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INVITED REVIEW

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

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Abstract Siderophore production and utilization is one of the major strategies deployed by bacteria to get access to iron, a key nutrient for bacterial growth. The biological function of siderophores is to solubilize iron in the bacterial environment and to shuttle it back to the cytoplasm of the microorganisms. This uptake process for Gram-negative species involves TonB-dependent transporters for translocation across the outer membranes. In *Escherichia coli* and many other Gram-negative bacteria, ABC transporters associated with periplasmic binding proteins import ferrisiderophores across cytoplasmic membranes. Recent data reveal that in some siderophore pathways, this step can also be carried out by proton-motive force-dependent permeases, for example the ferrichrome and ferripyochelin pathways in *Pseudomonas aeruginosa*. Iron is then released from the siderophores in the bacterial cytoplasm by different enzymatic mechanisms depending on the nature of the siderophore. Another strategy has been reported for the pyoverdine pathway in *P. aeruginosa*: iron is released from the siderophore in the periplasm and only siderophore-free iron is transported into the cytoplasm by an ABC transporter having two atypical periplasmic binding proteins. This review presents recent findings concerning both ferrisiderophore and siderophore-free iron transport across bacterial cytoplasmic membranes and considers current knowledge about the mechanisms involved in iron release from siderophores.

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Introduction

Iron is absolutely required by almost all living organisms, because it is a cofactor for a large number of important enzymes, involved in many fundamental cellular processes, including electron transfer, cell respiration, and superoxide metabolism. Although iron is extremely abundant on Earth, the solubility of iron is very low at physiological pH in aerobic environments: the presence of oxygen rapidly oxidizes iron(II) into insoluble ferric oxyhydroxides with a solubility product of 10^{-39}. Iron in the environment of microorganisms infecting a host is in complex with biological macromolecules such as hemes, metalloenzymes, ferritins (iron storage proteins), or transferrin and lactoferrin (two proteins involved in iron transport). The concentration of free iron at neutral pH in human and animal body fluids is estimated to be about 10^{-18} M (Raymond et al. 2003). Indeed, the free iron concentration is far too low to support bacterial growth in most microorganism biotopes.

To survive and compete, microorganisms evolved multiple means of obtaining iron and these systems are essential for bacterial pathogenicity (Miethke and Marahiel 2007; Schalk 2013). The most diverse and broadly distributed iron uptake mechanisms used by microorganisms are ferrisiderophore acquisition systems. Siderophores are small organic chelators (molecular weight between 150 and 2000 Da) with a very high affinity for iron (Hider and Kong 2011a). They are synthesized by bacteria and secreted into their environment, where they efficiently solubilize and chelate iron (Hider and Kong 2011a). Once

the ferrisiderophore complex is formed, it is shuttled back into the bacteria via specific transport proteins. To reach the cytoplasm of Gram-negative bacteria, ferrisiderophore complexes have to cross both the outer and cytoplasmic (inner) membranes and the periplasm. Uptake across the first membrane involves TonB-dependent transporters (TBDT), and across the inner membrane ABC transporters associated with periplasmic binding proteins (PBP) or proton-motive force-dependent permeases. The molecular mechanisms involved in the translocation of ferrisiderophores across the outer membranes (OMs) of Gram-negative bacteria have been the subject of many reviews the last decade and will not be discussed here in detail [for the more recent reviews see (Krewulak and Vogel 2008; Fairman et al. 2011; Schalk et al. 2012)]. The present review focuses on the fate of ferrisiderophores in the bacterial periplasm and cytoplasm after import across the outer membranes. In particular, the wide diversity of ferrisiderophore outcomes and its dependence on the nature of the chelators and the bacteria species are illustrated.

Siderophores

More than 500 different siderophores have now been identified with very different chemical structures (Hider and Kong 2011a). Hexacoordinate complexes dominate iron coordination chemistry, so it is not surprising that the hexadentate structure is the most common siderophore form. The coordination ligands are mostly hydroxamate and catechol functions, attached to either linear or cyclic scaffolds, to form a hexadentate structure (Fig. 1). Siderophores with lower denticity (tetradentate, tridentate, and bidentate) are also produced by microorganisms, but these chelators cannot alone achieve full Fe(III) coordination, and complexes with higher siderophore:Fe(III) stoichiometry have been described (Spasojevic et al. 2001). In general, hexadentate siderophores have a much higher affinity for Fe(III) than do tetradentate siderophores, which have a higher iron affinity than bidentate siderophores (Albrecht-Gary and Crumbliss 1998). The two strongest chelators are the triscatecholate-trilactone derivatives bacillibactin and enterobactin, which have formation constants of 10^{48} and 10^{49} M^{-1}, respectively (Loomis and Raymond 1991; Dertz et al. 2006). All siderophores possess a higher affinity for iron(III) than for iron(II). Recent work has also shown that siderophores are able to chelate metals other than iron and play a key role in bacterial metal tolerance (Braud et al. 2009a, b; Schalk et al. 2011; Hannauer et al. 2012). For many siderophores, a large range of closely related structures have been reported. For example, more than 60 analogs of pyoverdines (Fuchs et al. 2001), 21 analogs of desferrichrome, 21 analogs of enterobactin, and 20 analogs of ferrioxamine have been described (Hider and Kong 2011a).

To adapt to variable environmental conditions, many microorganisms produce more than one siderophore and are able to use several others (xenosiderophores) produced by other microorganisms present in their environment. To exploit xenosiderophores, the bacteria only express the import proteins necessary to capture these ferrisiderophores from their environment and no siderophore biosynthesis occurs. For example, *Pseudomonas aeruginosa*, a Gram-negative bacterium, produces two major siderophores, pyoverdine and pyochelin (Fig. 1), but are able to use at least five heterologous siderophores: cepabactin, ferrichome, enterobactin, ferrioxamine, and citrate (Poole and McKay 2003; Llamas et al. 2006; Schalk 2008; Hannauer et al. 2010). Bacteria probably produce and use multiple siderophores to be more competitive in their different biotopes and the strategies employed are undoubtedly driven by evolution (Lee et al. 2012). The complexity of these strategies also illustrates the high importance of iron for microorganisms.

Translocation across the outer membrane by TonB-dependent transporters

Ferrisiderophore complexes are recognized at the cell surface of Gram-negative bacteria by specific outer membrane transporters called TonB-dependent transporters (TBDT; for reviews on these proteins see (Krewulak and Vogel 2008; Fairman et al. 2011; Schalk et al. 2012). Very briefly, the biological function of TBDT is to import siderophore-iron complexes from the extracellular medium into the periplasm. They are composed of a 22-stranded antiparallel transmembrane β-barrel (Figs. 2, 3), and the lumen of the barrel is filled with the N-terminal globular domain (called the plug, hatch, or cork) (Schalk et al. 2012). The binding site is always located on the extracellular face of the transporter, exposed to the solvent, and is constituted of residues of both the plug and the β-barrel domains. The proton-motive force of the inner membrane provides the energy necessary for translocation of ferrisiderophores through the lumen of TBDT (Schalk et al. 2012). This energy is transferred to TBDT in the outer membrane by the TonB complex (hence the name TonB-dependent transporters), which although in the inner membrane spans the periplasm.

