed by binding of O_2 under aerobic conditions (81). This has led to the suggestion of a ROS mediated pathway for *B. japonicum* Irr degradation in the presence of heme. The available data indicates that heme binding to both sites of *B. japonicum* Irr is required for efficient degradation of the protein. The HRM is not limited to *B. japonicum* Irr, having also been identified in Irr proteins from *Nitrobacter*, *Xanthobacter* and *Magnetospirrilum* (61), suggesting a similar mechanism of iron regulation in these organisms.

In the absence of both of its substrates, ferrochelatase binds Irr with high affinity, thereby competing with DNA binding and alleviating regulatory activity. However, binding of protoporphyrin IX to ferrochelatase causes dissociation of its complex with Irr. Therefore, when the rate of heme synthesis outstrips the availability of iron, Irr is released, downregulating iron-dependent biosynthetic pathways and

activating genes involved in iron acquisition. Once the concentration of Fe^{2+} in the labile iron pool increases sufficiently such that metalation of protoporphyrin IX is coordinated with its synthesis, heme is inserted into Irr, targeting the protein for oxidative degradation and therefore ensuring that regulatory activity is abrogated. It is thought that this system of regulation allows the rate of iron uptake to be matched to metabolic need under varying conditions, rather than simply maintaining the labile iron pool at a concentration determined by the affinity of Fe^{2+} for the transcriptional regulator (74).

Iron acquisition by bacteria

Despite its natural abundance in the earth's crust, iron is often a growth-limiting micronutrient for bacteria due to the insolubility of the Fe^{3+} ion at neutral pH, which limits the dissolved iron concentration to 1.4 x 10^{-9} M under aerobic conditions (82). To counter the low bioavailability of iron in many environments, bacteria have evolved high affinity iron acquisition pathways. Whilst these are often targeted by host immune systems or competing bacteria to limit growth (83), they are also under the control of the global regulators described above to enable expression to be repressed should iron availability exceed cellular requirement (84). Iron uptake in bacteria has been extensively studied with the ultimate aim of preventing infection by targeting iron metabolism. Here we survey the main features whilst referring the interested reader to several recent reviews (85-88).

Siderophore mediated iron uptake

The most widely distributed iron acquisition strategy under aerobic conditions is the secretion of siderophores (89). These are small molecule chelators (150-2000 Da) (90) with high affinity for Fe^{3+} (K_d in the range 10^{-20} to 10^{-49} M) that acquire iron from the extra-cellular environment (85). Over 500 examples have been characterized to date falling in to 3 main classes, the catechols, hydroxamates and α-hydroxycarboxylates, defined according to the nature of the iron ligating moiety (89). Examples containing more than one of the aforementioned iron ligating groups are termed mixed siderophores.

Siderophore synthesis is non-ribosomal but occurs in the cytoplasm meaning that in Gram negative bacteria their export and, in most cases, utilization of the sequestered iron requires transport across both the cytoplasmic and periplasmic membranes. There appears to be the greatest diversity in the proteins involved in the export across the cytoplasmic membrane with examples belonging to both ABC transporter (91) and Major Facilitator Superfamily (MFS) (92) classes reported. Export across the outer membrane is mediated by TolC-like efflux pumps (93).

Once secreted from the cell siderophores acquire iron either by outcompeting host proteins such as transferrin, or by the solubilization of Fe^{3+} from iron containing minerals. Import across the outer membrane is mediated by porins (Figure 4A) composed of 22 stranded β-barrels and an extracellular facing 'plug' domain that binds the iron loaded siderophore with high (typically nM) affinity. The TonB/ExbBD energy transducing complex spans the periplasmic space and connects the porin to the cytoplasmic membrane potential, allowing active transport of the substrate.

Once internalized, periplasmic binding proteins (Figure 4B) act as chaperones delivering the ferric siderophore complex to the cytoplasmic membrane. Here ABC transporters (Figure 4D) couple transport across the inner membrane to ATP hydrolysis. Many bacteria are able to utilize multiple siderophores to satisfy their requirement for iron including 'xenosiderophores'; those which the organism is unable to synthesize but can internalize and extract iron from (94). A general trend is that the outer membrane porins show specificity for their cognate siderophore whilst the inner membrane ABC transporters have greater flexibility in substrates tolerated. Therefore the genomes of Gram negative bacteria encode a greater number of outer membrane porins for siderophore uptake than ABC transporters dedicated to the same task (95).

Once the loaded siderophore has been translocated to the cytoplasm the iron is typically released via reduction to Fe^{2+} (95) for which the chelators have lower affinity. A possible exception to this are the hexadentate triscatechelates, which form the most stable Fe^{3+} complexes of all siderophores, stabilizing this oxidation state to such an extent that the midpoint of the Fe^{3+}/Fe^{2+} couple is in the range -600 to -750 mV (versus SHE). It is thought that esterase-mediated hydrolysis of the backbone, resulting in three bidentate catechol

units, is required for iron release. This raises the midpoint potential of the chelated iron to around -350 mV, which is accessible to intracellular reductants such as NADH (E_m ~ -320 mV) (85,95). Other exceptions to the scheme outlined above are known, most notably for the pyoverdines, the principle siderophores of some pseudomonads, where reductive iron release occurs in the periplasm (96).

Extraction of iron from heme

In the case of many pathogenic bacteria heme represents an important source of iron since it accounts for some 75% of the iron content of mammals (97). The heme acquisition pathway shows many parallels to siderophore uptake, perhaps reflecting the insolubility and potential toxicity of both heme and Fe^{3+}.

In some cases, such as the Has system of *Pseudomonas aeruginosa*, heme scavenging proteins termed hemophores are secreted to the extracellular environment (98). These proteins ligate heme via the sidechains of conserved His and Tyr residues (99,100). In contrast to siderophores, they deliver the extracted heme to outer membrane heme binding proteins and are not themselves reimported to the cell. The outer membrane proteins bind heme via two histidine residues and have a lower intrinsic affinity for heme than hemophores. However, formation of the hemophore/outer membrane binding protein complex induces a conformational change in the hemophore, lowering its affinity for heme and ensuring transfer in the desired direction (101).

In other systems, such as Phu also from *P. aeruginosa*, the outer membrane receptors acquire heme directly from host proteins (102). Whilst PhuR, the outer membrane heme binding protein of Phu, employs His/Tyr ligation of heme (103), it appears that His/His ligation is more common amongst these proteins (86). In either case they bind heme with pM affinity and are able to extract it from host proteins such as hemoglobin or the hemoglobin-haptoglobin complex (86).

The outer membrane heme-binding proteins are associated with 22-25 stranded β-barrel porins (Figure 4A). These are also coupled to the cytoplasmic membrane potential by the TonB/ExbBD complex. As with siderophores, heme is shuttled to the inner membrane by periplasmic binding proteins (Figure 4C) and imported to the cytoplasm by ABC transporters (Figure 4D) (86). Once located in the cytoplasm heme can be directly incorporated in to bacterial proteins, but is more commonly subjected to oxidative degradation by heme oxygenases to liberate the iron (104). Heme acquisition systems are subject to negative regulation by the iron-dependent transcriptional regulators to avoid iron overload, but expression is also linked to sensing of heme availability by hemophores via extra-cytoplasmic function σ factors (105).

Uptake of ferrous iron

Under acidic and/or anaerobic conditions, iron is predominantly in the soluble ferrous oxidation state. Consequently, bacteria have evolved mechanisms for the direct uptake of iron in this form. The solubility of Fe^{2+} means that active transport across the outer membrane of Gram negative bacteria is not required, and it enters the periplasm by free diffusion through porins (106). Several systems have been demonstrated to import Fe^{2+} into the cytoplasm, including MntH (107), ZupT (108), YfeABCD (109), FutABC (110), EfeUOB (111) and Feo, but, of these, only Feo appears both widespread and dedicated to the transport of Fe^{2+} (106).

Feo was first identified in *E. coli* where the operon encodes three proteins, FeoA, FeoB and FeoC (112). However, it seems that FeoC is limited to the γ-proteobacteria (88) and the most commonly occurring (54% of sequenced genomes) feo gene organization consists of only *feoAB*, whilst 11% of sequenced bacterial genomes contain *feoB* alone (106). FeoB is a ~80 kDa membrane protein containing 7-12 transmembrane helices (106). A cytoplasmic domain located at the N-terminus has been shown to bind and hydrolyze GTP (113-115), with hydrolysis thought to be activated by K^+ (116). At present it is unclear whether this supports active transport of the Fe^{2+} substrate or is used to signal the energy status of the cell. This GTPase domain is linked to the membrane spanning helices by a GDP Dissociation Inhibitor domain (117) and switch regions thought to alter conformation upon nucleotide binding. The mechanism by which FeoB transports Fe^{2+} remains elusive, but is thought to be mediated by binding of the metal to the sulfur atoms of Cys and Met residues located in the transmembrane helices (106).

Both *feoA* and *feoC* encode small (~8 kDa) hydrophilic proteins. FeoA is a basic protein with isoelectric point at around pH 9.0, consistent with localization to the inner leaf of the cytoplasmic membrane (118). The protein displays significant homology to SH3 domains and possess the same fold (119). This has led to the suggestion that protein-protein interactions between FeoA and the GTPase domain of FeoB regulate the rate of nucleotide hydrolysis. Whilst deletion of *feoA* has been shown to result in a 60% reduction in Fe^{2+} transport (88), direct interaction between FeoA and FeoB has not yet been demonstrated. FeoC adopts the winged helix-turn-helix fold (120,121) common in DNA binding domains, and from its structure has been predicted to be a repressor of transcription (118,122). However, DNA binding activity of FeoC remains to be demonstrated.

The Feo system exemplifies the complex interplay of iron and O_2 metabolism that is likely a universal characteristic of bacteria. Under anaerobic conditions the expression of ferric import systems decreases due to an increase of Fe^{2+}-Fur. The *feo* operon is also negatively regulated by Fur, thereby preventing iron overload. However at typical intracellular iron concentrations, the combined positive regulation of *feo* by ArcA and FNR alleviates Fur-mediated repression (15). In this way anaerobic conditions lead to the repression of ferric iron uptake systems whilst the expression of *feo*, the importer matched to the most likely available iron source, has been reported to increase 3-fold under anaerobic conditions (88).

Iron uptake in Gram positive bacteria

The iron acquisition pathways of Gram positive bacteria show significant similarity to the Gram-negative systems described above despite the absence of an outer membrane and periplasmic space. Both siderophore bound iron and heme are transported across the cell membrane by ABC transporters, whilst the Feo system is employed for the import of ferrous iron (87,98). Iron is also extracted from internalized heme by heme oxygenase enzymes (123,124).

Clearly there is no requirement for either outer membrane porins or periplasmic binding proteins. However, heme is unable to diffuse across the 15-80 nm of the peptidoglycan cell wall. Transport of heme across the cell wall is mediated by a series of proteins anchored at the cell surface. The Isd heme uptake pathway of *Staphylococcus aureus* is the most extensively studied of the Gram positive systems and is thought to be representative of the general mechanism these bacteria employ for heme uptake (87). Four proteins are required for the transfer of heme across the cell wall to the IsdE/F ABC transporter complex. These are anchored to the cell surface by the sortases SrtA and SrtB (125,126). In each of the four surface anchored proteins, heme is bound at NEAr iron Transporter (NEAT) domains containing conserved YXXXXY domains in which the leading Tyr serves as a ligand to the heme iron (127). IsdB and IsdH extract heme from host proteins whilst IsdA and IsdC shuttle the extracted heme to the ABC transporter complex with IsdC acting as the central conduit for transfer to IsdE/F (128). The unidirectional transfer of substrate is driven by the increasing affinity for heme of sequential NEAT domains in the shuttle pathway (129).

Tyrosine is an unusual heme ligand amongst heme binding proteins in general, but is prevalent among the proteins involved in bacterial heme acquisition. The hemophores and periplasmic binding proteins of the Gram negative bacteria, in addition to those involved in transfer of heme across the cell wall in the Gram positive case, all utilize tyrosine as a ligand, suggesting that its properties may be particularly suited to the capture and transfer of heme.

Iron storage in bacteria

Iron acquired via the mechanisms described above initially enters the labile iron pool. The existence of an intracellular pool of iron not bound to proteins was initially postulated on thermodynamic grounds (130). Since iron utilizing proteins typically bind the metal with K_d of the order 10^{-8} to 10^{-7} M, it was argued that a population of free metal with concentration greater than this must exist to prevent dissociation. This was presumed to be composed of Fe^{2+} as a result of the reducing environment of the cytoplasm, and the requirement for rapid ligand exchange; the kinetic lability of Fe^{2+} complexes being typically 10^4 times greater than their Fe^{3+} counterparts.

Despite its critical importance in iron homeostasis, the chemical composition of the labile iron pool remains the source of considerable debate, in part due to the difficulty of defining the speciation of intracellular iron. Siderophores,

amino acids, citrate and low molecular weight thiols have all been proposed as candidate ligands. Whole cell Mössbauer spectroscopy provides the most direct empirical insight. The feature assigned to the labile iron pool has parameters typical of high spin ferrous iron and is commonly interpreted as resulting from oxygen and nitrogen ligation (131). The relative affinities for Fe^{2+} and intracellular abundance of the proposed chelators makes citrate the most likely candidate of the oxygen donor ligands listed above. However, glutathione (or equivalent low molecular weight thiols such as mycothiol in the actinobacteria or bacillithiol in the firmicutes) is predicted to outcompete citrate at typical cytoplasmic concentrations and pH, leading to the counterproposal that the labile iron pool is dominated (up to 80%) by $[Fe(H_2O)_5GSH]^{2+}$ or similar complexes (12). The prevalence of water in the coordination sphere of the Fe^{2+} would likely result in the high spin electronic configuration reported by Mössbauer spectroscopy, despite the presence of a thiol ligand. Therefore, on balance, it seems likely that thiol coordinated Fe^{2+} constitutes a major component of the labile iron pool.

Intriguingly, a very recent report suggests that polyphosphate acts as a hexadentate chelator of iron *in vivo*. Not only does this inorganic polymer act as a repressor of the Fenton reaction by saturating the coordination sphere of the metal, it has also been shown to act as an intracellular buffer of free iron (132). The extent to which this inorganic macromolecule contributes to either the labile iron pool or the long-term iron storage capacity of bacterial cells remains to be established.

The primary purpose of the labile iron pool is thought to be to ensure correct metalation of the iron proteome which has been estimated to account for 60% of intracellular iron in cells grown on iron replete (50 μM) liquid media (133). However, as a result of the scarcity of iron, and despite the potentially catastrophic consequences of the Fenton reaction (Equation 2), when the concentration of the labile iron pool exceeds this metabolic requirement, the excess is not simply excreted from the cell via efflux mechanisms. Rather, dedicated iron storage proteins belonging to the ferritin superfamily are employed to sequester the metal in a non-reactive state, which can be re-mobilized to satisfy cellular requirements during iron starvation. The signal pathway triggering the release of these iron stores remains to be elucidated but it is reasonable to assume that the initial event would be depletion of the labile iron pool, leading to de-metalation of iron-dependent transcriptional regulators.

