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Review Article

Iron assimilation and storage in prokaryotes

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Introduction

Iron is an essential element for most living organisms. Its roles in microbial physiology are numerous. Iron is a constituent of all haem enzymes, which include cytochromes and hydroperoxidases. The common type of ribonucleotide reductase contains iron, and non-iron nitrogenases require an iron protein in a complex for their activity (Robson *et al.*, 1986). However, exceptions do exist; for example, certain lactobacilli devoid of haem and containing a cobalt form of ribonucleotide reductase appear to have no iron requirement for their growth (Archibald, 1983).

Iron, the fourth most-represented element in the earth's crust, is abundant in the environment and should not be a limiting factor for bacterial growth. However, in the presence of oxygen and at a non-acidic pH, iron is particularly insoluble and tends to precipitate as ferric hydroxides. Therefore, bacteria have evolved various powerful systems to overcome this low solubility of external iron (Lankford, 1973). Besides its insolubility, another problem associated with the metabolism of iron resides in its ability to react with reduced forms of oxygen (hydrogen peroxide and superoxide), leading to the production of deleterious free radicals responsible for lipid peroxidation, as well as for alterations in protein and nucleic acids (Flitter *et al.*, 1983). Therefore, in order to avoid such toxicity, iron homeostasis is strictly controlled and results from a co-ordinated integration of assimilation, utilization and storage of this element. Iron uptake is an obvious step to be regulated in response to variations in environmental iron concentration, and has been actively studied in the last decade. The first part of this paper is a non-exhaustive overview of iron assimilation and its control in prokaryotes. The storage of iron within bacteria is also important, particularly in properly tuning the flux of iron required by iron-proteins involved in metabolic reactions. The breakdown and turnover of iron-proteins could lead to an increase in the pool of intracellular free ionic iron that would need to be detoxified. On the other hand, stored iron could be used for the construction of active iron-containing enzymes during an increase in growth rate. It is widely accepted that the amount of an element 'must be regulated by controlled absorbtion' (Underwood, 1971) and thus could explain why much less attention has been accorded to the function of iron storage in bacteria. A review of iron storage in prokaryotes comprises the second part of this paper. Finally, an interesting situation concerning iron uptake and storage by eukaryotic organelles (mitochondria in all cells and plastids in plants), thought to have evolved from prokaryotic progenitors (Gray, 1989), is covered at the end of this review.

Iron assimilation by prokaryotes

Because a tremendous amount of information is available in the literature concerning this topic, a rapid overview will be presented prior to focussing on regulation at the molecular level.

At high levels of environmental iron ($>10\,\mu M$), low-affinity systems are responsible for passive iron uptake and they are poorly understood. High-affinity transport systems operate when iron is limited in the environment and these systems have been extensively studied over the past 30 years. This interest comes probably in part because of the importance that iron acquisition by microbial pathogens from the host plays during infection (Byers, 1987; Crosa, 1989; Byers *et al.*, 1991). Among these high-affinity transport systems, often associated (but not always) with pathogenicity, siderophores (Lankford, 1973) represent low molecular mass carriers (400 to 1000 Da), marked by a very high affinity for ferric iron. Siderophores have highly divergent structures but can be classified into two main groups: phenolates, with

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enterobactin as a classic member, and hydroxamates, with aerobactin as an example. Siderophores are secreted into the environment of cells where they bind ferric iron. Ferrisiderophore assimilation is achieved by specific receptors at the cell surface associated with other periplasmic and cytoplasmic membrane proteins (Bagg & Neilands, 1987*a*); iron reaches the cytoplasm either by internalization of the ferrisiderophore itself or of the iron alone.

Besides these highly specific siderophore systems, some microbes have developed the ability to take up iron directly from naturally occuring iron-binding acids like pyruvate, malate, isocitrate and citrate (Archibald & De Voe, 1980), the latter being by far the most studied of these systems (see below). In mycobacteria, under iron-deficient conditions, iron can be transported into cells as ferric salicylate (Messenger *et al.*, 1986); the role of this system, however, is restricted because it does not operate in the presence of phosphate (Ratledge *et al.*, 1974).

Siderophore-mediated iron assimilation

Iron starvation leads to the synthesis of native siderophores by bacteria and to the concomitant induction of the specific transport system responsible for ferrisiderophore reception and internalization. Beside specific transport systems, other systems for transport of exogenous siderophores can also be induced.

In this section, siderophore-mediated uptake systems of *Escherichia coli* will be reviewed, prior to a brief look at this process in other bacteria. Siderophore biosynthesis requires the expression of different genes, often organized in an operon. Enterobactin synthesis needs the products of genes *entA,B,C* to produce 2,3-hydroxybenzoic acid from chorismic acid, and the products of genes *entD,E,F,G* to catalyse production of one enterobactin molecule from three molecules of 2,3-dihydrobenzoic acid and L-serine (reviewed in Crosa, 1989). All these genes are located on the chromosome. In the case of aerobactin, the pColV-K30 plasmid [Gibson & Magrath, 1969; reviewed in Crosa (1989) and in Bagg & Neilands (1987*a*)] harbours genes in an operon responsible for the synthesis of this hydroxamate siderophore. The genes *iucA,B,C,D* encode, respectively, synthetase, acetylase, synthetase and oxygenase. In addition, the gene *iutA*, also belonging to the aerobactin operon, codes for the specific receptor of ferrienterobactin. Instead of being plasmid-encoded, the aerobactin system is encoded on the chromosome in some invasive *E. coli* (Valvano & Crosa, 1984, 1988; Valvano *et al.*, 1986). The assimilation of all phenolate and hydroxamate siderophores in *E. coli* requires two proteins, TonB and ExbB, thought to reside within the cytoplasmic membrane (Postle & Good, 1983). Their necessity for iron uptake has been made evident by mutations in *tonB* (Hantke & Braun, 1978) and *exbB* (Eick-Helmerich *et al.*, 1987; Hantke & Zimmermann, 1981). Genetic data have also shown that the TonB protein interacts with outer-membrane receptors (Hantke & Braun, 1978; Braun *et al.*, 1987). These outer-membrane receptors confer specificity to the iron transport systems. In other words, for each ferrisiderophore, a specific receptor is found in the outer membrane: ferrichrome is recognized by FhuA, coprogen and ferric rhodotorulate by FhuE, ferric aerobactin by IutA and ferric enterobactin by FepA (reviewed by Bagg & Neilands, 1987*a*). Then, a set of proteins are required to define a permease responsible for the translocation and internalization of the ferrisiderophore. Two groups of proteins are involved. The first is composed of the proteins FhuC,D,B, and this system is responsible for translocation accross the inner membrane of all ferric hydroxamates. These proteins share all the characteristics of periplasmic transport systems (Ames, 1986; Braun *et al.*, 1983; Burkhardt & Braun, 1987; Coulton *et al.*, 1987; Fecker & Braun, 1983; Köster & Braun, 1989). The FhuD protein is probably the ferrihydroxamate-binding protein. FhuC faces the cytosol and shares a strong sequence homology with ATP-binding proteins (Burkhardt & Braun, 1987; Coulton *et al.*, 1987) suggesting its involvement in energy coupling. FhuB is the transmembrane component responsible for the translocation of ferrihydroxamates across the membrane.