Each TBDT recognizes and transports a specific siderophore, or in some cases, a few structurally related siderophores, but never siderophores with different chemical structures (Ferguson et al. 2000; Mislin et al. 2006; Greenwald et al. 2009; Hoegy et al. 2009, 2010). This is associated with a strong correlation between the number of

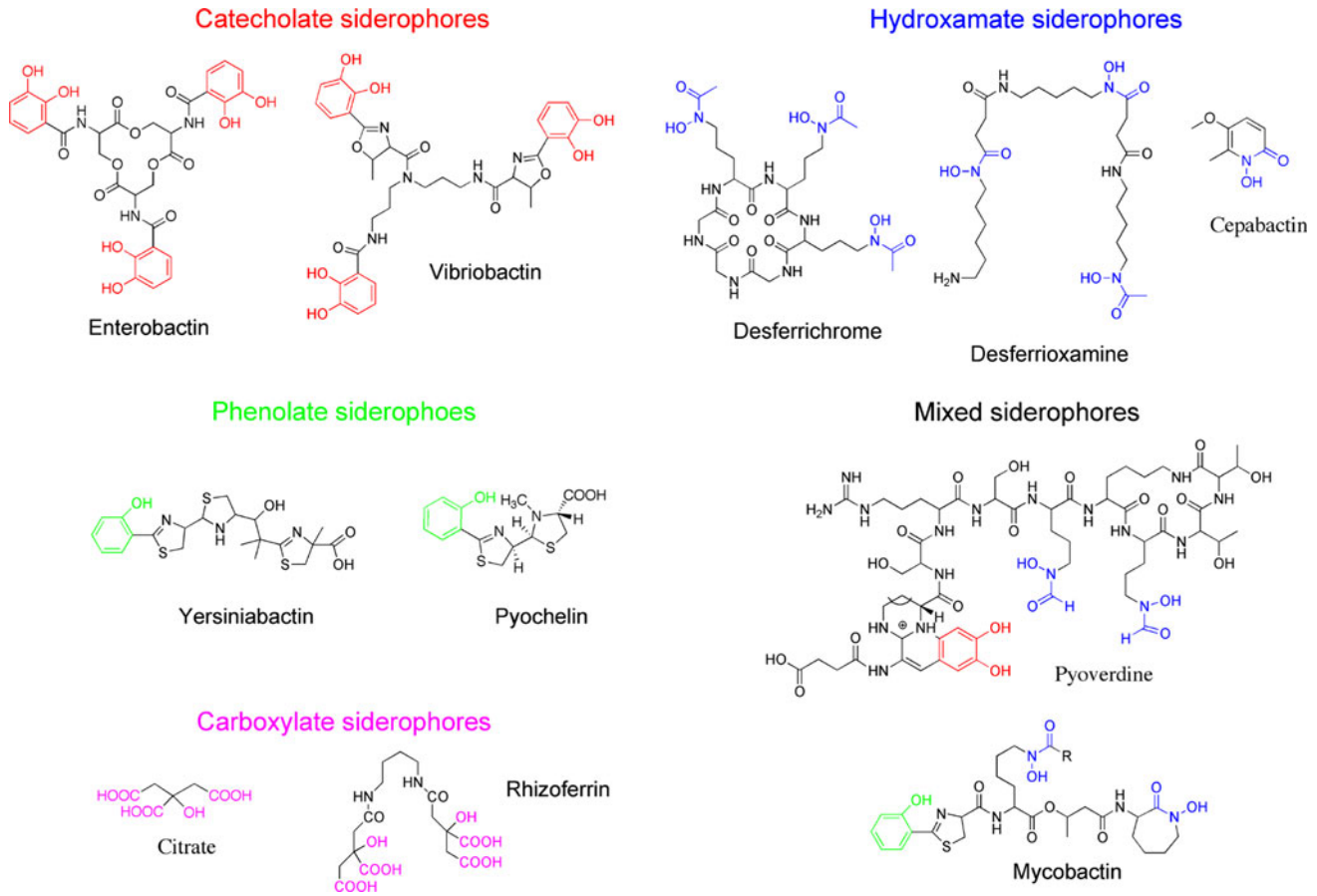

Fig. 1 Examples of siderophores

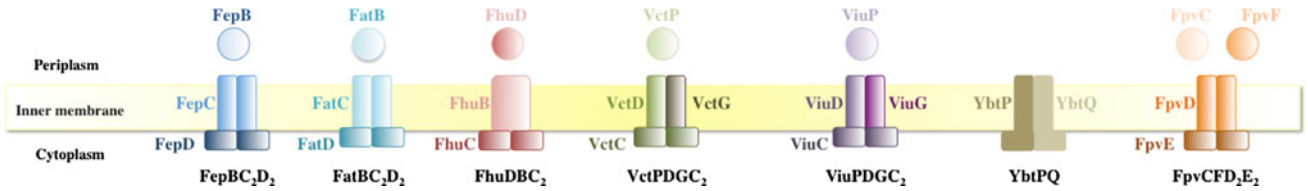

Fig. 2 ABC transporters involved in iron uptake by siderophores in Gram-negative bacteria. $FepBC_2D_2$ is involved in enterobactin pathway in *E. coli*; $FatBC_2D_2$ in enterobactin pathway in *V. anguillarum*; $FhuDBC_2$ in ferrichrome pathway in *E. coli*; $VctPDGC_2$ and $ViuPDGC_2$ in vibriobactin and enterobactin pathways in *V. cholera*; YbtPQ in yersiniabactin pathway in *Y. pestis* and $FpvCFD_2E_2$ in pyoverdine pathway in *P. aeruginosa*

siderophores able to be used by a bacterium and the number of genes encoding iron-regulated TBDTs in its genome. Genome sequencing has revealed that many bacteria have numerous genes encoding these transporters (Blanvillain et al. 2007; Schauer et al. 2008). In the *P. aeruginosa* genome, there are 11 genes coding for TBDTs and involved in iron uptake (Llamas et al. 2006), and *Escherichia coli* has eight genes coding for siderophore-iron TBDTs.

Translocation of ferrisiderophores across the cytoplasmic membrane by ABC transporters

In most reported iron uptake mechanisms, once the ferrisiderophore is in the periplasm, it binds to the siderophore-periplasmic binding protein (PBP) component of an ATP-binding cassette (ABC) transporter. The ferrisiderophore is then imported into the cytoplasm via interaction of the ferrisiderophore-PBP complex with the permease

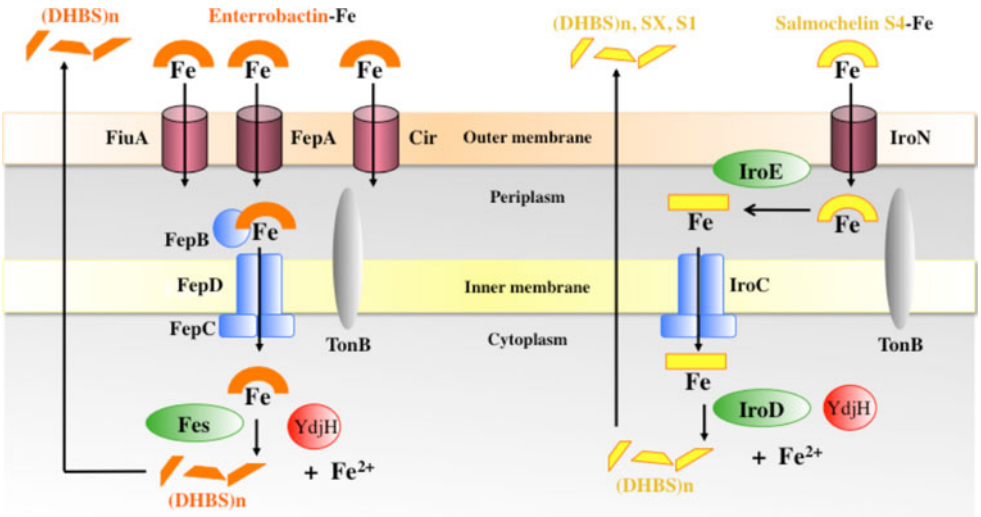

Fig. 3 Enterobactin pathway in *E. coli* and Salmochelin pathway in *E. coli*. TBDT transporters are represented in *pink*, the TonB proteins in *grey*, ferrisiderophore import ABC transporters with the corresponding PBP in blue, enzymes involved in siderophore hydrolysis in *green* and in iron reduction in *red*. For details and an explanation, refer to the text

components of the ABC transporter (Hvorup et al. 2007). ATP hydrolysis is coupled to this transport.