Ferritins are found in all kingdoms of life (134). Most animal cells contain only 24-meric heteropolymers of ferritins (135). These are composed of H- and L-chains, which, respectively, contain and lack a catalytic site for iron oxidation, but which are isostructural and can thus co-assemble in different proportions depending on the organism/tissue. In contrast, bacterial genomes commonly encode multiple predicted ferritins of different classes. These include prokaryotic analogues of the animal ferritins called Ftns, heme containing 24-meric ferritins, called Bfrs, that are unique to bacteria, and mini-ferritins, which are dodecamers that have only been identified in prokaryotes. All prokaryotic ferritin subunits contain a catalytic center for the oxidation of iron and assemble into homopolymers (134)

All ferritins share a 4 α-helical bundle structural motif and all except the L-chain units of animal cells contain di-iron catalytic sites, called ferroxidase centers, for the oxidation of iron (136) (Figure 5). These are described in more detail below for each class of the bacterial proteins. Typical ferritins self-assemble into cage-like structures. The mini-ferritins form dodecamers of tetrahedral 3 3 2 symmetry with internal and external diameters of 4.5 and 9 nm respectively (Figure 5A), and possess additional helical elements at the N-terminus and 2-fold axis (137). All other cage-forming ferritins possess only a short fifth helix (E) at the C-terminus, altering the packing geometry. As a result they assemble into larger rhombic dodecahedral cages possessing octahedral 4 3 2 symmetry with internal and external diameters of 8 and 12 nm, respectively (138) (Figure 5C). All of these cage-like structures are permeated by channels at the vertices of their packing motifs that span the protein coat, connecting the interior cavity to bulk solution. The 4-fold channels of 24-meric ferritins are lined by the E helices, but variants in which this helix is missing are still competent to form assemblies with iron-storing capability. Rather, assembly of the protein is impaired by the disruption of residues at the C-terminus of helix D (139).

The channels located at the 3-fold axes have been demonstrated to constitute the route of iron entry into animal ferritins (140).

Comparatively little work has been reported on iron entry into the proteins from prokaryotes. Whilst some may also utilize the 3-fold channel (141), the so-called B-channels are used in at least a subset (142). These channels, which are found almost exclusively in prokaryotic ferritins, are formed at the 2-fold axis at the intersection between 3 monomeric units.

The proposal of dedicated routes for the transportation of Fe^{2+} from bulk solution through the protein coat to the site of oxidation has faced resistance due to the existence of a channel directly linking the ferroxidase center to bulk solution (143). However, there is increasing evidence that networks of carboxylate residues with conformational flexibility play key roles in Fe^{2+} transfer in all cage-forming ferritins (141,142,144-146). All ferritins sequester Fe^{2+} from solution and utilize an electron accepting co-substrate, such as O_2 or H_2O_2, to drive its oxidation to the Fe^{3+} state. This oxidized product is then translocated to the interior cavity where it is stored as a hydrated ferric oxy mineral similar to ferrihydrite. Up to several thousand iron atoms per protein can be stored in this way. However, the molecular architecture of the catalytic centers carrying out this chemistry, and the mechanistic detail of how it is achieved, varies between the different classes of bacterial protein (147).

Expression of the mini-ferritins is usually regulated by σ factors under nutritional stress, or in response to oxidative stress (148), whilst that of the 24meric examples is usually controlled by iron-responsive transcriptional regulators. However, unlike systems for iron uptake, this cannot be achieved by a mechanism of direct repression under high concentrations of free iron. For example under low iron conditions in *E. coli*, production of Ftn and Bfr proteins is repressed by the small RNA RyhB (34), which binds to *ftn* and *bfr* mRNAs (as well as many others), affecting translation through a number of mechanisms that include inhibiting translation and promoting mRNA degradation. RyhB is repressed by Fur so that, at elevated iron concentrations, the metalated protein down-regulates RyhB, leading to increased levels of Ftn and Bfr proteins. It has also been reported that expression of *ftnA* can be induced by Fur in a RhyB-independent manner (35). The mRNA-binding global regulator CsrA plays an important role in iron homeostasis, through its repression of genes such as *bfr* and *dps* (149), expression of which are not required under exponential, minimal stress conditions. In *rhizobiales*, *bfr* expression is directly repressed by Irr under iron limitation, with RirA implicated in de-repression as iron availability increased (72). However, in some examples of cyanobacteria, iron storage is not positively regulated by increasing iron concentration (150,151). These observations further illustrate the complexity of cellular iron regulation.

Iron oxidation in Ftns

The Ftns are the closest analogues to the eukaryotic ferritins found in bacteria and are also widely distributed among archaea. The crystal structures of several examples are available, including that of the most intensively studied, FtnA of *E. coli* (138). These reveal an asymmetric di-iron ferroxidase center with similar architecture to that of the H-chain ferritins from animals (Figure 5D). The predicted high affinity site (site A) is coordinated by a bidentate Glu (17 in *E. coli* FtnA numbering), His53 and bridging Glu50 that also coordinates the predicted lower affinity site (site B). Coordination of the second site is completed by monodentate Glu94 and, in most examples, a second Glu (Glu130 in *E. coli*). This residue also ligates a third metal binding site (site C) whose coordination is completed by a further three monodentate Glu residues (Glu49, 126 and 129). A conserved Tyr residue (Tyr24) is also located close to site B and forms a hydrogen bond to one of the site B ligands (Glu94).

In vitro studies of recombinantly expressed proteins have been employed to interrogate the mechanism by which Ftns lay down a mineral core within their interior cavity, and have revealed marked similarity to that of their counterparts from eukaryotes. Under aerobic conditions, and in the absence of alternative co-substrate such as H_2O_2, O_2 binds to the freshly occupied di-Fe^{2+} center resulting in the rapid formation of a di-Fe^{3+}-peroxo intermediate that is detectable via a transient absorbance feature in the wavelength range 600-650 nm (152). Hydrolysis of this intermediate results in the formation of a ferric-oxo species thought to be the precursor of the mineral core, which is not stably bound at the ferroxidase center (153). It remains to be demonstrated how the oxidized product is transported from the site of oxidation to the cavity, though this may involve the

growth of iron-oxo clusters from carboxylate sidechains located on the inner surface of the protein coat in close proximity to the ferroxidase centers.

The effect of substitutions of site C residues suggest it is involved in ferroxidase center activity in some instances, although the role of both site C and the conserved nearby tyrosine residue appear variable between different proteins (147). Some examples of Ftn exhibit a stoichiometry of their iron/oxygen chemistry that is greater than 2:1 and is affected, together with the rate of iron oxidation, by disruption of site C, suggesting a role for this site in Fe^{2+} oxidation/catalytic turnover. In others, the site appears to function to regulate the rate of flux of the oxidized product out of the ferroxidase center, such that flux is greater in the absence of site C. A role has been postulated for the conserved Tyr as a 'molecular capacitor' providing, together with the three Fe^{2+} ions bound at sites A-C, four reducing equivalents enabling the direct reduction of O_2 to H_2O (154). However, whilst every reported example of an H-chain like ferritin contains a Tyr residue at the equivalent position to Tyr24 of *E. coli* FtnA, the effect of substitution of this residue, e.g. by Phe, is variable (147), suggesting that its function is variable. Furthermore, whilst some data support a role for conserved ferritin Tyr residues as electron donors this is not always the case. In some instances, observation of di-Fe^{3+} peroxo species requires that assays be performed with a large excess of Fe^{2+} over ferroxidase center sites (155). These are precisely the conditions under which site C would be expected to be occupied and involvement of a third Fe^{2+} ion and oxidation of a Tyr residue would result in the direct formation of H_2O. However, the observation of a di-Fe^{3+} peroxo species that decays to form the di-Fe^{3+} center and H_2O_2, indicate that H_2O is not formed and, therefore, that the conserved Tyr does not function as a reductant.

Regardless of the route of iron exit from Ftn ferroxidase centers, it is apparent that oxidized iron is translocated from here into the interior of the protein, regenerating empty binding sites, facilitating catalytic turnover. Furthermore, the rate of this flux is increased by further incoming Fe^{2+} substrate. This 'displacement' model of core formation is directly analogous to that proposed for eukaryotic ferritins (153), although the effect of helix E deletion on the ability to generate a mineral core is different between the two classes of protein (139,156,157) which may reflect different routes of Fe^{3+} exit from the catalytic centers.

Iron oxidation in Bfrs

The most striking difference between the Ftns and Bfrs is the presence in the latter of 12 heme groups, located at the monomer-monomer interface of each of the subunit dimers that make up the 12 faces of the rhombic dodecahedral protein assembly. *In vitro* data indicate that the presence or absence of these prosthetic groups has little effect on the rate of iron uptake by the protein (158), particularly at low iron loadings. Instead, they are thought to promote the reductive mobilization of the mineral core (159) via their interaction with a small [2Fe-2S] cluster containing ferredoxin, called Bfd (160) that is differentially expressed from *bfr* despite its adjacent location on many bacterial genomes.

The coordination of iron at the ferroxidase center also differs significantly between Bfr and Ftn. The catalytic center of the former is almost symmetric (Figure 5D) with each metal ion coordinated by two bridging Glu residues (51 and 127, *E. coli* protein residue numbering), a His (54 at site A and 130 at site B) and a monodentate Glu (18 at site A and 94 at site B) (161). The *E. coli* protein remains the most extensively characterized example of Bfr and, here at least, the difference in iron coordination at the ferroxidase center relative to other ferritins has an impact on the mechanism (though this is not the case for all, see below). Rather than releasing oxidized iron from the ferroxidase center into the interior of the protein, iron bound here appears to be a stable cofactor regardless of oxidation state (162), presumably as a consequence of the increased coordination number. Nevertheless, *in vitro* assays of iron mineralization activity demonstrate that the protein is able to lay down a mineral core containing up to 2800 equivalents of iron (163). Therefore oxidized iron must be deposited in the interior of the protein via a route other than the displacement mechanism employed by the Ftns and other ferritins.

Crystallographic studies identified an iron binding site, Fe_{IS}, located on the inner surface of the protein that is important for function (161). This, together with a network of aromatic residues, including the tyrosine conserved in other classes of ferritin (Tyr25 in this instance), deliver electrons

into the ferroxidase center, generating Fe^{3+} within the protein cage in the process (164,165). The reduced ferroxidase center then reacts with a further oxidizing equivalent completing the catalytic cycle. Unlike the Ftns, the stoichiometry of the Fe:O_2 reaction is 4:1, consistent with H_2O_2 being a far more effective co-substrate than O_2 for Bfr (166).

To a first approximation, the ligation of iron at the ferroxidase center of *Pseudomonas aeruginosa* Bfr (BfrB[a]) is identical to that in the *E. coli* protein. However, the structure of the protein derived from crystals subjected to different soaking conditions demonstrated conformational flexibility in residue His130 (167). Whilst this residue acts as an ligand to iron in site B for structures in which the ferroxidase center is occupied, these sites are vacant in crystals formed from the protein as isolated and His130 in these structures is rotated relative to those with metal containing active sites such that it would be unable to bond to a metal ion located at site B. These observations led to the proposal that the ferroxidase center of *P. aeruginosa* Bfr behaves as a gated pore for iron entry to the protein and a displacement mechanism of core formation akin to that of the Ftns. It is noteworthy that the rate at which the *P. aeruginosa* and *E. coli* Bfr proteins oxidize Fe^{2+} following binding of the metal to apo ferroxidase centers is similar, but the former is able to lay down a mineral core at a rate far greater than the latter, consistent with mechanistic differences between them. The structure-function relationships governing these differences has not yet been resolved.

The roles of Ftn and Bfr varies between organisms

In *E. coli*, an *ftnA* deletion mutant exhibited marked impairment of growth compared to the wild type strain on transfer from iron-replete to iron-deficient conditions (168). This phenotype was not observed for the *bfr* mutant, suggesting a role other than iron storage for this protein, possibly in oxidative stress response. In contrast, deletion of the *bfrB* gene in *P. aeruginosa* severely impairs the ability of the organism to accumulate iron as FtnA does not sequester a mineral core even in the absence of Bfr. Deletion of *bfd* or disruption of the Bfr:Bfd interaction elicits an iron starvation response, even under iron-replete conditions due to irreversible deposition of iron within the BfrB core (169). Therefore, it appears that the roles of Ftn and Bfr are reversed in the two organisms and this may correlate with the reported differences in mineralization mechanism. A similarly variable picture is emerging from studies of ferritins in other organisms. For example, in *Salmonella enterica*, Bfr appears to be the major iron store (170), while, in *Mycobacterium tuberculosis*, Ftn (previously known as BfrB[a]) is important for virulence (171) and under high iron levels, while Bfr (BfrA) appears to be important for recycling iron under low iron levels (172). In the strictly anaerobic sulfate-reducing bacterium *Desulfovibrio vulgaris*, Bfr plays an important role in protecting the organism from O_2, which is normally toxic to such bacteria (173).

Iron oxidation by Dps/Dpr proteins

The Dps (DNA-binding proteins under starvation) proteins are composed of 12 identical α-helical subunits (rather than 24) and are consequently also known as mini-ferritins. They are significantly upregulated during stationary phase or periods of oxidative stress (174). In addition to consuming the Fenton reagents Fe^{2+} and H_2O_2, they bind non-specifically to DNA (175,176). This provides a physical barrier and can induce a crystalline transition in the nucleoid (177,178), both of which are thought to protect against oxidative damage. The affinity of these proteins for DNA is thought to be due to a 'tail' at the N-terminus of the peptide that is rich in positively charged residues providing a favorable electrostatic interaction (179-181). Dps proteins protect against multiple stress factors but require both DNA binding and ferroxidase activity in all cases (181). We note that homologues of Dps proteins have been identified in nutritionally deficient stationary phase cultures that exhibit antioxidant activity but do not bind to DNA. These proteins, termed Dpr, are under the control of transcriptional regulators that respond to redox status/oxidative stress e.g. PerR in *Streptococcus pyogenes* (182) or RitR in *Streptococcus pneumoniae* (183).

The subunit arrangement of Dps 12mer mini-ferritins results in a change in the symmetry of the channels penetrating the protein coat (2-fold channels and two classes of 3-fold channel) compared to the 24mer proteins. One of the classes of 3-fold channel is unique to these proteins, whilst the second is similar to the 3-fold channels of other ferritins, and is thought to constitute the route of iron entry (179,184). The location and structure of

the ferroxidase center is also unique among the cage-forming ferritins. Rather than being buried within the 4 α helical bundle, it is located at the interface between the two protomers of each subunit dimer. In the majority of structural models derived from diffraction data, this site contains only a single ion coordinated by conserved carboxylate and histidine residues (184-186). The first reported example was from the Dps of *Listeria innocua*, with iron ions coordinated by Glu62 and Asp58 of one protomer and His31 of its partner within the subunit dimer (184) (Figure 5B).

A di-iron form of the catalytic site, modeled by placing an iron ion at the position of a nearby ordered water, suggested that Glu62 might bridge the two metals, with His43 from the same protomer as His31 being the only other potential ligand. In the few cases where two metal ions have been observed at the ferroxidase center, the second metal has a significantly larger temperature factor than its surroundings indicating significant lability of this site (187). Attempts to assess iron binding by fluorescence quenching indicated 24 equivalents of iron per protein upon addition of Fe^{3+} but only 12 equivalents when titrating with Fe^{2+} (188). This has led to the proposal that the di-iron site is only formed as an intermediate in the oxidation reaction of Dps, in contrast to the 24mer cages where the occupancy of both sites is thought to be a prerequisite for rapid reactivity with either O_2 or H_2O_2. Consistent with a role in combating oxidative stress, the Dps centers utilize H_2O_2 as the co-substrate for Fe^{2+} oxidation, being significantly less reactive towards O_2 (189).