The second protein system to be described is responsible for ferric-phenolate siderophore uptake (reviewed in Earhart, 1987). It also has similarities with periplasmic permease (Ames, 1986) and requires FepB, a periplasmic protein which binds phenolate siderophores (Pierce *et al.*, 1983; Pierce & Earhart, 1986; Elkins & Earhart, 1989), FepC, an inner-membrane ATP-binding protein (Pierce & Earhart, 1986; Ozenberger *et al.*, 1987; Shea & McIntosh, 1991) and two hydrophobic proteins, FepD and FepG, encoded in the same operon as *fepC* (*fepDGC*) (Chenault & Earhart, 1991; Shea & McIntosh, 1991).

Siderophore production for iron assimilation is not a trait restricted to *E. coli*. Many other bacteria use this method for acquisition of iron. The more well-defined system concerns essentially bacteria pathogenic for plants and animals. Siderophore production by *Pseudomonas* strains has been widely studied: two molecules, pseudobactin and pyochelin are synthesized (reviewed in Crosa, 1989). The ferric-pseudobactin receptor, PupA, from *Pseudomonas putida* WCS 358, has been characterized recently (Bitter *et al.*, 1991). Mycobacteria, including *Mycobacterium leprae*, are able to produce myobactin, a lipid-soluble siderophore (Hall & Ratledge, 1984) and exochelin, which is found in the culture medium of

starved *Myobacterium smegmatis* (Macham & Ratledge, 1975; Ratledge, 1984). Most of the mesophilic *Aeromonas* species produce amonabactin and/or enterobactin (Barghouti *et al.*, 1989). Recently, an amonabactin biosynthetic gene (*amoA*) from *Aeromonas hydrophila*, has been cloned and sequenced (Barghouti *et al.*, 1991), revealing its homology with *E. coli entC*. Ferrioxamines, a group of hydroxamate siderophores, are produced by members of the genera *Erwinia* and *Hafnia*. They are also taken up efficiently by bacteria which not produce them, such as *E. coli*, *Yersinia enterocolitica* and *Serratia marscesens*. FoxA, a receptor protein for ferrioxamine, has recently been characterized in *Y. enterocolitica* (Bäumler & Hantke, 1992*b*) and a lipoprotein, PCP_{YE}, which facilitates uptake of this siderophore, has been cloned and sequenced (Bäumler & Hantke, 1992*a*). This protein is homologous to PCP_{Hi}, a lipoprotein of *Haemophilus influenzae* (Deich *et al.*, 1988).

The marine fish pathogen *Vibrio anguillarum* has its virulence mediated by the pJM1 plasmid (Crosa,1980, and reviewed in Crosa, 1989). The siderophore produced by this bacterium is known as anguibactin and resembles pyochelin. The molecular characterization of the iron transport system encoded by pJM1 has recently been reported (Köster *et al.*, 1991). It shares remarkable homology with other iron transport systems described in members of the *Enterobacteriaceae*. However, besides the probable existence of a Fur-like negative repressor (see below) in *V. anguillarum*, a *trans*-acting factor, Taf, is also required for the transcriptional activation and full expression of the iron transport system found on pJM1 (Tolmasky & Crosa, 1984; Tolmasky *et al.*, 1988).

Phytopathogenic bacteria also produce siderophores for iron assimilation (Loper & Buyer, 1991). Although their role in virulence is largely unknown, there is one case, *Erwinia chrysanthemi*, for which production of the catechol siderophore chrysobactin (Persmark *et al.*, 1989) contributes to systemic virulence (Enard *et al.*, 1988). *Erwinia carotovora* subsp. *carotovora* W3C105 has recently been shown to produce aerobactin and a ferric aerobactin receptor encoded by a chromosomal fragment; however, a functional aerobactin acquisition system is not necessary for the pathogenicity of this bacterium (Ishimaru & Loper, 1992).

Iron-dicitrate transport system

Besides siderophore-mediated iron assimilation, organic acids are also able to bind external iron and facilitate its assimilation by bacteria. Of these organic acids, citrate-dependent iron uptake by *E. coli* K12 has received much attention over the last 10 years (Hussein *et al.*, 1981). The *fec* operon is responsible for iron-dicitrate uptake. It has been cloned and sequenced (Staudenmaier *et al.*, 1989). The FecA protein is the outer-membrane receptor. FecB is a protein found in the periplasmic space, while FecC and D are very hydrophilic proteins found in the cytoplasmic membrane. FecE is also a cytoplasmic-membrane protein, sharing two regions of sequence homology with nucleotide-binding proteins. The TonB protein is also required for transport of iron(III)-dicitrate (Zimmermann *et al.*, 1984). Therefore, it appears that the organization of the iron(III)-dicitrate transport system in *E. coli* is very reminiscent of that described above for ferrisiderophore uptake. However, the iron-dicitrate transport system has the pecularity of being induced by both iron and citrate (Hussein *et al.*, 1981; Zimmermann *et al.*, 1984). A large amount of iron in the medium suppresses the system, because it is under control of the Fur repressor (see below), like the ferrisiderophore systems. The regulatory mechanisms of the iron-dicitrate system have recently been reviewed (Braun & Hantke, 1991). Iron(III)-dicitrate is the inducer and does not need to be internalized to switch-on the *fec* operon. It just requires to be transported across the outer membrane via the action of the FecA and TonB proteins. The inducer could bind to a transmembrane signalling protein, which could be the product of *fecR* encoded upstream of the *fec* operon. An activator, encoded by *fecI*, also found upstream of *fec*, could be bound to this transmembrane protein in the uninduced state, and be released by iron(III)-dicitrate, leading to its binding onto the operator found upstream of *fecA*, and consequently to initiation of transcription of the *fec* operon.

E. coli is not the only bacterium that uses citrate as a shuttle for iron assimilation. *Mycobacterium smegmatis* (Messenger & Ratledge, 1982), *Neisseria meningitidis* (Archibald & De Voe, 1980) and *Pseudomonas aeruginosa* (Harding & Royt, 1990) can also do so. However, *P. aeruginosa* also uses citrate as a carbon source while in *E. coli* and *N. meningitidis*, dissociation of the metal and ligand occurs within the cell envelope and only iron enters the cell.