Bacterial ABC transporters involved in ferrisiderophore import commonly consist of five structural domains (Fig. 2): a periplasmic binding protein, two transmembrane polypeptides that form a channel through which the ferrisiderophore passes through, and two nucleotide binding subunits that hydrolyse ATP. These permeases and ATPases are usually assembled from four separate polypeptides. This is the case of the ferrienterobactin ABC transporter in *E. coli*, $FepBC_2D_2$ (FepB being the PBP, and the dimers $FepC_2$ and $FepD_2$ forming the permease and the ATPAses, respectively) (Shea and McIntosh 1991) (Fig. 3), and the ferrienterobactin ABC transporter from *Vibrio anguillarum*, $FatBC_2D_2$ (FatB, PBP; $FatC_2$, permease and $FatD_2$, ATPase). Nevertheless, some ABC transporters have a different oligomeric organization (Fig. 2). In the hydroxamate siderophore ABC transporter from *E. coli*, $FhuDBC_2$, the FhuB dimer is fused into one polypeptide chain forming the permease, but like the other ATP-binding domains, two copies of FhuC assemble to form a dimer and FhuB is the PBP (Mademidis et al. 1997; Mademidis and Koster 1998). Moreover, the two ABC transporters involved in iron acquisition by vibriobactin and enterobactin in *Vibrio cholera* have the following stoichiometry: $VctPDGC_2$ and $ViuPDGC_2$ (Wyckoff et al. 2007; Wyckoff and Payne 2011). Both systems consist of a monomeric PBP (VctP and ViuP) which delivers the ferrisiderophore to the permease complex composed of two distinct integral membrane permease proteins (VctDG and ViuDG) and two identical ATPases (VctC and ViuC). Another atypical ABC transporter is YbtPQ involved in iron uptake by yersiniabactin, a phenolate-thiazoline siderophore that is related to pyochelin in *Yersinia pestis* (Perry and Fetherston 2011). YbtP and YbtQ are similar inner membrane proteins, and both polypeptides contain an N-terminal membrane-spanning domain corresponding to the permease moiety and a C-terminal ATPase; the two probably function as a heterodimer in the transport of ferriyersiniabactin (Fetherston et al. 1999; Perry and Fetherston 2011). It is still unclear whether a PBP is required for this uptake system: there is no identifiable candidate within the yersiniabactin locus. At last, $FpvCFD_2E_2$, an ABC transporter involved in iron acquisition by pyoverdine in *P. aeruginosa*, has two PBPs, FpvC, and FpvF (Brillet et al. 2012). Sequence alignments clearly show that FpvC and FpvF belong to two different sub-groups of PBPs (Brillet et al. 2012). FpvC appears to be a metal-binding PBP, whereas FpvF has homology with ferrisiderophore binding PBPs. In vivo cross-linking assays and incubation of purified FpvC and FpvF resulted in the formation of complexes between the two proteins (Brillet et al. 2012). These complexes were able to bind ferripyoverdine in vitro.

All the ATP-binding domains of all these ABC transporters contain a Walker A motif (GxxGxGKS/T where x can be any amino acid), the Walker B motif (hhhD where h is a hydrophobic amino acid), the signature sequence that is unique to the ABC transporter family (LSGGQQ/R/KQR), the switch region that contains a His residue, and the Q-loop that has a conserved Gln (Krewulak and Vogel 2008). BtuBCD, the vitamin B12 importer from *E. coli* (Borths et al. 2002; Locher et al. 2002), and HmuUV, the heme ABC transporter in *Y. pestis* (Woo et al. 2012), are currently the only ABC transporters for which there are high-resolution visualizations of the structure. So far, no ABC transporter involved in ferrisiderophore import has been crystallized or the structure solved. However, the structures of two PBPs have been solved: FhuD, the ferrichrome PBP in *E. coli* (Clarke et al. 2000, 2002); and CeuE, the ferrienterobactin PBP in *Campylobacter jejuni* (Muller et al. 2006). They are composed of two lobes

separated by a deep cleft that harbors the substrate-binding site [for reviews on the structures of these proteins see (Krewulak and Vogel 2008; Chu and Vogel 2011)].

Ferrisiderophore transport across the cytoplasmic membrane exhibits less specificity than that across the outer membrane, and PBP-dependent ABC transporters may have some flexibility in their ligand specificities. The ferric hydroxamate uptake system of *E. coli* facilitates the transport of various hydroxamate siderophores, including ferrichrome, coprogen, ferrioxamine B, and aerobactin, each of which requires its cognate TBDT at the outer membrane (FhuA, FhuE, FhuF, and Iut, respectively) (Braun et al. 1998). FepB, the PBP of the ABC transporter FepBCD in *E. coli*, transports both ferrienterobactin and ferridihydroxybenzylserine across the inner membrane (Elkins and Earhart 1989; Stephens et al. 1995). By contrast, bacteria may also express two ABC transporters for just one siderophore. It is the case of *Vibrio cholerae*, which, to import iron, uses the catechol siderophores vibriobactin (Wyckoff et al. 2007) that is synthesized and secreted, and enterobactin, a xenosiderophore (Mey et al. 2002). Ferrivibriobactin is transported across the outer membrane by its TBDT ViuA (Butterton and Calderwood 1994) and ferrienterobactin by VctA or IrgA (Mey et al. 2002). These siderophores are transported across the inner membrane by one of two periplasmic binding protein-dependent ABC transporters, $VctPDGC_2$ or $ViuPDGC_2$ (Mey et al. 2002), and not just by one as for the siderophore pathways in *E. coli*.

Translocation of ferrisiderophores across the inner membrane by proton-motive force-dependent permeases

There is recent evidence that not only ABC transporters but also permeases are involved in ferrisiderophore translocation across the cytoplasmic membranes. Cuiv et al. (2004) were the first to demonstrate, through various different approaches, that the permease RhtX alone is involved in the transport of ferrirhizobactin 1021, a hydroxamate siderophore produced by *Sinorhizobium meliloti*. Its gene, *rhtX*, maps in the region encoding rhizobactin 1021 biosynthesis genes in *S. meliloti*, and this chromosomal locus has no obvious import ABC transporter potentially involved in ferrisiderophore translocation across the inner membrane. The second example in the literature of a permease involved in ferrisiderophore transport across the inner membrane is FptX for the uptake of ferripyochelin by *P. aeruginosa*: *fptX* maps close to the gene cluster encoding enzymes for pyochelin biosynthesis and ferripyochelin import across the outer membrane. In this region, there is no confirmed cytoplasmic membrane transporter of the ABC transporter family. Mutation of *fptX* by allelic replacement delayed, but did not entirely abolish, pyochelin utilization (Michel et al. 2007). It appears that, in the absence of FptX, ferripyochelin may enter the cytoplasm by an alternative transporter or accumulates in the periplasm with a slower kinetic. By contrast, the uptake of iron by enantiopyochelin [an enantiomer of the *P. aeruginosa* siderophore pyochelin (Youard et al. 2007; Hoegy et al. 2009; Brillet et al. 2011)] into the cytoplasm of *Pseudomonas fluorescens* was found to involve a classical PBP-dependent ABC transporter ($FetCD_2E_2$) (Reimmann 2012). Another example is FiuB for the uptake of ferrichrome across the inner membrane in *P. aeruginosa* (Hannauer et al. 2010). In *E. coli*, the ABC transporter $FhuDBC_2$ is responsible for this step (Mademidis et al. 1997; Mademidis and Koster 1998), but no homologue of this ABC transporter could be found in the *P. aeruginosa* genome. Mutation of FiuB permease completely abolished ferrichrome uptake (Hannauer et al. 2010). Finally, YbtX in *Y. pestis* also shares similarities with RhtX, FiuB, and FptX (Fetherston et al. 1999; Perry and Fetherston 2011); however, a *Y. pestis fbtX* mutant is not defective in iron uptake via yersiniabactin siderophore (Fetherston et al. 1999; Perry and Fetherston 2011). Note that in such mutants, the YbtPQ ABC transporter also encoded at the same locus may perform this step of the transport in the absence of YbtX. Therefore, the function of YbtX remains unclear.