Fe storage in Cyanobacteria

A survey of the distribution of iron storage proteins in cyanobacterial genomes revealed significant differences to other bacteria, with only around 12% of genomes containing a homolog of FtnA. A great many of the genomes of marine picocyanobacteria (*Prochlorococcus* and *Synechococcus*) contain a distinct class of ferritin that differs from the classic Ftn proteins in that the coordinating sidechains that make up site C are absent in the peptide chain. An example from *Synechococcus sp* CC9311, *Syn*Ftn, was found to be upregulated in response to exposure to elevated concentrations of copper (88). Furthermore, several of the marine picocyanobacteria possess genes encoding homologs of both *Syn*Ftn and FtnA. Together these observations suggest that *Syn*Ftn may have a role in oxidative or general stress response rather than iron homeostasis. *In vitro* characterization of this protein demonstrated that, whilst the mineral core is generated via the typical displacement of oxidized iron from the catalytic center, the oxidation of this site proceeds via a mixed valent Fe^{2+}/Fe^{3+} intermediate not previously observed during ferritin activity (or indeed the oxidation of any other O_2-activated diiron protein save one), where di-Fe^{2+} sites are oxidized directly to di-Fe^{3+} peroxo species. The Fe^{2+}/Fe^{3+} intermediate oxidizes to a metastable di-Fe^{3+} form in ~10 s at atmospheric O_2 concentration. This breaks down to release mineral product to the protein interior and regenerate apo sites able to bind further equivalents of Fe^{2+} and initiate another reaction cycle. In further contrast to other bacterial Ftns, the di-Fe^{2+} form of *Syn*Ftn ferroxidase centers is unreactive towards H_2O_2, utilizing only O_2 as co-substrate (190).

Whilst the genomes of many cyanobacteria lack homologs of any of the characterized 24mer ferritins, homologs of the mini ferritins appear to be widespread (191) and these have been shown to have roles in iron homeostasis, in addition to oxidative stress response (192). Some genomes encode multiple examples. Amongst the most extensively studied are those of *Nostoc punctiforme*, a filamentous cyanobacterium in which the majority of cells in filaments are in a vegetative state and perform photosynthesis, but around 5% form heterocysts – differentiated cells that perform a N_2 fixing function. *N. punctiforme* encodes five Dps homologs (193), annotated NpDps1-5 (194), that are differentially transcribed depending on cell type. Of these, NpDps1-3 have been designated typical Dps like proteins based on sequence homology (195), with NpDps2 predominantly expressed in photosynthetic vegetative cells and the others predominantly in heterocysts. As with the Dps proteins of pathogens, they also use H_2O_2 as the preferred oxidant. Whilst this group of proteins exhibit some degree of co-regulation, individual proteins are also thought to be upregulated in response to a variety of environmental cues. NpDps1 is expressed in response to low temperature (196), whilst NpDps2 confers resistance to oxidative stress induced both by exogenous H_2O_2 (191) and high light levels (194), and is also expressed in response to heat

shock. NpDps5 appears to perform a similar role to NpDps2, conferring resistance to both H_2O_2 (197) and light induced oxidative stress (194), but is also involved in iron homeostasis. The ligation of the ferroxidase center in this protein differs markedly from canonical Dps proteins and closely resembles that of bacterial Bfrs discussed above (191). Finally NpDps4 possesses unusually His-rich ligation of iron at the ferroxidase center and utilizes only O_2 and not H_2O_2 as an oxidant for iron (198). Accordingly a role for this protein has been proposed as an O_2 scavenger within heterocysts where nitrogenase activity requires that a microoxic (< 10 μM O_2) environment be maintained (199). Based on sequence comparisons to other Dps proteins, it has been suggested that this type of reaction center, which is common amongst, but restricted to, the cyanobacteria (198) be classified as the His-type ferroxidase center.

Iron storage in Encapsulins

Encapsulins are large macromolecular assemblies, similar in structure to virus capsids. They are composed of proteins possessing the HK97 fold, a ubiquitous fold among proteins forming virus shells and other large compartments. (200). Two major classes of encapsulin cage architecture have been reported, distinguished by their triangulation number, T. The faces of the encapsulin are composed of regular hexagonal and pentagonal units, with curvature to create the enclosed 3D structure introduced by the latter. T defines the distance separating pentagonal units and therefore the size of the protein cage. Those with triangulation number T = 1 have the smallest possible enclosed volume and are composed of 60 identical subunits (201), whilst larger cages composed of 180 subunits possess a triangulation number T = 3 (202). Diameters range from 24 – 32 nm. Very recently, a new type of encapsulin was reported, from the bacterium *Quasibacillus thermotolerans*, which is larger still, with a diameter of 42 nm and novel T = 4 topology (203).

These large assemblies have the ability to encapsulate cargo proteins, which are targeted to the capsid by short C-terminal sequences (204). Among the cargo proteins of encapsulins are ferritin-like proteins. These encapsulated ferritins (EncFtn) are members of the ferritin superfamily that possess ferroxidase activity but do not themselves assemble to form cages (205). Whilst they are not as ubiquitous as their cage-forming counterparts, they have been identified in a wide range of bacterial and archaeal species from diverse environments (206). In all cases, these EncFtn proteins assemble into dimers; most assemble further to form annular pentamers of dimers (Figure 6B). As a result, all lack the intrinsic ability to solubilize mineral cores, requiring localization within encapsulin cages in order to do so (207,208). Due to their greater size, encapsulin complexes containing EncFtn are capable of storing at least 4 times (205,207) (and in the case of the *Q. thermotolerans* encapsulin, ~10 times) the amount of iron associated with the classical ferritins described above.

Most EncFtn proteins differ from the other members of the ferritin superfamily in that the protein monomer essentially consists of two antiparallel α-helices, with an additional shorter helix at the C-terminus. The classic 4 α-helical motif of the ferritins is achieved by the association of these subunits into dimers. The *Q. thermotolerans* EncFtn is distinct in that its subunit consists of a four α-helical bundle, which assembles into dimers.

The di-iron ferroxidase center has an approximate 2-fold symmetry axis (Figure 6C), with each of the two monomers contributing identical ligand sets (c.f. the case with the Dps proteins). In *Q. thermotolerans* EncFtn, each iron is coordinated by a bridging Glu, and two His residues. In most others, each monomer provides a bridging Glu such that there are two equivalent Glu residues bridging the metals. Each iron is also ligated by a His and a bidentate Glu, with the two additional ligands located on the same monomer. The hydroxyl of a Tyr residue is located 4.5 Å from each of the irons of the ferroxidase center in most structures but their significance is not known, as the mechanism of iron oxidation at EncFtn centers remains to be elucidated (206). Whilst these proteins have been demonstrated to support the catalytic oxidation of Fe^{2+} in the presence of O_2, and this has been shown to be inhibited by Zn^{2+}, it is not known whether O_2 or H_2O_2 is the preferred substrate of EncFtn.

Efflux of iron from the cell

Due to the fact that iron has long been viewed as a growth-limiting nutrient, mechanisms of iron export from bacterial cells are a relatively

under researched area. However, it is apparent that under certain circumstances simply downregulating iron acquisition may not be sufficient to ensure cellular survival. Chief among these is ROS assault, which arises from the close link between oxidative stress and elevated levels of intracellular iron mediated by the Fenton reaction. In some cases at least, countering this assault necessitates the active removal of iron from the cell, but the discovery of the efflux systems responsible is a relatively recent development (209). Consequently the understanding of these systems lacks the mechanistic detail available for the molecules of iron sensing, import and storage. However the main features of the four known classes of bacterial iron efflux systems are outlined below.

P-type ATPases are cytoplasmic membrane proteins that consist of a transmembrane domain containing 6-8 helices, an ATP binding domain and a soluble actuator domain. Examples with iron exporting activity belong to the P_{1B4} family and have been identified in *Bacillus subtilis* (PfeT) (210), *Listeria monocytogenes* (FrvA) (211), *M. tuberculosis* (CtpD) (212), the group A *Streptococci* (PmtA) (213,214) and *Sinorhizobium meliloti* (Nia) (215). Where the regulator of transcription has been identified, it is Fur and/or PerR, indicating the dual role in iron-mediated and peroxide stress response.

Cation diffusion facilitator (CDF) metal ion transporters are ubiquitous among prokaryotes and eukaryotes, with a wide range of cations transported. The proteins consist of 6 transmembrane helices with a histidine-rich loop interconnecting transmembrane helices 4 and 5. A soluble cytoplasmic domain is located at the C-terminus. Little is known about the factors influencing metal ion selectivity, but iron exporting activity has been reported for examples from *E. coli* (YiiP or FieF) (216), *P. aeruginosa* (AitP) (217) and *Shewonella oneidensis* (FeoE) (218). Unlike the P-type ATPase systems, the transcriptional regulators of their expression have yet to be identified.

Major facilitator superfamily proteins function in the transmembrane transport of cations, but the mechanism by which they achieve this is not well understood. They are made up of two domains, each consisting of 6 transmembrane helices. IceT of *Salmonella typhimurium* (219) is the only reported example with iron exporting activity, and is under the transcriptional control of the BaeSR system that regulates antibiotic resistance and efflux.

Membrane-bound ferritins do not form cages and are therefore are not *bone fide* ferritins (Figure 6A). However they contain a ferritin-like domain at the N-terminus that has ferroxidase activity (220). Located on the cytoplasmic side of the membrane, this domain is required for iron transport. The C-terminal domain is membrane spanning and has significant sequence homology to the vacuolar iron transporters such as VIT1 of *Arabidopsis thaliana*. Reported examples are found in the α-proteobacteria *Agrobacterium tumefaciens* (221) and *B. japonicum* (220) where they are thought to be important in oxidative stress response during the infection of plants. Annotated as MbfA, their transcription is under the control of Irr.

Concluding remarks

In this review we have attempted to provide an overview of the current understanding of iron detoxification by bacteria, as summarized in Figure 7. The mode of operation of the Fe^{2+} binding transcriptional regulators Fur and DtxR are now understood in molecular detail and a great many genes under their control have been identified. Work is now underway unravelling the complex interplay between these and other regulators involved in response to oxidative and nutritional stress, and a great deal of progress is being made in this area. Whilst no crystal structures are yet available for the iron responsive transcriptional regulators of the α–proteobacteria, Irr and RirA, the mechanism by which they use iron containing prosthetic groups to sense the concentration of the metal has been established, as has the molecular basis of their ability to also sense O_2. Also, an understanding of the interplay between these two regulators and the genes that they control is emerging. The common thread between all is a downregulation of iron acquisition pathways and upregulation of iron storage systems in response to elevated iron concentrations (Figure 7).

The greater number of ferritins encoded in bacterial genomes compared to those of animals possibly reflects the greater need for bacterial cells to respond to a variety of environmental stresses that are linked to iron, from iron deprivation to ROS- and RNS-induced oxidative stress. Reported growth inhibition of deletion mutants compared to wild type strains of various bacteria consistently

support the notion that ferritin minerals are viable stores of nutritionally available iron.

A recent study of *E. coli* revealed that exponentially growing cells contain a significant proportion of iron in the reduced state, with ferric mineral iron only accumulating in stationary phase (131). This fascinating result highlights the importance of precise physiological conditions in determining the extent to which the quota of iron within *E. coli* cells is oxidized to the ferric state. It suggests that the redox state of intracellular iron in bacterial cells is a more subtle balance of the oxidoreductase activity of ferritins and the reducing environment created by low molecular weight thiols than has previously been appreciated. These observations were rationalized in terms of an expansion of the 'respiratory shield' hypothesis originally proposed for mitochondria. In essence, diffusion of O_2 across either the mitochondrial, or in this instance, the cytoplasmic membrane is prevented by its consumption during respiration. Thus, the enzymes of the respiratory chain form a shield, creating a microaerobic environment in the interior matrix/cytoplasm that protects O_2-sensitive proteins and cofactors from damage during normal respiratory function. The static dissolved O_2 concentration inside mitochondria has been estimated at around 1 μM (222) and that in the cytoplasm of bacterial cells is assumed to be similar during exponential growth. This emphasizes an important difference between the environments in which the ferritins of bacteria and animals operate. Respiration in animal cells is restricted to mitochondria and ferritins located in the cytosol are therefore exposed to a significantly greater O_2 concentration than their bacterial counterparts for which peroxide would logically be expected to be an available co-substrate for iron oxidation.

A topical debate in the field of ferritin research is the existence or otherwise of a 'universal' mechanism of iron oxidation. This was proposed based on similarities between different ferritins in terms of their mineralized iron products, their iron binding stoichiometries, and common intermediates that are formed during Fe^{2+} oxidation/mineralization (223). The above considerations would argue for variation between bacterial and animal ferritins based on availability of potential substrates. Furthermore, the existence of multiple well described mechanisms, including the very recent discovery of extremely unusual iron-O_2 chemistry in the cyanobacterial ferritin *Syn*Ftn (190), which share only the broadest characteristics, provides ample evidence that such variation exists even within bacterial ferritins. Nature never fails to impress with the different ways in which it has found solutions to similar, if not identical, problems. The encapsulated ferritins provide the most recently discovered and a particularly striking example of the variety of solutions to the problems posed by iron.