Other systems for iron assimilation

The utilization of siderophores and organic acids as iron chelators for assimilation of this element are not the only iron uptake mechanisms used by prokaryotes. Certain pathogenic bacteria, such as *Neisseria gonorrhoeae* and *Neisseria meningitidis*, possess receptor proteins for lactoferrin or transferrin (iron transporter proteins in eukaryotes) allowing the use of their iron (Mickelsen & Sparling, 1981). In the serum of vertebrates, free haemoglobin and haem are not abundant because they are complexed by haptoglobin and haemopexin. Certain haemolytic bacteria are able to acquire iron from these complexes (Francis *et al.*, 1985; Dyer *et al.*, 1987, Stull,

1987; Zakaria-Meehan *et al.*, 1988). *Haemophilus influenzae*, an important human pathogen, is also dependent on an iron supply for virulence, but does not produce siderophores under iron-deficient conditions, nor is it able to use iron from siderophores produced by other bacteria (Morton & Williams, 1990; Pidcock *et al.*, 1988; Williams *et al.*, 1990). Instead, *H. influenzae* uses haem, complexed to albumin, haemopexin and haptoglobin, and iron from ferric-transferrin and ferric-lactoferrin as iron sources. These iron complexes bind to outer-membrane proteins (Morton & Williams, 1990; Schryvers, 1989; Hanson & Hansen, 1991). Furthermore, two iron-repressed periplasmic proteins have been identified recently (Harkness *et al.*, 1992). One of them is homologous to Fbp, the major iron-binding protein of *N. gonorrhoeae* and *N. meningitidis*. Motile aeromonads capable of infecting fishes also use haem present in the host as an iron source (Massad *et al.*, 1992). *Aeromonas* species produce various cytolytic toxins, some of which are important in virulence (Chakraborty *et al.*, 1987; Howard & Buckley, 1986)), and this could be a way to access iron in haem by disrupting the cells (Byers *et al.*, 1991). Recently, in *Serratia marcescens*, a system was reported for iron assimilation which requires neither siderophore nor receptor protein production and which is independent of the TonB/ExbB functions (Zimmermann *et al.*, 1989).

Fur: a key regulatory protein in the control of iron uptake

The high-affinity iron transport systems described above are generally activated under iron starvation conditions, as proposed many years ago (Garibaldi & Neilands, 1956). The evidence for an iron-binding repressor, inhibiting siderophore synthesis at high intracellular iron concentration, comes from a mutant of *Salmonella typhimurium* which constitutively overexpresses iron-regulated outer-membrane proteins (Ernst *et al.*, 1978). This mutation, termed *fur* (ferric uptake regulation), was then obtained and mapped from *E. coli*, and the corresponding gene was cloned and sequenced (Bagg & Neilands, 1985; Hantke, 1981, 1984; Schaffer *et al.*, 1985). The Fur protein is unusually rich in histidine and has a molecular mass of 17 kDa; it lacks significant homology to any known DNA-binding proteins. A Fur overproducer plasmid has been constructed, allowing a large amount of pure Fur protein to be obtained (Wee *et al.*, 1988); its DNA-binding properties have been characterized (De Lorenzo *et al.*, 1987) and an 'iron box' defined (Bagg & Neilands, 1987*a*). Furthermore, it has been shown, using the operator/promoter of the aerobactin operon, and an *in vitro* transcription/translation system, that Fur acts as a classical negative repressor which blocks the aerobactin promoter with Fe(II), or some other metals, as a co-repressor (Bagg & Neilands, 1987*b*). Fur has also been postulated as a positive regulator in the case of expression control of *ird* (Hantke, 1987), of the iron-dependent superoxide dismutase encoded by *sodB* (Niederhoffer *et al.*, 1989) and of the catabolic pathways allowing growth on succinate, fumarate and acetate (Hantke, 1987). Regulatory studies have also shown that the *fur* gene of *E. coli* could have its expression controlled by its own product in a feedback mechanism, as well as by the cAMP–CAP (catabolite-activator protein) system (De Lorenzo *et al.*, 1988).

Structural studies of Fur using NMR have demonstrated its folding (Saito & Williams, 1991; Saito *et al.*, 1991*a*, *b*). The structure/function relationship has also been recently adressed by a biochemical approach, showing the existence of two domains (Coy & Neilands, 1991): the N-terminal domain of the protein is responsible for DNA-binding and the C-terminal domain binds to metal ions.

The repression of siderophore production and assimilation by the Fur protein under iron-sufficient conditions seems to be a mechanism conserved in various bacterial species, including *Yersinia pestis*, *Shigella dysenteriae*, *Vibrio cholerae* and *Corynebacterium diphtheriae* (Staggs & Perry, 1991).

Iron deposition and storage in prokaryotes

After assimilation, iron can be used to supply metabolic requirements and/or stored internally in various forms. Mechanisms of iron deposition and storage, as well as functions played by the different compounds involved in these processes, have been investigated much less than iron assimilation, and are reviewed below.

Bacteriomagnetite

Amorphous colloidal species of iron hydroxides have been found associated with bacterial surfaces (Ferris *et al.*, 1989; Ghiorse, 1984), but the most characterized metal deposition in prokaryotes is magnetite (Fe_3O_4), in the form of small membrane-bound magnetosomes (Blakemore, 1975, 1982; Frankel *et al.*, 1979). The morphology of these magnetosomes has been studied mainly in *Aquaspirillum magnetotacticum*, where they are truncated octahedrons (Blakemore, 1982; Matsuda *et al.*, 1983) with a thin dusting of amorphous iron on their surface (Mann *et al.*, 1984). The magnetosomes are arranged in one or more lines along the cell axis, leading to alignment of the cell to the geomagnetic field (Blakemore, 1982). Each magnetite crystal is surrounded by a lipid bilayer membrane harbouring two unique proteins (Gorby *et al.*, 1988).

Bacterioferritin

Because of the insolubility and toxicity of iron in the presence of oxygen, it is likely that all aerobic organisms have evolved a class of ubiquitous iron-storage proteins, the ferritins, able to sequester a few thousand iron atoms in their central cavity, in a soluble, non-toxic, bioavailable form (Grossman *et al.*, 1992).