RhtX, FptX, and FiuB appear to be members of a novel family of permeases that function as single-subunit transporters of siderophores at the inner membrane to facilitate the uptake of ferrisiderophore complexes. They are found in a variety of species, including *S. meliloti* and *P. aeruginosa*. These transporters show similarity to a number of uncharacterized proteins, which are all encoded proximal to genes that are either known to be or predicted to be involved in iron acquisition (Cuiv et al. 2004). It is striking that none of the members of this novel family of permeases has associated proteins that function as chaperones of the ferrisiderophores across the periplasm. In these iron uptake pathways, the ferrisiderophore complexes must be either transferred directly from the TBDTs to the inner membrane permeases or diffuse freely in the periplasm before being caught by the permease-binding site. These permeases also share similarity with the permeases of the AmpG family from *E. coli*. AmpG is a permease for muropeptides involved in cell wall recycling and that generates signal molecules for the induction of β-lactamase; it is dependent on the proton-motive force (Jacobs et al. 1994; Cheng and Park 2002). No such proton-motive force-dependence has been demonstrated for RhtX, FptX, or FiuB. Clearly, further investigations are necessary for this permease family, and in particular, to assess their dependence on the proton-motive force, to elucidate the mechanism of transfer of

ferrisiderophores across the periplasm and the inner membrane, and to identify other siderophore pathways using these transporters.

Mechanisms of iron release from siderophores

Ferrisiderophore complexes are thermodynamically very stable, and the mechanism of iron release from siderophores is a complex issue. Three mechanisms have been proposed: hydrolysis of the siderophore, proton-assisted dissociation of the complex, and reduction of the metal center [siderophores having a lower affinity for Fe(II) than for Fe(III)] (Barchini and Cowart 1996; Albrecht-Gary and Crumbliss 1998; Dhungana et al. 2005). Siderophore hydrolysis has a major disadvantage: the metabolic cost associated with the constant production of siderophores by bacteria is substantial. A proton-assisted mechanism for iron release would involve an extremely low pH, and this is not consistent with the intracellular pH of bacteria. The iron reduction model faces the problem that the redox potentials of many siderophores are much more negative than those of most biological reducing agents, including ascorbate, glutathione, and NADH (Creutz 1981; Williams and Yandel 1982; Millis et al. 1993); consequently, iron reduction for ferrisiderophore dissociation would have to involve specific bacterial reductases or a strong Fe(II) chelator, which could shift the redox potential of the siderophore complex to a more positive value or provide a thermodynamic driving force for reduction through the formation of a highly stable Fe^{2+} complex (Mies et al. 2006; Harrington and Crumbliss 2009). Nevertheless, this has been confirmed by in vitro analysis of the dissociation of ferrioxamine B by glutathione or ascorbate in the presence of the iron(II) chelator, bathophenanthroline sulfonate (BPDS) (Mies et al. 2006). In this study, a mechanism was proposed in which a ternary complex is formed between ferrioxamine B and BPDS in a rapid pre-equilibrium step, followed by a rate-limiting reduction of the ternary complex by glutathione or ascorbate. Reduction is followed by a rapid ligand exchange step by which iron is released from ferrioxamine B to form a Fe(II)-$(BPDS)_3$. The existence of this mechanism in vivo has not yet been demonstrated. Indeed, bacterial ferrisiderophore dissociation mechanisms have received little attention until now and only a very few siderophore pathways have been investigated: those involving the siderophores enterobactin, salmochelin, ferrichrome, and their analogs. These studies indicate that iron is released either by hydrolysis, or by modification of the siderophore scaffold, and/or by reduction of the coordinated ferric iron; they also reveal that this can occur in either the cytoplasm or the periplasm, depending on the siderophore pathway.

Ferrisiderophore dissociation in the bacterial cytoplasm

For most iron uptake pathways, iron is released from siderophores in the cytoplasm after translocation of the ferrisiderophore complexes across the cytoplasmic membrane by either ABC transporters or permeases. The efficiency of reduction is largely dependent on the ferrisiderophore redox potentials (see above), which differ enormously between different structural siderophore classes and according to local proton activity and binding equilibria of oxidized and reduced species. For the ferrienterobactin pathway in *E. coli* (Fig. 3), after translocation of ferrienterobactin across the outer and inner membranes by FepA or FiuA and Cir (TBDTs) and $FepBC_2D_2$ (ABC transporter), a cytoplasmic esterase, Fes, hydrolyzes the siderophore to release the metal ion (Brickman and McIntosh 1992). Equivalent enzymes, IroE and IroD, have been identified in *E. coli* and *Salmonella* for the uptake of iron via salmochelin S4, a double C-glucosylated enterobactin (Lin et al. 2005; Zhu et al. 2005). Ferrisalmochelin S4 is taken up by *E. coli* by IroN (TBDT) (Fig. 3) (Hantke et al. 2003) and may be cleaved in the periplasm by IroE, a periplasmic hydrolase, to form the linear trimer, ferrisalmochelin S2, which is taken up by IroC (Zhu et al. 2005). In the cytoplasm, ferrisalmochelin S2 binds IroD and is cleaved to give the dimer salmochelin S1, the monomer salmochelin and 2,3-dihydroxybenzoylserine (Zhu et al. 2005). It also has been suggested that periplasmic IroE only hydrolases salmochelin and that the apo siderophore is exported (Lin et al. 2005). Further work is necessary to identify the exact role of IroE. *Bacillus subtilis*, a Gram-positive bacterium, secretes the cathecholic trilactone siderophore bacillibactin for ferric iron scavenging. BesA (previously called Yuil) was the first trilactone hydrolase to be described in a Gram-positive bacterium: it catalyzes ferribacillin hydrolysis leading to cytosolic iron release (Miethke et al. 2006; Abergel et al. 2009). Recent data suggest that this cathecholate siderophore hydrolysis may be associated with iron reduction, at least in *E. coli*, by the NADPH-dependent reductase YdjH (Miethke et al. 2011). Proteins of this family are widespread among bacteria and often associated with siderophore utilization. YdjH can catalyze the release of iron from a variety of iron chelators, including ferritricatecholates and ferridicitrate, displaying the greatest efficiency for the hydrolyzed ferrienterobactin complex ferri(2,3-dihydroxybenzoylserine)$_3$ (Miethke et al. 2011). In this case, YqjH catalyzes reductive iron release in a step that directly follows the trilactone backbone hydrolysis of ferric enterobactin (Miethke et al. 2011).