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References

1. Beinert, H., Holm, R. H., and Munck, E. (1997) Iron-sulfur clusters: Nature's modular, multipurpose structures. *Science* **277**, 653-659
2. Gamba, I., Codola, Z., Lloret-Fillol, J., and Costas, M. (2017) Making and breaking of the O-O bond at iron complexes. *Coord. Chem. Rev.* **334**, 2-24
3. Berks, B. C., Ferguson, S. J., Moir, J. W. B., and Richardson, D. J. (1995) Enzymes and associated electron transport systems that catalyse the respiratory reduction of nitrogen oxides and oxyanions. *BBA-Bioenergetics* **1232**, 97-173
4. Kappler, U., and Maher, M. J. (2013) The bacterial SoxAX cytochromes. *Cell. Mol. Life Sci.* **70**, 977-992
5. Poulos, T. L. (2014) Heme enzyme structure and function. *Chem. Rev.* **114**, 3919-3962

6. Zhang, Y. F., Sen, S., and Giedroc, D. P. (2020) Iron acquisition by bacterial pathogens: Beyond Tris-catecholate complexes. *Chembiochem* **21**, 1955-1967
7. Touati, D. (2000) Iron and oxidative stress in bacteria. *Arch. Biochem. Biophys.* **373**, 1-6
8. Imlay, J. A. (2013) The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nat. Rev. Microbiol.* **11**, 443-454
9. Reniere, M. L. (2018) Reduce, induce, thrive: Bacterial redox sensing during pathogenesis. *J. Bacteriol.* **200**, e00128-18
10. Chandrangsu, P., Rensing, C., and Helmann, J. D. (2017) Metal homeostasis and resistance in bacteria. *Nat. Revs. Microbiol.* **15**, 338-350
11. Chandrangsu, P., Loi, V. V., Antelmann, H., and Helmann, J. D. (2018) The role of bacillithiol in Gram-positive firmicutes. *Antiox. Red. Signal.* **28**, 445-462
12. Hider, R. C., and Kong, X. L. (2011) Glutathione: a key component of the cytoplasmic labile iron pool. *Biometals* **24**, 1179-1187
13. Keyer, K., and Imlay, J. A. (1996) Superoxide accelerates DNA damage by elevating free-iron levels. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13635-13640
14. Jacques, J. F., Jang, S., Prevost, K., Desnoyers, G., Desmarais, M., Imlay, J., and Masse, E. (2006) RyhB small RNA modulates the free intracellular iron pool and is essential for normal growth during iron limitation in *Escherichia coli*. *Mol. Microbiol.* **62**, 1181-1190
15. Beauchene, N. A., Mettert, E. L., Moore, L. J., Keles, S., Willey, E. R., and Kiley, P. J. (2017) O_2 availability impacts iron homeostasis in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 12261-12266
16. Koh, E. I., Robinson, A. E., Bandara, N., Rogers, B. E., and Henderson, J. P. (2017) Copper import in *Escherichia coli* by the yersiniabactin metallophore system. *Nat. Chem. Biol.* **13**, 1016
17. Schaffer, S., Hantke, K., and Braun, V. (1985) Nucleotide-sequence of the iron reglulatory gene *fur*. *Mol. Gen. Gen.* **200**, 110-113
18. Baichoo, N., and Helmann, J. D. (2002) Recognition of DNA by Fur: a reinterpretation of the Fur box consensus sequence. *J. Bacteriol.* **184**, 5826-5832
19. Escolar, L., deLorenzo, V., and Perez Martin, J. (1997) Metalloregulation *in vitro* of the aerobactin promoter of *Escherichia coli* by the Fur (ferric uptake regulation) protein. *Mol. Microbiol.* **26**, 799-808
20. Pecqueur, L., D'Autreaux, B., Dupuy, J., Nicolet, Y., Jacquamet, L., Brutscher, B., Michaud-Soret, I., and Bersch, B. (2006) Structural changes of *Escherichia coli* ferric uptake regulator during metal-dependent dimerization and activation explored by NMR and x-ray crystallography. *J. Biol. Chem.* **281**, 21286-21295
21. Perard, J., Coves, J., Castellan, M., Solard, C., Savard, M., Miras, R., Galop, S., Signor, L., Crouzy, S., Michaud-Soret, I., and de Rosny, E. (2016) Quaternary structure of Fur proteins, a new subfamily of tetrameric proteins. *Biochemistry* **55**, 1503-1515
22. Pohl, E., Haller, J. C., Mijovilovich, A., Meyer-Klaucke, W., Garman, E., and Vasil, M. L. (2003) Architecture of a protein central to iron homeostasis: crystal structure and spectroscopic analysis of the ferric uptake regulator. *Mol. Microbiol.* **47**, 903-915
23. Fuangthong, M., and Helmann, J. D. (2003) Recognition of DNA by three ferric uptake regulator (Fur) homologs in *Bacillus subtilis*. *J. Bacteriol.* **185**, 6348-6357
24. Sarvan, S., Charih, F., Askoura, M., Butcher, J., Brunzelle, J. S., Stintzi, A., and Couture, J. F. (2018) Functional insights into the interplay between DNA interaction and metal coordination in ferric uptake regulators. *Sci. Rep.* **8**, 7410
25. Sarvan, S., Butcher, J., Stintzi, A., and Couture, J. F. (2018) Variation on a theme: investigating the structural repertoires used by ferric uptake regulators to control gene expression. *Biometals* **31**, 681-7044
26. Mills, S. A., and Marletta, M. A. (2005) Metal binding characteristics and role of iron oxidation in the ferric uptake regulator from *Escherichia coli*. *Biochemistry* **44**, 13553-13559

27. Deng, Z. Q., Wang, Q., Liu, Z., Zhang, M. F., Machado, A. C. D., Chiu, T. P., Feng, C., Zhang, Q., Yu, L., Qi, L., Zheng, J. G., Wang, X., Huo, X. M., Qi, X. X., Li, X. R., Wu, W., Rohs, R., Li, Y., and Chen, Z. Z. (2015) Mechanistic insights into metal ion activation and operator recognition by the ferric uptake regulator. *Nat. Commun.* **6**, 7642
28. Choi, J., and Ryu, S. (2019) Regulation of iron uptake by fine-tuning the iron responsiveness of the iron sensor Fur. *Appl. Environ. Microbiol.* **85**, e03026-18
29. Delany, I., Rappuoli, R., and Scarlato, V. (2004) Fur functions as an activator and as a repressor of putative virulence genes in *Neisseria meningitidis*. *Mol. Microbiol.* **52**, 1081-1090
30. Seo, S. W., Kim, D., Latif, H., O'Brien, E. J., Szubin, R., and Palsson, B. O. (2014) Deciphering Fur transcriptional regulatory network highlights its complex role beyond iron metabolism in *Escherichia coli*. *Nat. Commun.* **5**, 4910
31. Yu, C. X., and Genco, C. A. (2012) Fur-mediated activation of gene Transcription in the human pathogen *Neisseria gonorrhoeae*. *J. Bacteriol.* **194**, 1730-1742
32. Pinochet-Barros, A., and Helmann, J. D. (2020) *Bacillus subtilis* Fur is a transcriptional activator for the PerR-repressed *pfeT* gene, encoding an iron efflux pump. *J. Bacteriol.* **202**, e00697-19
33. McHugh, J. P., Rodriguez-Quinones, F., Abdul-Tehrani, H., Svistunenko, D. A., Poole, R. K., Cooper, C. E., and Andrews, S. C. (2003) Global iron-dependent gene regulation in *Escherichia coli* - A new mechanism for iron homeostasis. *J. Biol. Chem.* **278**, 29478-29486
34. Masse, E., and Gottesman, S. (2002) A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4620-4625
35. Nandal, A., Huggins, C. C. O., Woodhall, M. R., McHugh, J., Rodriguez-Quinones, F., Quail, M. A., Guest, J. R., and Andrews, S. C. (2010) Induction of the ferritin gene (*ftnA*) of *Escherichia coli* by Fe^{2+}-Fur is mediated by reversal of H-NS silencing and is RyhB independent. *Mol. Microbiol.* **75**, 637-657
36. Isabella, V., Wright, L. F., Barth, K., Spence, J. M., Grogan, S., Genco, C. A., and Clark, V. L. (2008) *cis*- and *trans*-acting elements involved in regulation of *norB* (*norZ*), the gene encoding nitric oxide reductase in *Neisseria gonorrhoeae*. *Microbiology* **154**, 226-239
37. Butcher, J., Sarvan, S., Brunzelle, J. S., Couture, J. F., and Stintzi, A. (2012) Structure and regulon of *Campylobacter jejuni* ferric uptake regulator Fur define apo-Fur regulation. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 10047-10052
38. Carpenter, B. M., Gilbreath, J. J., Pich, O. Q., McKelvey, A. M., Maynard, E. L., Li, Z. Z., and Merrell, D. S. (2013) Identification and characterization of novel *Helicobacter pylori* apo-Fur-regulated target genes. *J. Bacteriol.* **195**, 5526-5539
39. Davies, B. W., Bogard, R. W., and Mekalanos, J. J. (2011) Mapping the regulon of *Vibrio cholerae* ferric uptake regulator expands its known network of gene regulation. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 12467-12472
40. Embree, M., Qiu, Y., Shieu, W. D., Nagarajan, H., O'Neil, R., Lovley, D., and Zengler, K. (2014) The iron stimulon and Fur regulon of *Geobacter sulfurreducens* and their role in energy metabolism. *Appl. Environ. Microbiol.* **80**, 2918-2927
41. Neto, J. F. D., Lourenco, R. F., and Marques, M. V. (2013) Global transcriptional response of *Caulobacter crescentus* to iron availability. *BMC Genomics* **14**, 549
42. Diaz-Mireles, E., Wexler, M., Sawers, G., Bellini, D., Todd, J. D., and Johnston, A. W. B. (2004) The Fur-like protein Mur of *Rhizobium leguminosarum* is a Mn^{2+}-responsive transcriptional regulator. *Microbiology* **150**, 1447-1456
43. Fillat, M. F. (2014) The Fur (ferric uptake regulator) superfamily: Diversity and versatility of key transcriptional regulators. *Arch. Biochem. Biophys.* **546**, 41-52
44. Touati, D., Jacques, M., Tardat, B., Bouchard, L., and Despied, S. (1995) Lethal oxidative damage and mutagenesis are generated by iron in *Δfur* mutants of *Escherichia coli* - protective role of superoxide dismutate. *J. Bacteriol.* **177**, 2305-2314
45. Berges, M., Michel, A. M., Lassek, C., Nuss, A. M., Beckstette, M., Dersch, P., Riedel, K., Sievers, S., Becher, D., Otto, A., Maass, S., Rohde, M., Eckweiler, D., Borrero-de Acuna, J. M., Jahn, M.,

Neumann-Schaal, M., and Jahn, D. (2018) Iron regulation in *Clostridioides difficile*. *Front. Microbiol.* **9**, 3183

46. Pohl, E., Holmes, R. K., and Hol, W. G. J. (1999) Crystal structure of the iron-dependent regulator (IdeR) from *Mycobacterium tuberculosis* shows both metal binding sites fully occupied. *J. Mol. Biol.* **285**, 1145-1156
47. Pappenheimer, A. M. (1977) Diphtheria toxin. *Ann. Rev. Biochem.* **46**, 69-94
48. Boyd, J., Oza, M. N., and Murphy, J. R. (1990) Molecular cloning and DNA sequence analysis of a diphtheria tox iron-dependent regulatory element (DtxR) from *Corynebacterium diphtheriae*. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5968-5972
49. Schmitt, M. P., and Holmes, R. K. (1991) Iron-dependent regulation of diphtheria toxin and siderophore expression by the cloned *Corynebacterium diphtheriae* repressor gene *dtxR* in *C. diphtheriae* C7 strains. *Infect. Immun.* **59**, 1899-1904
50. Tao, X. and Murphy, J. R. (1994) Determination of the minimal essential nucleotide sequence for diphtheria tox repressor binding by in vitro affinity selection. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 9646-9650
51. White, A., Ding, X. C., vanderSpek, J. C., Murphy, J. R., and Ringe, D. (1998) Structure of the metal-ion-activated diphtheria toxin repressor tox operator complex. *Nature* **394**, 502-506
52. Pohl, E., Holmes, R. K., and Hol, W. G. J. (1999) Crystal structure of a cobalt-activated diphtheria toxin repressor-DNA complex reveals a metal-binding SH3-like domain. *J. Mol. Biol.* **292**, 653-667
53. Tao, X., Boyd, J., and Murphy, J. R. (1992) Specific binding of the diphtheria tox regulatory element DtxR to the tox operator requires divalent heavy metal ions and a 9-base pair interupted palindromic sequence. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5897-5901
54. Spiering, M. M., Ringer, D., Murphy, J. R., and Marletta, M. A. (2003) Metal stoichiometry and functional studies of the diphtheria toxin repressor. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 3808-3813
55. Ding, X., Zeng, H., Schiering, N., Ringe, D., and Murphy, J. R. (1996) Identification of the primary metal ion-activation sites of the diphtheria, tox repressor by X-ray crystallography and site-directed mutational analysis. *Nat. Struct. Biol.* **3**, 382-387
56. D'Aquino, J. A., Tetenbaum-Novatt, J., White, A., Berkovitch, F., and Ringe, D. (2005) Mechanism of metal ion activation of the diphtheria toxin repressor DtxR. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18408-18413
57. Posey, J. E., Hardham, J. M., Norris, S. J., and Gherardini, F. C. (1999) Characterization of a manganese-dependent regulatory protein, TroR, from *Treponema pallidum*. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 10887-10892
58. Do, H., Makthal, N., Chandrangsu, P., Olsen, R. J., Helmann, J. D., Musser, J. M., and Kumaraswami, M. (2019) Metal sensing and regulation of adaptive responses to manganese limitation by MtsR is critical for group A *streptococcus* virulence. *Nucl. Acids Res.* **47**, 7476-7493
59. Cheng, Y. Q., Yang, R. J., Lyu, M. Y., Wang, S. W., Liu, X. C., Wen, Y., Song, Y., Li, J. L., and Chen, Z. (2018) IdeR, a DtxR family iron response regulator, controls iron homeostasis, morphological differentiation, secondary metabolism, and the oxidative stress response in *Streptomyces avermitilis*. *Appl. Environ. Microbiol.* **84**, e01503-18
60. Chao, T. C., Becker, A., Buhrmester, J., Puhler, A., and Weidner, S. (2004) The *Sinorhizobium meliloti fur* gene regulates, with dependence on Mn(II), transcription of the *sitABCD* operon, encoding a metal-type transporter. *J. Bacteriol.* **186**, 3609-3620
61. Rudolph, G., Hennecke, H., and Fischer, H. M. (2006) Beyond the Fur paradigm: iron-controlled gene expression in rhizobia. *FEMS Microbiol. Revs.* **30**, 631-648
62. Rodionov, D. A., Gelfand, M. S., Todd, J. D., Curson, A. R. J., and Johnston, A. W. B. (2006) Computational reconstruction of iron- and manganese-responsive transcriptional networks in alpha-proteobacteria. *PLoS Comp. Biol.* **2**, 1568-1585
63. Hamza, I., Chauhan, S., Hassett, R., and O'Brian, M. R. (1998) The bacterial Irr protein is required for coordination of heme biosynthesis with iron availability. *J. Biol. Chem.* **273**, 21669-21674