Bacterioferritins have been purified and characterized from *E. coli* (Yariv *et al.*, 1981), *Azotobacter vinelandii* (Stiefel & Watt, 1979), *Azotobacter chroococcum* (Chen & Crichton, 1982), *Pseudomonas aeruginosa* (Moore *et al.*, 1986), *Nitrobacter winogradskyi* (Kurokawa *et al.*, 1989) and the cyanobacterium *Synechocystis* PCC 6803 (Laulhère *et al.*, 1992). They are multimeric (24-mers) proteins with subunits arranged in a 432 symmetry, resembling ferritin from eukaryotes (Smith *et al.*, 1989; Harrison *et al.*, 1991). Some important differences, however, do exist between eukaryotic and prokaryotic ferritins. Bacterioferritins contain haem residues (1 haem per 2 to 8 subunits) and in certain cases their identity with previously characterized cytochromes has been demonstrated [cytochrome *b*-1 in *E. coli* (Smith *et al.*, 1988); cytochrome *b*-557.5 in *A. vinelandii* (Stiefel & Watt, 1979); cytochrome *b*-559 in *N. winogradskyi* (Kurokawa *et al.*, 1989)]. The axial ligands of haem have recently been shown to be methionine residues in bacterioferritin from *P. aeruginosa* (Cheesman *et al.*, 1990). Bacteroferritin iron-cores are amorphous due to the presence of a high amount of inorganic phosphate within them (Fe atoms:P atoms varying from 1/1 to 1/2), but this property depends upon the composition of the medium rather than upon the protein shell (Mann *et al.*, 1986, 1987; Harrison *et al.*, 1991). The complete amino acid sequences of *E. coli* and *A. vinelandii* bacterioferritin have been determined by cloning and sequencing the corresponding *bfr* gene (Andrews *et al.*, 1989; Grossman *et al.*, 1992). Partial amino acid sequences are also available for *N. winogradskyi* (Kurokawa *et al.*, 1989) and *Synechocystis* PCC 6803 (Laulhère *et al.*, 1992) bacterioferritins. These data and immunological results (Andrews *et al.*, 1991) have pointed out the conservation of bacterioferritins among various bacterial species. However, they share a low sequence homology (between 24 and 29%) with eukaryotic ferritins, which could, nevertheless, be compatible with a common origin (Grossman *et al.*, 1992). Only one type of subunit has been described for bacterioferritin, although a new gene has been characterized recently in *E. coli*, sharing a greater sequence homology with the eukaryotic H-ferritin chain than the *bfr* gene (Izuhara *et al.*, 1991); furthermore, in *A. vinelandii*, two non-identical subunits of bacterioferritin have been described (Harker & Wullstein, 1985). The question concerning bacterioferritin heteropolymers, as for ferritin in eukaryotes, is still open.

The function of bacterioferritin *in vivo* remains obscure. A *bfr* mutation was found not to affect growth of *E. coli* (as reported by S. C. Andrews, Conference communication), and the amount of bacterioferritin subunit appears to be unchanged in *Synechocystis* PCC 6803 whatever the iron status of the cells, even under iron starvation conditions (Laulhère *et al.*, 1992). This raises the question of whether or not bacterioferritin synthesis responds to excess iron. A mechanism for the control of *E. coli* BFR synthesis involving anti-sense RNA expression regulated by the Fur protein has been proposed (Andrews *et al.*, 1989). A structure reminiscent of the iron responsive element (IRE), which is involved in the translational regulation of ferritin synthesis in response to iron in eukaryotic cells, has also been described in the case of *A. vinelandii bfr* (Grossman *et al.*, 1992). No experimental data supporting these regulatory hypotheses have been reported.

Other compounds participating in iron storage

In *E. coli* bacterioferritin iron represents no more than 1% of the total cellular iron and the bulk of iron in the iron-rich cells is in the form of aggregates (Bauminger *et al.*, 1980), which could be associated with a novel form of iron-protein (Matzanke *et al.*, 1989). More recently, Laulhère *et al.* (1992) have shown that the bulk of iron in *Synechocystis* PCC 6803 is associated with low molecular mass (<10 kDa) compounds of unknown composition, which fulfil the storage function. These authors hypothesized that the abundance of saturable low molecular mass molecules, which could vary according to the cellular iron content (Bauminger *et al.*,1980), may control the fate of newly imported iron. In the case of an iron overload, the small molecule storage pool would take care of the excess iron and bacterioferritin would hardly be involved. On the other hand, in the case of iron deficiency, a rapid overflow of iron from the saturable low molecular mass molecules is conceivable and bacterioferritin would buffer this release of iron prior to its use for metabolic requirements.

Iron uptake and storage by organelles of eukaryotic cells

Mitochondria present in all eukaryotic cells and plastids present in plant cells have evolved from prokaryotic progenitors that invaded primitive eukaryotic cells (Gray, 1989). For this reason, it is relevant to report in this review our knowledge of iron uptake and storage by these organelles. The means by which iron is taken up by

mitochondria is not well documented and no information is available for iron assimilation by plastids. Iron ferritin can be taken up in low amounts by mitochondria from hepatocytes (Sibille *et al.*, 1989) but the major pathway by which iron is assimilated by mitochondria involves mainly an AMP-Fe complex and, to a lower extent, ATP-Fe (Weaver & Pollack, 1990). Two kinds of mitochondrial receptors are involved: one that accepts iron from ATP with low efficiency and one from AMP with high efficiency.

No reports have appeared concerning iron-storage molecules in mitochondria. In contrast, it is well established that in plants, ferritins are localized in chloroplasts and non-green plastids, although they are encoded by nuclear genes (Seckback, 1982; Lescure *et al.*, 1991). Interestingly, it has been reported that plant ferritins have an amino acid sequence closely related to animal ferritin sequences and not to bacterioferritin sequences, including those of cyanobacteria, thought to be the ancestors of plastids; relics of a bacterioferritin gene are still present on the chloroplast genome, however (Laulhère *et al.*, 1992; Ragland *et al.*, 1990).

Conclusion

Iron assimilation is well documented in various model systems, particularly *E. coli*. It is clear that the knowledge gained from these systems will help to understand, and eventually to control, iron uptake by pathogenic bacteria, in order to have new tools to treat important diseases. The function and regulation in bacteria of the expression of the iron-storage protein ferritin is unclear, and deserves further study. Iron assimilation by eukaryotic organelles of prokaryotic type (i.e. mitochondria and plastids) is also an important area that has not been intensively scrutinized. The study of iron assimilation and storage in prokaryotes has provided much information related to a wide range of scientific activities, ranging from molecular genetics to pathology and agronomy, and more remains to be discovered.

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References

AMES, G. F. L. (1986). Bacterial periplasmic transport systems: structure, mechanism and evolution. *Annual Review of Biochemistry* **55**, 397–425.

ANDREWS, S. C., HARRISON, P. M. & GUEST, J. R. (1989). Cloning, sequencing, and mapping of the bacterioferritin gene (*bfr*) of *Escherichia coli* K-12. *Journal of Bacteriology* **171**, 3940–3947.

ANDREWS, S. C., FINDLAY, J. B. C., GUEST, J. R., HARRISON, P. M., KEEN, J. N. & SMITH, J. M. A. (1991). Physical, chemical and immunological properties of the bacterioferritins of *Escherichia coli*, *Pseudomonas aeruginosa* and *Azotobacter vinelandii*. *Biochimica et Biophysica Acta* **1078**, 111–116.

ARCHIBALD, F. (1983). *Micrococcus lysodeikticus*, an organism not requiring iron. *FEMS Microbiology Letters* **19**, 29–32.

ARCHIBALD, F. & DE VOE, I. W. (1980). Iron acquisition by *Neisseria meningitidis*. *Infection and Immunity* **27**, 322–334.