In *E. coli*, iron release from desferrichrome (Fig. 4a), coprogen and ferrioxamine also occurs in the cytoplasm and involves iron reduction by the enzyme FhuE (Matzanke

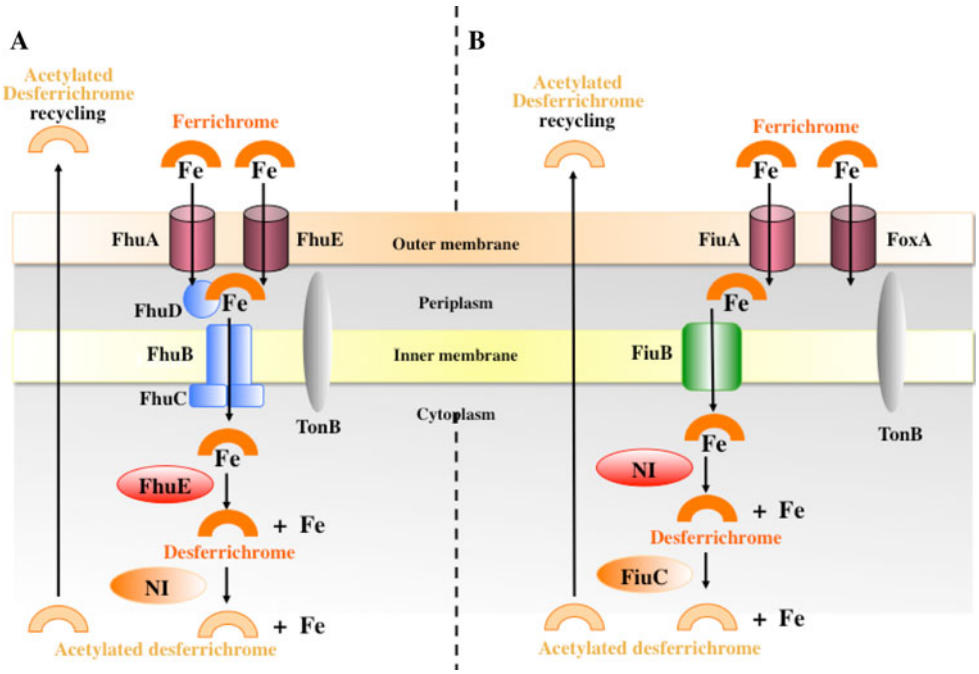

Fig. 4 Ferrichrome pathway in *E. coli* and in *P. aeruginosa*. All TBDT transporters are represented in *pink*, the TonB proteins in *grey*, ferrisiderophore import ABC transporters with the corresponding PBP in *blue* and permeases in *green*, enzymes involved in siderophore acetylation in orange and in iron reduction in *red*. For details and an explanation, refer to the text. *NI* non-identified

et al. 2004). Iron reduction and dissociation from desferrichrome is followed by acetylation of the siderophore to decrease its affinity for iron, and its excretion into the growth medium (Hartman and Braun 1980). The amino acid sequence of FhuE does not show significant similarities to sequences of any other known proteins. However, in *P. aeruginosa*, *Rhizobium leguminosarium* and *Rhizobium meliloti*, homologues of the FhuE gene are found (Capela et al. 2001; Llamas et al. 2006). In *P. aeruginosa* (Fig. 4b), as in *E. coli*, iron dissociation from desferrichrome involves acetylation by FiuC and probably iron reduction (Hannauer et al. 2010). Like in *E. coli*, acetylated desferrichrome is recycled into the extracellular medium by an unknown mechanism (Hannauer et al. 2010).

All these different strategies to release iron from the siderophore in the bacterial cytoplasm seem to require both an iron reduction step and inactivation or complete hydrolysis of the siderophore to prevent metal chelation in the cytoplasm.

Ferrisiderophore dissociation in bacterial periplasm

Iron release from the siderophore pyoverdine has been observed in the periplasm in the case of *P. aeruginosa* (Fig. 5): the iron is released from the chelator by a process involving no chemical degradation or modification of pyoverdine, but apparently based on iron reduction (Greenwald et al. 2007; Yeterian et al. 2010). Apo pyoverdine is then recycled from the periplasm into the extracellular medium by the efflux pump PvdRTOpmQ and is able to chelate another iron ion in the microorganism's environment (Schalk et al. 2002; Imperi et al. 2009; Yeterian et al. 2010). This system has the advantage of reutilizing intact siderophores and, thus, avoiding the substrate and energy costs associated with their de novo biosynthesis. The involvement of a reduction step was shown by work with a pyoverdine-Ga complex (Greenwald et al. 2007; Yeterian et al. 2010). Gallium has the same coordination as iron but only exists in the Ga(III) form and, therefore, cannot be reduced. The pyoverdine-Ga complex accumulated in the bacterial periplasm when incubated with *P. aeruginosa* and no metal-siderophore dissociation or pyoverdine recycling into the extracellular medium were observed; this suggests that with iron, there is a reduction step allowing metal release from the pyoverdine-Fe(III) complex. The proteins involved in this iron reduction have not been identified. However, an ABC transporter $FpvCFD_2E_2$ with two PBPs (FpvC and FpvF, already described above, Fig. 2) have been shown to be involved in this iron uptake pathway (Brillet et al. 2012). Deletion of $FpvCFD_2E_2$ partially inhibited cytoplasmic uptake of ^{55}Fe in the presence of pyoverdine and markedly slowed the in vivo kinetics of iron release from the siderophore. Since FpvC appears to be a metal-binding PBP whereas FpvF has homology with ferrisiderophore binding PBPs, the current model is that both FpvC and FpvF participate in the periplasmic dissociation of ferripyoverdine: there may be a dissociation mechanism in which FpvC-FpvF-ferripyoverdine dissociates into FpvC-Fe and FpvF-pyoverdine, and then $FpvD_2E_2$ transports siderophore-free iron across the inner membrane. In support of this model, mass-

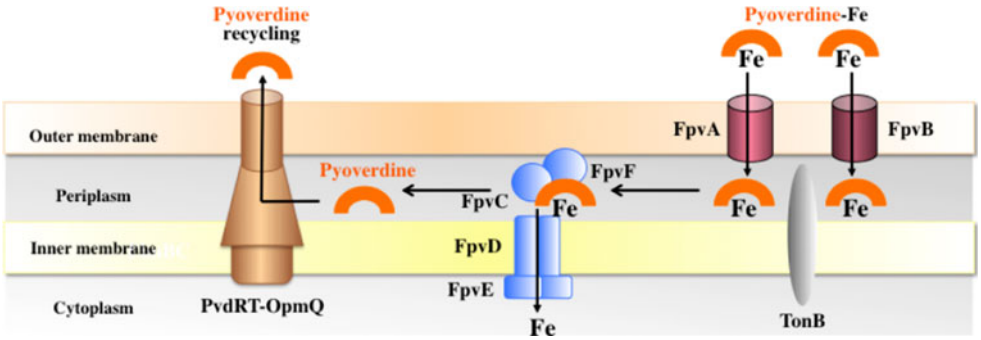

Fig. 5 Pyoverdine pathway in *P. aeruginosa*. TBDT transporters are represented in pink, the TonB proteins in *grey*, ferrisiderophore import ABC transporters with the corresponding PBPs in *blue* and the siderophore efflux system in *brown*. For details and an explanation, refer to the text

spectrometry analyses clearly showed that apo pyoverdine binds more efficiently to FpvF than to FpvC (Brillet et al. 2012). How FpvC and FpvF contribute to the ferripyoverdine dissociation process has not been elucidated. Does the binding of these two PBP to the ferrisiderophore complex shift the redox potential of the ferripyoverdine complex to a more positive value or does FpvC provide a thermodynamic driving force for reduction through the formation of a highly stable FpvC-Fe(II) complex? These questions remain unresolved and further studies are necessary to elucidate this mechanism and identify the reductase.