64. Todd, J. D., Wexler, M., Sawers, G., Yeoman, K. H., Poole, P. S., and Johnston, A. W. B. (2002) RirA, an iron-responsive regulator in the symbiotic bacterium *Rhizobium leguminosarum*. *Microbiology* **148**, 4059-4071
65. Santos, J. A., Pereira, P. J. B., and Macedo-Ribeiro, S. (2015) What a difference a cluster makes: The multifaceted roles of IscR in gene regulation and DNA recognition. *Biochim. Biophys. Acta* **1854**, 1101-1112
66. Pellicer Martinez, M. T., Crack, J. C., Stewart, M. Y. Y., Bradley, J. M., Svistunenko, D. A., Johnston, A. W. B., Cheesman, M. R., Todd, J. D., and Le Brun, N. E. (2019) Mechanisms of iron- and O_2-sensing by the [4Fe-4S] cluster of the global iron regulator RirA. *Elife* **8**, e47804
67. Todd, J. D., Sawers, G., Rodionov, D. A., and Johnston, A. W. B. (2006) The *Rhizobium leguminosarum* regulator IrrA affects the transcription of a wide range of genes in response to Fe availability. *Mol. Genet. Genom.* **275**, 564-577
68. Yeoman, K. H., Curson, A. R. J., Todd, J. D., Sawers, G., and Johnston, A. W. B. (2004) Evidence that the *Rhizobium* regulatory protein RirA binds to cis-acting iron-responsive operators (IROs) at promoters of some Fe-regulated genes. *Microbiology* **150**, 4065-4074
69. Pellicer Martinez, M. T. P., Martinez, A. B., Crack, J. C., Holmes, J. D., Svistunenko, D. A., Johnston, A. W. B., Cheesman, M. R., Todd, J. D., and Le Brun, N. E. (2017) Sensing iron availability via the fragile [4Fe-4S] cluster of the bacterial transcriptional repressor RirA. *Chem. Sci.* **8**, 8451-8463
70. Chao, T. C., Buhrmester, J., Hansmeier, N., Puhler, A., and Weidner, S. (2005) Role of the regulatory gene *rirA* in the transcriptional response of *Sinorhizobium meliloti* to iron limitation. *Appl. Environ. Microbiol.* **71**, 5969-5982
71. Todd, J. D., Sawers, G., and Johnston, A. W. B. (2005) Proteomic analysis reveals the wide-ranging effects of the novel, iron-responsive regulator RirA in *Rhizobium leguminosarum* bv. *viciae*. *Mol. Gen. Genom.* **273**, 197-206
72. Costa, D., Amarelle, V., Valverde, C., O'Brian, M. R., and Fabianoa, E. (2017) The Irr and RirA proteins participate in a complex regulatory circuit and act in concert to modulate bacterioferritin expression in *Ensifer meliloti* 1021. *Appl. Environ. Microbiol.* **83**, e00895-17
73. O'Brian, M. R. (2015) Perception and homeostanc control of iron in the *Rhizobia* and related Bacteria. in *Ann. Rev. Microbiol., Vol 69* (Gottesman, S. ed.). pp 229-245
74. Qi, Z. H., and O'Brian, M. R. (2002) Interaction between the bacterial iron response regulator and ferrochelatase mediates genetic control of heme biosynthesis. *Mol. Cell* **9**, 155-162
75. Rudolph, G., Semini, G., Hauser, F., Lindemann, A., Friberg, M., Hennecke, H., and Fischer, H. M. (2006) The iron control element, acting in positive and negative control of iron-regulated *Bradyrhizobium japonicum* genes, is a target for the Irr protein. *J. Bacteriol.* **188**, 733-744
76. Qi, Z. H., Hamza, I., and O'Brian, M. R. (1999) Heme is an effector molecule for iron-dependent degradation of the bacterial iron response regulator (Irr) protein. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 13056-13061
77. Singleton, C., White, G. F., Todd, J. D., Marritt, S. J., Cheesman, M. R., Johnston, A. W. B., and Le Brun, N. E. (2010) Heme-responsive DNA binding by the global iron regulator Irr from *Rhizobium leguminosarum*. *J. Biol. Chem.* **285**, 16023-16031
78. Bhubhanil, S., Ruangkiattikul, N., Niamyim, P., Chamsing, J., Ngok-ngam, P., Sukchawalit, R., and Mongkolsuk, S. (2012) Identification of amino acid residues important for the function of *Agrobacterium tumefaciens* Irr protein. *FEMS Microbiol. Lett.* **35**, 68-77
79. White, G. F., Singleton, C., Todd, J. D., Cheesman, M. R., Johnston, A. W. B., and Le Brun, N. E. (2011) Heme binding to the second, lower-affinity site of the global iron regulator Irr from *Rhizobium leguminosarum* promotes oligomerization. *FEBS J.* **278**, 2011-2021
80. Yang, J. H., Ishimori, K., and O'Brian, M. R. (2005) Two heme binding sites are involved in the regulated degradation of the bacterial iron response regulator (Irr) protein. *J. Biol. Chem.* **280**, 7671-7676

81. Kobayashi, K., Nakagaki, M., Ishikawa, H., Iwai, K., O'Brian, M. R., and Ishimori, K. (2016) Redox-dependent dynamics in heme-bound bacterial iron response regulator (Irr) protein. *Biochemistry* **55**, 4047-4054
82. Ratledge, C., and Dover, L. G. (2000) Iron metabolism in pathogenic bacteria. *Ann. Rev. Microbiol.* **54**, 881-941
83. Ellermann, M., and Arthur, J. C. (2017) Siderophore-mediated iron acquisition and modulation of host-bacterial interactions. *Free Radical Bio. Med.* **105**, 68-78
84. Andrews, S. C., Robinson, A. K., and Rodriguez-Quinones, F. (2003) Bacterial iron homeostasis. *FEMS Microbiol. Revs.* **27**, 215-237
85. Miethke, M. (2013) Molecular strategies of microbial iron assimilation: from high-affinity complexes to cofactor assembly systems. *Metallomics* **5**, 15-28
86. Huang, W. L., and Wilks, A. (2017) Extracellular Heme Uptake and the Challenge of Bacterial Cell Membranes. in *Ann. Rev. Biochem., Vol 86* (Kornberg, R. D. ed.). pp 799-823
87. Sheldon, J. R., and Heinrichs, D. E. (2015) Recent developments in understanding the iron acquisition strategies of gram positive pathogens. *FEMS Microbiol. Rev.* **39**, 592-630
88. Lau, C. K. Y., Krewulak, K. D., and Vogel, H. J. (2016) Bacterial ferrous iron transport: the Feo system. *FEMS Microbiol. Rev.* **40**, 273-298
89. Hider, R. C., and Kong, X. L. (2010) Chemistry and biology of siderophores. *Nat. Prod. Rep.* **27**, 637-657
90. Schalk, I. J., Mislin, G. L. A., and Brillet, K. (2012) Structure, function and binding selectivity and stereoselectivity of siderophore-iron outer membrane transporters in *Curr. Top. Membr.* (Lutsenko, S., and Arguello, J. M. eds.). pp 37-66
91. Furrer, J. L., Sanders, D. N., Hook-Barnard, I. G., and McIntosh, M. A. (2002) Export of the siderophore enterobactin in *Escherichia coli*: involvement of a 43 kDa membrane exporter. *Mol. Microbiol.* **44**, 1225-1234
92. Imperi, F., Tiburzi, F., and Visca, P. (2009) Molecular basis of pyoverdine siderophore recycling in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U.S.A* **106**, 20440-20445
93. Wilson, B. R., Bogdan, A. R., Miyazawa, M., Hashimoto, K., and Tsuji, Y. (2016) Siderophores in iron metabolism: From mechanism to therapy potential. *Trends Mol. Med.* **22**, 1077-1090
94. McRose, D. L., Seyedsayamdost, M. R., and Morel, F. M. M. (2018) Multiple siderophores: bug or feature? *J. Biol. Inorg. Chem.* **23**, 983-993
95. Schalk, I. J., and Guillon, L. (2013) Fate of ferrisiderophores after import across bacterial outer membranes: different iron release strategies are observed in the cytoplasm or periplasm depending on the siderophore pathways. *Amino Acids* **44**, 1267-1277
96. Greenwald, J., Hoegy, F., Nader, M., Journet, L., Mislin, G. L. A., Graumann, P. L., and Schalk, I. J. (2007) Real time fluorescent resonance energy transfer visualization of ferric pyoverdine uptake in *Pseudomonas aeruginosa* - A role for ferrous iron. *J. Biol. Chem.* **282**, 2987-2995
97. Smith, A. D., and Wilks, A. (2012) Extracellular heme uptake and the challenges of bacterial cell membranes in *Curr.Top. Membr.* (Lutsenko, S., and Arguello, J. M. eds.). pp 359-392
98. Braun, V., and Hantke, K. (2011) Recent insights into iron import by bacteria. *Curr. Opin. Chem. Biol.* **15**, 328-334
99. Letoffe, S., Deniau, C., Wolff, N., Dassa, E., Delepelaire, P., Lecroisey, A., and Wandersman, C. (2001) Haemophore-mediated bacterial haem transport: evidence for a common or overlapping site for haem-free and haem-loaded haemophore on its specific outer membrane receptor. *Mol. Microbiol.* **41**, 439-450
100. Deniau, C., Gilli, R., Izadi-Pruneyre, N., Letoffe, S., Delepierre, M., Wandersman, C., Briand, C., and Lecroisey, A. (2003) Thermodynamics of heme binding to the HasA(SM) hemophore: Effect of mutations at three key residues for heme uptake. *Biochemistry* **42**, 10627-10633
101. Krieg, S., Huche, F., Diederichs, K., Izadi-Pruneyre, N., Lecroisey, A., Wandersman, C., Delepelaire, P., and Welte, W. (2009) Heme uptake across the outer membrane as revealed by crystal structures of the receptor-hemophore complex. *Proc. Natl. Acad. Sci. U.S.A* **106**, 1045-1050

102. Smith, A. D., and Wilks, A. (2015) Differential contributions of the outer membrane receptors PhuR and HasR to heme acquisition in *Pseudomonas aeruginosa*. *J. Biol. Chem.* **290**, 7756-7766
103. Smith, A. D., Modi, A. R., Sun, S. F., Dawson, J. H., and Wilks, A. (2015) Spectroscopic determination of distinct heme ligands in outer-membrane receptors PhuR and HasR of *Pseudomonas aeruginosa*. *Biochemistry* **54**, 2601-2612
104. Wilks, A., and Ikeda-Saito, M. (2014) Heme Utilization by pathogenic bacteria: Not all pathways lead to biliverdin. *Acc. Chem. Res.* **47**, 2291-2298
105. Llamas, M. A., Imperi, F., Visca, P., and Lamont, I. L. (2014) Cell-surface signaling in *Pseudomonas*: stress responses, iron transport, and pathogenicity. *FEMS Microbiol. Rev.* **38**, 569-597
106. Sestok, A. E., Linkous, R. O., and Smith, A. T. (2018) Toward a mechanistic understanding of Feo-mediated ferrous iron uptake. *Metallomics* **10**, 887-898
107. Makui, H., Roig, E., Cole, S. T., Helmann, J. D., Gros, P., and Cellier, M. F. M. (2000) Identification of the *Escherichia coli* K-12 Nramp orthologue (MntH) as a selective divalent metal ion transporter. *Mol. Microbiol.* **35**, 1065-1078
108. Grass, G., Franke, S., Taudte, N., Nies, D. H., Kucharski, L. M., Maguire, M. E., and Rensing, C. (2005) The metal permease ZupT from *Escherichia coli* is a transporter with a broad substrate spectrum. *J. Bacteriol.* **187**, 1604-1611
109. Perry, R. D., Mier, I., and Fetherston, J. D. (2007) Roles of the Yfe and Feo transporters of *Yersinia pestis* in iron uptake and intracellular growth. *Biometals* **20**, 699-703
110. Katoh, H., Hagino, N., and Ogawa, T. (2001) Iron-binding activity of FutA1 subunit of an ABC-type iron transporter in the cyanobacterium *Synechocystis* sp strain PCC 6803. *Plant Cell Physiol.* **42**, 823-827
111. Cao, J., Woodhall, M. R., Alvarez, J., Cartron, M. L., and Andrews, S. C. (2007) EfeUOB (YcdNOB) is a tripartite, acid-induced and CpxAR-regulated, low-pH Fe2+ transporter that is cryptic in Escherichia coli K-12 but functional in E-coli O157 : H7. *Mol. Microbiol.* **65**, 857-875
112. Kammler, M., Schon, C., and Hantke, K. (1993) Characterization of the ferrous iron uptake system of Escherichia-coli. *J. Bacteriol.* **175**, 6212-6219
113. Marlovits, T. C., Haase, W., Herrmann, C., Aller, S. G., and Unger, V. M. (2002) The membrane protein FeoB contains an intramolecular G protein essential for Fe(II) uptake in bacteria. *Proc. Natl. Acad. Sci. U.S.A* **99**, 16243-16248
114. Seyedmohammad, S., Born, D., and Venter, H. (2014) Expression, purification and functional reconstitution of FeoB, the ferrous iron transporter from *Pseudomonas aeruginosa*. *Protein Expres. Purif.* **101**, 138-145
115. Smith, A. T., and Sestok, A. E. (2018) Expression and purification of functionally active ferrous iron transporter FeoB from *Klebsiella pneumoniae*. *Protein Expres. Purif/* **142**, 1-7
116. Ash, M. R., Guilfoyle, A., Clarke, R. J., Guss, M., Maher, M. J., and Jormakka, M. (2010) Potassium-activated GTPase reaction in the G protein-coupled ferrous iron transporter B. *J. Biol. Chem.* **285**, 14594-14602
117. Eng, E. T., Jalilian, A. R., Spasov, K. A., and Unger, V. M. (2008) Characterization of a novel prokaryotic GDP dissociation inhibitor domain from the G protein coupled membrane protein FeoB. *J. Mol. Biol.* **375**, 1086-1097
118. Cartron, M. L., Maddocks, S., Gillingham, P., Craven, C. J., and Andrews, S. C. (2006) Feo - Transport of ferrous iron into bacteria. *Biometals* **19**, 143-157
119. Su, Y. C., Chin, K. H., Hung, H. C., Shen, G. H., Wang, A. H. J., and Chou, S. H. (2010) Structure of *Stenotrophomonas maltophilia* FeoA complexed with zinc: a unique prokaryotic SH3-domain protein that possibly acts as a bacterial ferrous iron-transport activating factor. *Acta Crystallogr. F* **66**, 636-642
120. Hung, K. W., Juan, T. H., Hsu, Y. L., and Huang, T. H. (2012) NMR structure note: the ferrous iron transport protein C (FeoC) from *Klebsiella pneumoniae*. *J. Biomol. NMR* **53**, 161-165

121. Hung, K. W., Tsai, J. Y., Juan, T. H., Hsu, Y. L., Hsiao, C. D., and Huang, T. H. (2012) Crystal structure of the *Klebsiella pneumoniae* NFeoB/FeoC complex and roles of FeoC in regulation of Fe^{2+} transport by the bacterial Feo system. *J. Bacteriol.* **194**, 6518-6526
122. Maddocks, S. E., and Oyston, P. C. F. (2008) Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. *Microbiology* **154**, 3609-3623
123. Skaar, E. P., Gaspar, A. H., and Schneewind, O. (2004) IsdG and IsdI, heme-degrading enzymes in the cytoplasm of *Staphylococcus aureus*. *J. Biol. Chem.* **279**, 436-443
124. Skaar, E. P., Gaspar, A. H., and Schneewind, O. (2006) *Bacillus anthracis* IsdG, a heme-degrading monooxygenase. *J. Bacteriol.* **188**, 1071-1080
125. Mazmanian, S. K., Skaar, E. P., Gaspar, A. H., Humayun, M., Gornicki, P., Jelenska, J., Joachmiak, A., Missiakas, D. M., and Schneewind, O. (2003) Passage of heme-iron across the envelope of *Staphylococcus aureus*. *Science* **299**, 906-909
126. Marraffini, L. A., Ton-That, H., Zong, Y. N., Narayana, S. V. L., and Schneewind, O. (2004) Anchoring of surface proteins to the cell wall of *Staphylococcus aureus* - A conserved arginine residue is required for efficient catalysis of sortase A. *J. Biol. Chem.* **279**, 37763-37770
127. Pilpa, R. M., Robson, S. A., Villareal, V. A., Wong, M. L., Phillips, M., and Clubb, R. T. (2009) Functionally distinct NEAT (NEAr Transporter) domains within the *Staphylococcus aureus* IsdH/HarA protein extract heme from methemoglobin. *J. Biol. Chem.* **284**, 1166-1176
128. Muryoi, N., Tiedemann, M. T., Pluym, M., Cheung, J., Heinrichs, D. E., and Stillman, M. J. (2008) Demonstration of the iron-regulated surface determinant (Isd) heme transfer pathway in *Staphylococcus aureus*. *J. Biol. Chem.* **283**, 28125-28136
129. Moriwaki, Y., Terada, T., Caaveiro, J. M. M., Takaoka, Y., Hamachi, I., Tsumoto, K., and Shimizu, K. (2013) Heme binding mechanism of structurally similar iron-regulated surface determinant near transporter domains of *Staphylococcus aureus* exhibiting different affinities for heme. *Biochemistry* **52**, 8866-8877
130. Williams, R. J. P. (1982) Free manganese(II) andiron(II) cations can act as intracellular cell controls. *FEBS Lett.* **140**, 3-10
131. Wofford, J. D., Bolaji, N., Dziuba, N., Outten, F. W., and Lindahl, P. A. (2019) Evidence that a respiratory shield in *Escherichia coli* protects a low-molecular-mass Fe^{2+} pool from O_2-dependent oxidation. *J. Biol. Chem.* **294**, 50-62
132. Beaufay, F., Quarles, E., Franz, A., Katamanin, O., Wholey, W.-Y., and Jakob, U. (2020) Polyphosphate functions *in vivo* as an iron chelator and Fenton reaction inhibitor. *mBio* **11**, e01017-01020
133. Hristova, D., Wu, C. H., Jiang, W., Krebs, C., and Stubbe, J. (2008) Importance of the maintenance pathway in the regulation of the activity of *Escherichia coli* ribonucleotide reductase. *Biochemistry* **47**, 3989-3999
134. Bradley, J. M., Moore, G. R., and Le Brun, N. E. (2017) Diversity of Fe^{2+} entry and oxidation in ferritins. *Curr. Opin. Chem. Biol.* **37**, 122-128
135. Theil, E. C., Behera, R. K., and Tosha, T. (2013) Ferritins for chemistry and for life. *Coord. Chem. Revs.* **257**, 579-586
136. Andrews, S. C. (2010) The Ferritin-like superfamily: Evolution of the biological iron storeman from a rubrerythrin-like ancestor. *Biochim. Biophys. Acta* **1800**, 691-705
137. Zeth, K., Offermann, S., Essen, L. O., and Oesterhelt, D. (2004) Iron-oxo clusters biomineralizing on protein surfaces: Structural analysis of *Halobacterium salinarum* DpsA in its low- and high-iron states. *Proc.Natl. Acad. Sci. U.S.A.* **101**, 13780-13785
138. Stillman, T. J., Hempstead, P. D., Artymiuk, P. J., Andrews, S. C., Hudson, A. J., Treffry, A., Guest, J. R., and Harrison, P. M. (2001) The high-resolution X-ray crystallographic structure of the ferritin (EcFtnA) of *Escherichia coli*; Comparison with human H ferritin (HuHF) and the structures of the Fe^{3+} and Zn^{2+} derivatives. *J. Mol. Biol.* **307**, 587-603