BAGG, A. & NEILANDS, J. B. (1985). Mapping of a mutation affecting regulation of iron uptake systems in *Escherichia coli* K12. *Journal of Bacteriology* **161**, 450–453.

BAGG, A. & NEILANDS, J. B. (1987*a*). Molecular mechanism of regulation of siderophore-mediated iron assimilation. *Microbiological Reviews* **51**, 509–518.

BAGG, A. & NEILANDS, J. B. (1987*b*). Ferric uptake regulation protein acts as a repressor, employing iron(II) as a cofactor to bind the operator of an iron transport operon in *Escherichia coli*. *Biochemistry* **26**, 5471–5477.

BARGHOUTI, S., YOUNG, R., OLSON, M. O., ARCENEAUX, J. E. L., CLEM, L. W. & BYERS, B. R. (1989). Amonabactin, a novel tryptophan- or phenylalanine-containing phenolate siderophore in *Aeromonas hydrophila*. *Journal of Bacteriology* **171**, 1811–1816.

BARGHOUTI, S., PAYNE, S. M., ARCENEAUX, J. E. L. & BYERS, B. R. (1991). Cloning, mutagenesis and nucleotide sequence of a siderophore biosynthetic gene (*amoA*) from *Aeromonas hydrophila*. *Journal of Bacteriology* **173**, 5121–5128.

BAUMINGER, E. R., COHEN, S. G., DICKSON, D. P. E., LEVY, A., OFER, S. & YARIV, J. (1980). Mössbauer spectroscopy of *Escherichia coli* and its iron-storage protein. *Biochimica et Biophysica Acta* **623**, 237–242.

BÄUMLER, A. J. & HANTKE, K. (1992*a*). A lipoprotein of *Yersinia enterocolitica* facilitates ferrioxamine uptake in *Escherichia coli*. *Journal of Bacteriology* **174**, 1029–1035.

BÄUMLER, A. J. & HANTKE, K. (1992*b*). Ferrioxamine uptake in *Yersinia enterocolitica*: characterization of the protein receptor FoxA. *Molecular Microbiology* **6** (in the press).

BITTER, W., MARUGG, J. D., DE WEGER, L. A., TOMMASSEN, J. & WEISBEEK, P. J. (1991). The ferric-pseudobactin receptor PupA of *Pseudomonas putida* WCS 358: homology to TonB-dependent *Escherichia coli* receptors and specificity of the protein. *Molecular Microbiology* **5**, 647–655.

BLAKEMORE, R. P. (1975). Magnetotactic bacteria. *Science* **190**, 377–379.

BLAKEMORE, R. P. (1982). Magnetotactic bacteria. *Annual Review of Microbiology* **36**, 217–238.

BRAUN, V. & HANTKE, K. (1991). Genetics of bacterial iron transport. In *Handbook of Microbial Chelates*, pp. 107–138. Edited by G. Winkelmann. Boca Raton: CRC Press.

BRAUN, V., GROSS, R., KÖSTER, W. & ZIMMERMANN, L. (1983). Plasmid and chromosomal mutants of the iron(III)-aerobactin transport system of *Escherichia coli*. Use of streptonigrin for selection. *Molecular and General Genetics* **192**, 131–139.

BRAUN, V., HANTKE, K., EICK-HELMERICH, K., KOSTER, W., PRESSLER, U., SAUER, M., SCHAFFER, S., SCHOFFLER, H., STAUDENMAIER, H. & ZIMMERMANN, L. (1987). Iron transport system in *Escherichia coli*. In *Iron Transport in Microbes, Plants and Animals*, pp. 35–51. Edited by G. Winkelmann, D. van der Helm & J. B. Neilands. Weinheim: VCH Publishers.

BURKHARDT, R. & BRAUN, V. (1987). Nucleotide sequence of the *fhuC* and *fhuD* genes involved in iron(III) hydroxamate transport: domains in FhuC homologous to ATP binding proteins. *Molecular and General Genetics* **209**, 49–55.

BYERS, B. R. (1987). Pathogenic iron acquisition. *Life Chemical Report* **4**, 143–159.

BYERS, B. R., MASSAD, G., BARGHOUTI, S. & ARCENAUX, J. E. L. (1991). Iron acquisition in the motile aeromonads: siderophore-dependent and -independent systems. *Experientia* **47**, 416–418.

CHAKRABORTY, T., HUHLE, B., HOF, H., BERGBAUER, H. & GOEBEL, W. (1987). Marker exchange mutagenesis of the aerolysin determinant in *Aeromonas hydrophila* demonstrates the role of aerolysin in *A. hydrophila* associated systemic infections. *Infection and Immunity* **55**, 2274–2280.

CHEESMAN, M. R., THOMSON A. J., GREENWOOD, C., MOORE, G. R. & KADIR, F. (1990). Bis-methionine axial ligation of haem in bacterioferritin from *Pseudomonas aeruginosa*. *Nature, London* **346**, 771–773.

CHEN, M. & CRICHTON, R. R. (1982). Purification and characterization of a bacterioferritin from *Azotobacter chroococcum*. *Biochimica et Biophysica Acta* **707**, 1–6.

CHENAULT, S. S. & EARHART, C. C. F. (1991). Organisation of genes encoding membrane proteins of the *Escherichia coli* ferrienterobactin permease. *Molecular Microbiology* **5**, 1405–1413.

COULTON, J. W., MASON, P. & ALLAT, D. D. (1987). *fhuC* and *fhuD* genes for iron(III)-ferrichrome transport in *Escherichia coli* K-12. *Journal of Bacteriology* **169**, 3844–3849.

COY, M. & NEILANDS, J. B. (1991). Structural dynamics and functional domains of the Fur protein. *Biochemistry* **30**, 8201–8210.

CROSA, J. H. (1980). A plasmid associated with virulence in the marine fish pathogen *Vibrio anguillarum* specifies an iron-sequestering system. *Nature, London* **284**, 566–568.

CROSA, J. H. (1989). Genetics and molecular biology of siderophore mediated iron transport in bacteria. *Microbiological Reviews* **53**, 517–530.

DE LORENZO, V., WEE, S., HERRERO, M. & NEILANDS, J. B. (1987). Operator sequences of the aerobactin operon of plasmid ColV-K30 binding the ferric uptake regulation (*fur*) repressor. *Journal of Bacteriology* **169**, 2624–2630.

DE LORENZO, V., HERRERO, M., GIOVANNINI, F. & NEILANDS, J. B. (1988). Fur (ferric uptake regulation) protein and CAP (catabolite-activator protein) modulate transcription of *fur* gene in *Escherichia coli*. *European Journal of Biochemistry* **173**, 537–546.

DEICH, R. A., METCALF, B. J., FINN, C. W., FARLEY, J. E. & GREEN, B. A. (1988). Cloning of genes encoding a 15,000-dalton peptidoglycan-associated outer membrane lipoprotein and an antigenically related 15,000-dalton protein from *Haemophilus influenzae*. *Journal of Bacteriology* **170**, 489–498.