The pyoverdine pathway is the only described example of iron uptake via siderophores involving iron release in the bacterial periplasm, and not in the cytoplasm, followed by transport of siderophore-free iron across the inner membrane. It would be interesting to determine whether similar mechanisms are present in other pathways.

The cytoplasmic iron pool

Iron release from siderophores mostly involves an iron reduction step. The iron used in the bacterial cytoplasm is mostly in the form of Fe(II) (ferrous iron), for example for incorporation into iron-requiring enzymes and incorporation into ferritins (iron storage proteins). However, this iron form is also Fenton active and thus deleterious. Therefore, it is likely that the ferrous iron after dissociation from the siderophore is bound by a chaperone or other Fe(II) chelator for sheltered intracellular transfer of the ion to the storage protein ferritin or to other metalloproteins. What compounds are available to coordinate cytoplasmic Fe^{2+}? An oligomeric sugar phosphate has been identified as being able to bind iron intracellularly released from ferrichrome or ferrioxamine in *E. coli* (Bohnke and Matzanke 1995) and may serve as a Fe(II) chaperone in the bacterial cytoplasm. Citrate also binds Fe(II) under cytoplasmic conditions, and Fe(II)-citrate has been suggested to be a major component of the labile iron cytoplasmic pool (Morley and Bezkorovainy 1983). However, Fe(II)-citrate is susceptible to autoxidation at pH 7.0 (Harris and Aisen 1973) and therefore it is likely that there is another ligand capable of coordinating Fe(II) in the cytoplasm. Glutathione is a candidate: it is widely distributed in the bacterial cytoplasm, and out-competes citrate for Fe(II) binding at pH 7.0 (Hider and Kong 2011b). Moreover, Fe(III) is rapidly reduced to Fe(II) in the presence of glutathione (Hamed et al. 1983), such that although the auto-oxidation of cytoplasmic Fe(II)-glutathione will occur at a slow rate, the resulting Fe(III) will be rapidly reduced back to Fe(II) (Hider and Kong 2011b). Therefore, Fe(II)-glutathione may be the most abundant form of iron in the bacterial cytoplasm (Hider and Kong 2011b).

Conclusion

For many years, the three iron uptake pathways in *E. coli* (ferrichrome, enterobactin, and citrate) were the dominant models in the field, and it was believed that the molecular mechanisms involved in these pathways would apply to all siderophores in all Gram-negative bacteria. However, in recent years, three pathways in *P. aeruginosa* (pyoverdine, pyochelin and ferrichrome), the yersiniabactin pathway in *Yersinia pestis*, and vibriobactin in *Vibrio cholera* have also been investigated in detail. The findings reveal uptake across the outer membrane by TBDT in all cases, but diverse molecular mechanisms in the downstream steps of iron release from the siderophore, with differences depending on the nature of the siderophore and the bacterial species. Analysis of numerous bacterial genomes suggests that the mechanisms at play are probably even more diverse. For example, the *Nitrosomonas europaea* genome contains 42 ferrisiderophore TBDT genes (Chain et al. 2003) indicating that this bacterium may use a large number of siderophores. Despite the plethora of TBDTs in *N. europaea*, genome annotation revealed only one set of three genes that were homologues of PBP-dependent ABC transporter systems. This single ABC transporter system may serve as the convergence point for transport of iron or ferrisiderophores across the cytoplasmic membrane. Either iron is released

from the siderophores in the bacterial periplasm of *N. europae* with only ferrous iron being imported into the cytoplasm by this single ABC transporter, or this transporter has a PBP recognizing a very wide range of siderophores. During evolution, bacteria have developed a large number of siderophore pathways using chelators with very different chemical structures, each requiring a corresponding TBDT for translocation across the outer membrane. It is entirely plausible that this is associated with a large diversity of molecular mechanisms for both the transport of iron by siderophores across the cytoplasmic membrane and ferrisiderophore dissociation. Indeed, these numerous siderophores produced by microorganisms have diverse chemical structures, various affinities and stoichiometries for iron chelation, and different redox potentials, consistent with there being numerous strategies involving different enzymes for extracting the metal. In this context, it is important to investigate iron acquisition by large numbers of disparate siderophores and by diverse bacteria if we are to get a full picture of all the strategies deployed by microorganisms to acquire iron.

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References

Abergel RJ, Zawadzka AM, Hoette TM, Raymond KN (2009) Enzymatic hydrolysis of trilactone siderophores: where chiral recognition occurs in enterobactin and bacillibactin iron transport. J Am Chem Soc 131:12682–12692. doi:10.1021/ja903051q

Albrecht-Gary AM, Crumbliss AL (1998) Coordination chemistry of siderophores: thermodynamics and kinetics of iron chelation and release. Metal Ions Biological Systems 35:239–327

Barchini E, Cowart RE (1996) Extracellular iron reductase activity produced by *Listeria monocytogenes*. Arch Microbiol 166:51–57

Blanvillain S, Meyer D, Boulanger A, Lautier M, Guynet C, Denance N et al (2007) Plant carbohydrate scavenging through tonb-dependent receptors: a feature shared by phytopathogenic and aquatic bacteria. PLoS ONE 2:e224

Bohnke R, Matzanke BF (1995) The mobile ferrous iron pool in *Escherichia coli* is bound to a phosphorylated sugar derivative. Biometals 8:223–230

Borths EL, Locher KP, Lee AT, Rees DC (2002) The structure of *Escherichia coli* BtuF and binding to its cognate ATP binding cassette transporter. Proc Natl Acad Sci USA 99:16642–16647

Braud A, Hannauer M, Mislin GLA, Schalk IJ (2009a) The *Pseudomonas aeruginosa* pyochelin-iron uptake pathway and its metal specificity. J Bacteriol 191:5317–5325. doi:10.1128/JB.00010-09

Braud A, Hoegy F, Jezequel K, Lebeau T, Schalk IJ (2009b) New insights into the metal specificity of the *Pseudomonas aeruginosa* pyoverdine-iron uptake pathway. Environ Microbiol 11:1079–1091. doi:10.1111/j.1462-2920.2008.01838.x

Braun V, Hantke K, Koster W (1998) Bacterial iron transport: mechanisms, genetics, and regulation. Met Ions Biol Syst 35:67–145

Brickman TJ, McIntosh MA (1992) Overexpression and purification of ferric enterobactin esterase from *Escherichia coli*. Demonstration of enzymatic hydrolysis of enterobactin and its iron complex. J Biol Chem 267:12350–12355

Brillet K, Reimmann C, Mislin GLA, Noël S, Rognan D, Schalk IJ, Cobessi D (2011) Pyochelin enantiomers and their outer membrane siderophore transporters in fluorescent Pseudomonads: structural bases of a unique enantiospecific recognition. J Am Chem Soc 133:16503–16509. doi:10.1021/ja205504z

Brillet K, Ruffenach F, Adams H, Journet L, Gasser V, Hoegy F et al (2012) An ABC transporter with two periplasmic binding proteins involved in iron acquisition in *Pseudomonas aeruginosa*. ACS Chem Biol, in press

Butterton JR, Calderwood SB (1994) Identification, cloning, and sequencing of a gene required for ferric vibriobactin utilization by *Vibrio cholerae*. J Bacteriol 176:5631–5638