139. Ohtomo, H., Ohtomo, M., Sato, D., Kurobe, A., Sunato, A., Matsumura, Y., Kihara, H., Fujiwara, K., and Ikeguchi, M. (2015) A physicochemical and mutational analysis of intersubunit interactions of *Escherichia coli* ferritin A. *Biochemistry* **54**, 6243-6251
140. Theil, E. C. (2011) Ferritin protein nanocages use ion channels, catalytic sites, and nucleation channels to manage iron/oxygen chemistry. *Curr. Opin. Chem. Biol.* **15**, 304-311
141. Bradley, J. M., Pullin, J., Moore, G. R., Svistunenko, D. A., Hemmings, A. M., and Le Brun, N. E. (2020) Routes of iron entry into, and exit from, the catalytic ferroxidase sites of the prokaryotic ferritin SynFtn. *Dalton Trans.* **49**, 1545-1554
142. Wong, S. G., Grigg, J. C., Le Brun, N. E., Moore, G. R., Murphy, M. E. P., and Mauk, A. G. (2015) The B-type channel is a major route for iron entry into the ferroxidase center and central cavity of bacterioferritin. *J. Biol. Chem.* **290**, 3732-3739
143. Ebrahimi, K. H., Hagedoorn, P. L., and Hagen, W. R. (2015) Unity in the biochemistry of the iron-storage proteins ferritin and bacterioferritin. *Chem. Revs.* **115**, 295-326
144. Williams, S. M., and Chatterji, D. (2017) Flexible aspartates propel iron to the ferroxidation sites along pathways stabilized by a conserved arginine in Dps proteins from *Mycobacterium smegmatis*. *Metallomics* **9**, 685-698
145. Behera, R. K., and Theil, E. C. (2014) Moving Fe^{2+} from ferritin ion channels to catalytic OH centers depends on conserved protein cage carboxylates. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 7925-7930
146. Masuda, T., Goto, F., Yoshihara, T., and Mikami, B. (2010) The universal mechanism for iron translocation to the ferroxidase site in ferritin, which is mediated by the well conserved transit site. *Biochem. Biophys. Res. Commun.* **400**, 94-99
147. Bradley, J. M., Moore, G. R., and Le Brun, N. E. (2014) Mechanisms of iron mineralization in ferritins: one size does not fit all. *J. Biol. Inorg. Chem.* **19**, 775-785
148. Haikarainen, T., and Papageorgiou, A. C. (2010) Dps-like proteins: structural and functional insights into a versatile protein family. *Cell. Mol. Life Sci.* **67**, 341-351
149. Pourciau, C., Pannuri, A., Potts, A., Yakhnin, H., Babitzke, P., and Romeo, T. (2019) Regulation of iron storage by CsrA supports exponential growth of *Escherichia coli*. *Mbio* **10**
150. Laulhere, J. P., Laboure, A. M., Vanwuytswinkel, O., Gagnon, J., and Briat, J. F. (1992) Purification, characterization and function of bacterioferritin from the cyanobacterium Synechocystis PCC-6803. *Biochem.* **281**, 785-793
151. Shcolnick, S., Summerfield, T. C., Reytman, L., Sherman, L. A., and Keren, N. (2009) The Mechanism of iron homeostasis in the unicellular cyanobacterium *Synechocystis* sp PCC 6803 and its relationship to oxidative stress. *Plant Physiol.* **150**, 2045-2056
152. Treffry, A., Zhao, Z. W., Quail, M. A., Guest, J. R., and Harrison, P. M. (1998) How the presence of three iron binding sites affects the iron storage function of the ferritin (EcFtnA) of *Escherichia coli*. *FEBS Lett.* **432**, 213-218
153. Bou-Abdallah, F., Yang, H., Awomolo, A., Cooper, B., Woodhall, M. R., Andrews, S. C., and Chasteen, N. D. (2014) Functionality of the three-site ferroxidase center of *Escherichia coli* bacterial ferritin (EcFtnA). *Biochemistry* **53**, 483-495
154. Ebrahimi, K. H., Hagedoorn, P. L., and Hagen, W. R. (2013) A conserved tyrosine in ferritin is a molecular capacitor. *Chembiochem* **14**, 1123-1133
155. Mohanty, A., Subhadarshanee, B., Barman, P., Mahapatra, C., Aishwarya, B., and Behera, R. K. (2019) Iron mineralizing bacterioferritin A from *Mycobacterium tuberculosis* exhibits unique catalase-Dps-like dual activities. *Inorg. Chem.* **58**, 4741-4752
156. Levi, S., Luzzago, A., Cesareni, G., Cozzi, A., Franceschinelli, F., Albertini, A., and Arosio, P. (1988) Mechanism of ferritin iron uptake - activity of the H-chain and deletion mapping of the ferro-oxidase site - a study of iron uptake and ferro-oxidase activity of human-liver, recombinant H-chain ferritins, and of 2 H-chain deletion mutants. *J. Biol. Chem.* **263**, 18086-18092

157. Ingrassia, R., Gerardi, G., Biasiotto, G., and Arosio, P. (2006) Mutations of ferritin H chain C-terminus produced by nucleotide insertions have altered stability and functional properties. *J. Biochem.* **139**, 881-885
158. Andrews, S. C., Le Brun, N. E., Barynin, V., Thomson, A. J., Moore, G. R., Guest, J. R., and Harrison, P. M. (1995) Site-directed replacement of the coaxial heme ligands of bacterioferritin generates heme-free variants. *J. Biol. Chem.* **270**, 23268-23274
159. Yasmin, S., Andrews, S. C., Moore, G. R., and Le Brun, N. E. (2011) A new role for heme, facilitating release of iron from the bacterioferritin iron biomineral. *J. Biol. Chem.* **286**, 3473-3483
160. Yao, H. L., Wang, Y., Lovell, S., Kumar, R., Ruvinsky, A. M., Battaile, K. P., Vakser, I. A., and Rivera, M. (2012) The structure of the BfrB-Bfd complex reveals protein-protein interactions enabling iron release from bacterioferritin. *J. Am. Chem. Soc.* **134**, 13470-13481
161. Crow, A., Lawson, T. L., Lewin, A., Moore, G. R., and Le Brun, N. E. (2009) Structural basis for iron mineralization by bacterioferritin. *J. Am. Chem. Soc.* **131**, 6808-6813
162. Kwak, Y., Schwartz, J. K., Huang, V. W., Boice, E., Kurtz, D. M., and Solomon, E. I. (2015) CD/MCD/VTVH-MCD studies of *Escherichia coli* bacterioferritin support a binuclear iron cofactor site. *Biochemistry* **54**, 7010-7018
163. Baaghil, S., Lewin, A., Moore, G. R., and Le Brun, N. E. (2003) Core formation in *Escherichia coli* bacterioferritin requires a functional. *Biochemistry* **42**, 14047-14056
164. Bradley, J. M., Svistunenko, D. A., Lawson, T. L., Hemmings, A. M., Moore, G. R., and Le Brun, N. E. (2015) Three aromatic residues are required for electron transfer during iron mineralization in bacterioferritin. *Angew. Chem. Int. Ed.* **54**, 14763-14767
165. Bradley, J. M., Svistunenko, D. A., Moore, G. R., and Le Brun, N. E. (2017) Tyr25, Tyr58 and Trp133 of *Escherichia coli* bacterioferritin transfer electrons between iron in the central cavity and the ferroxidase centre. *Metallomics* **9**, 1421-1428
166. Bou-Abdallah, F., Lewin, A. C., Le Brun, N. E., Moore, G. R., and Chasteen, N. D. (2002) Iron detoxification properties of *Escherichia coli* bacterioferritin - Attenuation of oxyradical chemistry. *J. Biol. Chem.* **277**, 37064-37069
167. Weeratunga, S. K., Lovell, S., Yao, H. L., Battaile, K. P., Fischer, C. J., Gee, C. E., and Rivera, M. (2010) Structural studies of bacterioferritin B from *Pseudomonas aeruginosa* suggest a gating mechanism for iron uptake via the ferroxidase center. *Biochemistry* **49**, 1160-1175
168. Abdul-Tehrani, H., Hudson, A. J., Chang, Y. S., Timms, A. R., Hawkins, C., Williams, J. M., Harrison, P. M., Guest, J. R., and Andrews, S. C. (1999) Ferritin mutants of *Escherichia coli* are iron deficient and growth impaired, and *fur* mutants are iron deficient. *J. Bacteriol.* **181**, 1415-1428
169. Eshelman, K., Yao, H. L., Hewage, A., Deay, J. J., Chandler, J. R., and Rivera, M. (2017) Inhibiting the BfrB: Bfd interaction in *Pseudomonas aeruginosa* causes irreversible iron accumulation in bacterioferritin and iron deficiency in the bacterial cytosol. *Metallomics* **9**, 646-659
170. Velayudhan, J., Castor, M., Richardson, A., Main-Hester, K. L., and Fang, F. C. (2007) The role of ferritins in the physiology of *Salmonella enterica* sv. *Typhimurium*: a unique role for ferritin B in iron-sulphur cluster repair and virulence. *Mol. Microbiol.* **63**, 1495-1507
171. He, X., Jiang, H. W., Chen, H., Zhang, H. N., Liu, Y., Xu, Z. W., Wu, F. L., Guo, S. J., Hou, J. L., Yang, M. K., Yan, W., Deng, J. Y., Bi, L. J., Zhang, X. E., and Tao, S. C. (2017) Systematic identification of *Mycobacterium tuberculosis* effectors reveals that BfrB suppresses innate immunity. *Mol. Cell. Prot.* **16**, 2243-2253
172. Khare, G., Nangpal, P., and Tyagi, A. K. (2017) Differential roles of iron storage proteins in maintaining the iron homeostasis in *Mycobacterium tuberculosis. PLoS One* **12**
173. Figueiredo, M. C. O., Lobo, S. A. L., Carita, J. N., Nobre, L. S., and Saraiva, L. M. (2012) Bacterioferritin protects the anaerobe *Desulfovibrio vulgaris* Hildenborough against oxygen. *Anaerobe* **18**, 454-458
174. Altuvia, S., Almiron, M., Huisman, G., Kolter, R., and Storz, G. (1994) The Dps promoter is activated by OxyR during growth and by IHF and a σS in stationary phase. *Mol. Microbiol.* **13**, 265-272

175. Almiron, M., Link, A. J., Furlong, D., and Kolter, R. (1992) A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. *Genes Develop.* **6**, 2646-2654
176. Azam, T. A., and Ishihama, A. (1999) Twelve species of the nucleoid-associated protein from *Escherichia coli* - Sequence recognition specificity and DNA binding affinity. *J. Biol. Chem.* **274**, 33105-33113
177. Frenkiel-Krispin, D., Ben-Avraham, I., Englander, J., Shimoni, E., Wolf, S. G., and Minsky, A. (2004) Nucleoid restructuring in stationary-state bacteria. *Mol. Microbiol.* **51**, 395-405
178. Wolf, S. G., Frenkiel, D., Arad, T., Finkel, S. E., Kolter, R., and Minsky, A. (1999) DNA protection by stress-induced biocrystallization. *Nature* **400**, 83-85
179. Grant, R. A., Filman, D. J., Finkel, S. E., Kolter, R., and Hogle, J. M. (1998) The crystal structure of Dps, a ferritin homolog that binds and protects DNA. *Nat. Struct. Biol.* **5**, 294-303
180. Ceci, P., Cellai, S., Falvo, E., Rivetti, C., Rossi, G. L., and Chiancone, E. (2004) DNA condensation and self-aggregation of *Escherichia coli* Dps are coupled phenomena related to the properties of the N-terminus. *Nucl. Acids Res.* **32**, 5935-5944
181. Karas, V. O., Westerlaken, I., and Meyer, A. S. (2015) The DNA-binding protein from starved cells (Dps) utilizes dual functions to defend cells against multiple stresses. *J. Bacteriol.* **197**, 3206-3215
182. Tsou, C. C., Chiang-Ni, C., Lin, Y. S., Chuang, W. J., Lin, M. T., Liu, C. C., and Wu, J. J. (2010) Oxidative stress and metal ions regulate a ferritin-like gene, dpr, in *Streptococcus pyogenes*. *Int. J. Med. Microbiol.* **300**, 259-264
183. Ulijasz, A. T., Andes, D. R., Glasner, J. D., and Weisblum, B. (2004) Regulation of iron transport in *Streptococcus pneumoniae* by RitR, an orphan response regulator. *J. Bacteriol.* **186**, 8123-8136
184. Ilari, A., Stefanini, S., Chiancone, E., and Tsernoglou, D. (2000) The dodecameric ferritin from *Listeria innocua* contains a novel intersubunit iron-binding site. *Nat. Struct. Biol.* **7**, 38-43
185. Kauko, A., Pulliainen, A. T., Haataja, S., Meyer-Klaucke, W., Finne, J., and Papageorgiou, A. C. (2006) Iron incorporation in *Streptococcus suis* Dps-like peroxide resistance protein Dpr requires mobility in the ferroxidase center and leads to the formation of a ferrihydrite-like core. *J. Mol. Biol.* **364**, 97-109
186. Roy, S., Gupta, S., Das, S., Sekar, K., Chatterji, D., and Vijayan, M. (2004) X-ray analysis of *Mycobacterium smegmatis* Dps and a comparative study involving other Dps and Dps-like molecules. *J. Mol. Biol.* **339**, 1103-1113
187. Ren, B., Tibbelin, G., Kajino, T., Asami, O., and Ladenstein, R. (2003) The multi-layered structure of Dps with a novel di-nuclear ferroxidase center. *J. Mol. Biol.* **329**, 467-477
188. Su, M. H., Cavallo, S., Stefanini, S., Chiancone, E., and Chasteen, N. D. (2005) The so-called *Listeria innocua* ferritin is a Dps protein. Iron incorporation, detoxification, and DNA protection properties. *Biochemistry* **44**, 5572-5578
189. Zhao, G. H., Ceci, P., Ilari, A., Giangiacomo, L., Laue, T. M., Chiancone, E., and Chasteen, N. D. (2002) Iron and hydrogen peroxide detoxification properties of DNA-binding protein from starved cells - A ferritin-like DNA-binding protein of *Escherichia coli*. *J. Biol. Chem.* **277**, 27689-27696
190. Bradley, J. M., Svistunenko, D. A., Pullin, J., Hill, N., Stuart, R. K., Palenik, B., Wilson, M. T., Hemmings, A. M., Moore, G. R., and Le Brun, N. E. (2019) Reaction of O_2 with a diiron protein generates a mixed-valent Fe^{2+}/Fe^{3+} center and peroxide. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 2058-2067
191. Ekman, M., Sandh, G., Nenninger, A., Oliveira, P., and Stensjo, K. (2014) Cellular and functional specificity among ferritin-like proteins in the multicellular cyanobacterium *Nostoc punctiforme*. *Environ. Microbiol.* **16**, 829-844
192. Li, X., Sandh, G., Nenninger, A., Muro-Pastor, A. M., and Stensjo, K. (2015) Differential transcriptional regulation of orthologous dps genes from two closely related heterocyst-forming cyanobacteria. *FEMS Microbiol Lett.* **362**
193. Meeks, J. C., Elhai, J., Thiel, T., Potts, M., Larimer, F., Lamerdin, J., Predki, P., and Atlas, R. (2001) An overview of the genome of Nostoc punctiforme, a multicellular, symbiotic cyanobacterium. *Photosynth. Res.* **70**, 85-106