DYER, D. W., WEST, E. P. & SPARLING, P. F. (1987). Effects of serum carrier proteins on the growth of pathogenic *Neisseria* with haem bound iron. *Infection and Immunity* **55**, 2171–2175.

EARHART, C. F. (1987). Ferrienterobactin transport in *Escherichia coli*. In *Iron Transport in Microbes, Plants and Animals*, pp. 67–84. Edited by G. Winkelmann, D. van der Helm & J. B. Neilands. Weinheim: VCH Publishers.

EICK-HELMERICH, K., HANTKE, K. & BRAUN, V. (1987). Cloning and expression of the *exbB* gene of *Escherichia coli* K12. *Molecular and General Genetics* **206**, 246–251.

ELKINS, M. F. & EARHART, C. F. (1989). Nucleotide sequence and regulation of *Escherichia coli* gene for ferrienterobactin transport protein FepB. *Journal of Bacteriology* **171**, 5443–5451.

ENARD, C., DIOLEZ, A. & EXPERT, D. (1988). Systemic virulence of *Erwinia chrysanthemi* 3937 requires a functional iron assimilation system. *Journal of Bacteriology* **170**, 2419–2426.

ERNST, J. F., BENNETT, R. L. & ROTHFIELD, L. I. (1978). Constitutive expression of the iron enterochelin and ferrichrome uptake systems in a mutant strain of *Salmonella typhimurium*. *Journal of Bacteriology* **135**, 928–934.

FECKER, L. & BRAUN, V. (1983). Cloning and expression of the *fhu* genes involved in iron(III)-hydroxamate uptake by *Escherichia coli*. *Journal of Bacteriology* **156**, 1301–1314.

FERRIS, F. G., TAZAKI, K. & FYFE, W. S. (1989). Iron oxides in acid mine drainage environments and their association with bacteria. *Chemical Geology* **74**, 321–330.

FLITTER, R. W., ROWLEY, D. A. & HALLIWELL, B. (1983). Superoxide dependent formation of hydroxyl radicals in the presence of iron salts. *FEBS Letters* **158**, 310–312.

FRANCIS, R. T., BOOTH, J. W. & BECKER, R. R. (1985). Uptake of iron from hemoglobin and the hemoglobin–haptoglobin complex by hemolytic bacteria. *Journal of Biochemistry* **17**, 767–773.

FRANKEL, R. B., BLAKEMORE, R. P. & WOLFE, R. S. (1979). Magnetite in fresh-water bacteria. *Science* **203**, 1355–1356.

GARIBALDI, J. A. & NEILANDS, J. B. (1956). Formation of iron binding compounds by microorganisms. *Nature, London* **177**, 526–527.

GHIORSE, W. C. (1984). Biology of iron- and manganese-depositing bacteria. *Annual Review of Microbiology* **38**, 515–550.

GIBSON, F. & MAGRATH, D. J. (1969). The isolation and characterization of a hydroxamic acid (aerobactin) formed by *Aerobacter aerogenes* 62-1. *Biochimica et Biophysica Acta* **192**, 175–184.

GORBY, Y. A., BEVERIDGE, T. J. & BLAKEMORE, R. P. (1988). Characterization of the bacterial magnetosome membrane. *Journal of Bacteriology* **170**, 834–841.

GRAY, M. W. (1989). The evolutionary origins of organelles. *Trends in Genetics* **5**, 294–299.

GROSSMAN, M. J., HINTON, S. M., MINAK-BERNERO, V., SLAUGHTER, C. & STIEFEL, E. I. (1992). Unification of the ferritin family of proteins. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 2419–2423.

HALL, R. M. & RATLEDGE, C. (1984). Myobactins as chemotaxonomic characters for some rapidly growing mycobacteria. *Journal of General Microbiology* **130**, 1883–1892.

HANSON, M. S. & HANSEN, E. J. (1991). Molecular cloning, partial purification, and characterization of a haemin-binding lipoprotein from *Haemophilus influenzae* type b. *Molecular Microbiology* **5**, 267–278.

HANTKE, K. (1981). Regulation of ferric iron transport in *E. coli*: isolation of a constitutive mutant. *Molecular and General Genetics* **182**, 288–292.

HANTKE, K. (1984). Cloning of the repressor protein gene of iron regulated system in *E. coli* K12. *Molecular and General Genetics* **197**, 337–341.

HANTKE, K. (1987). Selection procedure for deregulated iron transport mutants (*fur*) in *E. coli* K12: *fur* not only affects iron metabolism. *Molecular and General Genetics* **210**, 135–139.

HANTKE, K. & BRAUN, V. (1978). Functional interaction of the *tonA–tonB* receptor system. *Journal of Bacteriology* **135**, 190–197.

HANTKE, K. & ZIMMERMANN, L. (1981). The importance of the *exbB* gene for vitamin B12 and ferric iron transport. *FEMS Microbiology Letters* **12**, 31–35.

HARDING, R. A. & ROYT, P. W. (1990). Acquisition of iron from citrate by *Pseudomonas aeruginosa*. *Journal of General Microbiology* **136**, 1859–1867.

HARKER, A. R. & WULLSTEIN, L. H. (1985). Evidence of two nonidentical subunits of bacterioferritin from *Azotobacter vinelandii*. *Journal of Bacteriology* **162**, 651–655.

HARKNESS, R. E., CHONG, P. & KLEIN, M. H. (1992). Identification of two iron-repressed periplasmic proteins in *Haemophilus influenzae*. *Journal of Bacteriology* **174**, 2425–2430.

HARRISON, P. M., ANDREWS, S., ARTYMIUK, P. J., FORD, G. C., GUEST, J. R., HIRZMANN, J., LAWSON, D. M., LIVINGSTONE, J. C., SMITH, J. M. A., TREFFRY, A. & YEWDALL, S. J. (1991). Probing structure–function relations in ferritin and bacterioferritin. *Advances in Inorganic Chemistry* **36**, 449–485.

HOWARD, S. P. & BUCKLEY, J. T. (1986). Molecular cloning and expression in *Escherichia coli* of the structural gene for the hemolytic toxin aerolysin from *Aeromonas hydrophila*. *Molecular and General Genetics* **204**, 289–295.

HUSSEIN, S., HANTKE, K. & BRAUN, V. (1981). Citrate-dependent iron transport system in *Escherichia coli* K-12. *European Journal of Biochemistry* **117**, 431–437.

ISHIMARU, C. A. & LOPER, J. E. (1992). High affinity uptake systems present in *Erwinia carotovora* subsp. *carotovora* include the hydroxamate siderophore aerobactin. *Journal of Bacteriology* **174**, 2993–3003.

IZUHARA, M., TAKAMUNE, K. & TAKATA, R. (1991). Cloning and sequencing of an *Escherichia coli* K12 gene which encodes a polypeptide having similarity to the human ferritin H subunit. *Molecular and General Genetics* **225**, 510–513.