Capela D, Barloy-Hubler F, Gouzy J, Bothe G, Ampe F, Batut J et al (2001) Analysis of the chromosome sequence of the legume symbiont *Sinorhizobium meliloti* strain 1021. Proc Natl Acad Sci USA 98:9877–9882

Chain P, Lamerdin J, Larimer F, Regala W, Lao V, Land M et al (2003) Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. J Bacteriol 185:2759–2773

Cheng Q, Park JT (2002) Substrate specificity of the AmpG permease required for recycling of cell wall anhydro-muropeptides. J Bacteriol 184:6434–6436

Chu BC, Vogel HJ (2011) A structural and functional analysis of type III periplasmic and substrate binding proteins: their role in bacterial siderophore and heme transport. Biol Chem 392:39–52. doi:10.1515/BC.2011.012

Clarke TE, Ku SY, Dougan DR, Vogel HJ, Tari LW (2000) The structure of the ferric siderophore binding protein FhuD complexed with gallichrome. Nat Struct Biol 7:287–291

Clarke TE, Braun V, Winkelmann G, Tari LW, Vogel HJ (2002) X-ray crystallographic structures of the *Escherichia coli* periplasmic protein FhuD bound to hydroxamate-type siderophores and the antibiotic albomycin. J Biol Chem 277:13966–13972

Creutz C (1981) The complexities of ascorbate as a reducing agent. Inorg Chem 20:4449–4452

Cuiv PO, Clarke P, Lynch D, O'Connell M (2004) Identification of *rhtX* and *fptX*, novel genes encoding proteins that show homology and function in the utilization of the siderophores rhizobactin 1021 by *Sinorhizobium meliloti* and pyochelin by *Pseudomonas aeruginosa*, respectively. J Bacteriol 186:2996–3005

Dertz EA, Xu J, Stintzi A, Raymond KN (2006) Bacillibactin-mediated iron transport in *Bacillus subtilis*. J Am Chem Soc 128:22–23

Dhungana S, Anderson DS, Mietzner TA, Crumbliss AL (2005) Kinetics of iron release from ferric binding protein (FbpA): mechanistic implications in bacterial periplasm-to-cytosol Fe^{3+} transport. Biochemistry 44:9606–9618

Elkins MF, Earhart CF (1989) Nucleotide sequence and regulation of the *Escherichia coli* gene for ferrienterobactin transport protein FepB. J Bacteriol 171:5443–5451

Fairman JW, Noinaj N, Buchanan SK (2011) The structural biology of beta-barrel membrane proteins: a summary of recent reports. Curr Opin Struct Biol 21:523–531. doi:10.1016/j.sbi.2011.05.005

Ferguson AD, Braun V, Fiedler HP, Coulton JW, Diederichs K, Welte W (2000) Crystal structure of the antibiotic albomycin in complex with the outer membrane transporter FhuA. Protein Sci 9:956–963

Fetherston JD, Bertolino VJ, Perry RD (1999) YbtP and YbtQ: two ABC transporters required for iron uptake in *Yersinia pestis*. Mol Microbiol 32:289–299

Fuchs R, Schafer M, Geoffroy V, Meyer JM (2001) Siderotyping: a powerful tool for the characterization of pyoverdines. Curr Top Med Chem 1:31–57

Greenwald J, Hoegy F, Nader M, Journet L, Mislin GLA, Graumann PL, Schalk IJ (2007) Real-time FRET visualization of ferric-pyoverdine uptake in *Pseudomonas aeruginosa*: a role for ferrous iron. J Biol Chem 282:2987–2995

Greenwald J, Nader M, Celia H, Gruffaz C, Geoffroy V, Meyer JM et al (2009) FpvA bound to non-cognate pyoverdines: molecular basis of siderophore recognition by an iron transporter. Mol Microbiol 72:1246–1259

Hamed MY, Silver J, Wilson MT (1983) Studies of the reactions of ferric iron with gluthatione and some related thiols. Inorg Chem Acta 78:1–11

Hannauer M, Barda Y, Mislin GL, Shanzer A, Schalk IJ (2010) The ferrichrome uptake pathway in *Pseudomonas aeruginosa* involves an iron release mechansim with acylation of the siderophore and a recycling of the modified desferrichrome. J Bacteriol 192:1212–1220. doi:10.1128/JB.01539-09

Hannauer M, Braud A, Hoegy F, Ronot P, Boos A, Schalk IJ (2012) The PvdRT-OpmQ efflux pump controls the metal selectivity of the iron uptake pathway mediated by the siderophore pyoverdine in *Pseudomonas aeruginosa*. Environ Microbiol 14:1696–1708. doi:10.1111/j.1462-2920.2011.02674

Hantke K, Nicholson G, Rabsch W, Winkelmann G (2003) Salmochelins, siderophores of Salmonella enterica and uropathogenic *Escherichia coli* strains, are recognized by the outer membrane receptor IroN. Proc Natl Acad Sci USA 100:3677–3682

Harrington JM, Crumbliss AL (2009) The redox hypothesis in siderophore-mediated iron uptake. Biometals 22:679–689. doi:10.1007/s10534-009-9233-4

Harris DC, Aisen P (1973) Facilitation of Fe(II) autoxidation by Fe(II) complexing agents. Biochim Biophys Acta 329:156–158

Hartman A, Braun V (1980) Iron transport in *Escherichia coli*: uptake and modification of ferrichrome. J Bacteriol 143:246–255

Hider RC, Kong X (2011a) Chemistry and biology of siderophores. Nat Prod Rep 27:637–657. doi:10.1039/b906679a

Hider RC, Kong XL (2011b) Glutathione: a key component of the cytoplasmic labile iron pool. Biometals 24:1179–1187. doi:10.1007/s10534-011-9476-8

Hoegy F, Lee X, Noël S, Mislin GL, Rognan D, Reimmann C, Schalk IJ (2009) Stereospecificity of the siderophore pyochelin outer membrane transporters in fluorescent Pseudomonads. J Biol Chem 284:14949–14957. doi:10.1074/jbc.M900606200

Hoegy F, Gwynn MN, Schalk IJ (2010) Susceptibility of *Pseudomonas aeruginosa* to catechol-substituted cephalosporin is unrelated to the pyochelin-Fe transporter FptA. Amino Acids 38:1627–1629. doi:10.1007/s00726-009-0353-5

Hvorup RN, Goetz BA, Niederer M, Hollenstein K, Perozo E, Locher KP (2007) Asymmetry in the structure of the ABC transporter-binding protein complex BtuCD-BtuF. Science 317:1387–1390

Imperi F, Tiburzi F, Visca P (2009) Molecular basis of pyoverdine siderophore recycling in *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 106:20440–20445. doi:10.1073/pnas.0908760106

Jacobs C, Huang LJ, Bartowsky E, Normark S, Park JT (1994) Bacterial cell wall recycling provides cytosolic muropeptides as effectors for beta-lactamase induction. EMBO J 13:4684–4694

Krewulak KD, Vogel HJ (2008) Structural biology of bacterial iron uptake. Biochim Biophys Acta 1778:1781–1804

Lee W, van Baalen M, Jansen VA (2012) An evolutionary mechanism for diversity in siderophore-producing bacteria. Ecol Lett 15:119–125. doi:10.1111/j.1461-0248.2011.01717

Lin H, Fischbach MA, Liu DR, Walsh CT (2005) In vitro characterization of salmochelin and enterobactin trilactone hydrolases IroD, IroE, and Fes. J Am Chem Soc 127:11075–11084