194. Moparthi, V. K., Li, X., Vavitsas, K., Dzhygyr, I., Sandh, G., Magnuson, A., and Stensjo, K. (2016) The two Dps proteins, NpDps2 and NpDps5, are involved in light-induced oxidative stress tolerance in the N_2-fixing cyanobacterium *Nostoc punctiforme*. *Biochim. Biophys. Acta* **1857**, 1766-1776
195. Howe, C., Ho, F., Nenninger, A., Raleiras, P., and Stensjo, K. (2018) Differential biochemical properties of three canonical Dps proteins from the cyanobacterium *Nostoc punctiforme* suggest distinct cellular functions. *J. Biol. Chem.* **293**, 16635-16646
196. Sato, N., Moriyama, T., Toyoshima, M., Mizusawa, M., and Tajima, N. (2012) The all0458/lti46.2 gene encodes a low temperature-induced Dps protein homologue in the cyanobacteria *Anabaena* sp PCC 7120 and *Anabaena variabilis* M3. *Microbiology* **158**, 2527-2536
197. Li, X., Mustila, H., Magnuson, A., and Stensjo, K. (2018) Homologous overexpression of NpDps2 and NpDps5 increases the tolerance for oxidative stress in the multicellular cyanobacterium *Nostoc punctiforme. FEMS Microbiol. Lett.* **365**, fny198
198. Howe, C., Moparthi, V. K., Ho, F. M., Persson, K., and Stensjo, K. (2019) The Dps4 from Nostoc punctiforme ATCC 29133 is a member of His-type FOC containing Dps protein class that can be broadly found among cyanobacteria. *PLoS One* **14,** e0218300
199. Moparthi, V. K., Moparthi, S. B., Howe, C., Raleiras, P., Wenger, J., and Stensjo, K. (2019) Structural diffusion properties of two atypical Dps from the cyanobacterium Nostoc punctiforme disclose interactions with ferredoxins and DNA. *Biochim. Biophy. Acta* **1860**, 148063
200. Giessen, T. W. (2016) Encapsulins: microbial nanocompartments with applications in biomedicine, nanobiotechnology and materials science. *Curr. Opin. Chem. Biol.* **34**, 1-10
201. Sutter, M., Boehringer, D., Gutmann, S., Guenther, S., Prangishvili, D., Loessner, M. J., Stetter, K. O., Weber-Ban, E., and Ban, N. (2008) Structural basis of enzyme encapsulation into a bacterial nanocompartment. *Nat. Struct. Mol. Biol.* **15**, 939-947
202. Akita, F., Chong, K. T., Tanaka, H., Yamashita, E., Miyazaki, N., Nakaishi, Y., Suzuki, M., Namba, K., Ono, Y., Tsukihara, T., and Nakagawa, A. (2007) The crystal structure of a virus-like particle from the hyperthermophilic archaeon *Pyrococcus furiosus* provides insight into the evolution of viruses. *J. Mol. Biol.* **368**, 1469-1483
203. Giessen, T. W., Orlando, B. J., Verdegaal, A. A., Chambers, M. G., Gardener, J., Bell, D. C., Birrane, G., Liao, M. F., and Silver, P. A. (2019) Large protein organelles form a new iron sequestration system with high storage capacity. *Elife* **8**, e46070
204. Contreras, H., Joens, M. S., McMath, L. M., Le, V. P., Tullius, M. V., Kimmey, J. M., Bionghi, N., Horwitz, M. A., Fitzpatrick, J. A. J., and Goulding, C. W. (2014) Characterization of a Mycobacterium tuberculosis Nanocompartment and Its Potential Cargo Proteins. *J. Biol. Chem.* **289**, 18279-18289
205. He, D. D., Hughes, S., Vanden-Hehir, S., Georgiev, A., Altenbach, K., Tarrant, E., Mackay, C. L., Waldron, K. J., Clarke, D. J., and Marles-Wright, J. (2016) Structural characterization of encapsulated ferritin provides insight into iron storage in bacterial nanocompartments. *Elife* **5**, e18972
206. He, D. D., Piergentili, C., Ross, J., Tarrant, E., Tuck, L. R., Mackay, C. L., McIver, Z., Waldron, K. J., Clarke, D. J., and Marles-Wright, J. (2019) Conservation of the structural and functional architecture of encapsulated ferritins in bacteria and archaea. *Biochem. J.* **476**, 975-989
207. McHugh, C. A., Fontana, J., Nemecek, D., Cheng, N. Q., Aksyuk, A. A., Heymann, J. B., Winkler, D. C., Lam, A. S., Wall, J. S., Steven, A. C., and Hoiczyk, E. (2014) A virus capsid-like nanocompartment that stores iron and protects bacteria from oxidative stress. *EMBO J.* **33**, 1896-1911
208. Giessen, T. W., and Silver, P. A. (2017) Widespread distribution of encapsulin nanocompartments reveals functional diversity. *Nat. Microbiol.* **2**, 17029.
209. Pi, H. L., and Helmann, J. D. (2017) Ferrous iron efflux systems in bacteria. *Metallomics* **9**, 840-851

210. Guan, G. H., Pinochet-Barros, A., Gaballa, A., Patel, S. J., Arguello, J. M., and Helmann, J. D. (2015) PfeT, a P_{1B4}-type ATPase, effluxes ferrous iron and protects *Bacillus subtilis* against iron intoxication. *Mol. Microbiol.* **98**, 787-803
211. Pi, H. L., Patel, S. J., Arguello, J. M., and Helmann, J. D. (2016) The *Listeria monocytogenes* Fur-regulated virulence protein FrvA is an Fe(II) efflux P_{1B4}-type ATPase. *Mol. Microbiol.* **100**, 1066-1079
212. Patel, S. J., Lewis, B. E., Long, J. E., Nambi, S., Sassetti, C. M., Stemmler, T. L., and Arguello, J. M. (2016) Fine-tuning of substrate affinity leads to alternative roles of *Mycobacterium tuberculosis* Fe^{2+}-ATPases. *J. Biol. Chem.* **291**, 11529-11539
213. VanderWal, A. R., Makthal, N., Pinochet-Barros, A., Helmann, J. D., Olsen, R. J., and Kumaraswami, M. (2017) Iron efflux by PmtA is critical for oxidative stress resistance and contributes significantly to group A *Streptococcus* virulence. *Infect. Immun.* **85** e00091-17
214. Turner, A. G., Djoko, K. Y., Ong, C. L. Y., Barnett, T. C., Walker, M. J., and McEwan, A. G. (2019) Group A *Streptococcus* co-ordinates manganese import and iron efflux in response to hydrogen peroxide stress. *Biochem. J.* **476**, 595-611
215. Zielazinski, E. L., Gonzalez-Guerrero, M., Subramanian, P., Stemmler, T. L., Arguello, J. M., and Rosenzweig, A. C. (2013) *Sinorhizobium meliloti* Nia is a P_{1B5}-ATPase expressed in the nodule during plant symbiosis and is involved in Ni and Fe transport. *Metallomics* **5**, 1614-1623
216. Grass, G., Otto, M., Fricke, B., Haney, C. J., Rensing, C., Nies, D. H., and Munkelt, D. (2005) FieF (YiiP) from *Escherichia coli* mediates decreased cellular accumulation of iron and relieves iron stress. *Arch. Microbiol.* **183**, 9-18
217. Salusso, A., and Raimunda, D. (2017) Defining the roles of the cation diffusion facilitators in Fe^{2+}/Zn^{2+} homeostasis and establishment of their participation in virulence in *Pseudomonas aeruginosa*. *Front. Cell. Infect. Microbiol.* **7**, 84
218. Bennett, B. D., Brutinel, E. D., and Gralnick, J. A. (2015) A ferrous iron exporter mediates iron resistance in *Shewanella oneidensis* MR-1. *Appl. Environ. Microbiol.* **81**, 7938-7944
219. Frawley, E. R., Crouch, M. L. V., Bingham-Ramos, L. K., Robbins, H. F., Wang, W. L., Wright, G. D., and Fang, F. C. (2013) Iron and citrate export by a major facilitator superfamily pump regulates metabolism and stress resistance in *Salmonella Typhimurium*. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 12054-12059
220. Sankari, S., and O'Brian, M. R. (2014) A bacterial iron exporter for maintenance of iron homeostasis. *J. Biol. Chem.* **289**, 16498-16507
221. Ruangkiattikul, N., Bhubhanil, S., Chamsing, J., Niamyim, P., Sukchawalit, R., and Mongkolsuk, S. (2012) *Agrobacterium tumefaciens* membrane-bound ferritin plays a role in protection against hydrogen peroxide toxicity and is negatively regulated by the iron response regulator. *FEMS Microbiol. Lett.* **329**, 87-92
222. Wofford, J. D., Bolaji, N., Dziuba, N., Outten, F. W., and Lindahl, P. A. (2019) Evidence that a respiratory shield in *Escherichia coli* protects a low-molecular-mass Fe(II) pool from O_2-dependent oxidation. *J. Biol. Chem.* **294**, 50-62
223. Hagen, W. R., Hagedoorn, P. L., and Ebrahimi, K. H. (2017) The workings of ferritin: a crossroad of opinions. *Metallomics* **9**, 595-605
224. Clarke, T. E., Ku, S. Y., Dougan, D. R., Vogel, H. J., and Tari, L. W. (2000) The structure of the ferric siderophore binding protein FhuD complexed with gallichrome. *Nat. Struct. Biol.* **7**, 287-291
225. Mattle, D., Zeltina, A., Woo, J. S., Goetz, B. A., and Locher, K. P. (2010) Two stacked heme molecules in the binding pocket of the periplasmic heme-binding protein HmuT from *Yersinia pestis*. *J. Mol. Biol.* **404**, 220-231
226. Woo, J. S., Zeltina, A., Goetz, B. A., and Locher, K. P. (2012) X-ray structure of the *Yersinia pestis* heme transporter HmuUV. *Nat. Struct. Mol. Biol.* **19**, 1310-1315
227. Kim, S., Lee, J. H., Seok, J. H., Park, Y. H., Jung, S. W., Cho, A. E., Lee, C., Chung, M. S., and Kim, K. H. (2016) Structural basis of novel iron-uptake route and reaction intermediates in ferritins from Gram-negative bacteria. *J. Mol. Biol.* **428**, 5007-5018

228. Lu, M., and Fu, D. (2007) Structure of the zinc transporter YiiP. *Science* **317**, 1746-1748

FOOTNOTES

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[a] BfrB is in fact the only Bfr found in *Pseudomonas aeruginosa*: the protein originally named BfrA was subsequently found to be of the Ftn class.

Figure 1

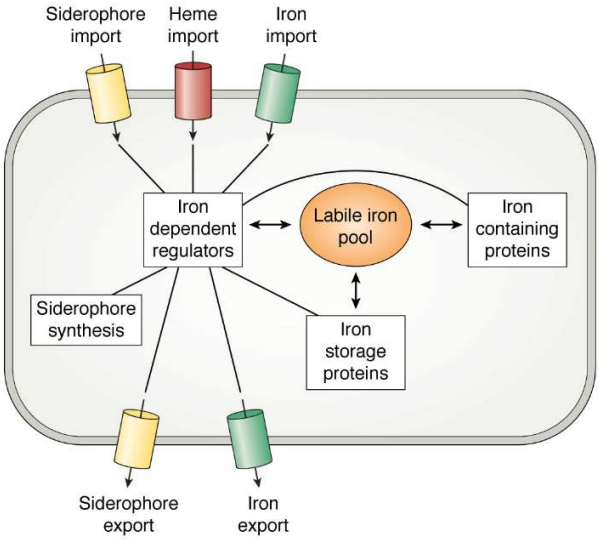

Figure 1. Routes of iron trafficking in bacterial cells. Heavy arrows depict intracellular movement of iron, light arrows movement of iron or iron-bearing compounds across the cell membrane, and lines connect the transcriptional regulators to systems under their control. When the concentration of the labile iron pool increases, iron, or an iron-containing group, binds to the transcriptional regulator. This leads to downregulation of processes such as siderophore synthesis, export of apo siderophores, import of Fe^{3+}-siderophores, heme import and Fe^{2+} uptake systems. Simultaneously, expression of iron containing and iron storage proteins is upregulated together, occasionally, with iron efflux pumps. Reduction in the labile iron pool leads to dissociation of iron/iron-containing groups from the regulators, resulting in the opposite transcriptional responses.