KÖSTER, W. L. & BRAUN, V. (1986). Iron hydroxamate transport of *Escherichia coli*: nucleotide sequence of the *fhuB* gene and identification of the protein. *Molecular and General Genetics* **204**, 435–442.

KÖSTER, W. L., ACTIOS, L. A., WALDBESER, L. S., TOLMASKY, M. E. & CROSA, J. H. (1991). Molecular characterization of the iron transport system mediated by the pJM1 plasmid in *Vibrio anguillarum* 775. *Journal of Biological Chemistry* **266**, 23829–23833.

KUROKAWA, T., FUKUMORI, Y. & YAMANAKA, T. (1989). *Nitrobacter winogradskyi* cytochrome *b*-559: a nonhaem iron-containing cytochrome related to bacterioferritin. *Biochimica et Biophysica Acta* **976**, 135–139.

LANKFORD, C. E. (1973). Bacterial assimilation of iron. *Critical Reviews in Microbiology* **2**, 273–331.

LAULHÈRE, J. P., LABOURÉ, A. M., VAN WUYTSWINKEL, O., GAGNON, J. & BRIAT, J. F. (1992). Purification, characterization and function of bacterioferritin from the cyanobacterium *Synechocystis* PCC 6803. *Biochemical Journal* **281**, 785–793.

LESCURE, A. M., PROUDHON, D., PESEY, H., RAGLAND, M., THEIL, E. C. & BRIAT, J. F. (1991). Ferritin gene transcription is regulated by iron in soybean cell cultures. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 8222–8226.

LOPER, J. E. & BUYER, J. S. (1991). Siderophores in microbial interactions on plant surfaces. *Molecular Plant–Microbe Interactions* **4**, 5–13.

MACHAM, L. P. & RATLEDGE, C. (1975). A new group of water-soluble iron-binding compounds from mycobacteria: the exochelins. *Journal of General Microbiology* **89**, 379–382.

MANN, S., FRANKEL, R. B. & BLAKEMORE, R. P. (1984). Structure, morphology and crystal growth of bacterial magnetite. *Nature, London* **310**, 405–407.

MANN, S., BANNISTER, J. V. & WILLIAMS, R. J. P. (1986). Structure and composition of ferritin cores isolated from human spleen, limpet (*Patella vulgata*) hemolymph and bacterial cells (*Pseudomonas aeruginosa*). *Journal of Molecular Biology* **188**, 225–232.

MANN, S., WILLIAMS, J. M., TREFFRY, A. & HARRISON, P. M. (1987). Reconstituted and native iron-cores of bacterioferritin and ferritin. *Journal of Molecular Biology* **198**, 405–416.

MASSAD, G., ARCENEAUX, J. E. L. & BYERS, B. R. (1991). Acquisition of iron from host sources by mesophilic *Aeromonas* species. *Journal of General Microbiology* **137**, 237–241.

MATSUDA, T., ENDO, J., OSSAKABE, N. & TONOMURA, A. (1983). Morphology and structure of biogenic magnetite particles. *Nature, London* **302**, 411–412.

MATZANKE, B. F., MÜLLER, G. I., BILL, E. & TRAUTWEIN, A. X. (1989). Iron metabolism of *Escherichia coli* studied by Mössbauer spectroscopy and biochemical methods. *European Journal of Biochemistry* **183**, 371–379.

MESSENGER, A. J. M. & RATLEDGE, C. (1982). Iron transport in *Mycobacterium smegmatis*: uptake of iron from ferric citrate. *Journal of Bacteriology* **149**, 131–135.

MESSENGER, A. J. M., HALL, R. M. & RATLEDGE, C. (1986). Iron uptake processes in *Mycobacterium vaccae* R877R, A Mycobacterium lacking myobactin. *Journal of General Microbiology* **132**, 845–852.

MICKELSEN, P. A. & SPARLING, P. F. (1981). Ability of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and commensal *Neisseria* species to obtain iron from transferrin and iron compounds. *Infection and Immunity* **33**, 555–564.

MOORE, G. R., MANN, S. & BANNISTER, J. V. (1986). Isolation and properties of the complex nonhaem-iron-containing cytochrome *B-557* (bacterioferritin) from *Pseudomonas aeruginosa*. *Journal of Inorganic Biochemistry* **28**, 329–336

MORTON, D. J. & WILLIAMS, P. (1990). Siderophore-independent acquisition of transferrin-bound iron by *Haemophilus influenzae* type b. *Journal of General Microbiology* **136**, 927–933.

NIEDERHOFFER, E. C., NARANJO, C. M. & FEE, J. A. (1989). Relationship of the superoxide dismutase genes, *sodA* and *sodB*, to the iron uptake (*fur*) regulon in *Escherichia coli* K-12. In *Metal Ion Homeostasis: Molecular Biology and Chemistry*, pp. 149–158. New York: Alan R. Liss.

OZENBERGER, B. A., NAHLIK, M. S. & MCINTOSH, M. A. (1987). Genetic organization of multiple *fep* genes encoding ferric enterobactin transport functions in *Escherichia coli*. *Journal of Bacteriology* **169**, 3638–3646.

PERSMARK, M., EXPERT, D. & NEILANDS, J. B. (1989). Isolation, characterization, and synthesis of chrysobactin, a compound with siderophore activity from *Erwinia chrysanthemi*. *Journal of Biological Chemistry* **264**, 3187–3193.

PIDCOCK, K. A., WOOTEN, J. A., DALEY, B. A. & STULL, T. L. (1988). Iron acquisition by *Haemophilus influenzae*. *Infection and Immunity* **56**, 721–725.

PIERCE, J. R. & EARHART, C. F. (1986). *Escherichia coli* K-12 envelope proteins specifically required for ferrienterobactin uptake. *Journal of Bacteriology* **166**, 930–936.

PIERCE, J. R., PICKETT, C. L. & EARHART, C. F. (1983). Two *fep* genes are required for ferrienterobactin uptake in *Escherichia coli* K-12. *Journal of Bacteriology*, **155**, 330–336.

POSTLE, K. & GOOD, R. F. (1983). DNA sequence of the *Escherichia coli tonB* gene. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 5235–5239.

RAGLAND, M., BRIAT, J. F., GAGNON, J., LAULHÈRE, J. P., MASSENET, O. & THEIL, E. C. (1990). Evidence for a conservation of ferritin sequences among plants and animals and for a transit peptide in soybean. *Journal of Biological Chemistry* **265**, 18339–18344.

RATLEDGE, C. (1984). Metabolism of iron and other metals by mycobacteria. In *The Mycobacteria – A Source Book*, part A, pp. 603–627. Edited by G. P. Kubica & L. G. Wayne. New York: Marcel Dekker.