Llamas MA, Sparrius M, Kloet R, Jimenez CR, Vandenbroucke-Grauls C, Bitter W (2006) The heterologous siderophores ferrioxamine B and ferrichrome activate signaling pathways in *Pseudomonas aeruginosa*. J Bacteriol 188:1882–1891

Locher KP, Lee AT, Rees DC (2002) The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism. Science 296:1793–1800

Loomis L, Raymond KN (1991) Solution equilibria of enterobactin complexes. Inorg Chem 30:906–911

Mademidis A, Koster W (1998) Transport activity of FhuA, FhuC, FhuD, and FhuB derivatives in a system free of polar effects, and stoichiometry of components involved in ferrichrome uptake. Mol Gen Genet 258:156–165

Mademidis A, Killmann H, Kraas W, Flechsler I, Jung G, Braun V et al (1997) ATP-dependent ferric hydroxamate transport system in *Escherichia coli*: periplasmic FhuD interacts with a periplasmic and with a transmembrane/cytoplasmic region of the integral membrane protein FhuB, as revealed by competitive peptide mapping. Mol Microbiol 26:1109–1123

Matzanke BF, Anemuller S, Schunemann V, Trautwein AX, Hantke K (2004) FhuF, part of a siderophore-reductase system. Biochemistry 43:1386–1392

Mey AR, Wyckoff EE, Oglesby AG, Rab E, Taylor RK, Payne SM (2002) Identification of the *Vibrio cholerae* enterobactin receptors VctA and IrgA: IrgA is not required for virulence. Infect Immun 70:3419–3426

Michel L, Bachelard A, Reimmann C (2007) Ferripyochelin uptake genes are involved in pyochelin-mediated signalling in *Pseudomonas aeruginosa*. Microbiology 153:1508–1518

Mies KA, Wirgau JI, Crumbliss AL (2006) Ternary complex formation facilitates a redox mechanism for iron release from a siderophore. Biometals 19:115–126

Miethke M, Marahiel MA (2007) Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev 71:413–451

Miethke M, Klotz O, Linne U, May JJ, Beckering CL, Marahiel MA (2006) Ferri-bacillibactin uptake and hydrolysis in *Bacillus subtilis*. Mol Microbiol 61:1413–1427

Miethke M, Hou J, Marahiel MA (2011) The siderophore-interacting protein YqjH acts as a ferric reductase in different iron assimilation pathways of *Escherichia coli*. Biochemistry 50:10951–10964. doi:10.1021/bi201517h

Millis KK, Weaver KH, Rabenstein DL (1993) Oxidation/reduction potential of glutathione. J Org Chem 58:4144–4146

Mislin GLA, Hoegy F, Cobessi D, Poole K, Rognan D, Schalk IJ (2006) Binding properties of pyochelin and structurally related molecules to FptA of *Pseudomonas aeruginosa*. J Mol Biol 357:1437–1448

Morley CG, Bezkorovainy A (1983) Identification of the iron chelate in hepatocyte cytosol. IRCS Med Sci 11:1106–1107

Muller A, Wilkinson AJ, Wilson KS, Duhme-Klair AK (2006) An [{Fe(mecam)}2]6- bridge in the crystal structure of a ferric enterobactin binding protein. Angew Chem Int Ed Engl 45:5132–5136

Perry RD, Fetherston JD (2011) Yersiniabactin iron uptake: mechanisms and role in *Yersinia pestis* pathogenesis. Microbes Infect 13:808–817. doi:10.1016/j.micinf.2011.04.008

Poole K, McKay GA (2003) Iron acquisition and its control in *Pseudomonas aeruginosa*: many roads lead to Rome. Front Biosci 8:d661–d686

Raymond KN, Dertz EA, Kim SS (2003) Enterobactin: an archetype for microbial iron transport. Proc Natl Acad Sci USA 100:3584–3588

Reimmann C (2012) Inner-membrane transporters for the siderophores pyochelin in *Pseudomonas aeruginosa* and enantio-pyochelin in *Pseudomonas fluorescens* display different enantioselectivities. Microbiology 158:1317–1324. doi:10.1099/mic.0.057430-0

Schalk IJ (2008) Metal trafficking via siderophores in Gram-negative bacteria: specificities and characteristics of the pyoverdine pathway. J Inorg Biochemi 102:1159–1169. doi:10.1016/j.jinorgbio.2007.11.017

Schalk IJ (2013) Innovation and originalities in the strategies developed by bacteria to get access to iron. Chembiochem 14:293–294. doi:10.1002/cbic.201200738

Schalk IJ, Abdallah MA, Pattus F (2002) Recycling of pyoverdin on the FpvA receptor after ferric pyoverdin uptake and dissociation in *Pseudomonas aeruginosa*. Biochemistry 41:1663–1671

Schalk IJ, Hannauer M, Braud A (2011) New roles for bacterial siderophores in metal transport and tolerance. Environ Microbiol 13:2844–2854. doi:10.1111/j.1462-2920.2011.02556

Schalk IJ, Mislin GL, Brillet K (2012) Structure, function and binding selectivity and stereoselectivity of siderophore-iron outer membrane transporters. Curr Top Membr 69:37–66. doi:10.1016/B978-0-12-394390-3.00002-1

Schauer K, Rodionov DA, de Reuse H (2008) New substrates for TonB-dependent transport: do we only see the 'tip of the iceberg'? Trends Biochem Sci 33:330–338. doi:10.1016/j.tibs.2008.04.012

Shea CM, McIntosh MA (1991) Nucleotide sequence and genetic organization of the ferric enterobactin transport system: homology to other periplasmic binding protein-dependent systems in *Escherichia coli*. Mol Microbiol 5:1415–1428

Spasojevic I, Boukhalfa H, Stevens RD, Crumbliss AL (2001) Aqueous solution speciation of Fe(III) complexes with dihydroxamate siderophores alcaligin and rhodotorulic acid and synthetic analogues using electrospray ionization mass spectrometry. Inorg Chem 40:49–58

Stephens DL, Choe MD, Earhart CF (1995) *Escherichia coli* periplasmic protein FepB binds ferrienterobactin. Microbiology 141(Pt 7):1647–1654

Williams KE, Yandel JK (1982) Outer-sphere electron-transfer reactions of ascorbate anions. Aust J Chem 35:1133–1144

Woo JS, Zeltina A, Goetz BA, Locher KP (2012) X-ray structure of the *Yersinia pestis* heme transporter HmuUV. Nat Struct Mol Biol. doi:10.1038/nsmb.2417

Wyckoff EE, Payne SM (2011) The Vibrio cholerae VctPDGC system transports catechol siderophores and a siderophore-free iron ligand. Mol Microbiol 81:1446–1458

Wyckoff EE, Mey AR, Payne SM (2007) Iron acquisition in *Vibrio cholerae*. Biometals 20:405–416

Yeterian E, Martin LW, Lamont IL, Schalk IJ (2010) An efflux pump is required for siderophore recycling by *Pseudomonas aeruginosa*. Environ Microbiol Report 2:412–418. doi:10.1016/j.febslet.2010.10.051

Youard ZA, Mislin GL, Majcherczyk PA, Schalk IJ, Reimmann C (2007) *Pseudomonas fluorescens* CHA0 produces enantio-pyochelin, the optical antipode of the *Pseudomonas aeruginosa* siderophore pyochelin. J Biol Chem 282:35546–35553

Zhu M, Valdebenito M, Winkelmann G, Hantke K (2005) Functions of the siderophore esterases IroD and IroE in iron-salmochelin utilization. Microbiology 151:2363–2372