Figure 2

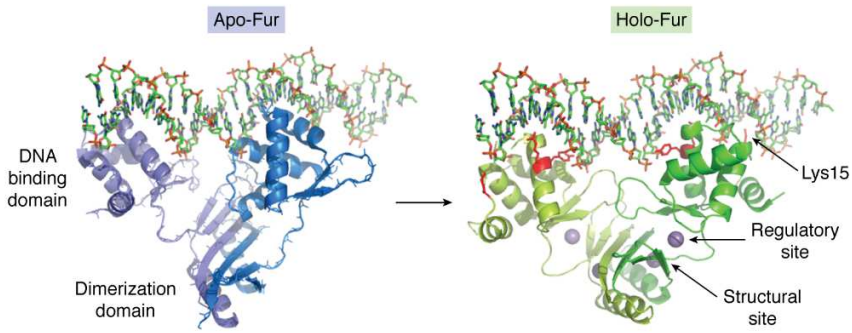

Figure 2. Domain movements induced by the binding of divalent metals to Fur. Binding of divalent metal ions to the regulatory site of Fur induces a rotation of the DNA binding domain relative to the dimerization domain, bringing the DNA recognition helices into more favorable alignment for binding to the Fur box. Residues K15, Y56 and R57, which form favorable interactions with the nucleotide, are highlighted in red. Reproduced from PDB depositions 4RAY and 4RB1 (27).

Figure 3

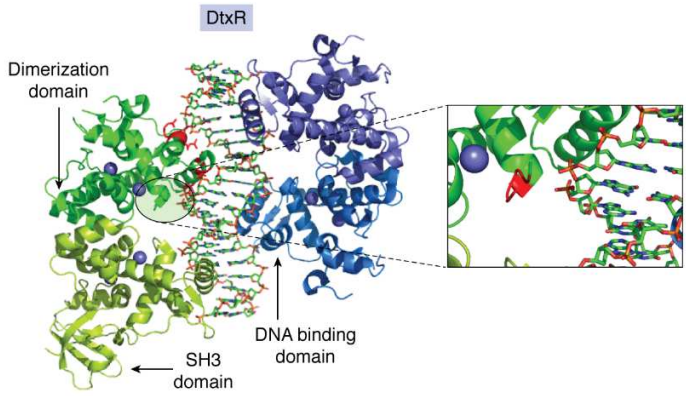

Figure 3. Binding of DtxR to a 21 base pair model oligonucleotide. Identical DtxR dimers bind to opposite faces of the nucleotide faces but only one of the four SH3-like domains is resolved crystalographically. The inset shows the N-terminal region of the protein with residues 3-6 highlighted in red. Upon binding of the regulatory metal ion the highlighted region undergoes a helix to coil transition that relieves what would otherwise be an unfavorable steric interaction between protein and DNA. Also highlighted in red are residues R27, A28, R29, T40, S42, R47, R50 and R60, which form favorable interactions with the nucleotide. Reproduced using PDB deposition 1C0W (52).

Figure 4

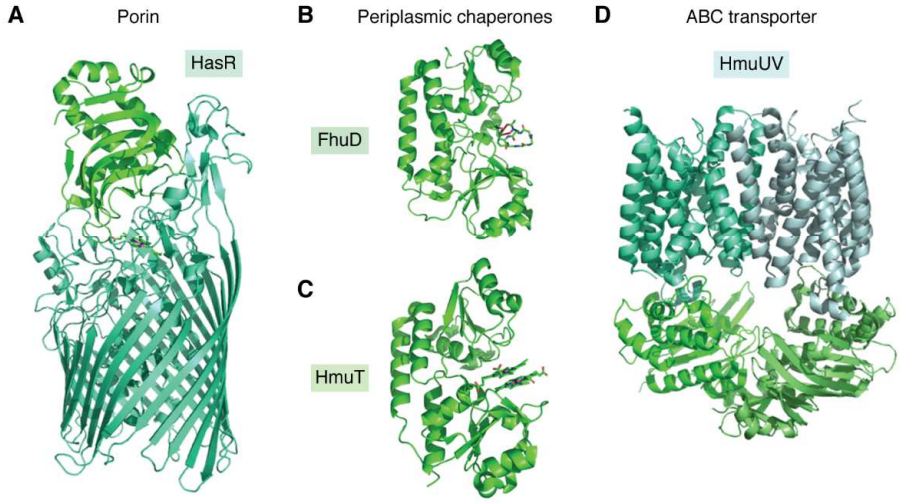

Figure 4. Structures of representative proteins involved in bacterial iron acquisition. HasR (A) a β-barrel porin involved in transport of heme across the periplasmic membrane in complex with HasA. The importers of siderophores exhibit very similar topology. Chaperone proteins FhuD (B) and HmuT (C) that shuttle siderophores and heme, respectively, across the periplasmic space. The ABC transporter HmuUV (D) that transports heme across the cytoplasmic membrane. ABC transporters involved in siderophore transport exhibit similar topology. Reproduced from PDB depositions 3CSL (101), 1EFD (224), 3NU1 (225) and 4G1U (226).

Figure 5

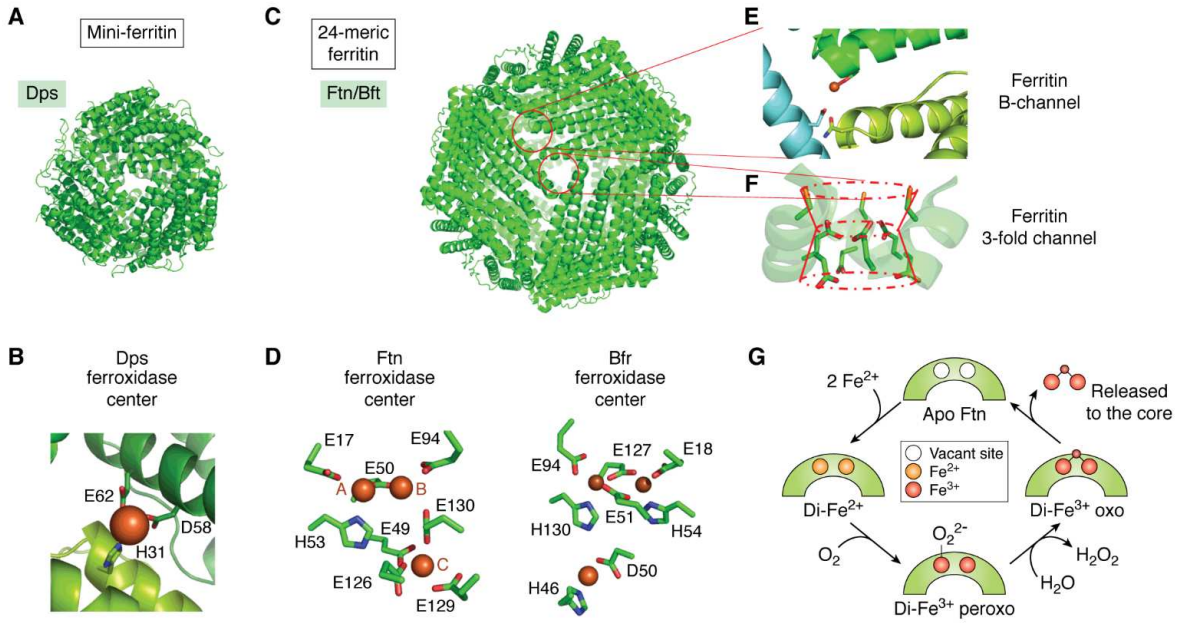

Figure 5. The bacterial ferritins. (A) The dodecameric assembly of *L. innocua* Dps (a mini-ferritin) viewed along one of the ferritin-like 3-fold channels. (B) Single iron ion observed bound to the *L. innocua* Dps ferroxidase center. (C) The 24-meric assembly adopted by both Ftn and Bfr viewed along the channel formed at the 3-fold symmetry axis. (D) The ligands to iron bound at the ferroxidase center of a typical bacterial Ftn together with the associated site C (left hand side) compared to the more symmetrical iron binding environment in *E. coli* Bfr and the distinct coordination environment of the iron ion located on the inner surface of the protein (right hand side). In Ftn, the higher affinity site A has a higher coordination number than site B. (E) Expanded view of the ferritin B-channel showing Fe^{2+} bound to D132 of one monomer with the potential ligands D30 and N63 of the two other monomers forming the channel also highlighted. (F) Side view of the ferritin 3-fold channel showing the conserved Cys (top), Glu (middle) and Asp (bottom) residues thought to guide the Fe^{2+} substrate toward the interior of the protein. (G) Schematic representation of the displacement mechanism that operates in some ferritins. Two equivalents of Fe^{2+} bind to the apo ferroxidase center. Oxygen (or peroxide) binds and is reduced to peroxide (or water) by the simultaneous oxidation of both Fe^{2+} ions to Fe^{3+}. Hydrolysis of the transient diferric peroxo intermediate liberates peroxide and forms a ferric-oxo precursor of the mineral core. This is displaced from the catalytic site completing the cycle by regenerating the apo ferroxidase center. Images produced using PDB depositions 1QGH (184) (Dps), 4ZTT (227) (Ftn) and 3E1P (161) (Bfr).

Figure 6

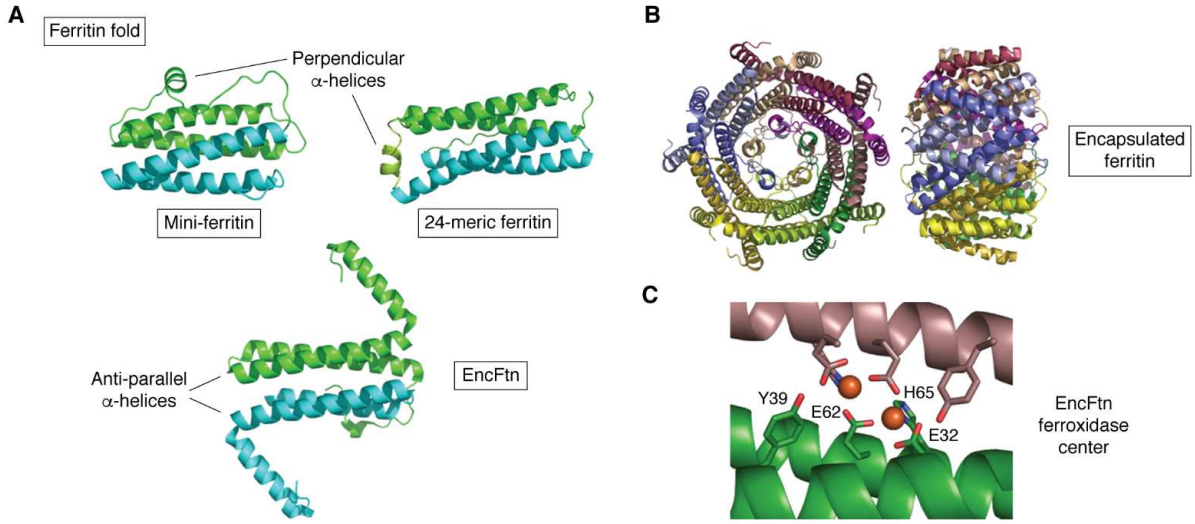

Figure 6. Encapsulated ferritins. (A) The ferritin fold is made up of 2 homologous pairs of anti-parallel α-helices (136) here colored green and cyan. In the true, cage forming, ferritins these are connected via a loop joining helices B and C. Short helices running perpendicular to the long axis of the bundle help to template cage formation in the mini-ferritins (top) or 24-meric examples (middle). Members of the superfamily that do not form cages, such as EncFtn (bottom), are associated with further extended secondary structure elements, such as the membrane spanning helices of MbfA or the large additional helices of EncFtn, which prevent assembly into cages. (B) The annular pentamer of dimers adopted by the majority of encapsulated ferritins. (C) The ferroxidase center of a typical encapsulated ferritin highlighting the non-crystallographic 2-fold symmetry of the iron environment. For clarity only the ligands provided by the lower of the two protomers have been labeled. Images produced using PDB deposition 5N5E (206) (EncFtn).

Figure 7

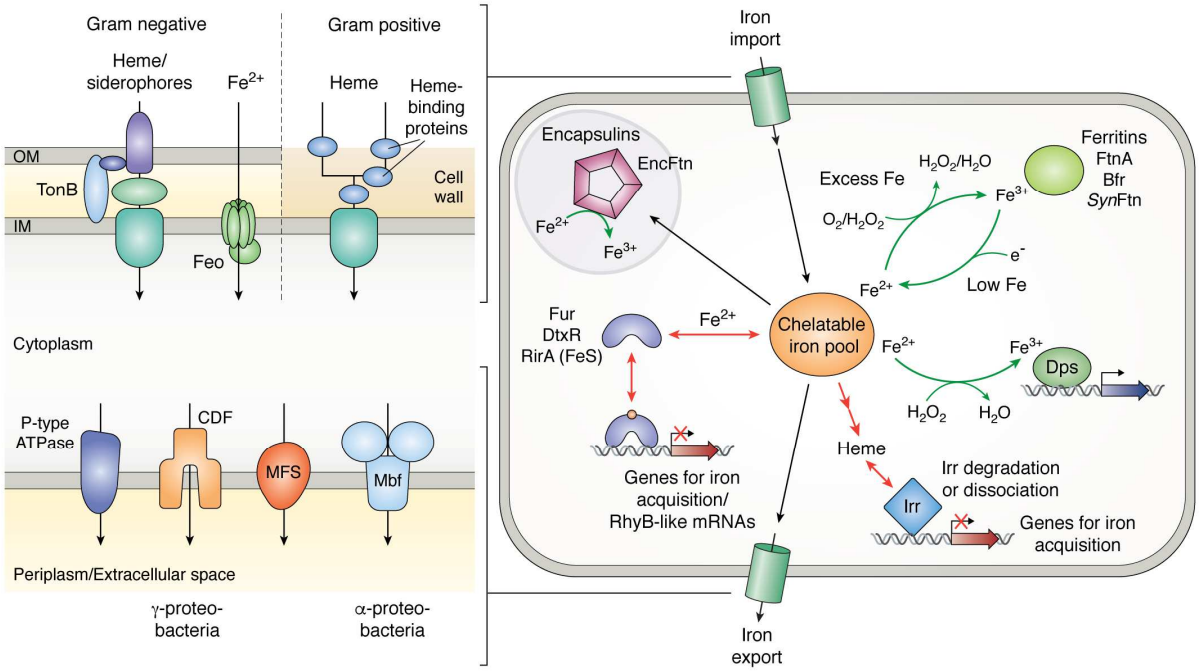

Figure 7. Schematic overview of the major components of iron-sensing and detoxification found in bacterial cells. Note that not all of these components are present in a single bacterial cell. Regulatory proteins are shown here as repressors, but, in some cases, can also act as activators. Encapsulins are large protein compartments that house EncFtn ferritin-like proteins. The fate of iron stored in encapsulins and in Dps proteins is not clear though it is likely that at some point it becomes bioavailable again. Ftn, Bfr and Dps do not appear to be distributed according to phyla. Fur is the transcriptional regulator in most bacteria but is replaced by DtxR/IdeR in some actinobacteria. In the α-proteobacteria, Fur plays a diminished role in iron homeostasis with the majority of these functions being performed by Irr. In some *rhizobiales* this is achieved in conjunction with a second global regulator, RirA. Import of siderophores and heme across the cytoplasmic membrane (IM) is performed by ABC transporters in all known cases and Feo is the major importer of Fe^{2+}. In Gram negative bacteria, heme and siderophores are imported to the periplasm by outer membrane (OM) porins, whilst a network of heme binding proteins transport this cofactor across the cell wall of the Gram-positive bacteria. Characterized Fe^{2+} export systems are rare but P-type ATPases are the most widely distributed. IceT of *Salmonella typhimurium* is the only example of the MFS characterized to date, whilst the CDF proteins are limited to γ-proteobacteria and the MbfA proteins to α-proteobacteria. YiiP from *E. coli* is the only Fe^{2+}efflux pump for which the structure has been solved (228).