RATLEDGE, C., MACHAM, L., BROWN, K. A. & MARSHALL, B. J. (1974). Iron transport in *Mycobacterium smegmatis*: a restricted role for salicylic acid in the extracellular environment. *Biochimica et Biophysica Acta* **372**, 39–51.

ROBSON, R. L., EADY, R. R., RICHARDSON, T. H., MILLER, R. W., HAWKINS, M. & POSTGATE, J. M. (1986). The alternative nitrogenase of *Azotobacter chroococcum*. *Nature, London* **322**, 388–390.

SAITO, T. & WILLIAMS, R. J. P. (1991). The binding of the ferric uptake regulation protein to a DNA fragment. *European Journal of Biochemistry* **197**, 43–47.

SAITO, T., WORMALD, M. R. & WILLIAMS, R. J. P. (1991*a*). Some structural features of the iron-uptake regulation protein. *European Journal of Biochemistry* **197**, 29–38.

SAITO, T., DULY, D. & WILLIAMS, R. J. P. (1991*b*). The histidines of the iron uptake regulation protein, Fur. *European Journal of Biochemistry* **197**, 39–42.

SCHAFFER, S., HANTKE, K. & BRAUN, V. (1985). Nucleotide sequence of the iron regulatory gene *fur*. *Molecular and General Genetics* **201**, 204–212.

SCHRYVERS, A. B. (1989). Identification of the transferrin- and lactoferrin-binding proteins in *Haemophilus influenzae*. *Journal of Medical Microbiology* **29**, 121–130.

SECKBACH, S. (1982). Ferreting out the secrets of plant ferritin – a review. *Journal of Plant Nutrition* **5**, 369–394.

SHEA, C. M. & MCINTOSH, M. A. (1991). Nucleotide sequence and genetic organization of the ferric enterobactin transport system: homology to other periplasmic binding protein-dependent systems in *Escherichia coli*. *Molecular Microbiology* **5**, 1415–1428.

SIBILLE, J. C., CIRIOLO, M., KONDO, H., CRICHTON, R. R. & AISEN, P. (1989). Subcellular localization of ferritin and iron taken up by rat hepatocytes. *Biochemical Journal* **262**, 685–688.

SMITH, J. M. A., QUIRK, A. V., PLANK, R. W. H., DIFFIN, F. M., FORD, G. C. & HARRISON, P. M. (1988). The identity of *Escherichia coli* bacterioferritin and cytochrome *b*-1. *Biochemical Journal* **255**, 737–740.

SMITH, J. M. A., FORD, G. C., HARRISON, P. M., YARIV, J. & KALB (GILBOA), A. J. (1989). Molecular size and symmetry of the bacterioferritin of *Escherichia coli*. X-ray crystallographic characterization of four crystal forms. *Journal of Molecular Biology* **205**, 465–467.

STAGGS, T. M. & PERRY, R. D. (1991). Identification and cloning of a *fur* regulatory gene in *Yersinia pestis*. *Journal of Bacteriology* **173**, 417–425.

STAUDENMAIER, J., VAN HOVE, B., YARAGHI, Z. & BRAUN, V. (1989). Nucleotide sequences of the *fecBCDE* genes and locations of the proteins suggest a periplasmic-binding-protein-dependent transport mechanism for iron(III) dicitrate in *Escherichia coli*. *Journal of Bacteriology* **177**, 2626–2633.

STIEFEL, E. I. & WATT, G. D. (1979). *Azotobacter* cytochrome b557.5 is a bacterioferritin. *Nature, London* **279**, 81–83.

STULL, T. L. (1987). Protein sources of haem for *Haemophilus influenzae*. *Infection and Immunity* **55**, 148–153.

TOLMASKY, M. E. & CROSA, J. H. (1984). Molecular cloning and expression of genetic determinants for the iron uptake system mediated by the *Vibrio anguillarum* plasmid pJM1. *Journal of Bacteriology* **160**, 860–866.

TOLMASKY, M. E., ACTIS, L. A. & CROSA, J. H. (1988). Genetic analysis of the iron uptake region of the *Vibrio anguillarum* plasmid pJM1: molecular cloning of genetic determinants encoding a novel *trans* activator of siderophore biosynthesis. *Journal of Bacteriology* **170**, 1913–1919.

UNDERWOOD, E. J. (1971). *Trace Elements in Human and Animal Nutrition*, 3rd edn, p. 15. New York: Academic Press.

VALVANO, M. A. & CROSA, J. H. (1984). Aerobactin iron transport genes commonly encoded by certain ColV plasmids occur in the chromosome of a human invasive strain of *Escherichia coli* K1. *Infection and Immunity* **46**, 159–167.

VALVANO, M. A. & CROSA, J. H. (1988). Molecular cloning, expression, and regulation in *Escherichia coli* K12 of a chromosome-mediated aerobactin iron transport system from a human invasive strain of *E. coli* K1. *Journal of Bacteriology*, **170**, 5529–5538.

VALVANO, M. A., SILVER, R. P. & CROSA J. H. (1986). Occurrence of chromosome- or plasmid-mediated aerobactin iron transport systems and hemolysin production among clonal groups of human invasive strains of *Escherichia coli* K1. *Infection and Immunity* **52**, 192–199.

WEAVER, J. & POLLACK, S. (1990). Two types of receptors for iron on mitochondria. *Biochemical Journal* **271**, 463–466.

WEE, S., NEILANDS, J. B., BITTNER, M. L., HEMMING, B. C., HAYMORE, B. L. & SEETHARAM, R. (1988). Expression, isolation and properties of Fur (ferric uptake regulation) protein of *Escherichia coli* K12. *Biology of Metals* **1**, 62–67.

WILLIAMS, P., MORTON, D. J., TOWNER, K. J., STEVENSON, P. & GRIFFITHS, E. (1990). Utilization of enterobactin and other exogenous iron sources by *Haemophilus influenzae*, *H. parainfluenzae* and *H. paraphrophilus*. *Journal of General Microbiology* **136**, 2343–2350.

YARIV, J., KALB, A. J., SPERLING, R., BAUMINGER, E. R., COHEN, S. G. & OFER, S. (1981). The composition and the structure of bacterioferritin of *Escherichia coli*. *Biochemical Journal* **197**, 771–775.

ZAKARIA-MEEHAN, Z., MASSAD, G., SIMPSON, L. M., TRAVIS J. C. & OLIVER, J. D. (1988). Ability of *Vibrio vulnificus* to obtain iron from hemoglobin-haptoglobin complexes. *Infection and Immunity* **56**, 275–277.

ZIMMERMANN, L., HANTKE, K. & BRAUN, V. (1984). Exogenous induction of the iron dicitrate transport system of *Escherichia coli* K12. *Journal of Bacteriology* **159**, 271–277.

ZIMMERMANN, L., ANGERER, A. & BRAUN, V. (1989). Mechanistically novel iron(III) transport system in *Serratia marcescens*. *Journal of Bacteriology* **171**, 238–243.